Critical Issues in Head and Neck Oncology

Key Concepts from the Sixth THNO Meeting

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Preface

The sixth Trends in Head and Neck Oncology (THNO-6) took place in the Meridien Hotel in Nice, France, November 2–4, 2017, and was organized by the same coordinating team as the fifth version with support of Pharma and practical logistical support of Congress Care. This time, the conference was endorsed by the European Head and Neck Society (EHNS) and the European Organization for Research and Treatment of Cancer (EORTC). As on previous occasions, the setup was educational, with a multidisciplinary focus. Case presentations, organized by colleagues from the Centre Antoine Lacassagne in Nice and some members of the coordinating team, induced a lively interaction between faculty and audience and underlined the importance of individualized patient care. Thanks to the dedication of all faculty members this book will be available within a year following the actual meeting, guaranteeing the most up-to-date information in this rapidly evolving field. We are most grateful to all faculty members for their efforts in realizing this important goal.

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Johannes Berkhof

Introduction

Human papillomavirus (HPV) is the main cause of cervical cancer and also causes a substantial number of cancers at other sites. It was recently estimated that approximately 29,000 oropharyngeal cancers and 8000 oral cavity and larynx cancers, occurring globally in year 2012, could be attributed to HPV and that about 80% of HPV-related head and neck cancer cases occurred in men [1]. Besides, an upward surge in HPV-associated oropharyngeal cancer has been observed in the United States (US) and some European countries in the last years, in particular in males [2–5]. US projections indicate that in 2020, oropharyngeal cancer will occur more frequently than cervical cancer [2]. The disproportionate burden and rising incidence of HPV-associated head and neck cancers in men has ignited discussion on the vaccination of boys. So far, most countries with a publicly funded HPV vaccination programme have targeted girls only since the main focus is on prevention of cervical cancer. The HPV-related burden in men is nowadays being recognized but a long-standing debate exists on whether there is sufficient evidence on the effects of the vaccine against cancer in men and whether the effects are large enough to justify the extra costs of vaccinating boys. In the following, I give an overview of the current evidence on the efficacy and expected impact of HPV vaccination in men and women with a focus on oropharyngeal cancer.

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HPV Vaccines

There are three HPV vaccines on the market registered for use from the age of 9 years. The vaccines are licensed for the prevention of lesions in the cervix, vulva, vagina, and anus, but not for the prevention of head and neck cancers. Cervarix® (GSK) is a bivalent vaccine that protects against HPV16 and HPV18 infections and also provides some cross-protection against a few other oncogenic HPV types [6–8]. Cervarix is registered for females and males in Europe and only for females in the US. Gardasil® (Merck & Co) is a quadrivalent vaccine that protects against HPV16 and HPV18 and also protects against HPV6 and HPV11, responsible for most cases of genital warts and recurrent respiratory papillomatosis. Gardasil is registered for females and males in both Europe and the US. Since 2016, a nonavalent vaccine Gardasil9® (Merck & Co) has become available with the main purpose to offer improved protection against cervical cancer and high-grade cervical dysplasia. For head and neck cancer prevention, the additional benefit of a nonavalent vaccine as compared to a bivalent or quadrivalent vaccine is limited as HPV16 accounts for about 80% of the HPV DNA positive cases and for about 90% of HPV DNA positive oropharyngeal cancers [9].

Early End-Points

The main reason that the current HPV vaccines are not licensed for the prevention of head and neck cancers is that clinical trials were only able to show an effect against cervical and other anogenital premalignant lesions [10–14]. Unlike anogenital cancers, HPV-positive head and neck cancers have no clearly visible premalignant end-point thus their histopathological progression remains poorly defined [15]. Moreover, the mean age of HPV-positive oropharyngeal cancer is above 50 years [16] and this means that if regulatory bodies demand a significant effect on cancer incidences from trials targeting adolescents and young adults, vaccine licensure against oropharyngeal cancer will be postponed for another three to four decades.

The only alternative to showing an effect on dysplasia is to measure oral HPV infections. However, establishing a link from oral HPV infection to cancer and precancer is hard, if not impossible. Studies will never be large enough to show an association between oral infection and invasive cancer. Furthermore, establishing a link between oral infection and subclinical dysplasia in healthy subjects seems ethically unfeasible. Nevertheless, case-control studies have provided strong support that HPV exposure is necessary for HPV-positive oropharyngeal cancer [16, 17] and it is widely accepted that HPV-positive head and neck cancers cannot develop without a preceding HPV infection.

The effect of HPV vaccination on the occurrence of oral infections has recently been studied in two populations. Participants in those studies were asked to collect rinse and gargle samples using a mouthwash. The first population consisted of women participating in a randomized trial with the bivalent vaccine [18]. The use of

randomization has the advantage that it minimizes bias related to demographic differences between the vaccine and the control arm. The effect of vaccination on oral vaccine-type HPV infections was estimated at 93% (1/2910 in vaccinated women versus 15/2924 in unvaccinated women). The second population was the National Health and Nutrition Examination Survey (NHANES), a representative subset of the US population. Two cross-sectional analyses on NHANES indicated that the occurrence of oral quadrivalent vaccine-type HPV infections was about 90% lower in vaccinated as compared to unvaccinated men and women [19, 20]. Limitations of the NHANES population are that vaccine status is self-reported and that subjects are not randomized with respect to vaccination. Regarding the latter, vaccine-associated effects were robust against confounders such as age, sex, sexual behaviour, smoking, and race [20]. The decision to get vaccinated may have been influenced by factors that were not measured, but it is unlikely that only unobserved confounders were responsible for the strong association between vaccination and oral infections. In another recent study, HPV16 and HPV18 specific antibodies in the oral mucosa of adult males were induced by vaccination, but the study was not able to demonstrate whether the antibody levels were sufficient to offer protection against incident infections [21]. To conclude, the current evidence on the effect of vaccines on infections and vaccine-induced antibodies in the oral region seems sufficient to include oropharyngeal cancer in the impact and cost-effectiveness assessments of vaccination strategies, but for vaccine licensure there is a need for more data on the effect of vaccination on infections and antibodies in the oral region.

Herd Effects

Most HPV vaccination programmes target girls because women experience the greatest HPV-related disease burden. Of all 630,000 new HPV-related cancers worldwide in 2016, 570,000 cases occurred in women [1]. Nevertheless, exclusion of boys from the programme has raised equity concerns because HPV-related cancers occur in, both, women and men. A widely used argument against sex-neutral vaccination is that vaccination of girls confers indirect protective effects or herd effects to men. This means that heterosexual men would be protected against HPV-associated diseases if the coverage of the girls' only vaccination programme is high. The required coverage level of a girls' only programme is, however, difficult to assess because herd effects depend on sexual network features and natural immunity after viral clearance [22].

For estimating herd effects, we usually rely on mathematical HPV infection models that describe the transmission of HPV in sexual networks. HPV infection models require many assumptions and can have a different architecture leading to uncertain and potentially inconsistent results. To study whether predictions provided by independent models were consistent, in a recent study, sixteen independent modelling teams provided estimates of the reduction in HPV16 and HPV18 under different vaccine coverage scenarios [23]. The results from the modelling teams were strikingly consistent despite the fact that models were developed in different

settings and calibrated to different data. A main result was that at 80% coverage of a girls' only programme, the HPV16 prevalence would decrease by 93% in women and by 83% in men. If both girls and boys were vaccinated with a coverage of 80%, HPV16 would virtually be eliminated from the population in most models. At a coverage of 60% among girls and boys, HPV16 would be reduced by 90%. Since the majority of immunization programmes shows coverage levels between 50 and 80%, sex-neutral vaccination is expected to reduce HPV16 and HPV18 prevalence to a very low level. Two important limitations of the models are that they only consider heterosexual networks and do not take differences in site-specific transmission into account. Those limitations are not likely to change the general message: sex-neutral vaccination can be important for reducing the prevalence of HPV to a very low level when a girls' only programme fails to achieve a coverage similar to those observed for paediatric vaccines.

A number of studies have emerged that aim to measure herd effects in real life data. In an Australian study on men attending a sexual clinic after a positive test for *Chlamydia trachomatis* [24], a significant reduction in the prevalence of the HPV types targeted by the quadrivalent vaccine from 18 to 7% was observed in Australian-born men before and after the start of the vaccination programme. In the last three calendar years of the study (2013–2015), the prevalence of the HPV types targeted by the vaccine was only 3%. As expected, no decrease in prevalence was observed for the HPV types that were not targeted by the vaccine. Another interesting study is a Finnish randomized trial where communities were either randomized to girls' only or sex-neutral vaccination with the bivalent vaccine [25]. A herd effect for HPV18 in cervical samples was observed in both study arms. A herd effect was not observed for HPV16 which may be related to the low vaccine coverage of 20% among boys and 45% among girls attending junior high school. The larger herd effect for HPV18 as compared to HPV16 in the Finnish trial concurs with intuition because HPV16 has a higher basic reproductive number than other HPV types [26, 27]. This means that a subject infected with HPV16 infects on average a larger number of susceptible subjects than a subject infected with another HPV type and hence it becomes more difficult to eliminate HPV16 from the population.

In a few years, it will be possible to measure herd effects in nationwide cervical cancer screening registries provided they are linked to vaccination registries. This information will be very important when developing cervical cancer screening algorithms for vaccinated cohorts, but its value for head and neck cancer prevention will be limited because herd effects observed in cervical cancer screening are expected to be different from herd effects in future head and neck cancers. The difference will be most pronounced in countries with a girls' only vaccination programme. Then, herd effects in unvaccinated women will be second-order indirect effects occurring because men have a lower probability of infecting unvaccinated women since they themselves will be indirectly protected by the girls' only vaccination programme. Therefore, mathematical models will still be needed to estimate the reduction of HPV infections in men and to facilitate decision-making on sex-neutral vaccination.

The Effect of Vaccinating Boys on Cancer in Men

Although herd effects are important to reduce HPV infections in the general population, the question remains whether vaccination of men would contribute sufficiently to the prevention of cancer in men to justify a sex-neutral vaccination programme. In a Dutch evidence synthesis study conducted in 2015 [28], the effect of vaccinating boys on future cancers in men was calculated. The cancers considered were cancers of the penis, anus, and anal canal, and squamous cell carcinomas of the oropharynx, including the base of tongue and tonsils (international classification of diseases 10th revision code C60, C21, and C01, C09 and C10). HPV aetiological fractions for the different tumour sites were obtained from several sources [29–31] and elevated cancer risks in homosexual and bisexual men (men having sex with men; MSM) as compared to heterosexual men were taken into account [32]. HPV-associated oral cavity and larynx carcinoma were not considered in this study because their burden is low relative to that of HPV-related oropharynx cancer [1]. The herd effects in men achieved when vaccinating girls only were estimated by a mathematical HPV transmission model [33]. The transmission model predicted that a 10% reduction of HPV16 or HPV18 among women would induce an 8% reduction of HPV16 or HPV18 among men. After taking these herd effects into account, the conclusion of the evidence synthesis study [28] was that vaccination of boys would still confer a substantial reduction in future cancer in men. At 60% vaccine coverage among girls, about 800 boys would need to be vaccinated to prevent an additional future cancer in men. Tumour site specific numbers were about 2000 boys for oropharyngeal cancer and anal cancer and 3500 for penile cancer. When the coverage in girls was increased to 90%, tumour-specific numbers were about 6500 boys for oropharyngeal cancer, 2600 for anal cancer, and nearly 30,000 for penile cancer. In the latter situation, the majority of the cancers prevented by vaccinating boys were anal cancers, which underscores the relevance of HPV vaccination for cancer prevention in MSM.

In a country with a girls' only vaccination programme and a coverage of 90%, sex-neutral vaccination can still be motivated as a strategy to prevent cancer in MSM, but targeted vaccination of adolescent and adult MSM has also been suggested. Targeted MSM vaccination is less costly than sex-neutral vaccination, but it is not effective in the subset of the HPV-positive MSM. Considering that HPV infections occur soon after the initiation of sexual debut, concerns can be raised with respect to the effectiveness of strategies for early identification of sexually naïve MSMs. Nonetheless, a modelling study indicated that targeted MSM vaccination may be cost-effective up to the age of 40 [34]. It is also important to understand that targeted MSM vaccination does not preclude sex-neutral vaccination and vice versa. After implementation of sex-neutral vaccination, targeted MSM vaccination may still be used as a catch-up for older age groups and as an option for MSM who spent their childhood in a different country.

Vaccination Coverage and Programme Resilience

Several modellers have pointed out that even when the coverage of a girls' only vaccination programme is low, it is more efficient to increase the uptake among girls than to vaccinate boys in order to reduce the HPV prevalence in the general population [35, 36]. This argument supports prioritization of efforts to increase the uptake among girls, but it is uncertain whether such efforts would be successful. So far, HPV vaccination programmes in most countries have achieved a coverage far below the 90% target level for paediatric vaccines. A main reason for the limited coverage among girls is that there are recurring concerns about vaccine safety and side effects [37, 38]. An alarming example is the HPV vaccination programme in Denmark where the vaccine coverage decreased from about 80 to 20% in 2015 as a result of an alleged association between HPV vaccine and Postural Orthostatic Tachycardia Syndrome (POTS) [39]. To assess whether these concerns are supported by data, the European Medicines Agency (EMA; www.ema.europa.eu) conducted a large study on the incidence of POTS and Complex Regional Pain Syndrome (CRPS). The EMA compared approximately 60,000 women vaccinated with Gardasil and 40,000 women vaccinated with Cervarix with placebo cohorts but did not find a significant association between adverse events and vaccination status. Besides, the POTS cases in Denmark were mainly observed in one centre suggesting considerable heterogeneity in the diagnosis of POTS.

The results from the EMA are reassuring, but the Danish example clearly indicates that HPV vaccination programmes are vulnerable. The sudden sharp decline in vaccine coverage that has happened in Denmark may happen in any other country as well. Sex-neutral vaccination has been suggested to make programmes more resilient against temporary changes in the vaccination coverage. A recent modelling study predicted that if the vaccine coverage was halved for a period of 5 years, then a sex-neutral vaccination would be about 12-fold more resilient than girls' only vaccination in terms of the percentage reduction in HPV prevalence in the female population [40]. Therefore, as long as HPV vaccine coverage is unpredictable, sex-neutral vaccination may be implemented to stabilize the impact of the programme against temporary variations in coverage.

Economic Considerations

So far, cost-effectiveness studies on sex-neutral vaccination have not yielded consistent results. Although some studies were positive, most studies recommended against sex-neutral vaccination [41]. An explanation for this finding is that some economic studies did not consider all non-cervical health outcomes in their main analysis. In a recent review, it was calculated that the standard measure in cost-effectiveness studies, that is the incremental cost-effectiveness ratio, would decrease 3.9-fold if all non-cervical disease had been taken into account, including oropharyngeal cancer and genital warts, as compared to cervical cancer only [41]. Of

course, negative results in cost-effectiveness studies are also strongly driven by the HPV transmission dynamics. A Canadian study illustrated that when herd effects from a girls' only programme are ignored, vaccination of boys is cost-effective even when only prevention of oropharyngeal cancer in men is considered [42]. Another commonly mentioned obstacle for sex-neutral vaccination is the high list price of the HPV vaccine. Sex-neutral vaccination is unlikely to be cost-effective at the current list price of the vaccine which varies between 100 and 160 euros per dose in high-income countries. For the costs of vaccination, however, a widely used containment strategy is tendering: health authorities use their purchasing power and the competition in the market of the vaccines to perform procurement procedures. This drives down the vaccine cost and enhances the sustainability of a programme. Experience with hepatitis B vaccines suggests that tendering may lead to strong price reductions over time [43]. Besides, in several Italian regions, tender-based HPV vaccine prices in the first 2 years after the vaccine became available were about 50% lower than the list price [44].

In a recent Dutch cost-effectiveness study [45], in which tender-based vaccine costs were set at about 65 euros for a 2 dose schedule and effects on cervical, vulvar, anal, penile, and oropharyngeal cancers were included, it was calculated that sex-neutral vaccination was cost-effective even when the coverage among girls increased up to 90%. Favourable cost-effectiveness results were also obtained in studies evaluating the vaccination of boys in the Norwegian programme and in the Italian programme when tender vaccine prices were used instead of list prices [46, 47]. All three studies used local input and therefore conclusions do not have to apply to all high-resource settings. However, altogether these findings at least suggest that countries should re-evaluate their economic argument for adopting a girls' only vaccination programme, preferably together with an analysis accounting for country-specific disease burden, country-specific and often tender-based vaccine price, achieved vaccine coverage in the girls' only programme, and the cost of administering vaccines.

Conclusions

The decision to switch from girls only vaccination to sex-neutral vaccination is more difficult to take than the decision to implement a girls' only vaccination programme. This is reflected in the speed at which decisions are taken. About 10 years ago, many countries implemented girls' only vaccination within 1 or 2 years after registration of the vaccine, but only a few of them have switched to sex-neutral vaccination in the meantime. A main argument used to support sex-neutral vaccination is that the burden of oropharyngeal cancer is disproportionate in men, but heterosexual men also benefit from the girls' only programme via herd effects. To facilitate decision-making, mathematical models have been used to assess the additional benefit and cost-effectiveness of vaccinating boys. With respect to sex-neutral vaccination, four conclusions from the models in the literature were that: (1)

sex-neutral vaccination may lead to near elimination of HPV16 and HPV18 when coverage levels are about 80%, (2) a girls' vaccination programme lowers the risk of cancer in men but sex-neutral vaccination still provides substantial extra protection against oropharyngeal and anal cancer when the coverage among girls is moderate, (3) sex-neutral vaccination makes a vaccination programme more robust against sudden changes in vaccine coverage, and (4) sex-neutral vaccination is likely to be cost-effective provided a low vaccine price that is negotiated by health authorities.

Models can be criticized and model-based evidence is graded lower than evidence from randomized trials and cohort studies. Nevertheless, in the coming years, evidence from cohort studies is unlikely to change our current perspective on the effect of vaccination on disease in men. A decision on sex-neutral vaccination will inevitably be taken under a certain degree of uncertainty, but since the incidence of oropharyngeal cancers is currently on the rise in men, such a decision should be both sound and timely.

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Cellular and Molecular Pathology in Head and Neck Cancer

Phil Sloan and Max Robinson

Introduction

Advances in technology and the advent of new therapies are driving the transformation of pathology services to provide molecular testing for head and neck cancer. Pathologists are increasingly playing an active role in clinical trials, particularly in the areas of companion biomarker diagnostic development and testing for patient stratification, biobanking and quality assurance. Higher standards and greater consistency of reporting in pathology is being achieved through the publication of internationally agreed pathology datasets (https://www.iccr-cancer.org/) and the WHO tumour classification [1]. Advances in computation are enabling the linking of datasets, so that patients can be tracked and more holistic information about clinical outcomes can be linked to pathological findings, as well as clinical interventional data. Computational biology and advances in molecular techniques are also enabling large scale studies of cancer cell genomes. Increasingly pathology and genomic services are being integrated and the implementation of digital pathology with the use of emerging artificial intelligence algorithms is allowing diagnostic services to be more effectively managed in a way that improves quality. All of these changes will affect the interactions between pathology and other disciplines involved in managing head and neck cancer.

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International Datasets and the WHO Classification

The International Collaboration on Cancer Reporting (ICCR) has formulated a new set of guidelines for head and neck cancer that are out to consultation at the time of writing. The aim of the ICCR project is to define Core and Optional items for incorporation into standards and datasets for histopathology reporting of cancers. At the same time a narrative text provides guidance and clarification for reporting pathologists, as well as citing relevant source literature. The ICCR guidelines are endorsed by several international and authoritative organisations and are generally aligned to national datasets such as those produced by the Royal Colleges of Pathologists of Australasia (RCPA) and the United Kingdom (RCP), the College of American Pathologists (CAP) and the Canadian Association of Pathologists-Association Canadienne des Pathologists (CAP-ACP), in association with the Canadian Partnership Against Cancer (CPAC) and the European Congress of Pathology (ECP). An advantage of the ICCR guidelines is that they will be freely available worldwide and if adopted universally will facilitate harmonisation of international reporting standards and clinical trials. There are nine sets of guidelines (Table 1) expected to be published in mid-2018.

Much has been written on the AJCC [2] and UICC TNM8 [3] staging manuals. The alignment between the two systems is a useful step forwards and will ensure greater global uniformity in staging. For the first time in the UICC manual, pathological staging and clinical staging are recorded separately for head and neck cancer in certain situations. Further, it is no longer possible to stage patients on the basis of clinical examination alone, as molecular testing for p16 is required for oropharyngeal cancer staging, for example. There are also changes in neck staging that are determined by histologically demonstrated extra-nodal extension. The ICCR guidelines on nodal dissection referred to above will be helpful in providing pathologists with exemplar images and descriptions that will assure higher consistence in pathological staging.

The World Health Organisation classification of head and neck tumours [1] was published towards the end of 2017 and provides an international gold standard that defines and describes the pathology and genetics of disease entities. In several instances diagnosis mandates the use of biomarkers, for example p16 immunohistochemistry for HPV associated oropharyngeal squamous carcinoma, and definitive

Table 1 ICCR head and neck datasets

Nasal cavity and paranasal sinuses
Major salivary gland
Oral cavity
Nasopharynx and oropharynx
Larynx, hypopharynx and trachea
Odontogenic tumours
Ear
Nodal excisions and neck dissection
Mucosal melanoma

pathological diagnosis can no longer be based on morphology alone. Several new entities have been accepted into the classification. Clinical experience of biological behaviour, responses to therapy and clinical outcomes for such rare entities can now be accrued. The most significant change to the 2017 edition is the recognition of human papillomavirus related squamous cell carcinoma as a distinct entity, which closely aligns with the latest UICC and AJCC staging systems. Other major changes involve descriptions of new entities in the sinonasal tract and salivary glands, as well as introduction of a new chapter on tumours and tumour like lesions of the neck. Odontogenic cysts are now included in the WHO classification and some controversial entities have been discarded or more logically classified throughout the text.

Molecular Sequencing

During the last 5 years, several studies have been published that have begun to define the molecular landscape of head and neck cancer. Several studies employing next generation sequencing (NGS), often involving whole exome sequencing have been reported. These studies have identified driver mutations in head and neck squamous cell carcinoma that could be potential targets for therapy. The Cancer Genome Atlas (TCGA) consortium [4] published a comprehensive molecular catalogue on head and neck squamous carcinoma in 2015. Frequent mutations of novel oncogenes that are targets for therapy were not, however, identified [5]. On the other hand, head and neck squamous cell carcinoma is characterized by numerous mutations that create neo-antigens, providing a rationale for the development of immunotherapeutic approaches.

Interestingly, analysis of TCGA data showed a relationship with patient age. Distinct mutational clusters were found in very young (19–40 years) as well as very old (>80 years) patients. In older patients four enriched pathways (Axon Guidance, ECM-Receptor Interaction, Focal Adhesion and Notch Signalling) that are only sporadically mutated in the other age groups were identified. By analogy to biological function the four pathways are supposed to regulate cell motility, tumour invasion and angiogenesis and may lead to less aggressive tumours in older age [6]. However, a disadvantage of NGS is that the mean sequencing coverage of ~80-fold results in limited sensitivity for the detection of tumour subclones. The value of NGS studies is greatly enhanced if clinical cohorts are selected that aim to address specific oncological issues such as the identification of tumours that are chemo/radio-resistant [7]. More studies are needed where the clinical cohorts are precisely defined by stage, subsite, aetiology and therapeutic response in order to elucidate molecular profiles that have clinical utility.

Whole genome sequencing has become progressively less expensive and is an attractive methodology because of the comprehensive genomic coverage that it allows. In the UK, the 100,000 Genomes project is an ambitious programme that aims to create a resource for research that potentially could make a step change in our understanding of the molecular basis of cancer, including head and neck cancer.

Table 2 Head and neck squamous carcinoma subsets [8]

Basal subtype—HPV−, high expression of EGFR/HER, hypoxia
Classical (CL subtype)—low expression of EGFR/HER
HPV+ CL
HPV− CL
Immune/mesenchymal (IM subtype) CD8+ infiltration
HPV+ IM
HPV− IM

Studies involving whole genome sequencing in head and neck cancer have already been published by Keck et al. 2015 [8] and these allowed head and neck cancer to be stratified into three subtypes (Table 2).

In the same year, a meta-analysis of whole genome sequencing data published by De Cecco et al. [9] separated head and neck cancer into six subtypes. Analysis was performed using different criteria to those of Keck et al. and was based on the tumours biological characteristics and de-regulated signalling pathways. De Cecco et al. designated the subtypes as immunoreactive, inflammatory, human papillomavirus (HPV)-like, classical, hypoxia- associated, and mesenchymal. Interestingly, adverse behaviour was associated with the hypoxia-associated and mesenchymal subtypes. These publications used differing but to some extent overlapping criteria. One of the limitations of the computational biology approach to analysis of large genomic datasets is that groups are defined by assumed biological behaviour and validation will be required before application of such data can be extended to individual patients. Nevertheless, WGS studies do indicate that that head and neck cancer has molecular subgroups and it is likely that the heterogeneity observed will be refined and ultimately will allow stratification for prognosis and therapy in the future.

Further analysis and validation studies are required to understand better the data from WGS platforms to allow the findings to translate into precision therapy for individual patients. Once a fuller understanding of the molecular pathology of head and neck cancer is gained, it is likely that the WGS will inform the development of gene panels that would have the advantages of greater sensitivity and specificity, lower cost, better turnaround time, reproducibility and the possibility of testing using formalin fixed paraffin embedded tissue. Alternatively, the cost of whole genome testing is falling year by year and turnaround times are shortening. It may be possible to introduce WGS into routine clinical services in the future. A current limitation is that fresh tissue must be used. Until this is resolved, biopsy samples would have to include adequate representative tissue for conventional histopathological sectioning as well as sufficient fresh tissue for WGS. This is against the trend for smaller biopsies preferred for clinical reasons, and would be highly problematic for laryngeal biopsies or small mucosal cancers, for example those arising in oral potentially malignant disorders. There are many other challenges to the implementation of WGS, not least the development of the bioinformatics analysis algorithms and expertise necessary to interpret sequence data for individual patients. Further, analysis of TCGA data relating to head and neck cancer to date has not identified obvious targets for specific drugs and currently there is no convincing

case for introduction of WGS for head and neck cancer into pathology services [5]. The most promising use of sequencing for head and neck cancer is in the identification of neo-antigens that could be used to develop personalised T- lymphocyte targeted therapy, but more research is needed to demonstrate efficacy.

Stromal Factors

Interplay between the cancer cells and the adjacent stroma is a significant determinant of behaviour and outcomes. Fibroblast heterogeneity is a poorly understood process but single cell genomic studies of head and neck cancer reveal two subpopulations of fibroblasts, one of which expresses smooth muscle actin (SMA) and one of which does not. The SMA expressing fibroblasts represent myofibroblasts whilst the other population represents normal and senescing fibroblasts. In vitro studies show that fibroblasts can be induced to express smooth muscle actin by TGF beta, indicating a reversible phenotype [10]. Furthermore in vitro, three fibroblast subpopulations can be identified that have distinctive genetic profiles. These are fibroblasts, myofibroblasts and senescent fibroblasts [11]. Importantly, fibroblast populations appear to have prognostic value [12]. Further studies of the tumour microenvironment are likely to provide insights into complex biological interactions that underpin cancer invasion and metastasis.

Matrix macromolecules are also an important determinant of cancer cell behaviour. In tongue carcinoma, the abundance of the tenascin C has been shown to be a significant prognostic factor [13]. In contrast to fibronectin which mediates fibroblast adhesion, tenascin C has been shown to have an anti-adhesive effect, facilitating cell migration in vitro [14]. In normal murine and human dorsal lingual epithelium, tenascin C has a distinctive pattern of distribution being located at the tips of the connective tissue papillae but not along the bases of the rete processes [15]. This distinctive pattern of distribution may relate to epithelial stem cell distribution and amplification divisions, facilitating cell flow along basement membrane or though cell signalling mechanisms. In a cohort of early stage tongue cancers, poor cumulative survival was associated with the presence of abundant stromal tenascin and fibronectin, whereas cellular tenascin did not distinguish the groups. This might be explained by the assembly and accumulation of tenascin in the extracellular matrix [13]. In addition to the matrix factors, epithelial-mesenchymal transition is a recognised process in head and neck cancer biology and elucidation of the pathways involved may lead to identification of future therapeutic targets.

Immunological Landscape

The introduction of immunotherapy into head and neck cancer practice offers a new range of therapeutic options. There is considerable interest in the use of programmed cell death 1 (PD-1)/programmed cell death ligand 1 (PD-L1) inhibitors in head and

neck cancer. The concept of precision medicine is that targeted therapies should be delivered with a rational biological basis and consequently regulatory bodies (e.g. FDA, USA; EMA, Europe; MHRA, UK) recommend the development of companion and complementary biomarkers for these class of drugs. Companion biomarkers act as 'gate-keepers' and govern the use of the drug, whereas, complementary tests guide clinical decisions, but not access to the treatment. For example, in non-small cell lung cancer the PD-1 inhibitor, pembrolizumab, has companion immunohistochemical tests for assessing PD-L1 expression in formalin-fixed paraffin-embedded tissue sections (Clones: Dako 22C3; Ventana SP263). Detection of PD-L1 on malignant cells, with a 'cut off' of 50% allows first line treatment with pembrolizumab, whereas, only 1% of malignant cell need to be positive for the treatment of recurrent disease (second line treatment; [16]. Nivolumab, another PD-1 inhibitor used in non-small cell lung cancer, has complementary immunohistochemical tests for PD-L1 expression (Clones: Dako 28-8; Ventana SP263), expression guides treatment decisions, but there are no absolute 'cut offs' that determine clinical utility [16]. In head and neck cancer, pembrolizumab and nivolumab have both been evaluated in clinical trials [17–20]. The manufacturers recommend complementary tests (pembrolizumab, Dako 22C3; nivolumab, Dako 28.8), however, there are issues around interpretation of the tests: should the pathologist report PDL-1 expression on malignant cells or malignant cell and immune cells? What are the optimal cut offs for drug efficacy? Checkmate 141 demonstrated that PDL-1 expression by at least 1% of malignant cells is associated with improved overall survival [18], whereas the Keynote trials showed that PDL-1 expression by tumour cells and immune cells is more effective at identifying the patients who are 'responders' [17, 19]. The reproducibility of scoring systems in head and neck cancer are yet to be established, but in lung cancer inter-laboratory variability is known to be problematic and the use of 'in vitro diagnostic devices' (IVD), as opposed to laboratory-developed assays, is recommended (NordiQC, 2018 (http://www.nordiqc.org/epitope.php?id=102)). The IVD manufacturers of PDL-1 tests are supporting pathologist training by 'face to face' engagement and online training modules. Participation in external quality assurance schemes is also recommended (http://www.nordiqc.org/epitope.php?id=102). In the future, it is likely that the delivery of immuno-onocology drugs, such as the PDL-1/PD1 inhibitors, will be supported by multiplex tests assessing broader 'immune activation' with complex algorithms predicting clinical efficacy [19].

Recently, immunogenomic studies have demonstrated heterogeneity between tumour types. In a large-scale study Thorsson et al. [21] identified six immune subtypes that are hypothesized to define immune response patterns impacting on prognosis. Immune subtypes differ by somatic aberrations, microenvironment, and survival characteristics.

Multiple control modalities of molecular networks have been shown to affect tumour-immune interactions. In a cohort of 10,000 cancers, six immune subtypes were identified. These are: wound healing, IFN-γ dominant, inflammatory, lymphocyte depleted, immunologically quiet, and TGF-beta dominant. Cancers were characterized by differences in macrophage or lymphocyte signatures, Th1:Th2 cell

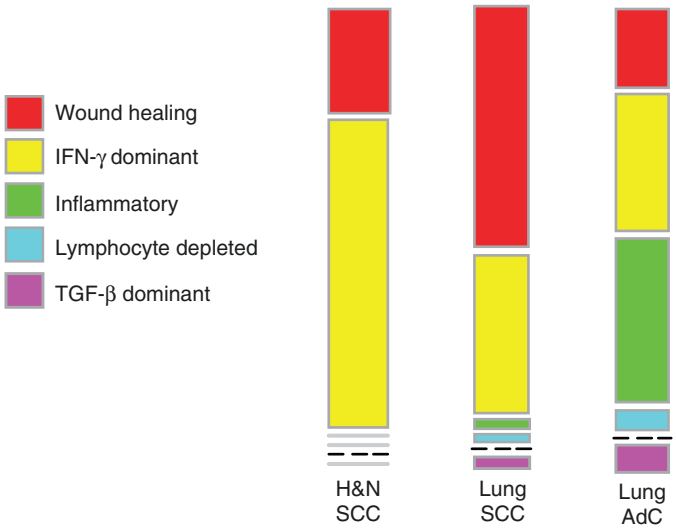

Fig. 1 Immune types of head and neck and lung cancer. The immune landscape of squamous carcinoma of lung is broadly similar to that of squamous cell carcinoma of the head and neck, whereas adenocarcinoma of the lung shows marked differences. Adapted from: Thorsson V, Immune Landscape of Cancer. Immunity. 2018;48:812–30

ratio, extent of intra-tumoural heterogeneity, aneuploidy, extent of neo-antigen load, overall cell proliferation, expression of immunomodulatory genes, and prognosis. Interestingly, head and neck squamous cell carcinoma shows two main immune patterns (>90%); most are IFN-γ dominant with wound healing slightly lower and other types rarely represented. The immune pattern is similar to lung squamous cell carcinoma but differs from lung adenocarcinoma significantly (Fig. 1). Interestingly, subpopulations of resident memory T cells (CD103) as tumour infiltrating lymphocytes have been demonstrated to regulate the magnitude of cytotoxic T cell responses in lung cancer [22]. It is likely that further research will elucidate the role of immune cells in head and neck cancer opening the door to the development of novel individualised T cell therapies.

Digital Pathology and Image Analysis

Digital pathology is increasingly being adopted for reporting worldwide, as it offers the advantages of developing larger hub laboratories that can offer a quality assured service with rapid turnaround times covering a wide geographical area, whilst allowing pathologists to remain close to the clinical teams in their hospital. At the same time, digital pathology is thought to be a solution to the shortfall in the pathology workforce, allowing clinical demand to be better matched to reporting capacity.

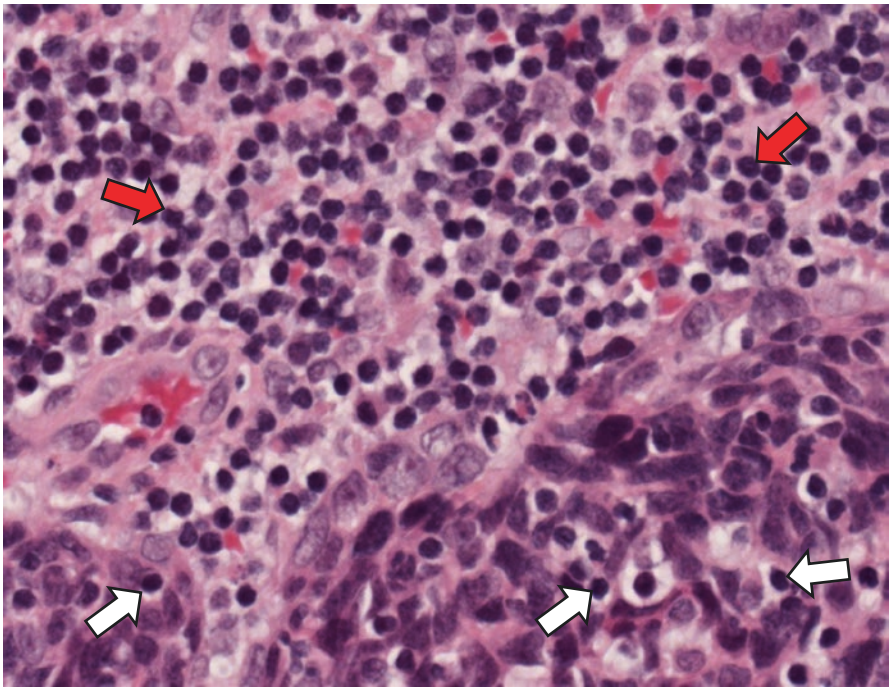

Fig. 2 Tumour infiltrating lymphocytes (white arrows) in HPV associated oropharyngeal carcinoma. Background mucosa associated lymphocytes (red arrows) are present in the top part of the photomicrograph

Further work is needed in the expanding field of digital pathology, particularly in the training of pathologists, validation of systems in the workplace, health economics, integrated LIMs systems and governance relating to the sharing and use of images. Image analysis is a logical extension of digital pathology and it offers the possibility of producing quantitative results on individual biopsies in a reproducible and inexpensive way.

The presence of tumour infiltrating lymphocytes (TILs, Fig. 2) has been shown to be prognostic in a variety of tumours including head and neck squamous carcinoma [23]. Numerous artificial intelligence systems are being developed currently for image analysis and these could potentially produce quantitative data for TILs using multiplex immunohistochemical imaging. It may be possible to use machine learning to identify TILs using routine haematoxylin and eosin stained sections, with validation by the pathologist. Alternatively, immunohistochemistry can be used to identify immune cell populations in an individual tumour and it may be easier and more accurate to quantitate such stained sections without pathologist annotation. Although still expensive and technically challenging, multiplexing allows a panel of immunomarkers to be used on a single section. In that way, background immune cells in a tumour can be quantitated and their spatial relationships can also be measured. Numerous combinations of immunomarkers are possible by

multiplexing and research is needed to evaluate these and develop suitable panels with diagnostic utility. Currently, multiplex immunohistochemistry is expensive, time consuming and technically challenging.

Biomarkers in Current Practice

For squamous cell carcinoma of the head and neck, HPV and EBER testing now form part of routine practice. Current guidelines mandate the use of p16 immunohistochemistry for oropharyngeal cancer. A number of laboratory protocols have been validated for HPV specific testing and these are discussed in chapter "HPV Assessment in Oropharynx Cancer: What is the Gold Standard?".

Molecular Pathology and Salivary Diagnostics

The 2017 WHO classification [1] now includes secretory carcinoma, characterised by the ETV6 rearrangement. Most malignant salivary tumours have been shown to possess characteristic molecular abnormalities, most frequently characteristic fusion genes (Table 3). Whilst for the most salivary tumours, diagnosis is based purely on histological appearances, increasingly a morpho-molecular approach is being used. It is important to sample salivary tumours thoroughly in the pathology laboratory because they can exhibit marked heterogeneity, sometimes with only one part showing characteristic diagnostic features. Immunohistochemistry is increasing applied to salivary tumours reflecting a change since the WHO 2005 classification where immunohistochemistry was regarded as generally unhelpful. Immunohistochemical identification of SOX 10 is useful for acinic cell and secretory carcinomas. However, positive staining may be seen in other salivary tumours

Table 3 Molecular pathology of selected salivary tumours

Salivary tumour	Molecular pathology
Adenoid cystic carcinoma	MYB(L1)-NFIB
Secretory carcinoma	ETV6-NTRK3, ETV6-
Mucoepidermoid carcinoma	CRTC1- or CRTC3-MAML2
Polymorphous carcinoma	PRKD1-3, PRKD1
Myoepithelial carcinoma	FGFR1-PLAG1, TGFBR3-PLAG1, ND4-PLAG1
Acinic cell carcinoma	HTN3-MSANTD3
Hyalinising clear cell carcinoma	EWSR1-ATF1
Salivary duct carcinoma	NCOA4-RET, HER2 gene amplification, TP53, PIK3CA, HRAS mutation PTEN loss/mutation
Carcinoma ex PSA	PLAG1, HMGA2
Others	Actionable genomic alterations

and SOX 10 should not be used as a sole diagnostic marker. DOG1 is expressed on the brush borders of acinic cell carcinoma cells and is highly specific in our hands, being able to distinguish between acinic cell carcinoma and secretory carcinoma. Where the diagnosis of secretory carcinoma is suspected on morphology, a panel showing SOX10 (+), DOG1 (−) and S100 (+) is useful to identify tumours for molecular testing. A combination of Cytokeratin7 and p63 is useful for identification of the luminal and myoepithelial layers in a variety of salivary tumours that exhibit double layered ductal differentiation. Other immunohistochemical markers may be useful, and those used for salivary lymphomas are outside the scope of this chapter. Molecular diagnostics are particularly useful in salivary carcinomas where high grade transformation has occurred and when the histological pattern consequently may be less clear. Molecular testing is also of value where a therapeutic target is identified. In this regard, the finding of the ETV6-NTRK3 fusion that characterises most secretory carcinomas is an essential perquisite to therapy targeting NTRK positive tumours. Although most secretory carcinomas are small and can be treated successfully by surgical removal, it is recognised that high grade transformation can occur and then NTRK targeted therapy, at present available as part of a global basket trial, STARTRK-2 (https://www.ignyta.com), may be an option.

The wide availability of next generation sequencing and fusion gene discovery platforms is likely to lead to more detailed morpho-molecular correlates that have prognostic value and can be used to guide therapy. Recently, for example, genomic differences were found that distinguish myoepithelial carcinoma arising de novo from myoepithelial carcinoma arising in pleomorphic adenoma. It was found that TGFB3- PLAG1 fusions characterise myoepithelial carcinoma de novo (a good prognosis group), whereas FGFR1-PLAG1 fusions identify myoepithelial carcinoma arising in pleomorphic adenoma which has a poorer prognosis. A diagnosis of invasive myoepithelial carcinoma arising in pleomorphic adenoma may indicate a more aggressive surgical and radiotherapy approach than would be used for myoepithelial carcinoma de novo.

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Part II
Prediction of Outcome

Oncogenomics/Proteomics of Head and Neck Cancer

Ruud H. Brakenhoff

Oncoproteomics

Besides RNA molecules, proteins are the biomolecules that form cellular structures and perform the majority of the work in cells. Activity of proteins is regulated by their expression level, but also, and in some cases primarily, by post-translational modifications such as phosphorylation, ubiquitination or by binding to co-factors or other proteins.

Proteins are ideal biomarkers as many methods in pathology are based on immunodetection of proteins. The key example in head and neck cancer diagnosis is the application of p16 immunostaining as a surrogate for human papillomavirus detection (see also below).

The methods to analyze a multitude, and preferably all, cellular proteins in a single experiment is indicated as 'proteomics' [1], a term in line with 'genomics': the large scale analysis of DNA, transcriptomics: the large scale analysis of messenger RNA, and metabolomics: the large scale analysis of metabolites. The working horse for proteomics approaches is mass spectrometry. The complex and expensive mass spectrometers are able to identify peptides on the basis of mass and charge, and fragment them to allow protein sequence analysis. In the early days proteins were separated on 1D or 2D gels, the protein spots were digested with trypsin and the peptides loaded on the mass spectrometer, identified and sequenced. The current equipment allows brute force approaches, and trypsin digested complex mixtures are directly loaded on mass spectrometers, with minimal isolation steps.

There are several challenges in proteomics. First challenge is the tremendous diversity in proteomes, The human genome consists of 20,000 genes but these can

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give rise to 100,000 transcripts by differential splicing and RNA editing, and give rise to over a million protein isoforms with all their post-translational modifications. Current mass spectrometry approaches allow detection of 6000–7000 proteins. Hence to detect all changes in protein composition and modification, a variety of depletion and enrichment methods is required to analyze pre-isolated parts of the cellular proteome.

The second challenge is the huge range of protein expression levels that may range over 10-log, with most interesting signaling molecules typically at the lower ranges.

The number of publications in the head and neck cancer field on proteomics is very limited. On the one hand this seems remarkable as proteins do the work and are ideal biomarkers for clinical applications. However in squamous cells, proteomics analyses are hampered by the high expression of structural proteins such as keratins. Moreover, a typical application at present is phosphoproteomics, methods to elucidate patterns of phosphorylation, which is highly relevant in the context of activated oncogenes and kinase inhibitors but somewhat less in HNSCC. However, there are applications at the horizon that relate to the molecular subclassifications of cancer, including head and neck cancer (see below), which might define a more prominent role of proteomics approaches in head and neck cancer research [2]. Also the introduction of immunotherapy and the search for neoantigens might propel developments in proteomics research [3, 4].

Head and Neck Cancer Classification

The very large majority of head and neck cancers (>90%) are squamous cell carcinomas (HNSCC) that arise in the mucosal linings of the upper aerodigestive tract, and in a single cell type, suggesting that it is a relatively homogeneous disease. However, HNSCC is remarkably heterogeneous, and this has led to several subclassifications of the disease in relation to anatomy, etiology, and clinical or molecular characteristics.

Anatomical Classification of Head and Neck Cancers

In combination with histology obtained from microscopic tumor examination, anatomical classification is used routinely in cancer registries to list some basic characteristics of neoplasms (morphology, behavior, and grading). These standardized registries are very helpful for epidemiological, health management, and clinical research. According to anatomical localization, four major sites of HNSCC (oral cancer, laryngeal cancer, oropharyngeal cancer and hypopharyngeal cancer) are distinguished, and based on ICD10 codes 13 subsites. The anatomical localization impacts treatment decisions as oral cancers are mostly treated by upfront surgery and oropharyngeal tumors by upfront chemoradiotherapy. Apart from site, stage of disease and histopathological observations after microscopic examination of

surgical specimen are used in clinical decision making. However, molecular characteristics are becoming increasingly more important.

Genetic Subclassification

Head and neck tumors arise by carcinogen exposure (smoking, excessive consumption of alcohol) but also by infection with the human papillomavirus (HPV), particularly in the oropharynx. HPV+ve tumors are distinct at the molecular and clinical level, with a much more favorable prognosis for HPV+ve oropharyngeal tumors. The difference in clinical characteristics is in fact so large that it has led to an adaptation in the TNM classification in the most recent 8th edition. In addition, there are several treatment de-escalation trials running that might lead to an adapted treatment in the future. Hence, at present a classification of HPV+ve versus HPV−ve disease has become standard for oropharyngeal cancer based on the clinical characteristics. At the molecular level HPV+ve and HPV−ve oropharyngeal cancers also present differently, which is the likely underlying cause for the clinical differences.

HPV−ve Tumors

Head and neck cancers are typically caused by mutations in tumor suppressor genes [5]. One of the driver genes is *CDKN2A* located on chromosome arm 9p encoding the $p16^{Ink4A}$ protein, which binds and disrupts the CyclinD/CDK4-6 complex that drives cells through the G1-S checkpoint. The $p16^{Ink4A}$ cell cycle inhibiting protein is frequently inactivated in HNSCC by mutation or methylation in combination with chromosomal loss, or by homozygous deletion [5]. A second driver is the *TP53* gene on chromosome 17p13 that is also frequently inactivated in HNSCC, mostly by missense mutations combined with allelic loss. Somatic mutations are found in 60–80% of the tumors [5]. Other frequently mutated genes are *NOTCH1* and *FAT1*, both likely implicated in the Wnt signaling pathway, and *NSD1* and *KMT2D*, both involved in epigenetic regulation [6].

These HPV−ve tumors are typically characterized by frequent copy number changes [5], but in the recent molecular profiling studies it has been shown that another subgroup of HPV−ve tumors exists that is characterized by very few copy number alterations. This subgroup of HPV−ve 'copy-number-silent' tumors is *TP53* wild type and typically display *HRAS* and *CASP8* mutations and has a more favorable prognosis [5]. In another previous study it has been shown that this group of tumors is mismatch repair proficient, diploid and seem to occur more frequently in females without a history of smoking and alcohol consumption [7]. At present we miss simple genetic assays that would allow analysis of DNA isolated from formalin-fixed paraffin-embedded tissue to further substantiate the clinical relevance of this subgroup of tumors in large cohorts.

HPV+ve Tumors

As described above, the human papillomavirus causes a subgroup of tumors, most particularly those that arise in the oropharynx. There are many HPV virus types that may cause cancer, but most prominent is HPV16, particularly when it concerns head and neck cancer. The virus encodes two oncogenes E6 and E7. The E7 gene binds the pocket proteins pRb, P130 and p107 thereby creating an S-phase environment in the cell. Usually an unscheduled S-phase with high E2F activity causes p14 inhibition of MDM2, and subsequently an increased level of p53. Increased levels of p53 will cause a cell cycle arrest and apoptosis, and to circumvent that stress response, the virus expresses the oncoprotein E6 that targets p53 for degradation. Hence, the virus hits precisely the same pathways as is noted in HPV−ve tumors, i.e. the p53-p21 pathway and the p16-cyclinD1/CDK4,6-Rb pathway.

Both E6 and E7 expression remains critical in HPV+ve HNSCC cell lines [8] and the cancer-associated phenotype caused by inactivation of the p53 and pRb pathways in oropharyngeal keratinocytes is at least cellular immortalization [9].

Tumor Classifications by Oncogenomics

Initially microarray hybridizations and later RNA sequencing studies allowed to identify all genes that are expressed in tissues. These gene expression profiles have been explored by several groups in different tumor types including HNSCC for prediction of tumor characteristics and prognosis. However, these studies also revealed new insights in tumor biology. In an early study reported in 2004, four expression subtypes in HNSCC emerged by cDNA microarray hybridization [10], resembling those in lung cancer found before [11]. These groups were termed later as basal, mesenchymal, atypical and classical gene expression subtypes on the basis of specific gene sets that are expressed. While HPV status was not included in the earliest report, an enrichment of HPV-associated gene expression was observed in the atypical subtype with elevated expression of *CDKN2A* ($p16^{Ink4A}$), *LIG1*, and the transcription factor *RPA2*. The basal gene expression profile showed signatures found in basal cells from airway epithelium (e.g. high *COL17A1* expression associated with the extracellular matrix production), and high expression of *TGFA*, *EGFR*, and *TP63* [11]. Genes associated with epithelial-to-mesenchymal transition (EMT) were enriched in the mesenchymal subtype, while genes enriched in the classical subtype were associated with exposure to cigarette smoke and xenobiotic metabolism. These four subtypes were identified again in a subsequent study using Illumina Expression BeadChips [12]. Although one cluster matched to the classical gene expression profile, the other clusters correlated less well with the former studies. This could illustrate the heterogeneity of HNSCC regarding gene expression, but may also indicate technical differences caused by the variety of used profiling platforms as well as differences in collecting and processing tumor samples [12].

Five subtypes of HNSCC, including two biologically distinct HPV subtypes were identified by Keck and coworkers in 2015 in a clinically homogeneous cohort of 134 locoregionally advanced HNSCCs with 44% HPV+ve cases. Gene expression profiling was carried out on Agilent 4x44Kv2 expression arrays and combined with previously published and publicly available data, generating a final data set consisting of more than 900 patients. One of the HPV+ve and HPV−ve subtypes showed an immune and mesenchymal phenotype, while the other HPV+ve and HPV−ve subgroups resembled the classical expression pattern according to prior nomenclature in other cancer types and molecular characteristics.

In a somewhat other approach using RNAseq profiling of HPV+ve oropharyngeal cancers, two expression subtypes could be identified, one characterized by keratin expression and indicated at HPV-KRT and one characterized by an immunological gene expression pattern identified as HPV-IMU [13]. These subgroups were verified in the TCGA data and there were suggestions for a different clinical outcome, but the sample size of the study was too low to find significant results [13].

Hence, several gene expression signatures have been established from microarray and RNAseq studies. However, none of these approaches could be implemented in the standard of care for HNSCC so far. A recent study demonstrated the use formalin-fixed paraffin embedded tumor tissue for mutation and transcriptional profiling by using Ion Torrent AmpliSeq cancer panel tNGS and NanoString gene expression assays [14]. However, in this pilot study, 230 cancer-relevant genes were analyzed and by unsupervised hierarchical clustering six groups of differentially expressed genes were identified in this dataset. It may be assumed that the ability to use fixed samples from the pathology archive will advance gene expression studies, but the limited gene set hampers cross-validation with other genome-wide studies [14].

The field moved on, and with novel elegant methods new insights were obtained. In a most recent sequencing study of almost 6,000 single cells, the malignant and non-malignant cells of 18 HPV−ve oral cancers were characterized in detail at the molecular level by RNA sequencing [15]. Also based on the sequencing data of single cells, the tumors could be classified in the expression groups previously assigned as atypical, mesenchymal, classical and basal. The authors further showed that the basal and mesenchymal subtype differed with respect to the number of fibroblasts in the tumor microenvironment, and when applying a correction algorithm for this variable, they could show that these in fact might belong to one subclass now indicated by the authors as ‘malignant-basal’. Whether the number of tumor-associated fibroblasts is indeed a mere numerical difference of fibroblasts in the tumor micro environment, or in fact may represent a key biological difference remains to be determined.

In summary, HNSCCs are classified by site and stage, but since the introduction of TNM8 also for molecular characteristics. These molecular classifications should be based primarily on basis of genetic characteristics [6] with a separation in HPV+ve oropharyngeal cancers, HPV−ve (oropharyngeal) cancers, and ‘copy-number-silent’ HNSCCs that are also HPV−ve. The HPV+ve oropharyngeal tumors can be separated on basis of expression subtype and indicated as HPV-KRT and HPV-IMU. The expression subtypes for the copy-number-silent’ tumors is at

present unclear, and perhaps the number of studies that really zoom in on this subgroup too limited.

Based on the latest insights the HPV−ve subgroup of tumors could be distinguished on basis of expression profiles as malignant-basal, classical and atypical. The situation for the expression subtypes in HPV−ve tumors is less consistent, and consequently the clinical relevance remains an enigma. However, this classification may gain importance by the recent finding that the classical subtype is characterized by mutations in a specific pathway that is also druggable [16, 17]. For the future a consistent molecular classification needs to be developed, and the variables that hamper this consistency identified.

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Targeted Next-Generation Sequencing in Head and Neck Cancer

Ingeborg Tinhofer

Optimization of Cancer Treatment Based on Cancer Genomics

Cancer is a disease initiated, propagated, and maintained by somatic genetic events. The advances in sequencing technology and bioinformatics analysis have facilitated the identification of distinctive genetic alterations that promote cancer cell growth by constitutive activation of cell signaling pathways or by inactivation of critical negative regulators of these networks. Detailed analysis of the genetic events in cancer by "next generation" sequencing (NGS) has boosted worldwide efforts of genome-wide personalized oncology aiming at identifying distinct molecular subgroups, novel actionable targets and predictive biomarkers. In parallel, small molecule inhibitors and antibodies have been developed that target particular oncogenic driver genes. These targeted agents may be equivalent or even inferior to current standard treatment in an unselected patient population but frequently induce impressive regression in tumors harboring the molecular target, demonstrating the value of "precision" medicine. The concept of targeted cancer therapy is strongly supported by a recent meta-analysis of 570 phase II studies including more than 32,000 patients [1]. This systematic review not only indicated that a personalized treatment strategy was independently associated with higher response rates, longer median progression-free and overall survival, but also that the worst outcomes were

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associated with use of targeted agents in a non-personalized manner [1]. Successful examples of personalized targeted treatment in routine cancer patient care include trastuzumab for human epidermal growth factor receptor type 2 (HER2)-expressing breast cancer [2], vemurafenib for melanomas that express mutated *BRAF* [3] and tyrosine kinase inhibitors for non-small cell lung cancer (NSCLC) with kinase-activating mutations in epidermal growth factor receptor (*EGFR*) [4].

Targeted Therapy of Head and Neck Cancer

Treatment of HNSCC at advanced stages remains a challenge. Despite multimodal treatment concepts including surgery, radiation and chemotherapy, <50% of patients with locally advanced HNSCC can be cured. Upon relapse, the disease characteristics change towards an even more aggressive and metastatic phenotype, with much lower responsiveness to currently available treatments. Conventional chemotherapy is active in recurrent/metastatic disease but does not confer a meaningful survival benefit. Among the molecular drugs directed against the tumor cell compartment, only antibodies blocking the epidermal growth factor receptor (EGFR) have been licensed so far. Primary or secondary resistance to EGFR blockade is frequent or will eventually develop [5]. Several driver mutations and other genomic aberrations have been associated with resistance to EGFR antibodies, including acquired *RAS* mutations [6] and activation of the c-Met signaling pathway [7, 8]. In addition, accumulating clinical and laboratory research points to a lack of antitumor activity of second-line cetuximab monotherapy in HPV-positive disease [9]. Certainly, further clinical trials incorporating the HPV status as stratification factor and including comprehensive molecular tumor profiling are needed to prospectively verify the predictive significance of the above-mentioned biomarkers for patients with recurrent/metastatic HNSCC who receive first- or second-line cetuximab-based palliative treatment [9].

As further targeted agents in development for treatment of HNSCC, inhibitors of mammalian target of rapamycin (mTOR) [10] and phosphoinositide 3-kinase (PI3K) [11] have shown activity in clinical trials. Lately, immune checkpoint inhibitors (ICI) have entered clinical development and antibodies directed against the immune-suppressive receptor programmed cell death protein 1 (PD-1) expressed by T cells were approved for treatment of recurrent/metastatic HNSCC. However, HNSCC tumors are known to use diverse strategies to escape immune surveillance, thus limiting the ability to fully exploit the immune system to achieve durable antitumor activity. Accordingly, there is an unmet need to develop novel treatment strategies and to improve the efficacy and response rates of existing therapies for HNSCC. The knowledge gained from recent comprehensive NGS studies on the landscape of genomic alterations in HNSCC and their functional role in disease progression and treatment resistance are expected to significantly improve future target identification and the development of molecular therapy.

Comprehensive NGS Studies Reveal a Complex Genetic Landscape in HNSCC

Imperative to exploiting the molecular vulnerabilities of HNSCC is the ability to identify potentially actionable genetic alterations. First in-depth insights in the complex genetic landscape of HNSCC were provided by two independent studies in which whole exome sequencing (WES) was used for detailed genome analysis of tumors from ~100 HNSCC cases in total [12, 13]. More recently, the Cancer Genome Atlas (TCGA) project produced a further large-scale NGS dataset from ~500 HNSCC tumors. All three studies confirmed the frequent occurrence of genetic alterations in *TP53*, *CDKN2A*, *PIK3CA* and *CCND1* already previously identified as driver genetic events in the neoplastic transformation of HNSCC [14–17]. In addition, novel mutations in genes involved in the differentiation program of squamous epithelium and the Notch/p63 axis (such as *NOTCH1*, *TP63* and *FBXW7*) were discovered. By application of advanced bioinformatics tools, novel molecular subgroups of HNSCC were identified. Loss-of-function alterations of the chromatin modifier *NSD1*, the *WNT* pathway genes *AJUBA* and *FAT1*, and activation of the oxidative stress factor *NFE2L2*, were found in a subgroup mainly comprised by laryngeal tumors [18]. Furthermore, a subgroup of oral cavity tumors was detected displaying infrequent copy number alterations in conjunction with activating mutations of *HRAS* or *PIK3CA*, coupled with inactivating mutations of *CASP8*, *NOTCH1* and *TP53* [18]. Comparative analysis of smoking-related and human papilloma virus (HPV)-driven carcinomas revealed clearly different genetic patterns in the two subgroups, an observation confirmed by further independent NGS studies [19–22]. Smoking-related HPV-negative HNSCC were associated with frequent mutations in *TP53* and *CDKN2A* as well as frequent copy number alterations including amplification of 3q26/28 and 11q13/22 [18]. Beside prevalent activating mutations in *PIK3CA*, the group of HPV-positive carcinomas was significantly enriched for *E2F1* amplifications and *TRAF3* deletions [18]. An overview of the frequencies of genetic alterations in distinct signaling pathways according to the HPV status is provided in Table 1.

As discussed recently by Liu and coworkers, collecting comprehensive clinical data was neither a primary objective nor a practical possibility in the TCGA project, given the worldwide scope and time constraints for sample accrual goals [23]. In addition, the type of treatment was not among the pre-specified items, which resulted in considerable heterogeneity in the collected patient cohorts. In the HNSCC subproject, almost all patients had been treated with curative intent. Sufficient fresh-frozen tumor material was necessary for the comprehensive NGS-based molecular characterization. This technical prerequisite resulted in a significant over-representation of tumors from the oral cavity and larynx for which surgical resection is the preferential treatment option in the curative setting. *Vice versa*, tumors located in the oro−/hypopharynx which are sometimes less well accessible to curative-intended surgery were under-represented. A substantial variation was also observed in the variables of the combination therapies, such as the sequence

Table 1 Frequencies of mutations/copy number alterations in genes associated with distinct signaling pathways, according to the HPV status

Pathway	Affected genes	HPV− (%)	HPV+ (%)	Pathway genes
Raf-Ras-Mek-Erk/Jnk	HRAS	7.0	1.4	KRAS HRAS BRAF RAF1 MAP3K1 MAP3K2 MAP3K3 MAP3K4 MAP3K5 MAP2K1 MAP2K2 MAP2K3 MAP2K4 MAP2K5 MAPK1 MAPK3 MAPK4 MAPK6 MAPK7 MAPK8 MAPK9 MAPK12 MAPK14 DAB2 RASSF1 RAB25
	DAB2	6.0	1.4	
	MAPK1	2.9	4.0	
	All pathway genes (N = 26)	*32.8*	*18.1*	
PI3K-Akt-mTor	PIK3CA	27.0	43.0	PIK3CA PIK3R1 PIK3R2 PTEN PDPK1 AKT1 AKT2 FOXO1 FOXO3 MTOR RICTOR TSC1 TSC2 RHEB AKT1S1 RPTOR MLST8
	PTEN	3.0	17.0	
	RICTOR	6.0	6.0	
	TSC1	0.7	6.0	
	All pathway genes (N = 17)	*44.6*	*63.9*	
RTK/growth factor	EGFR	15.0	4.0	FGFR3 EGFR DDR2 EPHA2 RET FGFR1 ERBB2 KIT IGF1R MET
	FGFR1	9.0	1.4	
	EPHA2	5.0	2.8	
	FGFR3	1.9	14.0	
	All pathway genes (N = 10)	*37.1*	*26.4*	
Cell cycle	CDKN2A	60.0	6.0	CDKN2A CDKN2B CCND1 MYC JAK2 CDK6 E2F5 RB1 CCNE1 CCND2 STAT1 RBL1 E2F1 RBL2 CDKN1B JAK1 CCNA1 CDK4 SRC STAT5B E2F3 STAT3 STAT2 CDK1 E2F7 STAT5A CCNB1 E2F4 E2F8 CDKN1A E2F2 CDK2 CDC25A E2F6
	CDKN2B	33.0	2.8	
	CCND1	27.0	6.0	
	MYC	12.0	1.4	
	JAK2	6.0	8.0	
	CDK6	5.0	1.4	
	E2F1	2.2	14.0	
	RB1	4.0	10.0	
	RBL1	2.9	7.0	
	STAT3	1.0	6.0	
	All pathway genes (N = 34)	*80.0*	*62.5*	
p53	TP53	82.0	10.0	TP53 MDM2
	MDM2	5.0	2.8	
	All pathway genes (N = 2)	*82.2*	*11.1*	
DNA damage response	ATR	11.0	8.0	CHEK1 CHEK2 RAD51 BRCA1 BRCA2 MLH1 MSH2 ATM ATR MDC1 PARP1 FANCF
	ATM	5.0	4.0	
	BRCA2	5.0	4.0	
	All pathway genes (N = 12)	*29.4*	*19.4*	

Table 1 (continued)

Pathway	Affected genes	HPV− (%)	HPV+ (%)	Pathway genes
Notch	NOTCH1	21.0	11.0	ADAM10 ADAM17 APH1A APH1B ARRDC1 CIR1 CTBP1 CTBP2 CUL1 DLL1 DLL3 DLL4 DTX1 DTX2 DTX3 DTX3L DTX4 EP300 FBXW7 HDAC1 HDAC2 HES1 HES5 HEYL ITCH JAG1 JAG2 KDM5A LFNG MAML1 MAML2 MAML3 MFNG NCOR2 NCSTN NOTCH1 NOTCH2 NOTCH3 NOTCH4 NRARP NUMB NUMBL PSEN1 PSEN2 PSENEN RBPJ RBPJL RFNG SNW1 SPEN HES2 HES4 HES7 HEY1 HEY2
	HES1	13.0	17.0	
	NOTCH2	10.0	0.0	
	FBXW7	9.0	6.0	
	EP300	7.0	13.0	
	SPEN	6.0	6.0	
	NOTCH3	5.0	6.0	
	NCOR2	5.0	4.0	
	All pathway genes (N = 55)	*72.8*	*69.4*	

The analysis of the frequency of gene alterations in the HPV− ($N = 415$) and HPV+ subgroups ($N = 72$) was performed in the TCGA HNSCC dataset available at cbioportal.org (PanCancer Atlas dataset, $N = 523$)

(induction, concomitant or adjuvant) and the type of chemotherapy as well as the dose of radiation. For a considerable fraction of patients, detailed information on treatment was even not provided. Despite these limitations, a recent comprehensive quality assessment of the clinicopathologic annotations in the TCGA program which was undertaken for the generation of the TCGA Clinical Data Resource (TCGA-CDR) for high-quality survival analyses [23] revealed no evidence of a systematic bias in the HNSCC cohort. Thus, the correlation of the genetic alterations and molecular subgroups identified in the TCGA project with outcome should reveal robust information on their prognostic value in HNSCC, at least for tumors of the oral cavity and larynx.

Frequencies and prognostic values of distinct somatic mutations and copy number alterations identified in the TCGA HNSCC dataset were corroborated by a large number of subsequent NGS studies using a more focused approach [20, 22, 24–30]. Targeted NGS-based genotyping revealed high prevalence of *NOTCH1* mutations and their independent association with poor outcome in a Chinese patient population of oral cavity cancers [31]. In a large single-center study in which the value of a targeted NGS platform for mutational analysis in an outpatient clinic setting was examined, *PIK3CA* amplification and oncogenic *RAS* mutations were established as prognostic factor of poor progression-free survival [25]. In our own studies in HNSCC patient cohorts uniformly treated with concurrent chemoradation [27] or surgery followed by adjuvant chemoradiation [22], we could validate the previously reported prognostic value of *TP53* mutations [32, 33]. We provided additional evidence that the association of *TP53* mutations with outcome significantly depended on the type of mutation [22]. We further established a significant association of

NOTCH1 mutations with improved efficacy of concurrent chemoradiation [27]. A further study in the model of laryngeal squamous cell carcinoma also showed that targeted NGS might also prove helpful in the early detection of patients at risk of progression from dysplasia to invasive carcinoma [34].

Besides shedding more light on the role of distinct genetic alterations/patterns in the pathogenesis of HNSCC, these studies also provided evidence that the prevalence of genetic alterations established by large NGS gene panels in FFPE HNSCC samples collected in clinical routine were comparable with the frequencies observed in the WES analysis of fresh-frozen tumors in a research project. They further showed that targeted NGS is feasible in the clinical setting [20, 25, 35, 36] and can be used for identification of patient subgroups for whom novel treatment intensification/de-escalation strategies will have to be developed. Below, we will discuss in more detail the main advantages and current limitations of targeted NGS compared to whole exome sequencing.

Precision Oncology by Targeted Panel Sequencing

Until recently, the most common clinically used sequencing platforms assessed a few hotspot variants in one or more frequently altered genes (i.e., *BRAF* V600E mutations in melanoma and *EGFR* L858R mutations in NSCLC). These assays have the potential to uncover the most extensively validated mutations in several tumor types. The idea of introducing more comprehensive cancer sequencing into routine clinical care was grounded on studies demonstrating the feasibility of integrating whole genome sequencing (WGS), WES and transcriptome sequencing into decision-making algorithms for patients with advanced or refractory cancer [37]. Since then, a large number of precision oncology programs [38, 39] and clinical trials implementing NGS analyses [1, 40, 41] have been developed. All available NGS strategies, ranging from targeted gene panels [36] through WES [39] covering all coding regions to WGS [42] across all bases of the human genome have been successfully used in a clinical setting. From these studies, it has become increasingly evident that a more extensive analysis of the genetic tumor profile compared to few hotspot mutations has several advantages. First, crucial alterations that are prevalent in one malignancy and predict response to available agents (i.e., *BRAF* V600E mutation in melanoma) may also occur at low frequency in other tumor types. Second, clinically relevant gene fusions, e.g. fusions involving *NTRK* genes for which novel, highly active inhibitors have recently been developed [43, 44], appear at low frequency across a wide range of tumors including head and neck cancers. Large structural alterations like these gene fusions cannot be detected with hotspot testing methods. Third, novel targeted agents continue to become available which display efficacy in molecular subtypes previously considered “undruggable” such as tumors with impaired p53 functions [45], amplification of c-Myc [46] or loss of master cell cycle regulators [47]. A significant proportion of patients may

Table 2 Targeted NGS versus whole exome sequencing: pros and cons

Targeted NGS	Whole exome sequencing
Advantages	
High depth of coverage	Detection of unknown variants
High overall exon coverage (>99%)	Detection of copy number alterations
Low DNA input required (down to 10 ng)	Favorable ratio of price/data output
Low costs	
Low efforts in bioinformatics	
Easily standardized	
Easy implementation in clinical routine	
Disadvantages	
Limited information (max. ~600 genes)	Higher DNA input (>500 ng)
Limited suitability for detection of complex alterations	Time-consuming work-flow High effort of bioinformatics
	Demanding clinical interpretation

therefore be excluded from potentially effective therapeutics based on too limited genetic profiling.

Current targeted NGS assays range from small panels of 20 to comprehensive panels of up to 600 genes. These assays have shown consistent results in the detection of single nucleotide variants (SNVs) and small insertion/deletions (indels) in solid tumors [48]. Targeted panels offer the advantage of high sequencing depth as well as high exon coverage (>99%) (Table 2). The depth of coverage represents the number of times a specific base has been sequenced and aligned to the reference genome. The exon coverage indicates the overall percentage of individual exons spanned by at least one sequencing read. These two variables are the most important factors for consistent calling of sequence variations. Most targeted panels have an average depth of coverage of 500×, thereby exceeding WES applications by an order of magnitude. Moreover, insufficient coverage in genomic regions rich in guanine/cytosine bases or repetitive regions is less frequently observed. Owing to the higher depth of coverage, targeted NGS also offer a lower threshold for uncovering intratumoral heterogeneity and genetic variants at low allele frequency. Targeted NGS thus represents a rapid and cost-effective tool with high accuracy, sensitivity and specificity for the detection of SNVs, indels and selected translocations, and can be quickly adapted to novel targets in a clinical setting.

Targeted gene panels also reduce the complexity of the bioinformatical analysis and the subsequent data interpretation by clinical experts. Targeted NGS can therefore easily be implemented in clinical trial protocols and the routine setting of molecular tumor boards. In fact, the vast majority of ongoing clinical trials of precision oncology rely on targeted NGS for the detection of actionable molecular targets. A further major advantage of panel sequencing approaches is their suitability of mutation analysis based on genomic DNA from archival formalin-fixed paraffin-embedded (FFPE) tumor tissue. Results from FFPE NGS-based panel sequencing have been shown to correlate well with the results obtained from fresh frozen tissue

[36]. Nonetheless, differences during fixation, paraffin-embedding and storage can have substantial effects on the accuracy of NGS results. FFPE is known for being a major source of sequencing artefacts, for example, by DNA cross-linking or cytosine deamination. The lack of a matched normal tissue control is another important factor that should be taken into consideration when analyzing the results from targeted NGS of tumor samples. The potential risk of misinterpreting pathogenic germline variants or reporting false-positive somatic alterations under this condition should not be ignored.

Altogether, targeted gene panels provide a fast and cost-efficient way to obtain a somatic tumor profile with a reasonable number of actionable alterations for rapid clinical interpretation. If these alterations are located in well-defined cancer-related genes for which a targeted therapy is available, such a focused NGS approach is ideally suited for the selection and stratification of patients in clinical trials and routine patient care. Several Clinical Laboratory Improvement Amendments (CLIA)-certified targeted gene panels for somatic characterisation of solid tumors are commercially available, and are optimally suited for implementation in a routine laboratory. However, they are limited by their omission of the vast majority of genomic information leading to non-detection of complex genomic aberrations or mutations in genes outside of the preselected panel. Obvious limitations of targeted panels include low sensitivity for detecting chromosomal CNVs and complex genomic rearrangements (Table 2).

Exploiting NGS Results for the Clinical Development of Targeted Therapies

The results from previous NGS studies in HNSCC were certainly crucial to better understand the pathophysiology of the disease and represent a valuable basis for the selection of novel therapy targets. However, they also revealed a major obstacle for future clinical development of molecular treatment of HNSCC. As most other cancer entities, HNSCC possess a large number of mutations that occur only at a low frequency (Fig. 1). Indeed, the analysis of the genomic profiles which we performed for this review in the TCGA HNSCC cohort available at cbioportal.org ($N = 504$, provisional dataset) revealed that only 63 genes (13%) of the top-500 most frequently affected genes were found to be altered in >10% of the total patient population. This phenomenon of so-called “long tail” mutations represent a significant challenge in the clinical development of targeted therapies since conducting clinical trials in small subsets of patients harboring distinct alterations in single genes can be logistically and technically challenging. Among the molecular alterations which were detected in >10% of the patients, only three genes (*PIK3CA*, *CCND1*, *EGFR*) are currently targetable by drugs either approved or in clinical development for treatment of HNSCC (Fig. 1).

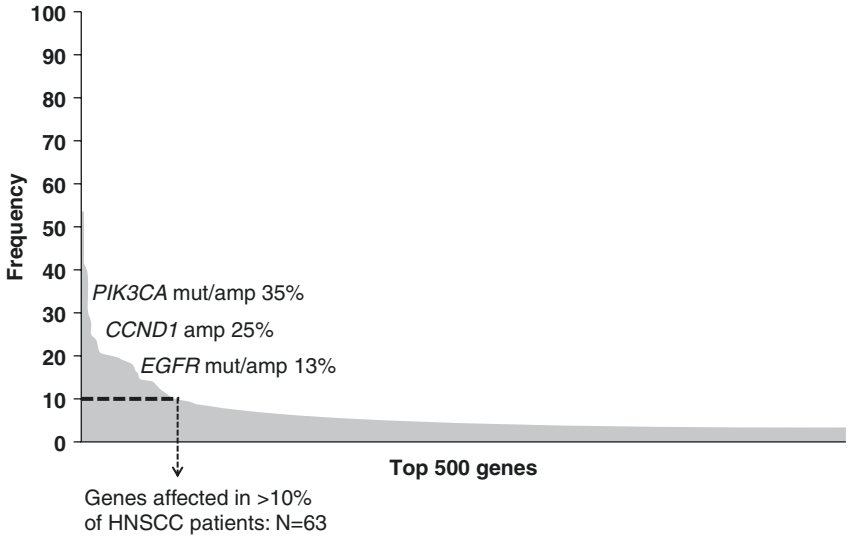

Fig. 1 Long tail plot of the distribution of genomic alterations in 504 HNSCC cases (TCGA, provisional dataset)

There are several strategies to address this challenge in the development of molecular treatment strategies: First, rather than screening for alterations in single key driver genes such as *EGFR* or *PI3KCA*, detection of genetic changes in further members of the same signaling pathway could identify a larger subgroup of patients with potential benefit from a given molecular treatment. Indeed, assessment of genes with key signaling function in the EGFR/Her2, FGFR or PI3K/AKT pathways in the TCGA HNSCC cohort (provisional dataset, $N = 504$) revealed a frequency of putative gain-of-function (GOF) alterations in 20%, 52% and 39% of patients, respectively (Table 3). These patient subgroups of considerable size might benefit from treatment with inhibitors of MEK, FGFR and PI3K/AKT/mTOR, respectively (Table 3). Furthermore, alterations in genes associated with distinct functions in DNA-repair processes including *BRCA2*, *RAD51*, *ATM, ATR* and Fanconi anemia (FA) genes are found in 29% of HNSCC patients. Disruptive alterations in these genes have been shown to define the so-called properties of "BRCAness" [49]—that is, traits that some sporadic cancers share with those occurring in either *BRCA1*- or *BRCA2*-mutation carriers and which are associated with high sensitivity to PARP inhibition [50]. Importantly, the patient subgroups displaying the four genotypes of distinct pathway alterations can be identified by sequencing analysis of only 5–22 genes (Table 3) and could thus easily be integrated in a gene panel for targeted NGS.

As second option, clinical development of molecular treatment in rare molecular subgroups could be integrated into international screening programs such as the collaborative European platform SPECTA (Screening cancer Patients for Efficient Clinical Trial Access) for state-of-the-art comprehensive molecular profiling

Table 3 Targeting genotypes/pathways rather than single alterations in HNSCC

Genotype	Phenotype	Genes	*N* (%)	Inhibitors
EGFR/HER2 pathway (*EGFR*, *ERBB2*, *HRAS*, *NRAS* and/or *BRAF*)	GOF	5	99 (20%)	MEK
FGFR pathway (genes encoding for FGFR receptors and/or ligands)	GOF	22	264 (52%)	pan-FGFR
PI3K/AKT pathway (PIK3CA, PIK3CB, AKT1, AKT2 and/or PTEN)	GOF	6	197 (39%)	PI3K/AKT, mTOR
BRCAness (*BRCA2*, Fanconi anemia (FA) genes, *RAD51*, *ATM* and/or *ATR*)	LOF	22	147 (29%)	PARP

The analysis of the frequency of distinct genotypes was performed in the TCGA HNSCC dataset publicly available at cbioportal.org (provisional cohort, $N = 504$). As gain-of-function (GOF) alterations leading to pathway activation were considered amplifications and activating mutations. Loss-of-function (LOF) alterations were defined as homozygous deletions or truncating mutations in the respective gene

combined with the collection of patient data and biological samples. Such approach would not only facilitate patient recruitment for clinical trials but also enable robust translational research in rare subsets of cancer. Last but not least, a shift away from cancer treatment by organ of origin to treatment of cancers according to their mutations, such as an ERBB2-amplified tumor or a PI3K-pathway mutant carcinoma could be envisioned. Recent clinical work illustrates the promise of such a "tumor-agnostic" targeted therapy [51]. In a basket study including pediatric and adult patients with 17 distinct tumor types that harbored rare chromosomal fusions involving *NTRK* genes [52], treatment with the Trk inhibitor larotrectinib produced durable responses in about 70% of the patients, regardless of their tumor's tissue of origin [52].

Intratumoral Genomic Heterogeneity in HNSCC: A Challenge for Personalized Medicine?

A mutational load ranking in the upper third of all tumor entities [53] and large interpatient genetic heterogeneity [18] are key features of HNSCC. Signs of high genetic instability are primarily detected in cases with a history of heavy smoking and alcohol consumption, most likely resulting from the extensive DNA damage that has been caused by tobacco carcinogen exposure for years. Exacerbating the complexity of the genetic landscape in HNSCC, intratumoral heterogeneity in terms of spatial and temporal differences in the mutational patterns of key driver genes can occur [54–56]. Identifying genetic targets for treatment based on a single biopsy is difficult in tumors displaying intratumoral heterogeneity. Circulating cell-free tumor DNA (ctDNA) in blood plasma might represent an interesting source for mutational analysis under this condition since it potentially represents the cumulative pool of mutant variants from the bulk tumor. In support of a diagnostic potential

of ctDNA in HNSCC, its detection has been reported in ~70% of HNSCC patients [57]. However, the number of mutant DNA fragments per mL of plasma are low (range: 1–100/mL) emphasizing the need of highly sensitive tools for ctDNA sequencing analysis. Various techniques are in use for targeted analysis of ctDNA including 'beads, emulsions, amplification, and magnetics' (BEAMing) [58], 'Safe-Sequencing System' (Safe-SeqS) [59], 'Cancer Personalized Profiling by deep Sequencing' (Capp-Seq) [60], 'and targeted next-generation sequencing (NGS) [26, 61]. Recently, concordance of genomic alterations between tumor tissue and ctDNA in the range of 15–95%, depending on the type of genetic variant and its allelic frequency in the primary tissue, have been reported in breast cancer [62]. Moreover, in lung cancer harboring *EGFR* T790 M associated with tyrosine kinase inhibitor resistance, sensitivity of plasma genotyping for detection of the mutant variant was only 70%. These data still argue against ctDNA genotyping as solitary diagnostic test of genetic alterations. Up to date, no systematic comparative analysis of tumor tissue and ctDNA has been presented for HNSCC so far. Thus, it is impossible to draw any conclusions on the potential of targeted genetic analysis based on ctDNA in HNSCC.

Conclusions

The use of molecular characteristics of individual tumors for tailoring personalized is becoming a reality in cancer treatment that may radically alter the standard course of clinical intervention. The applications of NGS technologies in large-scale profiling studies have largely supported world-wide efforts of elucidating the complex genomic landscape of HNSCC. Yet, the interplay among the identified genetic alterations and their biological effects in tumor development and treatment resistance remain largely unknown. Therefore, it will remain a challenge of the next decade to interpret the already available sequencing data, perform prospective biomarker-driven clinical trials and develop concepts of clinically applicable personalized therapy.

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Homologous Recombination Repair Function as a Predictor of Treatment Response

Kevin J. Harrington

Introduction

Spontaneous DNA damage is an inevitable consequence of cellular metabolism and proliferation. It is also triggered by exogenous agents such as ionising (e.g. X- and γ-ray) and non-ionising (e.g. ultraviolet) radiation and chemical toxins in the environment. The DNA damage response (DDR) is absolutely essential for the preservation of genomic integrity over the lifespan of an organism [1]. Therefore, during the development of cancer, disruption of the DDR is a key step that contributes to the acquisition of specific, so-called hallmarks of cancer [2], such as sustained proliferative signalling and resistance to cell death. These capabilities, in turn, increase levels of endogenous DNA damage and cause many cancers to have high levels of so-called replication stress. This, in turn, necessitates the acquisition of compensatory mechanisms that allow cancer cells to survive in a state of uncontrolled genomic instability that further accelerates the process of carcinogenesis.

Importantly, with modern technologies, these changes in cancer cells can be identified and have the potential to be used as biomarkers to predict treatment responses to standard therapies such as radiotherapy and chemotherapy. In addition, their presence may represent a type of molecular 'Achilles heel' that allows clinicians to attack tumors with a new generation of targeted agents. In both of these contexts, assessing the functionality of the DNA damage repair pathway offers significant opportunities for clinician scientists with an interest in a number of tumor types, including squamous cell cancer of the head and neck (SCCHN).

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Molecular Responses to Radiation-/Chemoradiation-Induced DNA Damage

The most lethal DNA lesions induced by radiotherapy (RT), with or without concomitant cisplatin-based chemotherapy, are double-stranded breaks (DSBs). Additionally, the reactive oxygen species produced by RT or chemoradiotherapy (CRT) can cause purine and pyrimidine lesions and single-stranded breaks (SSBs) in DNA [3], which can degenerate into DSBs if left unrepaired. These lesions are chemically identical to those generated endogenously during normal cellular metabolism and replication. Not surprisingly, therefore, there are specific mechanisms to detect and repair these breaks in the DNA: DSBs are repaired either by non-homologous end-joining (NHEJ) repair or, during S and G2 phases of the cell cycle, by high-fidelity homologous recombination repair (HRR). SSBs and base damage are repaired through the base excision repair pathway [4], but these mechanisms will not be considered further in this chapter.

RT- and CRT-induced DNA damage is sensed by different molecular complexes within the cell that are specific to the type of lesion that has been generated. These sensing mechanisms activate so-called apical DDR kinases, ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related (ATR), which integrate signals from different types of DNA damage and replication stress. Downstream checkpoint kinases, Chk1 and Chk2, transduce the signal to a wide variety of effector molecules which, depending on the context and level of damage, may cause cell cycle arrest at the G1/S (via cdc25A), intra-S (via cdc25A and cdc7), or G2/M (via cdc25C) checkpoints to allow time for repair (Fig. 1). They also play a pivotal role in activating DNA repair pathways: Chk1 activates crosslink repair via the Fanconi anaemia pathway, NHEJ via DNA–dependent protein kinase (DNA-PK) and HRR via Rad51; Chk2 activates HRR via BRCA1. They also promote chromosomal stability: Chk1 plays a role in replication fork stabilisation; Chk2 in mitotic spindle assembly [5–7].

RT-/CRT–induced DSBs are sensed by the MRN (MRE11, Rad50 and NBS1) complex, which recruits ATM dimers to the sites of DSBs, causing them to separate and activate by trans-autophosphorylation (i.e. each member of the dimer phosphorylates its partner molecule) [8]. ATM signals directly to p53 which increases transcription of p21/waf1 with subsequent inhibition of cdk2/cyclin E. Additionally, it activates Chk2, which also activates p53 (via a different phosphorylation site). Overall, in cells with intact p53, this results in cell cycle arrest at the G1/S checkpoint and subsequent repair of the DNA damage (or apoptosis if the damage is too significant or complex to repair) [4].

ATR [8, 9] is activated by a number of different DNA lesions, all of which converge in the formation of single-stranded DNA (ssDNA) coated with replication protein A (RpA) (Fig. 1). ATR functions as a complex with ATR interacting protein (ATRIP). ATRIP binds to RpA-coated ssDNA and the ATR-ATRIP kinase is activated by a number of key regulatory proteins (Rad17-RFC complex, the Rad9-Rad1-Hus1 (9-1-1) complex and topo-2 binding protein) which recognise the

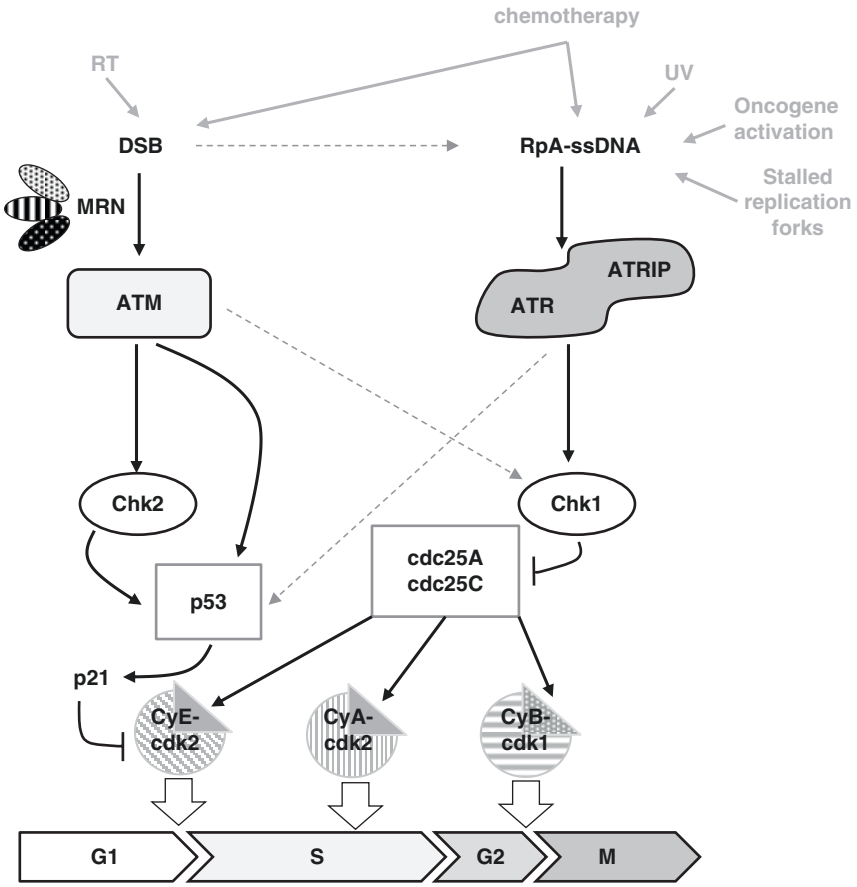

Fig. 1 Simplified diagrammatic representation of the DNA damage response. The ATM pathway is activated by DSBs, causing activation of Chk2 and p53 with subsequent G1 cell cycle arrest. Diverse inputs converging on RpA-coated ssDNA activate the ATR-ATRIP complex, with downstream phosphorylation of Chk1, amongst other targets, resulting in arrest in G2/M. The ATR pathway also plays an important role in S-phase progression and replication fork stabilisation. The substantial crosstalk between these pathways is indicated by the dotted arrows

junctions between ssDNA and dsDNA and stimulate ATR kinase activity. This activating complex occurs after a number of DNA lesions, including stalled replication forks/replication stress and DSB [9, 10].

The traditional view that DSBs activate ATM, Chk2 and G1 arrest and other DNA damage activates ATR, Chk1 and G2/M arrest is now viewed as being rather simplistic, in light of the potential redundancy between the pathways [11, 12]: ATM can activate ATR and Chk1 and Chk1 can activate p53. Furthermore, DSBs can activate ATR, through end-processing in an ATM-dependent and independent manner, and single strand lesions can degenerate into DSBs, activating ATM, in the event of replication fork collapse.

Measuring HRR Function to Predict Response to Radiotherapy/Chemoradiotherapy

Given the fact that unrepaired DSBs are the main lesions that determine responses of tumors and normal tissues to RT/CRT, attempts have been made to study their formation and resolution as a means of predicting responses to standard-of-care therapies. As discussed above, the protein Rad51 is a key component of HRR and forms foci at the site of DSBs that can be detected using immunofluorescence [13]. Two studies in patients with breast cancer have shown that Rad51 foci, as measured by immunofluorescence in tumor biopsies obtained after inducing DNA damage, can be used as a functional marker of DDR [13, 14]. In addition, in the former study, it was shown that delayed HRR, as determined by non-resolution of Rad51 foci, is able to predict for response to induction chemotherapy in patients with breast cancer.

We have undertaken a similar study in patients with SCCHN with the goal of investigating if delayed repair of DNA DSB can serve as a predictive biomarker of response to CRT in patients with locally advanced SCCHN [15]. Eligible patients had stage III/IV SCC of the oropharynx, with primary tumors that were visible and amenable to biopsy transorally. All patients received platin-based induction chemotherapy followed by CRT according to a standard regimen [16]. Response at 3 months after treatment was assessed using clinical examination and PET-CT scans. Residual disease as diagnosed on PET-CT scans was confirmed by biopsy. Patients underwent a baseline pre-treatment tumor biopsy and then a punch biopsy of the primary tumor under local anaesthetic between 24 and 30 h after administration of platinum during the first cycle of induction chemotherapy. Directly contiguous sections were cut for Rad51 and phosphorylated H2AX (γH2AX) analyses, the latter was performed to confirm that the biopsied tumors had been exposed to chemotherapy in concentrations sufficient to induce DNA damage. Three investigators blinded to all clinical data, including clinical response, counted the foci independently. The staining of nuclear foci was scored as follows. Between 100 and 500 cells in the sub-epithelial layer (invasive tumor) with any nuclear geminin (S-phase marker) staining were counted at representative areas. A cell with any nuclear geminin staining was scored positive and defined as being in S-phase of the cell cycle. Any geminin-positive cell in the sub-epithelial layer was considered by definition to be a tumor cell. Rad51 foci were only counted in geminin-positive cells. A cell was counted as Rad51-positive if there was at least one distinct focus per nucleus. γH2AX staining was performed on a contiguous section, with cells containing at least one focus considered positive to confirm that DNA damage had been inflicted. The mean number of foci counted by the three independent investigators was calculated. The percentage of geminin-positive cells that were also positive for Rad51 was calculated as the Rad51 score for pre- and 24-h post-treatment specimens. The difference in Rad51 score for the pre- and post-treatment specimens was then calculated. Tumor specimens with a $\leq$10% difference between pre- and post-

treatment specimens were deemed to have repaired the chemotherapy-induced DSBs, and were classified as Rad51-negative. Such tumors were defined as HRR competent. Tumors in which there was a >10% difference in Rad51 score in the pre- and post-treatment specimens were defined as Rad51-positive and HRR incompetent.

Thirteen pairs of samples were available for detailed analysis. Median Rad51 scores for pre- and post-treatment samples were 12% and 50%, respectively. The baseline level of Rad51 positivity before chemotherapy was considered to be due to spontaneous DNA damage, including that arising from replication stress. Based on the cut-off of persistence of Rad51 foci at < or >10% above baseline, three tumors were classified as Rad51-negative (HRR competent) and ten as Rad51-positive (HRR incompetent). At the 3-month post-treatment assessment, complete clinical and radiological responses (CR) to CRT were achieved for 10/13 (77%) patients; the remaining 3/13 (23%) patients had biopsy-proven evidence of persistent or progressive disease (PD). All three patients classified as having Rad51-negative/HRR competent tumors had PD and the ten patients with Rad51-positive/HRR incompetent tumors had a CR (Fig. 2). Two of five (40%) patients with HPV-negative disease and 100% of patients with HPV-positive disease had Rad51-positive/HRR incompetent tumors and had a CR. All three Rad51-negative/HRR competent tumors with PD were HPV-negative and would have been predicted to be in the poor prognosis group according to a standard classification [17].

In conclusion, this study demonstrates that failure to resolve Rad51 foci following platin-induced DNA damage can be used as an index of DSB repair functionality and can predict response following CRT for locally advanced SCCHN, albeit in a very small number of patients. Such an assay might hold promise as a potential biomarker for patient selection in trials of DDR pathway modulation, especially in patients with Rad51-negative/HRR competent tumors. In addition, this study provides direct clinical evidence that delayed DSB repair may, at least in part, explain the enhanced treatment sensitivity of HPV-positive SCCHN.

Targeting HRR Function to Sensitise Tumors to Radiotherapy/Chemoradiotherapy

The cell kill induced by radiotherapy relies on cells attempting to divide with unrepaired, damaged DNA. Cell death after irradiation is generally thought to occur through mitotic catastrophe [18, 19], or in some cases replicative senescence [20]. Mitotic catastrophe occurs when cells try to undergo mitosis in the presence of unresolved DNA damage: the resulting chromosomal missegregation or loss of chromatid fragments leads to abnormal cell division, resulting in a delayed form of cell death (sometimes after subsequent rounds of cell division). There is also increasing evidence

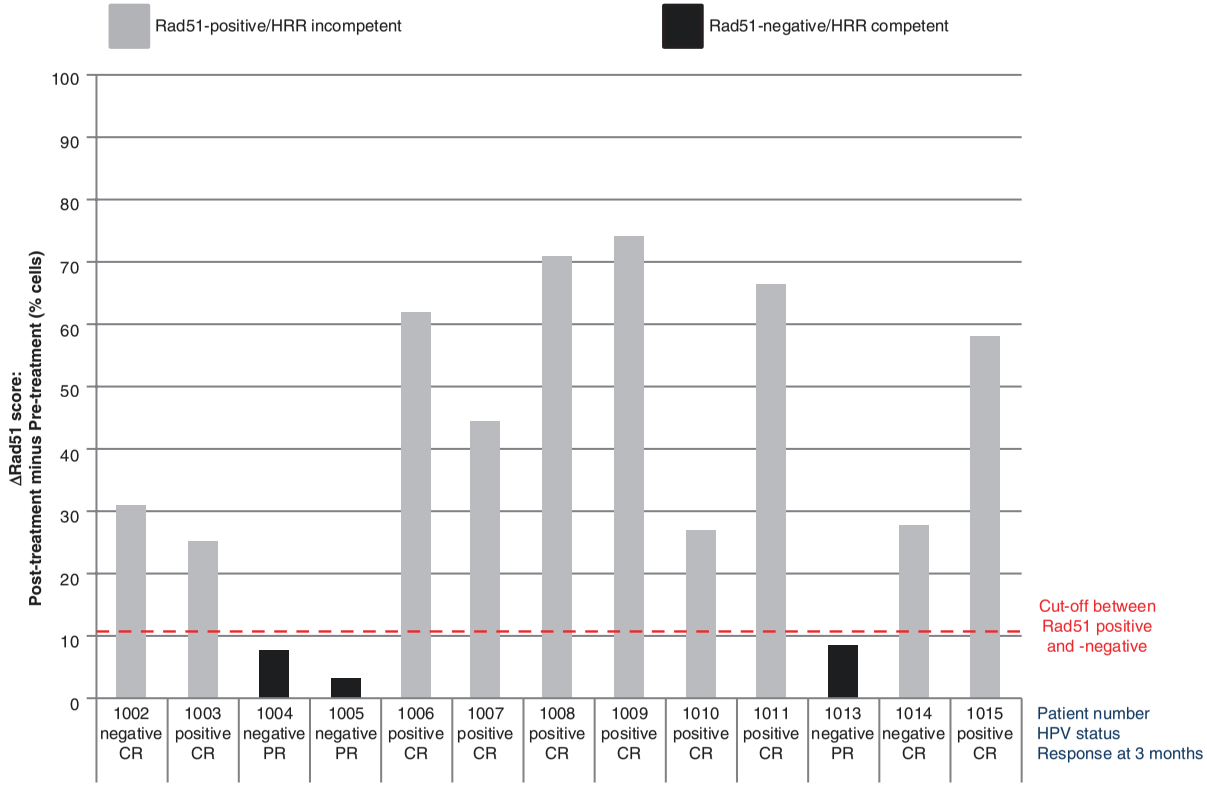

Fig. 2 Relationship between RAD51 scoring status and treatment outcome at 3 months post-treatment in 13 patients with locally advanced SCCHN. Rad51-negative/HRR competent tumors [indicated by black bars] were associated with partial response or progressive disease (PR), whereas Rad51-positive/HRR incompetent tumors [indicated by grey bars] were associated with complete remission (CR)

that aberrant mitoses are associated with the accumulation of cytoplasmic DNA fragments and micronuclei that may profoundly influence the consequences of cell death. In particular, there is a growing appreciation of the interplay between micronucleus generation and the development of anti-tumor immunity following RT/CRT [21, 22].

Inhibition of regulatory checkpoints prevents cell cycle arrest and blocks effective initiation/completion of DNA repair following DNA damage by radiation (and/or genotoxic chemotherapy). A significant proportion of tumors, including SCCHN, have lost normal p53 function [23] and, as a result, have impairment of the G1/S checkpoint. Cells lacking in p53 function rely heavily upon the ability of the ATR-Chk1 pathway to mediate intra-S and G2/M cell cycle arrest to allow HRR following RT/CRT. Therefore, therapeutic inhibition of the ATR-Chk1 axis has to potential to lead to impaired HRR function and subsequent mitosis with unrepaired DNA damage, resulting in mitotic catastrophe [24].

Therefore, G2/M checkpoint control can be seen as a very attractive target for drug development with the following therapeutic paradigm. After treatment with G2/M checkpoint inhibitors, p53 pathway-proficient cells (e.g. normal cells) will undergo effective cell cycle arrest, predominantly in G1, to allow DNA repair. As such, they will be relatively resistant to pharmacological inhibition of the G2/M cell cycle checkpoint—although cycling normal cells that have already passed the G1/S checkpoint will also be at risk of mitotic catastrophe after G2/M inhibition. In contrast, p53 pathway-deficient cancer cells will be especially sensitive to inhibition of the remaining S and G2 checkpoints [24] by G2/M checkpoint inhibitors and will pass through mitosis with unrepaired radiation-induced DNA damage, with resultant mitotic catastrophe and cell death. As such, in p53 pathway-defective tumor cells, G2/M checkpoint inhibitors may be capable of mediating a synthetically lethal interaction with ionising radiation (Fig. 3).

Targeting G2/M Checkpoint Control Through ATR or Chk1 Inhibition

The hypothesis outlined above that checkpoint inhibition has the potential to lead to radiosensitisation has been extensively supported by preclinical studies. Early indications of the promise of checkpoint inhibition came from observations that pharmacologically active concentrations of caffeine cause p53-deficient cells arrested by DNA damage in S or G2 to proceed through the cell cycle to a lethal mitotic event [25–28]. However, this was not a clinically translatable strategy because the doses of caffeine that would have been necessary to cause this effect in patients were not tolerable. A similar effect was noted from the non-selective protein kinase inhibitor, 7-hydroxystaurosporine (UCN-01) [29]. It was later discovered that UCN-01 inhibits Chk1 [30], which is likely responsible for this effect, and caffeine inhibits ATM and ATR.

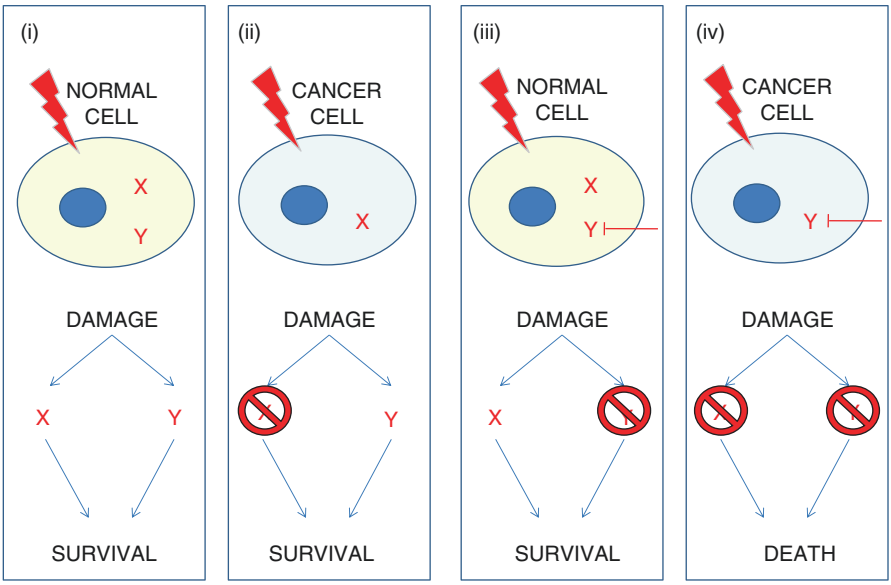

Fig. 3 Diagrammatic representation of synthetic lethality following combination radiotherapy and targeted drug treatment. (1) Following irradiation (red lightning flash) of a normal cell, the resulting DNA damage can be repaired by a number of different pathways which, for the sake of simplicity, are represented by X and Y. As a result, the normal cell survives radiation-induced damage. (2) In tumor cells, specific DNA repair pathways may be lost as part of the genomic instability associated with the malignant process (in this case, depicted as loss of pathway X). Nevertheless, upregulation of pathway Y (shown as a large Y) is sufficient to allow the tumor cell to survive the radiation-induced damage. (3) Following administration of a specific inhibitor of pathway Y, normal cells are able to survive irradiation by repair mediated by pathway X. (4) In contrast, administration of a pathway Y inhibitor to cancer cells blocks their only remaining means of repairing radiation-induced DNA damage and results in cancer-specific cell death or so-called "synthetic lethality"

ATR Inhibitors

ATR inhibitors (ATRi) may radiosensitize cancer cells through disruption of the intra-S and G2 checkpoints and possibly by interfering with the roles that ATR plays in the DSB response, replication fork stabilisation and the response to hypoxia [31, 32].

A number of small molecule inhibitors of the ATR kinase have been developed. In pre-clinical studies they have usually been investigated in combination with genotoxic chemotherapy or ionising radiation. As yet, there have been no published reports of ATR inhibitors in patients, although some agents are currently in early phase clinical trials.

VE-821 (Vertex Pharmaceuticals, UK, recently acquired by Merck KGaA) is the most extensively reported ATR inhibitor at present. It causes cell death and growth inhibition in cancer cell lines and synergises with DNA-damaging agents, including RT [33]. Sensitisation was seen in ataxia telangiectasia (AT) cells, and in normal

cells whose ATM function had been disrupted by a specific inhibitor, or whose p53 function had been reduced by small interfering RNA (siRNA) silencing or HPV E6 protein (which targets p53 for degradation). Additionally, ATR inhibition sensitises cells to the effect of hypoxia and reoxygenation both with and without radiation. The compound sensitized multiple pancreatic cancer cell lines to gemcitabine and radiation (under both oxic and hypoxic conditions): with evidence of maintained DNA damage and reduced homologous recombination (as shown by reduced Rad51 foci) [34].

A related compound, VE-822 (Vertex Pharmaceuticals, UK, recently acquired by Merck KGaA) sensitised pancreatic cancer cell lines to both radiation and gemcitabine in vitro, without apparent toxicity in normal cell lines. Additionally, it has demonstrated signs of efficacy in pancreatic tumor xenografts, with inhibition of radiation-induced Chk1 phosphorylation and a reduction in tumor growth when combined with radiation or gemcitabine-based chemoradiation in comparison to untreated or single-agent treated controls. VE-822 did not appear to cause any additional in-field radiation-induced toxicity to the small bowel [35].

AZD6738 is an orally active ATR inhibitor currently in phase I clinical trials. It has been shown to have in vitro growth inhibitory activity in a panel of human cancer cell lines [36] and radiosensitized multiple cancer cell lines to single radiation fractions in a manner that is independent of both p53 and BRCA2 status. Radiosensitization by AZD6738 to clinically-relevant doses of fractionated radiation has also been demonstrated in vitro using a 3D tumor spheroid model and, in vivo, AZD6738 radiosensitized by abrogating the radiation-induced G2 cell cycle checkpoint and inhibiting HRR. Mitosis with damaged DNA resulted in mitotic catastrophe as measured by micronucleus formation by live cell fluorescent-ubiquitination cell cycle imaging of cell cycle progression and nuclear morphology (Fig. 3). Induction of micronuclei was significantly more pronounced for AZD6738 compared to inhibition of the downstream kinase CHK1 alone at isoeffective doses.

Chk1 Inhibitors

A number of Chk1 inhibitors have been investigated in preclinical trials in cell lines and xenograft models and a smaller number have reached clinical trials. The first Chk1 inhibitor was UCN-01. Preclinical data with this agent showed enhancement of radiation and genotoxic chemotherapy-induced cytotoxicity which was selective for cells with dysfunctional p53 function [29]. UCN-01 progressed to a number of phase 1 trials as monotherapy [37] and in combination with DNA-damaging agents such as platinum [38], topoisomerase inhibitors [39] and nucleoside analogues [40, 41]. Clinical trial data showed a prolonged plasma half-life with multiple off-target effects that caused toxicity, including hyperglycaemia, cardiac arrhythmia, breathlessness/hypoxia, hypotension, nausea and diarrhoea. In phase II studies, UCN-01 was tested in combination with DNA-damaging chemotherapy but the reported response rates were not impressive and its development was terminated [42–44].

AZD7762 is a combined Chk1 and Chk2 inhibitor which radiosensitized tumor cells in a p53-dependent manner in vitro and in vivo in preclinical trials. Again the data on p53 selectivity were not entirely clear-cut [45, 46] (indicating that this may not be the most important focus for radiosensitization, perhaps relating to reduced Chk1 activation of HRR). This agent progressed to clinical trial as a single-agent and in combination with gemcitabine (NCT00413686) but its development was terminated prematurely because of concerns about toxicity (especially cardiological effects) [47].

We have evaluated the activity of a potent Chk1 inhibitor (SAR-020106) in combination with radiation in vitro and in vivo [48]. As with other agents in this class, SAR-020106 abrogated radiation-induced G2/M arrest and reduced clonogenic survival in p53-deficient, but not p53 wild-type, tumor cells. SAR-020106 promoted mitotic entry following irradiation in both p53-deficient and -proficient cells, but with significantly different results: p53-deficient cells tended to undergo apoptosis or become aneuploid, while p53 wild-type cells underwent a post-mitotic G1 arrest followed by subsequent normal cell cycle re-entry. Following combination SAR-020106 and radiation therapy, HRR-mediated DNA damage repair was inhibited at 4 h in p53-deficient and -proficient cell lines. At 24 h, there was a significant increase in the number of apoptotic cells only in the p53-deficient cell lines. In vivo efficacy was confirmed by delayed tumor growth and increased survival in a clinically relevant human head and neck squamous cell carcinoma xenograft model. This agent has not progressed to clinical studies.

Following on from the development of SAR-020106, we have tested an orally bioavailable Chk1 inhibitor (CCT244747) as a radiosensitiser and investigated whether a mechanistically rational triple combination of radiation/paclitaxel/Chk1 inhibitor delivered according to an optimised schedule would provide added benefit [49]. As with other Chk1 inhibitors, CCT244747 abrogated radiation-induced G2 arrest in p53-deficient SCCHN cell lines, HN4 and HN5, and caused cells to enter mitosis with unrepaired DNA damage. The addition of paclitaxel further increased cell kill and significantly reduced tumor growth in an HN5 xenograft model. Importantly, a lower dose of paclitaxel could be used when CCT244747 was included, therefore potentially limiting toxicity. Triple therapy reduced the expression of a number of markers of radioresistance. In cell survival studies in two different cell lines, the relatively radioresistant cell (HN5) was sensitised to a far greater degree than the more radiosensitive cell line (HN4) to the triple combination of radiation/paclitaxel/Chk1 inhibitor. Chk1 expression was also analysed in a panel of head and neck tumors and observed that primary tumors from HPV+ patients, who went on to recur post-radiotherapy, exhibited significantly stronger expression of total and activated Chk1. In keeping with the data on the resolution of Rad51 foci after induction platin-based chemotherapy [15] discussed above, this finding suggests that Chk1 may serve as a biomarker for identifying tumors likely to recur after RT/CRT.

Clinical trials have commenced of a molecule that is related to CCT244747. This agent SRA737 is an orally bioavailable Chk1 inhibitor and is being tested as a single-agent [NCT02797964] and in combination with either gemcitabine or gemcitabine/cisplatin doublet [NCT02797977].

Conclusions

HRR plays a central role in repairing DNA damage caused by anti-cancer therapies. In non-malignant cells, this is a highly desirable event in that it limits treatment-induced toxicities. However, in malignant cells, the ability to repair spontaneous or treatment-induced DNA damage represents a survival mechanism that can exert a serious negative effect on patient outcomes.

In recent years, we have begun to understand how we might be able to measure HRR functionality in cancer cells, both at baseline and in response to therapeutic perturbation, as a means of predicting treatment outcomes in response to standard-of-care therapies. Such tests have the potential to allow us to select patients for more or less intensive treatment (based on the tumor's ability to repair treatment-induced damage). For example, measuring the degree of resolution of platin-induced Rad51 foci within tumor cells appears to show significant promise as a means of predicting outcomes to chemoradiotherapy in locally advanced SCCHN.

In addition, our increasingly detailed understanding of the mechanisms of DNA repair has highlighted the potential value of targeting HRR using specific drugs. ATR and Chk1 inhibitors offer the prospect of inducing synthetic lethality in cancer cells, especially those such as SCCHN that have a tendency to be reliant on G2/M cell cycle checkpoint control. A number of clinical trials are ongoing at present and it is hoped that these will lead to new therapeutic strategies for patients with locally advanced and relapsed/metastatic SCCHN.

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Part III
Oral Cavity Cancer

Surgical Management of Oral Cavity Cancer

C. René Leemans and Sat Parmar

Introduction

Oral cancer counts for approximately half of all tumors that occur in the head and neck. 300,000 new cases of oral cavity tumors are reported worldwide and the incidence is increasing, also in young adults [1]. It is the sixth most common cancer worldwide. Tobacco and excessive alcohol use are the most common etiological factors in the Western world. The incidence in human papilloma virus-nduced tumors of the oral cavity is also rising and accounts for an estimated 5% of the total [2]. The clinical significance is still unclear. The incidence of oral cancer in females is also increasing. The incidence in South-East Asia, parts of South America and Eastern Europe is remarkably higher than in the western world. Smoking or chewing tobacco with betel quid and alcohol are held responsible.

Due to an increased knowledge of the carcinogenic process leading to squamous cell carcinoma of the oral cavity, the future may hold promise in terms of more tailored treatment approaches [3]. That having been said it is still surprising that two thirds of the patients present with advanced staged disease and very large tumors. It is therefore clear that the key to improved survival currently mainly lies in early detection. Thus efforts also need to be directed towards awareness among the general public of the early signs and symptoms [4].

The last decade has also witnessed increasing emphasis on quality of life after treatment for cancer and consequently surgeons have found procedures that preserve to the best of their ability form and function. Patients however, still prioritize cure of cancer as their main goal, with preservation of form and function and maintaining or improvement of quality of life after that [5]. Another aim is prevention or

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early detection of second primary cancers. Attempts in large trials to influence the incidences of second primary cancers by chemoprevention have thus far failed [6].

Several treatments for oral cancer exist. Surgery is the main treatment for cure for most of these patients, and radiation or chemoradiation is added in case the patient is at high risk for recurrence. Radiation as main treatment is used for some patients who cannot have surgery because of other medical problems. Biological therapies such as anti-EGFR antibodies in combination with radiation therapy have entered the arena. Also of note is photodynamic therapy that is currently used by some for tumours that are not deeply infiltrating. In stages I and II single modality treatment is preferable, consisting of either surgery or radiation therapy. For stages III and IV multimodal therapy is warranted consisting mainly of surgery and radiation therapy or for inoperable or unresectable tumors chemoradiation therapy. Treatment is dictated by tumor factors that can be determined by imaging, examination and histology, patient factors such as age, comorbidity and patient wishes and expectations, and physician, institutional, and provider factors.

Tumor Factors

The size of the lesion has a determinant impact on the treatment decision. Small and not too deeply infiltrating tumors of the oral cavity can be easily resected transorally, whereas more posterior located lesions or those that infiltrate deeply into the tongue or floor of mouth need greater access (Figs. 1 and 2).

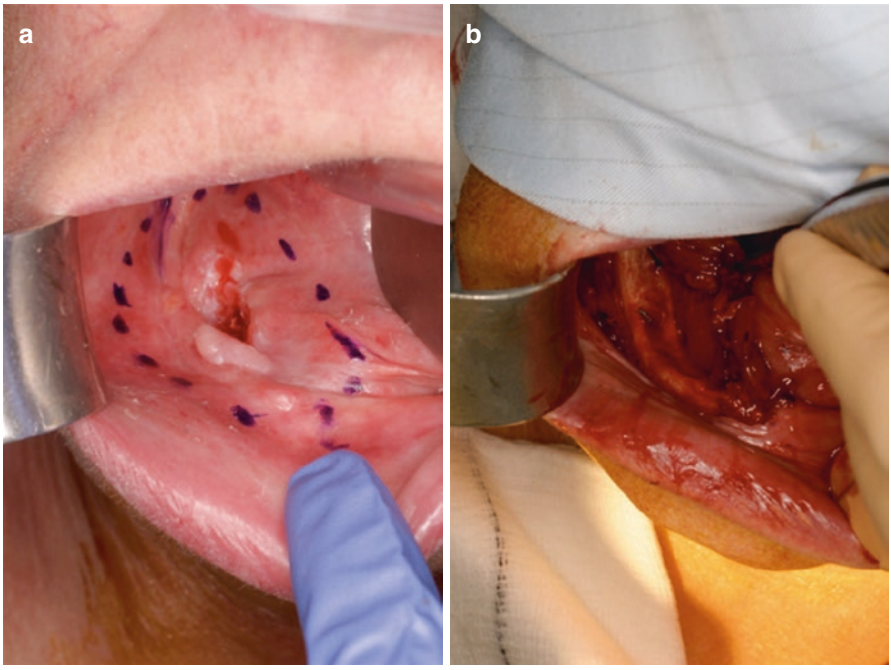

Fig. 1 (**a**, **b**) Peroral resection of a right-sided floor of mouth carcinoma

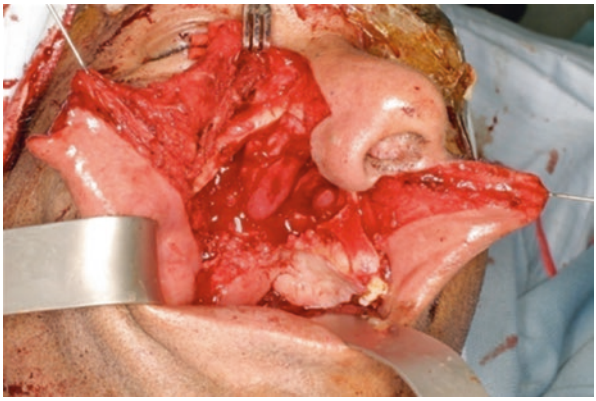

Fig. 2 Facial access for a right sided maxillary tumour

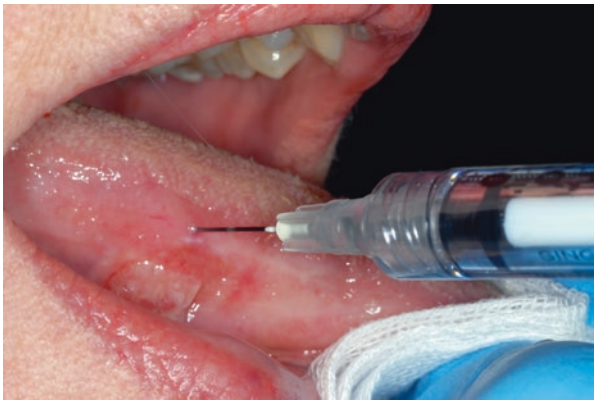

Fig. 3 Peritumoral injection of 99mTc-labelled colloidal albumin for sentinel node procedure

The status of the neck nodes is also important and when neck staging is high, distant metastasis should be ruled out. Other factors that determine treatment are the proximity to mandibular or maxillary bone, multiplicity and previous treatments as well as pathological characteristics of the tumor. The depth of invasion (DOI) in tongue cancers determines the incidence of occult nodal metastasis in the clinically negative neck. Tumors that are thicker than 4 mm have a distinctly higher neck metastatic rate [7]. DOI should be measured from an imaginary line connecting the levels of normal epithelium surrounding the tumor [8].

Treatment of the neck is vital, since it determines to a large extend the survival of the patient. For the thicker T1-lesions and T2-lesions and above, an elective neck dissection is often indicated. In lesions that can be resected transorally, a negative sentinel node procedure may justify a policy of observation towards the neck [9, 10] (Fig. 3).

Unresectable tumors or those that if resected would inflict great morbidity to the patient, may be treated with chemoradiation schedules [11]. The morbidity of these protocols is often significant. Salvage surgery for failure after these protocols is often very disappointing [12–15].

As said, most tumors of the oral cavity are treated surgically, even when they are large. Functional outcome is often acceptable. Tumors that have been non-radically

excised have close or positive margins or those that exhibit extranodal spread, need postoperative chemoradiation therapy as long as patients can tolerate this treatment [16].

Patient Factors

Co-morbidly rather than chronological age are important considerations, in managing patients with advanced oral cancer [17]. A lengthy operation, even with modern day anesthesiology, may be too much for patients with severe comorbidity, especially when microvascular reconstruction is necessary. Previous treatments in the same area may also contraindicate the ideal treatment and modifications have to be made.

Other Factors

The experience of the multidisciplinary team that has taken care of the patient with oral cancer and its experience and local setting, may be a determining factor in the management of such patients. It should be emphasized that patients with head and neck cancer need discussion within a multidisciplinary team in order to get the best available treatment proposal.

Surgical Access to Oral Cancer

Several approaches may be employed, depending on the factors described above. They include a perioral approach, a pull through approach, a lower cheek flap or upper cheek flap approach or a mandibulotomy approach. The choice depends on tumor size and site and location, as well as proximity to bone and the need for neck dissection and reconstructive surgery. Small superficial lesions may be resected per orally, with or without a marginal mandibular resection or an infrastructure maxillectomy. For larger lesions when communication with the neck after a neck dissection results, a flap reconstruction is necessary to re-establish an oral seal. Currently many institutions favor microvascular reconstruction by a fasciocutaneous flap for optimal postoperative outcome. Larger posteriorly located lesions may require a mandibulotomy for adequate exposure (Fig. 4).

Management of the mandible is an important consideration in the surgical treatment of oral cancer. If possible a marginal mandibulectomy (rim resection) is favored in case the tumor is close to the bone. This is feasible when the tumor is superiorly to the mylohyoid muscle and when the procedure is only needed for adequate margins. Minimal erosion of the cortex or the alveolar process does not contraindicate marginal resection. In the dentate patient with sufficient height of

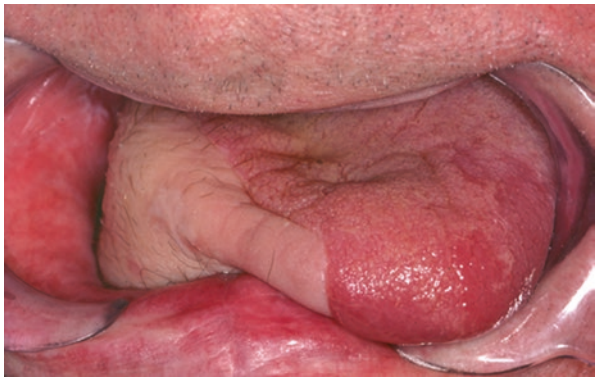

Fig. 4 End result after removal of T3 right sided oral tongue cancer that was reconstructed by a free radial forearm flap

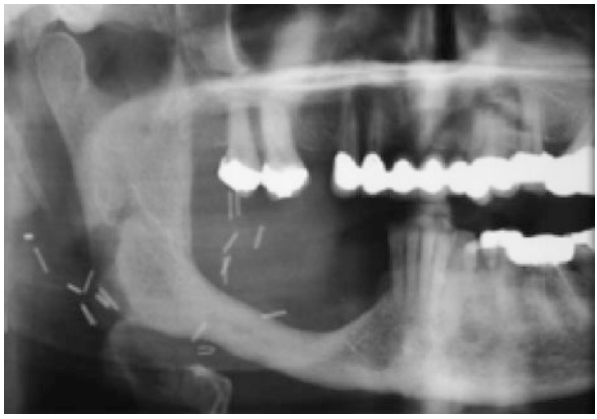

Fig. 5 Rim resection of mandible for SCC

mandible, it is also feasible to apply a marginal resection when there is limited invasion of the alveolar process. Marginal resections should be preferably boat-shaped in order to avoid points of increased tension and risk of fracture of the marginal segment (Fig. 5).

For tumors located posteriorly, for instance retromolar trigone lesions, a marginal resection including the coronoid process can be undertaken. In other cases, for instance if there is clear invasion of the cortex, a segmental resection is indicated.

The need for reconstruction and type of reconstruction obviously depends on the nature of the defect. For small superficial lesions primary closure or healing by secondary intention is appropriate. For larger lesions, a local flap, regional flap, or free flap is indicated. Free flaps can be fasciocutaneous, osteocutaneous or osteomyocutaneous, depending on the need for specific reconstructive tissue.

The ultimate reconstruction for an oral cavity cancer patient after surgery is placement of osseointegrated implants and dental rehabilitation. Using modern day osteocutaneous free flaps, this is a very achievable goal, but often takes many months or even years before the final result is obtained.

Outcome

Failure in treatment usually occurs locally or regionally, whereas few patients experience distant metastasis. Disease specific survival for T1 and T2 lesions is 94% and 76 %, respectively, while for the more advanced lesions this drops to 60%. Patients with a N1 positive neck have a disease specific survival (DSS) of approximately 60%, while those having N0 fare much better with a DSS of 84%. Advanced neck nodal disease results in a disappointing 46% survival. Cases with positive margins result in a distinctly worse outcome compared to cases with tumor-free margins, while the same is true for extranodal spread in the neck [17–19]. This is the reason that attempts have been made to improve results in those patients by adding chemotherapy to postoperative radiation therapy. Two large randomized trials, (EORTC 22931 and RTOG 9501) have been conducted and they have shown improved locoregional control [16]. Studies that report on quality of life after treatment for oral cancer are emerging and may be helpful in counseling the patient and when determining between equally efficacious treatment alternatives.

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Reconstruction in the Oral Cavity: When and How

Jim Higginson, Prav Praveen, Tim Martin, and Sat Parmar

Introduction

The oral cavity is a sensitive region, with many complex anatomical structures contributing to a range of vital physiological and social functions in a relatively small area. It forms part of and supports the airway, permits chewing, swallowing and enjoyment of food, allows both verbal and non-verbal communication, and forms a substantial part of the individual's social identity and self-identity. If these anatomical structures are disrupted or lost, reconstituting both form and function is a complex surgical challenge.

Oral cancer is common, and often presents at an advanced stage, frequently involving key structures within the oral cavity. The gold standard of treatment is complete surgical resection; adjuvant radiotherapy and or chemotherapy may also be indicated, depending on the stage and grade of disease. Resection of the tumour with adequate margins can result in loss of large amounts of tissue, and the more tissue is lost, the more complex the reconstructive challenge. However, the poor prognosis associated with positive resection margins means that it is unacceptable to perform the resection with a view to optimising the results of the reconstruction. The ablation must be oncologically determined, and then reconstruction planned and performed around the resultant defect to maximise quality of life for the patient.

Planning for reconstruction should take into account the available options and the ability of each option to restore form and function. The merits of each option should then be balanced against the complexity of that reconstruction, and discussed frankly with the patient and the multi-disciplinary team. Ideally, the simplest satisfactory option should be selected, but the complexity of head and neck reconstruction frequently precludes simpler surgical solutions, and revision surgery is generally

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much more technically challenging. The emphasis must be on selecting the optimal option at the first opportunity.

In this chapter we will discuss the options for various soft- and hard-tissue defects in the oral cavity, offer evidence and advice to guide decision-making, and present cases that represent our experience, both good and bad.

Reconstruction of Soft Tissue Defects

The oral mucosa is a specialised membrane that is flexible and sensitive, but sufficiently tough to withstand considerable force whilst chewing. There are no reliable donor sites from elsewhere in the body that can reconstitute this (although small amounts of mucosa can on occasion be procured from other sites in the mouth), but it is a widely-observed phenomenon that skin, when transferred into the mouth as part of a vascularised flap, adopts a more soft and pliable phenotype, although they retain histological features of skin. In addition, mesenchymal tissue such as muscle or fat, when secured in the mouth, acquires a mucosal covering within a matter of weeks. This process is known as mucosalisation, and is thought to represent seeding of mucosal stem cells onto the mesenchyma, but remains poorly understood.

Tongue and Floor of Mouth Defects

The lateral border of the anterior tongue and the floor of mouth are the oral cavity sub-sites most affected by oral squamous cell carcinoma. The tongue has many crucial roles in physiological and social function, and loss of these functions can have a great impact on quality of life. The tongue maintains the patency of the airway, articulates sound into intelligible speech, manipulates the food bolus and oral secretions during chewing and swallowing, and is exquisitely sensitive with densely packed touch, proprioceptive and taste receptors.

Floor of mouth pathology can be considered together with tongue malignancies, as resection of either site with sufficient margins frequently involves removing at least some tissue from the other site. The two sub-sites are intimately connected, and are best thought of as a functional unit that should be considered as a whole for optimal functional outcomes.

Aims of Treatment

The principle aim of treatment is to restore the patient to function as close to the pre-morbid state as possible. The extent to which this is possible is greatly dependent upon the amount of residual tissue following oncologically sound resection.

Principles of Lingual Reconstruction The two main aims are to maximise the function of the residual tongue, and to restore the bulk of the tongue. The relative importance of these depends on the size of the defect after reconstruction—the less bulk of native tissue remains, the more the reconstruction should focus on restoring bulk. This can often require over-reconstructing to allow for some post-operative atrophy of donor tissue.

Key aspects of maximising function include:

- Emphasising accurate reconstruction of the tongue tip, ensuring it is sufficiently mobile to allow contact with the premaxilla, a key factor in articulation of intelligible speech. Effective tip elevation correlates strongly with better functional outcome [1].
- Allowing sufficient mobility for the tongue to cleanse the lingual and buccal sulci, aiding movement of food and secretions posteriorly.
- Optimising sensation.

Principles of Reconstructing the Floor of Mouth Here, the key is to re-store a natural sulcal anatomy that allows free movement of lingual tissues. As such pliable, mobile tissue is required, but avoidance of excessive bulk is also important. Inset of flaps into the floor of mouth should avoid creating a 'sump', a concavity in which secretions collect, increasing the risk of salivary fistula during the recovery period, and forming an unpleasant food trap in the long-term.

However skilled the surgeon, the importance and complexity of lingual function means that the patient is likely to experience substantial worsening of their quality of life. A key role of the multi-disciplinary team in the work-up of patients with tongue or floor of mouth cancer is careful explanation of the changes that are likely to occur, and to manage expectations. An experienced speech and language therapist is invaluable here.

Options for Treatment

There are a number of evidence-based and common-sense principles that help guide decision-making when selecting a lingual reconstruction, but often it comes down to the preference and experience of the surgeon.

Primary Closure For very small defects—up to 2 cm—of the lateral tongue, primary closure can be achieved, however there is a significant risk that doing so will cause tethering of lingual function and caution should be exercised when considering this approach. In the floor of mouth, primary closure for all but the smallest resections is likely to result in significant tethering of tongue mobility.

Secondary Intention Historically, allowing a resection of a defect on the lateral tongue to granulate and heal by secondary intention led to contracture, scarring and immobilisation of the tongue that caused substantial tethering and compromise of

lingual function. However, the use of carbon dioxide lasers for oncological resection of smaller (T1/T2) lateral tongue tumours is allowing more wounds to be managed in this manner. Resection with CO_2 laser causes substantially less scarring and fibrosis than resection with 'cold steel' or electrocautery, and good outcomes can be achieved with a short procedure and a minimal hospital stay. The raw wound surface must be kept clean with chlorhexidine mouthwash to prevent secondary infection and associated risk of post-operative bleeding [2].

Local Flaps Pedicled flaps from local intra-oral tissue can provide a nice tissue match to fill smaller defects intra-orally, but because of the bulk of tissue that is frequently lost following resection of tongue and floor of mouth tumours, they are rarely indicated in practice. For smaller defects of the floor of mouth, a facial artery myomucosal (FAMM) pedicled flap may provide pliable soft tissue that avoids a second surgical site.

Regional Flaps Before the advent of free flaps, these were the gold standard for lingual and floor of mouth defects, in particular the pectoralis major myocutaneous flap was used extensively. However, they are bulky, have high morbidity, and are in fact more prone to vascular compromise than free flaps.

Free Flaps For hemiglossectomy defects, the reconstruction must reconstitute bulk, but must also allow free movement of the residual tongue, as the substantial redundancy within the lingual intrinsic musculature means that surprising function can be retained if half of the tongue remains. The advent of the radial forearm free flap in 1981 revolutionised lingual and floor of mouth reconstruction by providing a reliable source of tissue that satisfied most of the criteria for good quality results. Other flaps that may be considered include the medial sural artery perforator (MSAP) flap, the lateral forearm flap and the anterolateral thigh (ALT) flap.

The 'default' reconstruction is a rectangular flap sutured to the edges of the defect and folded under to form a neotongue and lingual sulcus. This inset is straightforward and reliable in most hands, and is our preferred approach but has some drawbacks that have led to many authors proposing modifications:

In 1994, Urken and Biller proposed a modification to hemiglossectomy reconstruction that shaped the donor radial forearm skin into a bilobed design, with the aim of promoting greater mobile independence of the lingual and floor of mouth components of the reconstruction (see Fig. 1) [3]. This bilobed concept has been used successfully with other donor free flaps, such as the ALT flap [4].

Davison et al. [5] proposed a different approach, in which they rotated the residual tongue tip and lengthened it with a Z-plasty, maximising the function of this crucial functional unit. They also suggested plication of the floor of mouth portion of the flap to aid sulcal cleansing and prevent formation of a sump (see Fig. 2).

Despite these and other technical modifications suggested in the literature, evidence evaluating outcomes after various reconstructive options is limited. One of the most comprehensive studies of function after reconstruction evaluated patient-related outcomes following reconstruction with the traditional rectangle radial fore-

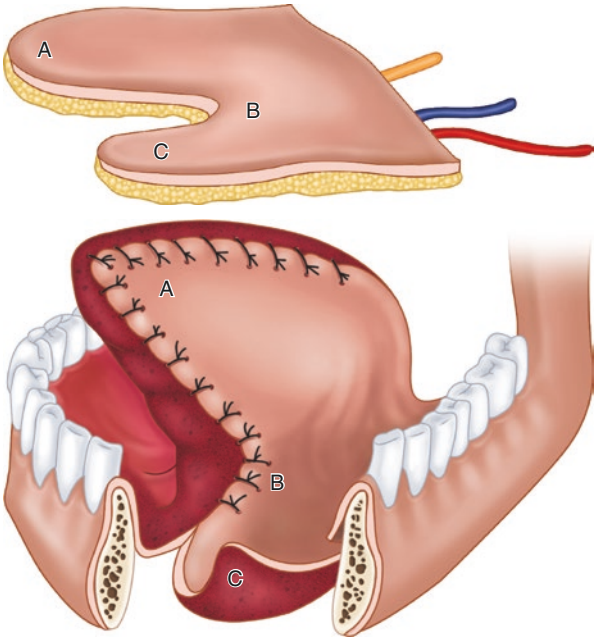

Fig. 1 Creating a bilobed shape from the harvested radial skin allows inset of the flap that allows greater independence of the lingual and floor of mouth components [3]. Note how the points A, B and C on the flap template (above) reconstitute the normal anatomy of tongue tip (A), junction of tongue and floor of mouth (B) and anterior floor of mouth (C) on inset (below)

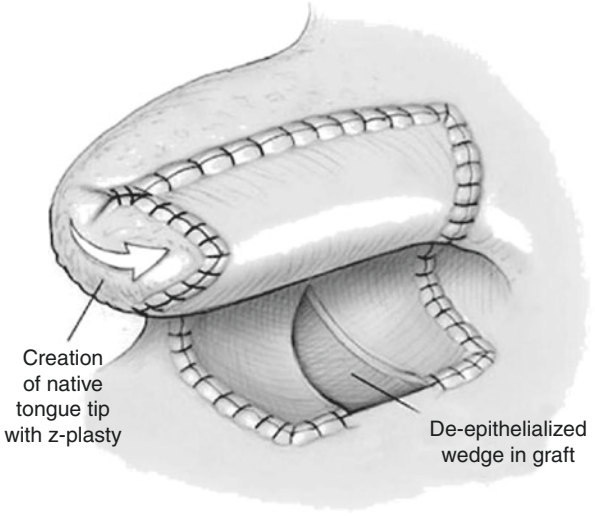

Fig. 2 This modification of the rectangular radial inset shows the maximisation of sensation of the residual tongue tissue by rotating and advancing the tongue tip, such that the whole tip is sensate. The edges of the de-epithelialised wedge are approximated with sutures, helping to eliminate the 'sump' effect [5]

arm free flap using carefully determined metrics [1]. The outcomes were found to be satisfactory, and in our view any reconstructions claiming superiority should be evaluated with equal exactitude, ideally in a prospective comparative study.

Sub-total and Total Glossectomy As noted in section "Aims of Treatment", when the pathology demands resection of a large amount of tongue tissue, functional outcomes are inevitably worse, and the emphasis of reconstruction moves towards

reconstitution of tissue bulk. With decreasing amounts of residual tissue, it is harder for the remnant to move the adynamic flap in a way that allows restoration of normal speech and lingual mobility. Instead, the emphasis is upon using free tissue to create a neotongue that can be mobilised by extrinsic muscles to contact the palate. This allows the patient improved articulation during speech, and allows them to direct food boluses posteriorly for a safe, effective swallow [6].

The choice of reconstruction in this context is often guided by surgical experience, but algorithms do exist to assist decision-making. Engel et al. proposed that hemiglossectomy defects be reconstructed as outlined above, that subtotal glossectomy defects (25–33% residual tongue tissue) be reconstructed with a pentagonal ALT fasciocutaneous flap, and total glossectomy (<25% residual tissue) be constituted with a pentagonal myocutaneous ALT flap. This pentagonal design reconstitutes bulk, and creates a mobile neotip that aids with function [7].

Other flap designs that achieve similar outcomes have been described. In particular, the 'Cathedral Tryptich' flap using an ALT flap reconstitutes adequate volume and acceptable function [8], as does the 'Mushroom' ALT flap [4]. Detailed functional evaluation comparing various total or sub-total glossectomy reconstruction techniques is lacking, however.

Ultimately, functional outcomes following loss of large volumes of lingual tissue remain suboptimal, and it is vital that the patient be adequately prepared for this in the pre-operative setting, with careful discussion between patient, carers, surgeons, speech and language therapist and nurse specialists.

Buccal Mucosa Defects

Like all other sites within the oral cavity, buccal mucosal malignancies are associated with smoking and alcohol consumption, although this site seems to be less frequently affected by these than the lateral tongue, floor of mouth or indeed the pharynx and larynx. Conversely, the use of smokeless tobacco, paan and betel quid is strongly associated with the development of cancer at this site. Also associated with the use of these substances is the development of the pre-malignant condition submucous fibrosis, which can lead to substantial trismus and is associated with a 4–8% risk of malignant transformation; these factors should be considered in all treatment plans.

In health, the buccal mucosa and underlying buccinator muscle have important roles in manipulation of the food bolus during chewing and swallowing. The tissues are elastic and expansile to accommodate food and mouth opening, but thin and pliable to minimise trauma in occlusion. Reconstructing all of these functions to minimise morbidity can be challenging. The thin tissues of this region can mean that advanced or endophytic cancers require resection of overlying skin to ensure oncological safety, causing significant aesthetic compromise. A further consideration is the opening of the parotid duct adjacent to the upper first molar tooth bilaterally—this is often involved in resection and the duct must be repositioned to allow free salivary drainage.

Where buccal cancers begin in or invade towards the anterior aspect of the mucosa, the resection may involve the oral commissure. This is important to consider in the pre-operative planning stage, as loss of the commissure presents a substantial reconstructive challenge. Oral competence is challenging to restore, leading to trouble with eating and social difficulties due to drooling. Microstomia is a common outcome of even the most favourable reconstructive options.

Aims of Treatment

The aims of treatment are guided by the nature of the defect following resection, and can be summarised as:

- Minimising trismus
- Maintaining facial contour and aesthetics
- Maintaining or restoring oral competence

Options for Treatment

Small Superficial Defects Where defects are small, and do not involve the overlying skin, the inherent elasticity of the remaining buccal mucosa can be used to close the defect primarily. This approach only works for defects of around 2 cm, as closure of larger defects can lead to trismus that markedly affects quality of life. For similarly small defects, if primary closure is not possible, allowing healing by secondary intention may be appropriate, although the risk of trismus is high.

Larger Superficial Defects If the defect is too large to allow primary closure, tissue must be recruited from elsewhere to allow coverage without compromising mouth opening. Local flaps such as the buccal fat or nasolabial flap can be used, or regional pedicled flaps such as the submental island or pectoralis major myocutaneous flaps offer different options to the surgeon.

The buccal fat pad is a distinct anatomical structure with its own thin fascial covering, separate from subcutaneous fat. It lies between the buccinator and masseter muscles, and has a rich anastomotic blood supply from branches of the maxillary, superficial temporal and facial arteries. It can be quickly and easily dissected, and provides a reliable source of tissue with minimal morbidity that rapidly mucosalises. Its anatomical proximity makes it a natural choice to consider for reconstruction of small to medium sized defects. However, it provides little bulk so is not suitable for deeper defects, is friable and easily damaged if handled carelessly, and can only reliably cover a defect of around 4 cm diameter [9].

Local and regional flaps prevent the morbidity of a distant donor site and help keep surgical complexity down, but there is some evidence that long-term mouth opening is less favourable with these than with free flap reconstruction [10]. If the defect is greater than around 5 cm in diameter, even if overlying skin is not involved, local and regional flaps are unlikely to be adequate, and so a free flap is indicated.

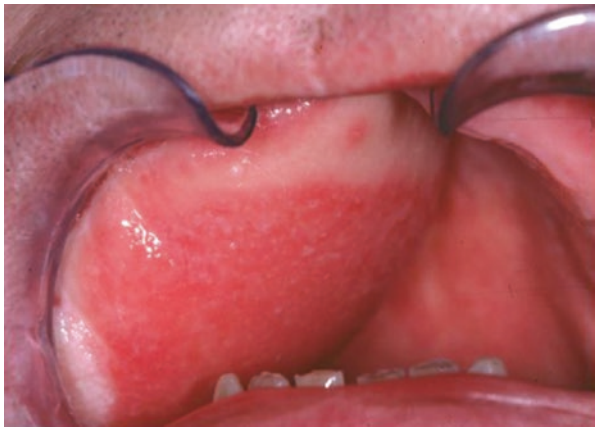

Fig. 3 This is an example of one of our cases where an excessively bulky ALT flap was used to reconstruct the buccal mucosa. The encroachment into the oral cavity is obvious, making it difficult to achieve good dental rehabilitation as the flap will catch in the occlusion

If a free flap is required to reconstruct intra-oral mucosa, the donor site must reliably provide a good quantity of thin, pliable skin to allow restoration of function. The radial forearm free flap is best placed to satisfy these criteria in most circumstances, and as such is well-established as the first choice for intra-oral buccal reconstruction. Mucosa from the contralateral cheek can be used as a free Facial Artery Myomucosal (FAMM) flap. This provides the best possible tissue match, but is disadvantaged by recreating a contralateral buccal defect that must be closed. In thin or cachectic patients, the anterolateral thigh (ALT) perforator flap may also be a good choice, but in the western setting the tissue provided is usually much too bulky for intra-oral lining [11]. An example of one of our cases where excess flap bulk resulted in a suboptimal result for the patient is seen in Fig. 3.

Full-thickness Defects Where external skin is incorporated in the resection, this must be reconstructed with careful attention to aesthetics. To facilitate aesthetic reconstruction, any facial aesthetic subunits that are involved should be resected in their entirety, so that transition between different skin tone and texture is as unobtrusive as possible. Skin must be provided, and the donor flap should be selected with consideration of the match with the resected skin. An example of this approach using an anterolateral thigh (ALT) free flap can be seen in Fig. 4.

Submucous Fibrosis If the patient presents with buccal mucosal cancer in the context of submucous fibrosis—usually associated with betel quid—the trismus can be marked even pre-operatively. Consideration should be given to release of the tissues and reconstruction of the contralateral side at the time of cancer surgery to allow return to normal function, though this adds substantially to the surgical complexity as coronoidectomy is often required in addition to soft tissue resection.

Commissure Defects As noted above, reconstruction of the oral commissure is key if this is to be lost as part of the resection. The most satisfactory aesthetic and functional outcomes are achieved by reconstitution of the oral aperture with local

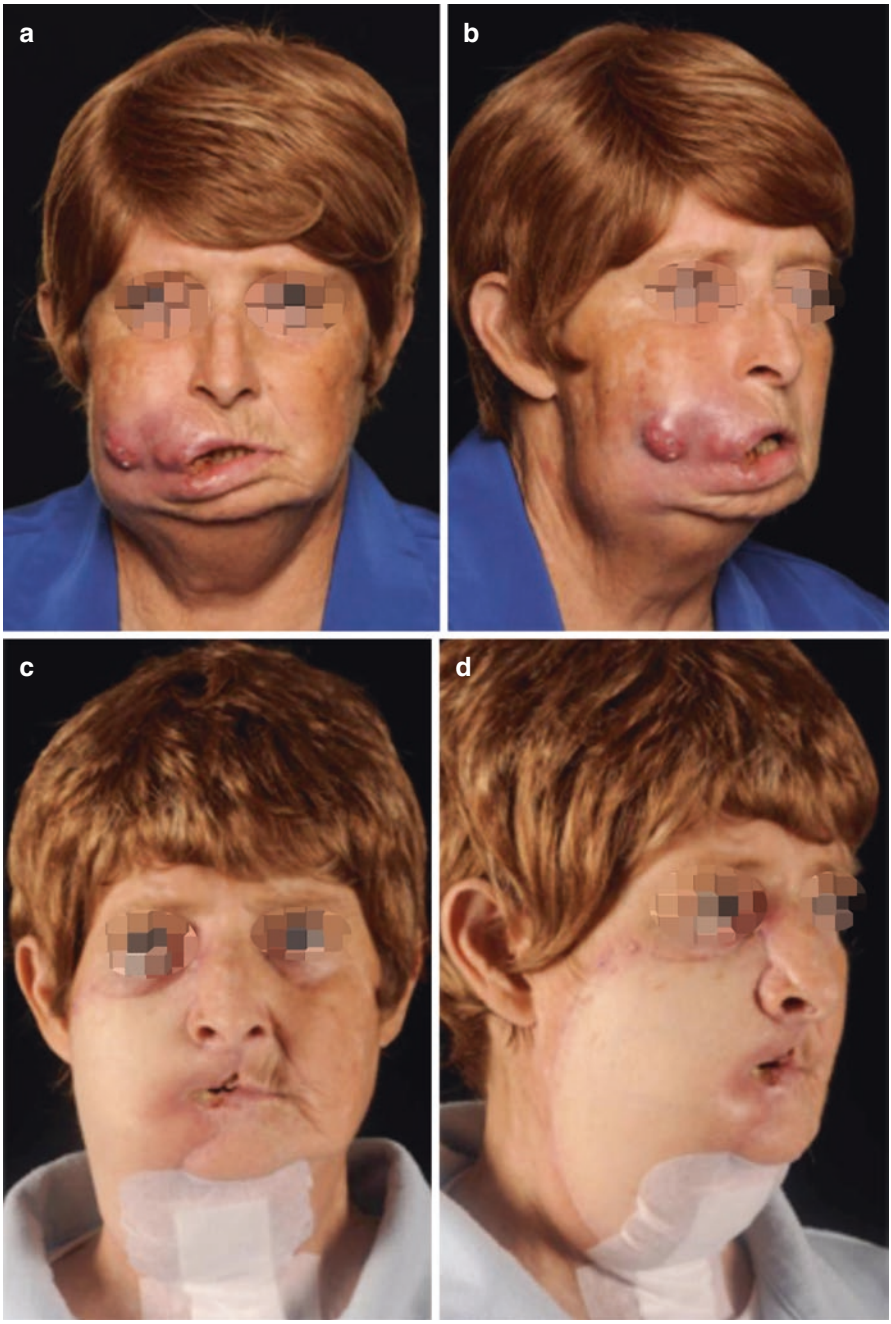

Fig. 4 Pre- and post-operative images of a patient with buccal cancer. The patient had a good quality of life before dying of distant metastases at one year. (**a**) Cancer of the buccal mucosa, invading through external skin. (**b**) Three quarter view: note the involvement of the oral commissure. (**c**) Following reconstruction with a large ALT flap, with resection expanded to take entire facial aesthetic subunits. (**d**) Three quarter view. Placing the transition between flap and local skin at the junction of aesthetic subunits helps to make the reconstruction less obtrusive

Fig. 5 The Therabite Jaw Motion Rehabilitation System by Atos Medical is a commonly used device for passive mobilisation of soft tissues in patients with trismus

flaps, such as the Abbe-Estlander or Gillies flaps. Even if cheek skin is to be reconstructed with a free flap, the sensate, dynamic reconstruction with local tissue is so advantageous that a combined approach is advisable. If the volume of tissue lost would result in unacceptable microstomia then there is no alternative but to reconstruct with a free flap, knowing that aesthetic and functional outcomes will be suboptimal (see Fig. 4).

Post-operative Care

All buccal resections and reconstructions are associated with significant risk of developing trismus, especially if post-operative radiotherapy is required. All patients should undergo intensive rehabilitation to mitigate this risk, using passive and active mouth opening exercise (see Fig. 5), though it should be noted that these exercises are frequently poorly tolerated by patients; the rationale and motivation for avoiding trismus should be carefully communicated in the pre-operative setting.

Soft Palate Defects

The soft palate is a dynamic structure that separates the oropharynx from the nasopharynx during speech and swallowing. Surgical resection leads to velopharyngeal insufficiency (VPI)—loss of this selective obturation of the nasopharynx. VPI is characterised by hypernasal speech, making communication difficult and resulting in negative social perceptions, and also by retrograde passage of food and oral secretions into the nasal cavity, causing discomfort and socially unacceptable nasal regurgitation.

Surgical resection of soft palate tumours results in complex defects of a structure that has vital dynamic function in speech, swallowing and airway protection. Reconstructing all of these functions presents a significant surgical challenge, and

whilst there are a number of options, there is no one reconstruction that offers ideal restoration of all of these functions.

The soft palate is anatomically a part of the oropharynx, rather than the oral cavity. Such distinctions are more than mere pedantry; the biology of oropharyngeal cancer is sufficiently different to oral cavity cancer to merit careful consideration of the treatment approach. Oral cavity cancer outcomes in terms of survival and quality of life are superior when the primary treatment is surgical [12], but this distinction is less clear in oropharyngeal tumours. Treatment with 'organ-preserving' primary chemoradiotherapy should be carefully considered with the multidisciplinary team before opting for surgical resection, with the associated functional compromise.

Aims of Treatment

If the multidisciplinary team feels that the most appropriate treatment is surgical resection, the aims of reconstructing the soft palate are to restore the barrier function of the soft palate, whilst still permitting nasal breathing:

- Maintain or restore velopharyngeal competence
- Separate nasopharynx from oropharynx
- Allow safe, effective swallowing (prevent retrograde passage of food)
- Facilitate intelligible speech, of normal character (prevent nasality)
- Maintain nasal patency
- Provide timely rehabilitation to allow rapid return to normal diet and speech.

Options for Treatment

Primary Closure and Secondary Intention Primary closure or allowing healing by secondary intention can be excellent options when defects are small. A rule of thumb is that if less than 25% of the palate has been resected, these simple options may be worth considering [13]. They have the advantage of being simple procedures, reducing operative time and morbidity, whilst allowing un-restricted function of the remaining structures where a bulky flap may actually impede function. However, primary closure or the scarring and contracture associated with healing by secondary intention may lead to stricture or tethering of the residual palate, so for larger defects other methods should be used.

Prosthesis The use of a palatal prosthesis was the standard of care before more advanced reconstructive techniques became available. They have the advantage of being simple and cheap to construct, and prosthetic rehabilitation affords good results in small defects where the residual anatomy allows retention of the device and has good dynamic function. Being removable, they allow ease of oncological surveillance at the primary site. However, from a patient perspective they can be

inconvenient, requiring frequent care and maintenance, and they require a degree of dexterity that not all patients are able to accomplish. If the patient has trismus, it may not be possible to use a removable prosthesis. Furthermore, the prosthesis can become uncomfortable if it causes or exacerbates existing mucositis—a particular concern in patients who have had or will have radiotherapy to the area. A final concern is that the use of a prosthesis results in delayed rehabilitation, as the prosthesis cannot be placed until healing is complete after surgery and radiotherapy.

Local Flaps Local flaps can provide a small amount of tissue, but have a major advantage in that they allow for a dynamic reconstruction. This can be an ideal option, but if the patient has had previous radiotherapy, or if oncologically-sound resection requires sacrifice of structures needed for the local flap, then they are unlikely to be a successful option.

Karle et al. [14] have reported good results by combining lateral pharyngeal wall flaps with a rotated palatal island flap to create a dynamic neovelopharynx following resection of the whole soft palate. The technique is shown in Fig. 6. However, the durability of this reconstruction following radiotherapy is un-proven, and previous radiotherapy is a contraindication to this flap as bone is left exposed, creating a high risk of osteoradionecrosis.

Regional Flaps Regional flaps employ tissue from sites further away, but do not require microvascular anastomosis. One example which has produced good results is the facial artery myomucosal (FAMM) flap, which uses buccal mucosa to reconstruct the soft palate, pedicled on the facial artery and the rich buccal vascular plexus. The donor defect is closed by advancing the buccal pad of fat [15]. Submental island flaps have also been described as having good results for smaller defects [16], though in male patients the coarse hair present on the cutaneous surface can be a substantial drawback.

Other regional flaps have been used, such as the pectoralis major myocutaneous flap and the latissumus dorsi flap. These both provide good quantities of tissue, but are adynamic, and are usually too bulky, causing ptosis and limiting how much movement is achievable by pairing with dynamic local flaps.

Microvascular Free Flaps The adynamic nature of free flaps can be overcome in part by combining them with local flaps [13]. The most commonly used free flap for soft palate reconstruction is the radial forearm free flap. It provides a generous amount of thin, soft, pliable skin, with an excellent track record for reliability and a long pedicle that allows ample room for inset in the oropharynx. It, along with all other free flaps, has the insurmountable drawback of being adynamic, and it replaces mucosal tissue with dry, potential hair-bearing keratinised skin.

For larger defects of 50–100% of the soft palate, the quality and quantity of tissue offered by a radial forearm flap makes it an excellent choice to restore bulk, and numerous techniques have been demonstrated that pair this thin flap with local, dynamic flaps to restore a degree of mobile function during swallowing. Seikaly

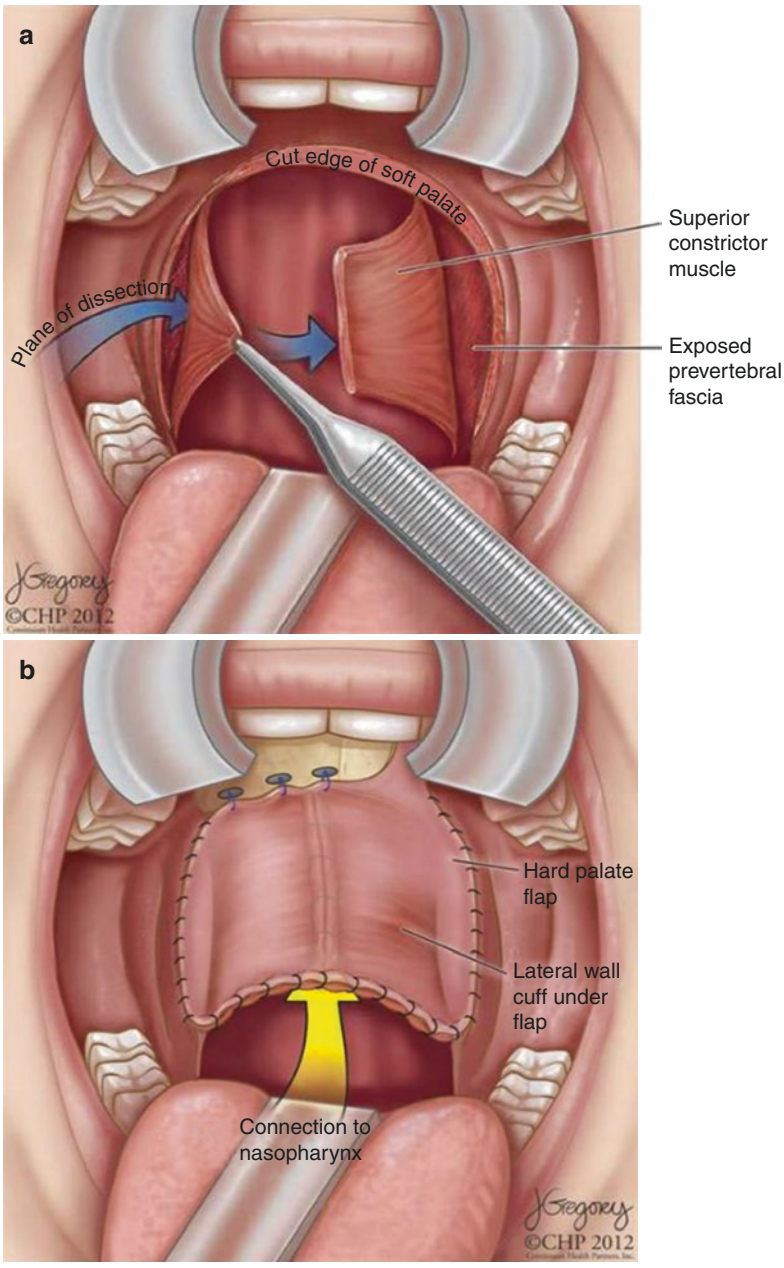

Fig. 6 Illustration of the palatal island and lateral pharyngeal wall flap reconstruction for oncological defects of the soft palate [14]. (**a**) Following resection of the soft palate, the lateral pharyngeal walls are elevated as myomucosal flaps with a posterior-based pedicle. The free edges are apposed to form a dynamic, muscular tube. (**b**) The muscular surface of the newly created tube is then covered with the rotated palatal island flap, and the inferior free edges suture together. The hard palate is left to heal by secondary intention, which is usually complete by 1 month

et al. [13] have shown excellent swallowing and speech outcomes in patients with defects of greater than 50% soft palate resection using a radial forearm sutured to local superior posterior myomucosal pharyngeal flaps (the Soft Palate Insufficiency Repair or 'SPIR' flap). The two layer closure allows separation of the nasopharynx from the oropharynx, apart from an aperture large enough to admit a nasogastric tube. This allows nasal breathing but prevents excessive nasality of speech and nasopharyngeal reflux during swallowing.

Reconstruction of Bony Defects

The oral cavity is partly encased by the mandible and the maxillary complex, two bony structures that have vital roles in the functions of the oral cavity and in facial aesthetics. Malignancies of the oral cavity frequently arise from mucosa with close anatomical relations to bone, and as such it is common for adequate oncological resection to require sacrifice of substantial amounts of bone. The functional and aesthetic morbidity arising from the loss of bony supporting structures is substantial and so these structures should be replaced. Reconstruction of the bony defect can be performed using alloplastic materials—such as acrylic obturators or titanium osteosynthesis plates—but the best material is autogenous bone, and a number of vascularised free flaps are suitable for this purpose, dependent on the nature of the defect and the status of the patient.

Until recently, the osteotomy would have to be judged 'by eye' intra-operatively and adjusted the defect. The rise of virtual pre-operative planning and rapid prototyping with 3-d printers means that the precise osteotomies required for a good result can be planned in advance and performed using a custom-made jig. This has allowed excellent aesthetic outcomes, saves time, and the planning software and models can be used as the focus of an informed discussion with the patient about expectations and outcomes following the procedure.

Mandibular Defects

Before the advent of osseous free flaps, functional outcomes for mandibular defects following oncological resection were poor. The head and neck reconstructive surgeon now has a wealth of reliable reconstructive options available, and the rising applicability of virtual planning and rapid prototyping to reconstruction continues to improve results. Osseous free flaps are now the gold standard in mandibular reconstruction. Soft tissue alone may be acceptable in smaller, lateral defects but evidence is lacking. Attempts to bridge bony continuity defects with reconstruction plates without vascularised bone frequently result in exposure of the plate and subsequent infection.

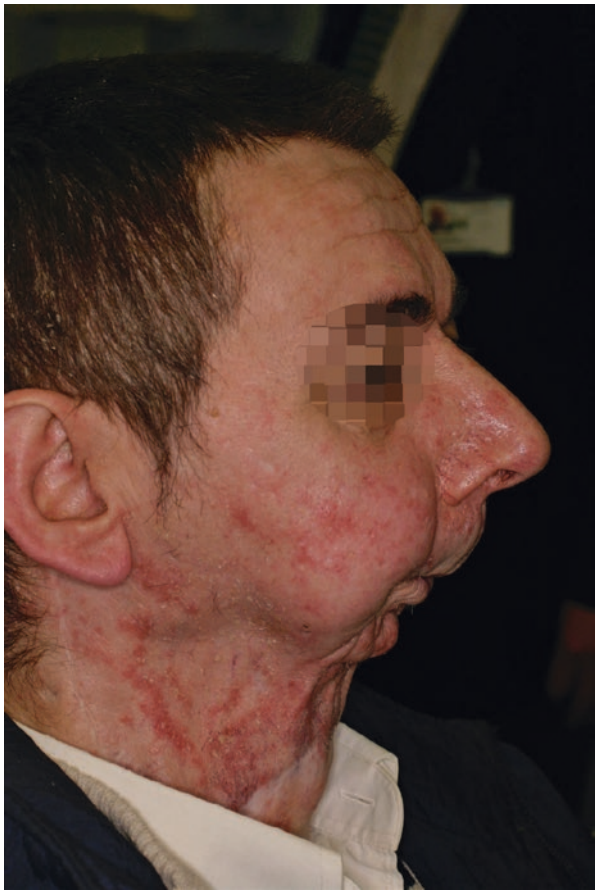

Fig. 7 A patient of ours with a failed anterior mandibular reconstruction, leading to the classic Andy Gump deformity and a poor quality of life for the patient

Decision making regarding reconstruction is guided by the nature of the defect. A number of classification systems for mandibular defects exist, notably the recent system proposed by Brown et al. [17], but none has been accepted universally. Our practice is to make decisions based on four key factors:

- *Is the defect anterior to the mental foramina?* Failure to reconstruct defects in the anterior mandible result in an 'Andy Gump' deformity, leaving patients with poor outcomes for speech, mastication, swallowing and aesthetics (see Fig. 7).
- *Does the patient have a reproducible dental occlusion?* If so, failure to reconstruct with bone will result in loss of dental function.
- *Does the patient have thin or frail soft tissues?* Patients with a fragile mucosal biotype and/or those patients who have had previous radiotherapy will need vascularised soft tissue for coverage along with the bony reconstruction.
- *Will the patient require post-operative radiotherapy?* A robust vascularised flap is the only reconstructive option that can reliably withstand a course of radiotherapy.

Choice of Flaps for Mandibular Reconstruction

The most important donor free flaps for mandibular reconstruction are:

- Fibular free flap,
- Iliac crest, pedicled on the deep circumflex artery (DCIA flap),
- Scapula/parascapular flap

Fibular Free Flap The fibular flap has established itself as the 'workhorse' flap for reconstruction of mandibular defects. It provides a reliable quantity—up to 25 cm—of high quality bone that will reliably accommodate osseointegrated implants and can be osteotomised to reconstitute mandibular anatomy. It has a long, reliable pedicle and can be harvested with muscular and skin paddles for coverage of intra-oral or extra-oral defects. It has the further advantage of being remote from the head and neck, allowing a two team approach which helps reduce surgical time. The morbidity associated with loss of the fibula is minimal, provided appropriate precautions are taken during the raising of the flap.

The flap is disadvantaged by a reliance on 'normal' vascular anatomy—a minority of patients will have an arterial supply to the foot that depends upon the peroneal artery, which is an absolute contraindication to the use of this flap, and mandates pre-operative investigation of the vasculature with Magnetic Resonance Angiography or similar imaging modality. Further disadvantages include a skin paddle that is less reliable than the bony component, an unaesthetic donor site scar, particularly if skin grafts are required to close defects arising from skin paddles, and the donor site can be slow to heal, requiring lengthy care from tissue viability experts (Figs. 8 and 9).

When choosing where to inset the osseous component of the flap, our practice was to reconstitute the lower border of the mandible to maximise aesthetics and symmetry. However, this meant that in order to provide dental reconstruction, the osseointegrated implants needed to be lengthy, causing technical difficulty and generating excessive torque forces. As such we now plate the bony component more towards the middle of the bony defect to achieve compromise between these two goals.

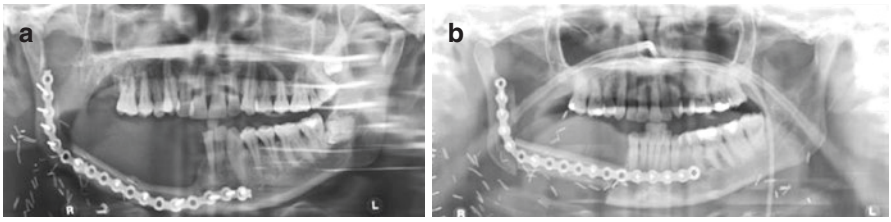

Fig. 8 Two post-reconstruction orthopantomograms, showing our initial low (**a**) and current higher (**b**) approach to inset of the fibula. The lower position affords a more aesthetic jawline, but compromises the placement of implants by requiring greater length and associated greater torque. The higher position allows easier dental rehabilitation

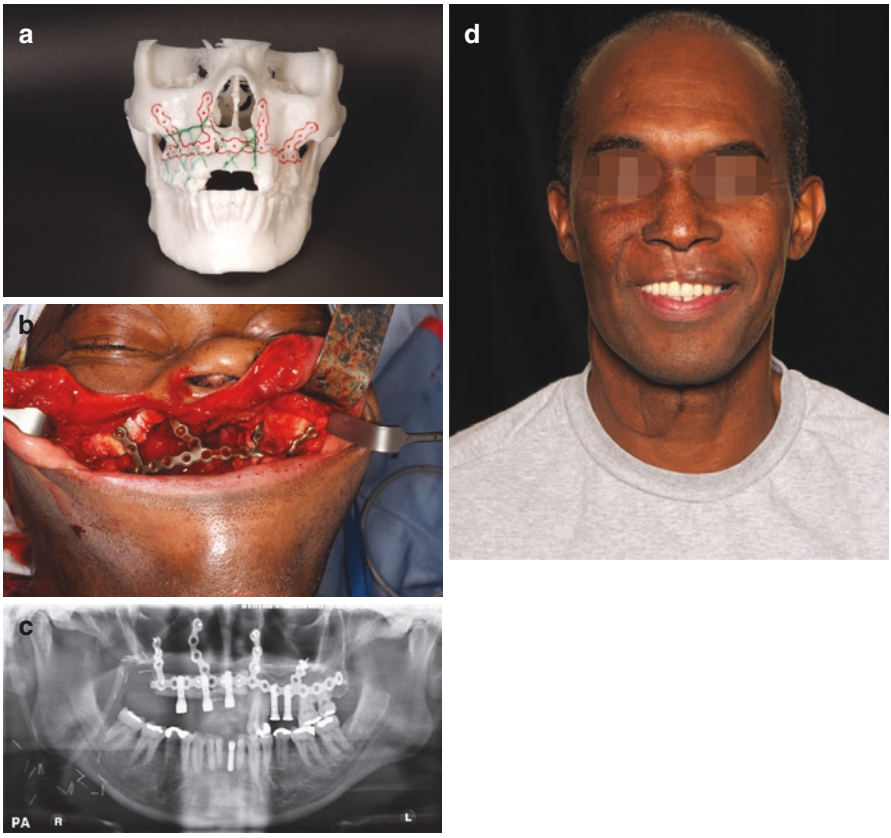

Fig. 9 A patient undergoing low maxillectomy for a maxillary tumour, with a fibular reconstruction and dental rehabilitation using osseointegrated implants. (**a**) Rapid 3-d printed prototype of the patient's skull created from a CT scan, showing the area to be resected (green) and the intended position of the custom osteosynthesis plates for fibula flap inset time (red). (**b**) Perioperative photograph showing the excellent fit of the custom-bent osteosynthesis plate. Having these made in advance greatly speeds up the process of flap inset, reducing flap ischaemic as well as overall operating time. (**c**) A post-operative orthopantomogram, showing the position of the maxillary bone, the customosteosynthesis plates and the osseointegrated dental implants. (**d**) Final result, showing excellent facial and dental aesthetics. The bent reconstructed patient has near normal oral and dental function

DCIA Flap The iliac crest is a rich source of material for both vascularised and non-vascularised osseous transfer. Since 1979, when it was discovered that the bone could be transferred using the deep circumflex iliac artery (DCIA) as the vascular pedicle, the DCIA flap has found widespread use for reconstruction of the mandible and maxilla. It provides a large amount of high quality bone up to 14 cm length that is well-suited to the acceptance of osseointegrated implants. The natural curve of the iliac crest bears a notable similarity to the geometry of the mandible, allowing inset of the flap with minimal modification. It can provide muscle and perforator-

based skin paddles for soft-tissue coverage, is at a remote site that allows two-team operating, and comes with minimal aesthetic or functional compromise in the long term [18].

However, questions have been raised about the reliability of the DCIA, and a meta-analysis of its use in mandibular reconstruction suggested that it was less reliable than other bony flaps (6.2% failure, compared with 3.4% for all other flaps). It has a short pedicle (6 cm), though this can be improved by harvesting the flap more posteriorly. Mobilisation in the post-operative period is painful and requires physiotherapy input. Perhaps the most notable drawback is the labour-intensive closure required, that must be performed meticulously or the patient is at risk of developing a substantial donor site hernia, which can be very diffcult to treat.

Scapular and Parascapular Flaps These two flaps are variations on the same theme, providing up to 14 cm of thin but high-quality bone from the lateral edge of the scapula, and flexible, reliable skin paddles pedicled on either the horizontal branch (Scapular) or descending branch (Parascapular) of the circumflex scapular artery. These two paddles may be taken in the same flap, and the separate pedicles afford considerable three-dimensional flexibility with respect to each other and the osseous component. The pedicle is short (3–4 cm) but reliable even in atherosclerotic patients, and of good diameter. With care during closure of the donor site, the long term morbidity is minimal.

The scapular system flaps have a major disadvantage in that to be raised the patient must be in the lateral decubitus position. This means that either the recipient site resection and neck dissection(s) must be performed with the patient in an awkward and unfamiliar orientation, or the patient must be moved from supine to decubitus and back during the procedure. Both options preclude two-team operating, and add substantially to the operative time.

Maxillary Defects

The aims of maxillary reconstruction are:

- to close artificial communications between nasal, oral, maxillary and orbital cavities created during ablative surgery,
- to reconstruct the dentition such that function is restored as close to normal as possible, and
- to restore aesthetics by reconstituting the width, height and projection of the resected tissues

High quality evidence to support decision making in maxillary reconstruction is lacking. As such, if bone is resected as part of the extirpation of a malignancy, the choice of reconstruction should be guided by the characteristics of the defect left after resection, patient preference, and surgical experience.

Having a clear understanding of the maxillectomy defect is important to guide surgical decision making. The most widely used classification system is that proposed by Brown et al. [19]. This system classifies the vertical extent of the defect numerically, and the horizontal extent of the defect alphabetically. The smaller and simpler defects can be managed with simple methods such as obturators, but the larger and more complex the defect, the more likely that the patient will require reconstruction with an osseous microvascular free flap.

Options for Reconstruction

In low defects—class I or II—obturators can provide a simple and satisfactory reconstruction. They are effectively acrylic partial or complete dentures with a vertical extension moulded to fit the defect, providing bulk for missing tissue and sealing off communication from the oral cavity into the maxillary sinus. They are simple, cheap and well tolerated by some patients, and allow for direct monitoring of the primary site for recurrence. However, they require a degree of dexterity to use, can be inconvenient to cleanse, and for larger defects provide inadequate support and are unstable.

Zygomatic implants can have a role in supporting dental prostheses, but they cannot seal off any communications between the oral cavity and other structures that were created during maxillectomy. The Zygomatic Implant Perforator (ZIP) flap approach uses a soft tissue flap (most commonly a radial forearm flap) to establish a seal, and a zygomatic implant is placed through this to support a dental prosthesis. Early results are promising [20].

For more posterior class II defects, a reconstruction with a soft tissue free flap may be adequate, as it seals the oroantral comumnication. A radial forearm flap is the most common choice [19], but other flaps have been reported. For larger defects, approaching or involving the orbit (class III and IV), simpler measures are unlikely to provide a satisfactory aesthetic or functional outcome, and the gold standard is now an osseous free flap, with alloplastic reconstruction of the orbital floor if needed.

Placement of free flaps increases surgical complexity and prevents direct monitoring of the wound bed for recurrence, but growing familiarity of these procedures and the increasing precision of modern imaging for both detection of recurrence and pre-operative virtual 3-dimensional planning a orded reconstruction means that these drawbacks are now largely theoretical.

Choice of Flaps for Maxillary Reconstruction

When repairing maxillectomy defects that require bony reconstruction, the armamentarium is larger than for the mandible. This partly reflects the variety of complex defects that can ensue following maxillectomy, but also reflects a shortage of high quality evidence.

Osseous or composite flaps

- DCIA
- Fibula
- Scapula
- Tip of scapula
- Radial

DCIA The DCIA provides bone with a natural geometry that fits well with class III and IV maxillectomy defects. It has also been shown to have the best rate of implant survival, and as such if osseointegrated implants are planned, this flap should be considered. However, aside from the drawbacks identified in the previous section, the short pedicle can make anastomosis challenging, as the maxillary position takes the flap further from reliable donor vessels in the neck.

Fibula As well as being the gold standard for reconstruction of the mandible, the fibula can be very useful for reconstruction of defects in the maxilla. Whilst the fibula is a long, thin bone, not immediately geometrically suited to maxillary defects, the rich periosteal blood supply means that with care the bone can be osteotomised and configured to fit the defect. If only a short amount of bone is needed, harvesting this from the distal end of the fibula provides a generous length of peroneal artery, helping to maintain a tension free anastomosis.

Scapular/Parascapular The advantages and disadvantages of the scapular system flaps as discussed above still apply when considering their use in the maxilla, though the short pedicle presents a challenge due to the more cranial position of the defect. If external skin coverage is required, this flap provides an excellent match in terms of tone and texture.

Tip of Scapula The scapula tip has natural geometry suited to reconstructing a low maxillectomy defect if placed horizontally, or a class III/IV defect if placed vertically (see Fig. 10). It is difficult to raise skin with it, but can come with muscle that rapidly mucosalises when used for intra-oral coverage. As it is based on the angular branch of the thoracodorsal artery, and does not require either branch of the circumflex scapular arteries, it has a much longer pedicle than the traditional scapular or parascapular flaps, which is advantageous for reaching more cranial positions without placing tension on the pedicle.

Osteocutaneous Radial Forearm Flap As discussed above, the radial forearm is a workhorse flap in the reconstruction of soft tissue defects, however it is possible to raise up to 10 cm of vascularised unicortical bone with a radial flap, which can be a useful option. However, the donor radius is at high risk of post-operative fracture and so great care must be taken with prophylactic compression plating and post-operative rehabilitation. Furthermore, the bone provided is thin, and does not take osseointegrated implants as well as other osseous flaps, though Fig. 11 shows that good results can be achieved.

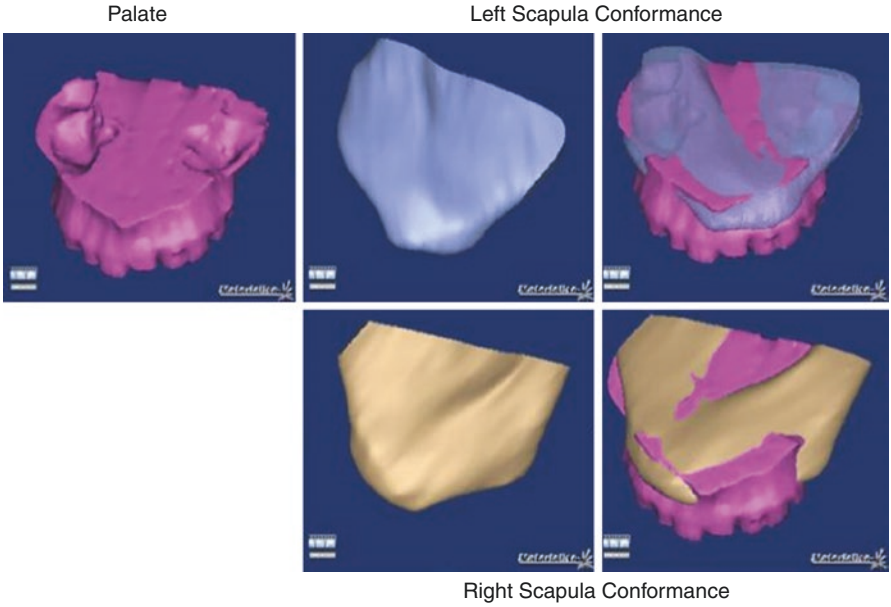

Fig. 10 This capture from a 3-dimensional virtual planning programme illustrates how well the tip of scapula conforms to the geometry of the maxilla (courtesy of Ralph Gilbert)

Dental Rehabilitation

The rising use of osseointegrated dental implants has revolutionised restorative dentistry, and they are now the gold standard for dental rehabilitation after oncological surgery to the oral cavity. Meticulous planning from the outset is essential to ensure the best functional and aesthetic outcome for the patient, and the multi-disciplinary team should include an experienced restorative dentist.

Titanium implants, if gently screwed into bone with good vascular supply, allow bone deposition on their surface, forming a direct structural and functional connection with the bone that allows the implant to bear masticatory forces nearly as large as those borne by natural dentition, although as there is no periodontal ligament, implants do not restore proprioception. They provide a base for a prosthetic superstructure, either an individual crown/bridge, or an implant-retained overdenture.

If the patient is undergoing a bony resection and reconstruction, the decision-making process for selecting the flap should consider whether the patient will require dental implants, as different osseous flaps have differing suitability for implants. The iliac crest has the best rate of suitability (83%), followed by the scapula (78%), fibula (67%) and radial osteocutaneous (21%) flaps [21].

Many patients undergoing bony resection will require post-operative adjuvant radiotherapy, and until recently it was unclear how the resultant reduction in bony vascularity a ected implant survival rates. A meta-analysis of 54 studies by

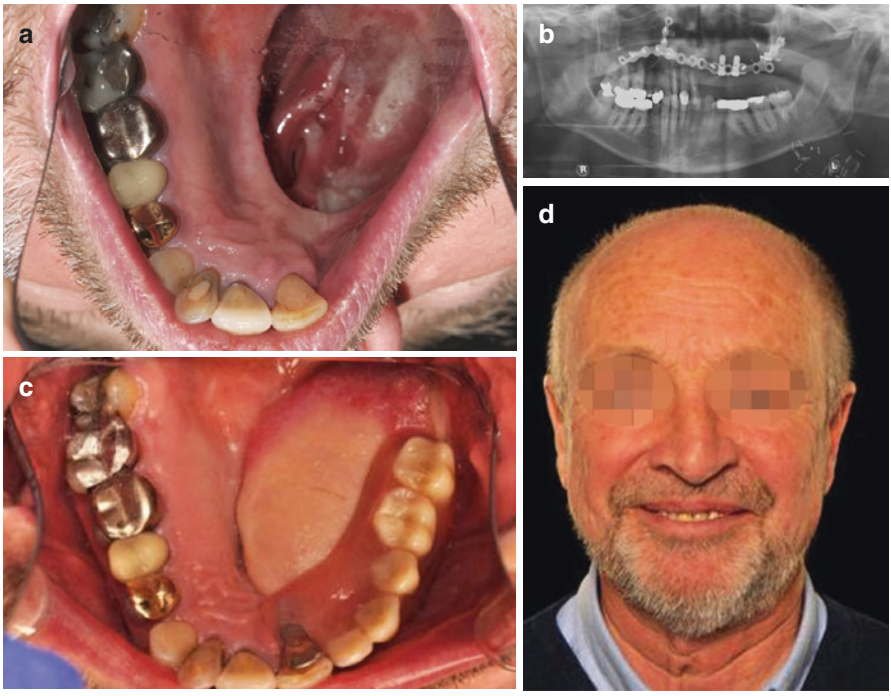

Fig. 11 Following Brown Class II maxillectomy, the patient wanted dental rehabilitation, but had aberrant vascular anatomy of the lower leg, preventing the use of a fibula flap. An osteocutaneous radial free flap was chosen, and the patient had an excellent result. (**a**) Maxillary defect. (**b**) Postoperative orthopantomogram showing placement of osteocutaneous radial free flap and dental implants. (**c**) Implant retained partial overdenture shown in situ, with underlying cutaneous component of the radial flap shown maintaining oroantral separation. (**d**) Good facial and dental aesthetic results, and the patient had excellent oral function

Chrcanavic et al. [22] showed that implant survival was reduced if they were placed shortly after radiotherapy. If they were placed before radiotherapy, or more than 12 months after radiotherapy had finished, there was no negative effect on survival. Survival was significantly better for implants in the mandible than those in the maxilla. The tendency for better implant survival with lower doses of radiation did not achieve statistical significance. Hyperbaric oxygen had no statistically significant effect on implant survival.

Zygomatic implants were initially designed as a way to retain dentures in patients with a severely resorbed maxilla, but have found use following maxillectomy for retention of obturators and dental prostheses, as discussed in section "Options for Reconstruction". They can be loaded immediately, which is good for patients and helps keeps costs down by only requiring a single procedure, but care should be taken during placement: the increased length of these implants creates unfavourable torque forces that can compromise the implants' survival.

In our unit, we have moved away from the use of zygomatic implants for reconstruction of oncological maxillectomy defects, as we find bony reconstruction and placement of intra-oral implants to provide more satisfactory results. We tend to limit their use to complex situations such as salvage or post-traumatic defects.

Conclusions

Surgical reconstruction following oncological resection in the oral cavity remains a significant surgical challenge, but the developments in microvascular surgery, 3-dimensional virtual planning, rapid prototyping and osseointegrated dental implantation have made substantial improvements in the quality of life the patient can expect. The best outcomes are achieved when an experienced surgeons make plans in consultation with the patient and with the other members of the multidisciplinary team, especially restorative dentists and speech and language therapists.

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Induction Chemotherapy: Does It Have a Place in Oral Cavity Cancer?

Jean-Pascal Machiels

In case of organ preservation treatment strategy, concomitant chemoradiation is the standard treatment for patients diagnosed with locally advanced squamous cell carcinoma of the head and neck (SCCHN). Induction chemotherapy (ICT) followed by radiotherapy is a second validated approach in patients with larynx and hypopharynx squamous cell carcinoma for whom total laryngectomy is required (see also Chapter "What Is the Optimal Larynx Preservation Approach and Who Are the Candidates?"). For other indications ICT remains investigational [1].

The Meta-Analysis of Chemotherapy for Head and Neck Cancer (MACH-NC) has shown that the addition of chemotherapy to the locoregional treatment improves the survival rate by 4.2% (95% CI: 2.8–5.6%) and by 2.2% (95% CI: 0.7–3.7%) at 5 and 10 years, respectively [2]. The highest benefit was observed when chemotherapy was administered concomitantly with radiation therapy, with an absolute survival increase of 6.4% (95% CI: 4.7–8.4%) at 5 years. In contrast, induction chemotherapy in the MACH-NC improves the 5-year survival rate by only 1.9% (95% CI: −0.7 to 4.3%) [3].

The best regimen for induction is the association of cisplatin, docetaxel, and 5-fluorouracil (TPF). In a meta-analysis, TPF has been shown to reduce progression, locoregional failure, distant failure, and death compared with cisplatin and 5-fluorouracil (PF), with HRs of 0.78 (95% CI: 0.69–0.87), 0.79 (95% CI: 0.66–0.94), 0.63 (95% CI: 0.45–0.89), and 0.72 (95% CI: 0.63–0.83), respectively. The absolute 5-year survival benefit of TPF over PF is 7.4% (95% CI: 2.3–12.5%). The MACH-NC included mainly induction clinical trials performed with PF [4].

The treatment of choice for locally advanced resectable oral cavity cancer is surgery followed by (chemo)radiation depending on the risk factors. In this chapter,

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we will review and discuss the role of induction chemotherapy in the treatment of squamous cell oral cavity cancer.

Data from Meta-Analyses

4331 patients with squamous cell oral cavity cancer were included in the MACH-NC [5]. Adding chemotherapy to the loco-regional treatment improved the overall survival rate by 5.1% (95% CI: 2–8.3%) at 5 years. The 5-year absolute survival benefit was 5.5%, 3.8%, and 6.9% for adjuvant, induction, and concomitant chemotherapy. However, only 11% of the oral cavity cancer patients of this meta-analysis were treated with surgery as loco-regional treatment. No studies that investigated TPF as induction regimen were included.

Lau and colleagues performed a systemic review and a cumulative meta-analysis to determine the benefit of ICT in the treatment of oral cavity cancer [6]. The selected clinical trials had (a) to include patients with untreated squamous cell oral cavity cancer and (b) to randomize patients between induction chemotherapy followed by the loco-regional treatment versus loco-regional treatment alone. Loco-regional treatment could be either (chemo)radiotherapy and/or surgery. The primary endpoint was overall survival. Secondary endpoints were disease-free survival, loco-regional recurrence, and distant metastasis. Twenty-seven randomized trials published between 1975 and 2015 met the inclusion criteria for a total number of 2872 patients. ICT did not improve overall survival (HR: 0.947, 95% CI: 0.85–1.05, p = 0.318), disease-free survival (HR: 1.05, 95% CI: 0.92–1.21, p = 0.462), or distant metastasis (HR: 0.626, 95% CI: 0.361–1.086, p = 0.096) compared to loco-regional treatment alone. However, there were significantly fewer loco-regional recurrences (HR: 0.778, 95% CI: 0.622–0.972, p = 0.027).

Induction Chemotherapy for “Unresectable” or “Unresected” Oral Cavity Cancer

No randomized trials have properly investigated whether ICT in case of unresectable or unresected oral cavity cancer could be beneficial [7]. Several trials have investigated TPF versus PF followed by (chemo)radiation [8]. Since the treatment of oral cavity cancer is mainly surgical, these studies included only 12.7% of oral cavity cancer patients. Therefore, it is unknown whether we can translate the general findings of these trials to oral cavity cancer. For example, in the Posner trial that investigated TPF versus PF as induction therapy before chemoradiation, 71 oral cavity cancer patients were included. According to the inclusion criteria patients should have been selected for an organ preservation approach or judged unresectable. For the subgroup of oral cavity cancer, median survival was 14 and 37 months in the PF and TPF groups, respectively (HR: 0.87, 95% CI: 0.47–1.6).

Induction Chemotherapy Before Surgery for Oral Cavity Cancer: Randomized Trials

Two randomized phase III trials investigated the role of ICT followed by surgery in patients with oral cavity cancers.

Licitra and colleagues randomized 198 oral cavity cancer patients between 3 cycles of PF followed by surgery versus surgery [9, 10]. This study included patients with T2–T4 (larger than 3 cm), N0–N2, M0 oral cavity cancer. Post-operative radiation therapy was administered to high risk (positive surgical margins and/or invasion of soft tissues of the face (cheek, chin) and/or more than three node metastases and/or extracapsular tumor spread). The primary endpoint was the occurrence of loco-regional or distant relapses. The secondary endpoints were objective response rate, overall survival, and toxicity. The study included the 198 patients in 10 years (initial target sample size: 258 patients). Three toxic deaths in the induction arm were recorded. Chemotherapy did not seem to induce additional surgical morbidities. The primary endpoint was not met. With a median follow-up of 11.5 years, there was no difference in the incidence of loco-regional relapse between chemotherapy and control group ($p = 0.6337$), nor in distant metastasis development ($p = 0.1527$). There was also no difference between groups in overall survival ($p = 0.3402$). Objective response rate and pathological response are described in Table 1. Patients with a pathological complete response (pCR) had higher probability of survival than those without with a 10-year overall survival of 76.2% versus 41.3% ($p = 0.0004$). Late toxicities in patients with a minimum follow up of 60 months (42 in each group) were similar between arms, except from fibrosis (cumulative incidence 40% versus 22%) and grade 2 dysphagia (14% versus 5%), both being less frequent in the chemotherapy arm. This observation was ascribed by the authors to less extensive surgery carried out in the chemotherapy group (31% versus 52% in control group) and fewer patients receiving postoperative radiotherapy (33% versus 46% in control group).

Zhong and colleagues randomized 256 patients between 2 cycles of TPF followed by surgery versus surgery [11]. Eligibility criteria included untreated stage III or IVA locally advanced resectable oral cavity cancer. The primary end point was

Table 1 Objective responses in the Licitra trial [8]

N = 82	Induction arm (%)	No induction arm
Clinical complete response	33	Non-applicable
Clinical partial response (>50%)	49	Non-applicable
Mandibulectomy	31	52%
Post-operative radiotherapy	33	46%
Complete pathologic response[a]	27	
Microscopic residual disease[b]	18	

[a]Complete response was defined as absence of any tumor cells

[b]Microscopic residual tumor cells: presence of scattered foci of a few tumor cells (20 pathologic slides)

Table 2 Objective response rate according to RECIST and pathological response in the Zhong trial [10]

N = 124	Induction arm (%)
Complete response	8.1
Partial response	72.6
Stable disease	16.9
Progressive disease	0.8
Favorable pathologic response[a]	27.7

[a]A favorable response was defined as absence of any tumor cells or presence of scattered foci of a few tumor cells (minimal residual disease with <10% viable tumor cells) (20 pathologic slides)

overall survival. Secondary end points included local control and safety. There were no unexpected toxicities, and induction chemotherapy did not increase perioperative morbidity. The clinical response rate to induction chemotherapy was 80.6%. Objective response rate and pathological response are described in Table 2.

After a median follow-up of 30 months, there was no significant difference in overall survival (HR: 0.977, 95% CI: 0.634–1.507) or disease-free survival (HR: 0.974, 95% CI: 0.654–1.45) between patients treated with and without TPF induction. Similar results were obtained after 70 months of follow-up [12]. Patients in the induction chemotherapy arm with a clinical response or favorable pathologic response (10% viable tumor cells) had superior overall survival as well as loco-regional and distant controls.

Marta and colleagues performed a meta-analysis with these two randomized clinical trials [13]. No significant overall benefit in favor of induction chemotherapy was found regarding loco-regional recurrence, disease-free survival and overall survival. A subgroup analysis of individual data from cN2 patients showed statistically significant overall survival benefit in favor of induction chemotherapy (HR: 0.42, 95% CI: 0.18–0.98). This analysis should be considered only to generate hypothesis since only 84 patients were eligible for this subgroup analysis and no difference for loco-regional control was found for cN2 patients.

Conclusions

Induction chemotherapy for squamous cell oral cavity cancer is not standard of care. The primary treatment remains surgery followed by (chemo)radiation if feasible. Further trials are needed in particular for patients with unresectable oral cavity tumor and in case of cN2 disease.

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Alternative Local Treatment in Oral Cavity Cancer: Photodynamic Therapy

Ing Bing Tan, Sharon D. Stoker, and Robert L. P. van Veen

Treatment of Oral Cancer

The standard treatment of early stage oral cancer is surgery with or without adjuvant radiotherapy. The more advanced lesions, not suitable for surgery, can be treated by chemoradiotherapy. Relatively good local response is achieved for the early stage lesions (5-year survival rates is 70–90%). Nevertheless, recurrent and residual disease does occur. In head and neck oncology in general, the incidence of second primary tumors is 20–30% and for recurrent disease even 10–50%. Previous treatment with surgery and/or radiotherapy limits the options for a second treatment. The prognosis for these patients with loco/regional recurrence is poor with 5-year survival rate of 20–40%. Therefore, there is a need for alternative treatment for these patients.

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Photodynamic Treatment: An Alternative Treatment

Several investigators have reported on results with photodynamic therapy (PDT) in patients with early stage oral cavity malignancies. They found good success rates for local control in carefully selected patients [1–8]. PDT is based on the interaction of three essential components: a photosensitizer i.e. a light sensitive drug, light of a specific wavelength and oxygen. The photosensitive drug is initially not toxic, but if activated by light it can locally interact with oxygen resulting in the formation of highly reactive oxygen species. Reactive oxygen species can readily oxidize biomolecules leading to cell death. The treatment effects of PDT against cancer is based on four mechanisms: direct cytotoxic effects on tumor cells, damage to the tumor vasculature with an acute vascular shut down, induction of an inflammatory reaction that can lead to development of a systemic immune response and apoptosis (see Fig. 1). The first-generation photosensitizers, hematoporphorin-derived photosensitizers (HpD or Photofrin) have shown its effectiveness.

Nowadays more potent second generation photosensitizers are used, which are activated by red light of longer wavelengths thus causing deeper tissue penetration depths, a higher singlet oxygen yield and with a shorter skin sensitivity to ambient light. However even with these newer sensitizers limitations are still encountered. The maximal depth of tissue penetration is ~8–10 mm for red light as compared to ~5 mm for first generation sensitizers. After systemic sensitizer administration patients are sensitive to light for approximately 2 weeks, especially for daylight. The photosensitizer is intravenously administered. The illumination has to be performed 96 h after the injection by a continuous wave (CW) laser with a wavelength of 652 nm.

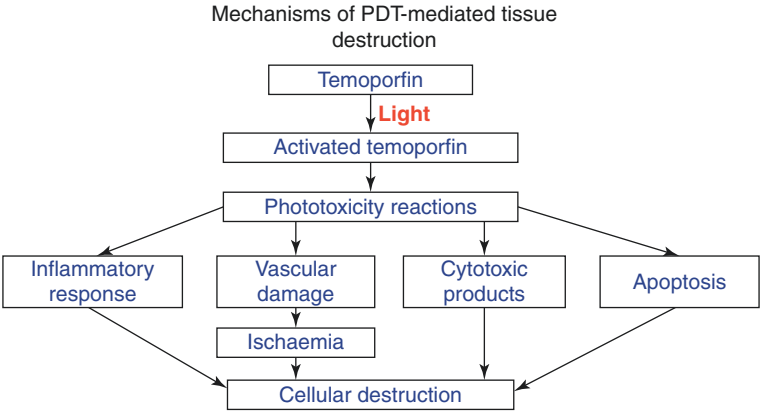

Fig. 1 Schematic view of the mechanism of PDT. The treatment effects of PDT against cancer is based on four mechanisms: direct cytotoxic effects on tumor cells, damage to the tumor vasculature with an acute vascular shut down, induction of an inflammatory reaction that can lead to development of a systemic immune response and apoptosis

PDT Experience

Over 30 years of research has been performed in The Netherlands Cancer Institute, initially only preclinical and soon followed by clinical applications. Since 1996 the department of head and neck surgery has participated in international multicenter studies. In 2006 the first PDT center in Europe was opened in Amsterdam, the Netherlands. Until now more than 500 patients with head and neck cancer (skin cancer excluded) are treated with PDT in this center. Currently ongoing studies include treatment planning for residual and recurrent nasopharynx and nasal cavity cancer, interstitial PDT for deep-seated tumors (later explained in more detail), and the combination of PDT and immunotherapy. These studies are performed in Indonesia and the Netherlands.

Photodynamic Therapy: The Treatment

The depth of penetration of the red light in Foscan PDT is ~8–10 mm. When taken into account a margin of 5 mm for curative treatment, tumors with a thickness of maximal 5 mm can be effectively treated. There is no restriction for the surface area of the tumor if the illumination of the entire tumor surface can be reached. Several special application tools have been developed for surface illumination, intraluminal illumination and interstitial illumination. These special applications will be described in more detail in the section on PDT techniques below. The major advantage of PDT is that it can be used after previous treatment with radiotherapy and/or surgery. Moreover, PDT can be repeated without accumulating toxicities and if used as primary treatment, all other options such as surgery and radiotherapy remain open.

Side Effects

After administration of the photosensitizer patients are sensitive to ambient light. They should go back into normal ambient light conditions gradually. It is important to realize that “light restriction” does not mean total darkness. Television and computer monitors are fine. A gradual increase in light exposure is important for photobleaching and breakdown of the photosensitizer. In case of total darkness, the sensitizer will not be broken down and the patient’s skin persists sensitive to light. The first few weeks direct bright sunlight should be avoided; this light is about ~100,000 lux (sunlight). However standard office light without any daylight entering the room is far less and is about ~400 lux which is an acceptable light intensity at day four after administration.

The most important side effect is swelling and pain. Proper management is extremely important. The following medication/intervention are advised as protocol standards in the treatment of PDT.

- Durogesic (Fentanyl) patch 25 mg
- Paracetamol/Codeine
- NSAID
- Dexamethasone
- Pain team if necessary

Patients Suitable for PDT

PDT can be used in patients with primary tumors, with multiple primary tumors, with recurrent or residual disease; however, PDT can also be employed in a palliative setting. Satisfying results with a complete response rate around 50% are reported in patients with no curative options left [5, 9]. Patients with recurrent or residual cancer of the nasopharynx or nasal cavity are also good candidates due to the difficult approach to these areas and the close surrounding critical structures that can easily be harmed by salvage surgery and or re-irradiation. Although PDT can be used as an effective treatment for different types of tumors in the head and neck, it is important to notice that patient selection is crucial. Only a few patients in each group are suitable for this treatment.

PDT Techniques

Surface Illumination

After injection of the photosensitizer the surface of the tumor including a 5 mm margin, is illuminated using a fiber optic micro-lens and CW laser light of 652 nm (2 W maximal output). The effective depth of PDT induced necrosis is around 8–10 mm. The surrounding healthy surface is also sensitive for light and should always be shielded from back-scattered light originating from the target area during the illumination (see Fig. 2). For shielding, pieces of moisturized green cloth are normally used.

With this surface illumination, patients with early (primary or recurrent) oral cavity and oropharynx cancer have been treated with good result [1–8]. Analyzing our institutional experience of early stage oral cavity and oropharynx neoplasms (Tis-T2) to identify the success rates for each subgroup according to T stage, primary or non-primary treatment and subsites in total, 170 patients with 226 lesions were found [7]. From these lesions, 95 were primary neoplasms, 131 were non-primaries (recurrences and multiple primaries).

Fig. 2 At the left a micro-lens fiber as used for the illumination of superficial tumor e.g. floor of mouth. On the left a surface PDT in the OR. The green cloth shields the healthy tissue from scattered light. A fixation-arm is used to position the micro-lens fiber on the correct distance and angle. The exposure time is 200 s for an output power of 100 mW/cm^2 delivering a total of 20 J/cm^2. Total OR time for these easy accessible tumors is about 45 min

The overall response rate was 90.7% with a complete response rate of 70.8%. Subgroup analysis identified oral tongue, floor of mouth sites with more favorable outcome. PDT has more favorable results with certain subsites and with previously untreated lesions. However, PDT can also find its place in treating lesions in previously treated areas.

Subject to debate is always the question whether PDT can be considered as an equal treatment as compared with transoral surgery (see below).

Photodynamic therapy (PDT) of early stage oral cavity tumors have been thoroughly reported. However, statistical comparison of PDT to the surgical treatment is not available in published literature. We have identified and matched cohorts of patients with early stage oral cavity cancers undergoing surgery (n = 43) and PDT (n = 55) from a single institute experience [1]. The groups are matched demographically and had the same pre-treatment screening and follow-up schedule. Both groups consisted only of tumors thinner than 5 mm to ensure comparability. The endpoints were local disease-free survival, disease free survival, overall survival and response to initial treatment. Local disease-free survival at 5 years was 67 and 74% for PDT and surgery groups, respectively [univariate HR = 1.9 (p = 0.26), multivariable HR = 2.7 (p = 0.13)]. Disease free survival at 5 years was 47 and 53% for PDT and surgery groups, respectively [univariate HR = 0.8 (p = 0.52), multivariable HR = 0.75 (p = 0.45)]. Overall survival was 83 and 75% for PDT and surgery groups, respectively [univariate HR = 0.5 (p = 0.19), multivariable HR = 0.5 (p = 0.17)]. In the PDT group, six patients (11%) and in the surgery group 11 patients (26%) had to receive additional treatments.

None of the tested parameters showed statistically significant difference. Although there is probably a selection bias due to the non-randomized design, this

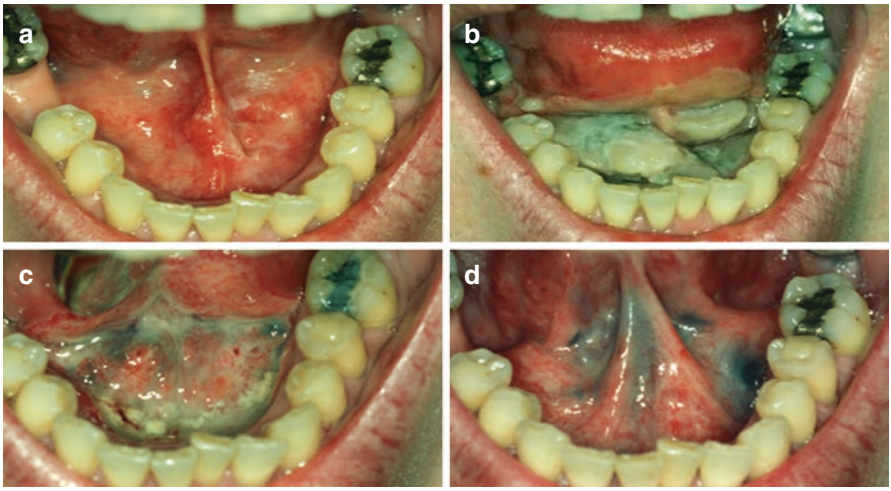

Fig. 3 Squamous cell carcinoma of the floor of the mouth T1N0 with adjacent erythroplakia. (**a**) tumor of the floor of the mouth (left sided around the insertion of the frenulum. (**b**) 1 week after illumination (tumor necrosis). (**c**) 2 weeks after illumination. (**d**) 2 months after illumination, there was no obstucion of the ductus of the salivary glands

study shows that PDT of early stage oral cavity cancer is comparable in terms of disease control and survival to transoral resection and can be offered as an alternative to surgical treatment. Another study performed in the Netherlands at the same period showed similar results [10].

Especially for certain indications, PDT can be of interest due to the limited damage of the tissues on the long-term. In case of a tumor of e.g. the anterior floor of mouth radical resection can be difficult, due to the proximity of the mandible (often with own dentition) (see Fig. 3). Marginal mandibulectomy (with loss of dentition) is often indicated to reach clear margins. PDT allows for preservation of function and cosmetic considerations in these cases (see Fig. 4).

Interstitial Illumination

Interstitial Photodynamic Therapy (iPDT) has been developed in the Netherlands Cancer Institute for the treatment of deep-seated tumors with a thickness exceeding >5 mm. The light sources are implanted within the tumor volume. For this type of PDT cylindrical diffusers are used (see Fig. 5). Prior to iPDT the light dose delivered to the tumor volume can be virtually simulated in 3D using CT and MR imaging. After delineation of the tumor on MRI, the emission profiles of the virtually positioned fibers are simulated with a modified computer program as used for brachytherapy to ensure full light dose coverage throughout the tumor volume. This approach provides information on the minimal amount of cylindrical sources needed, their length and their optimal location within the tumor [11].

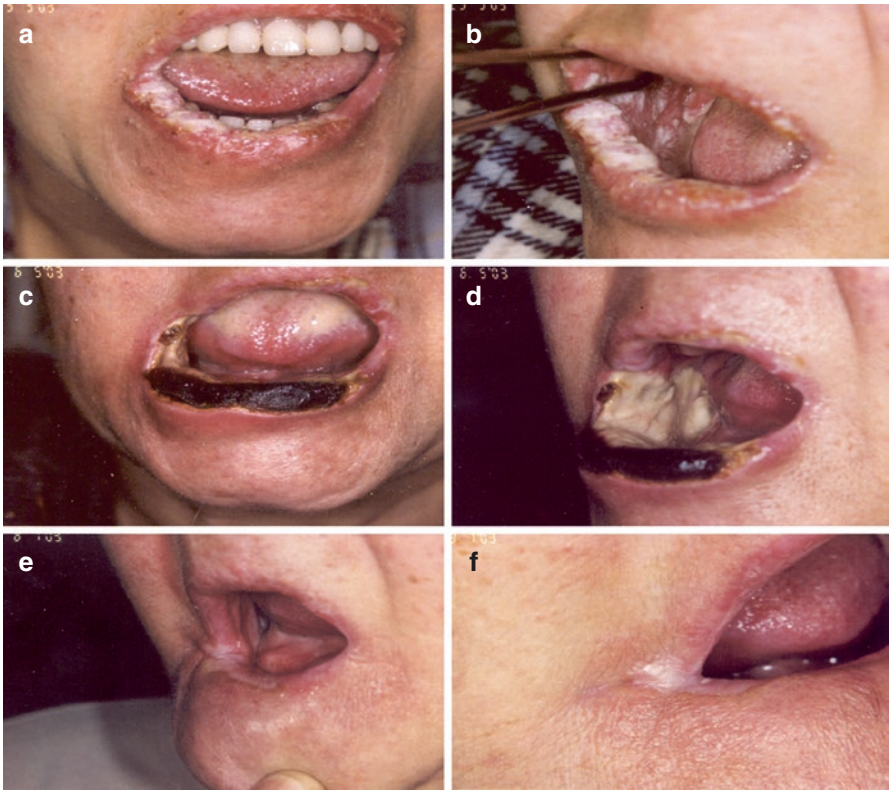

Fig. 4 Recurrent disease of the lip, previously a T1N0 carcinoma lower lip and T1N0 carcinoma upper lip for which surgical excision was performed. (**a**, **b**) recurrent invasive carcinoma of the lower lip, buccal mucosa and lower alveolus. Simultaneously she had a carcinoma in situ of the floor of mouth. (**c**, **d**) 3 weeks post-illumination. (**e**, **f**) 3 months post-illumination: no function loss and complete response (biopsy proven)

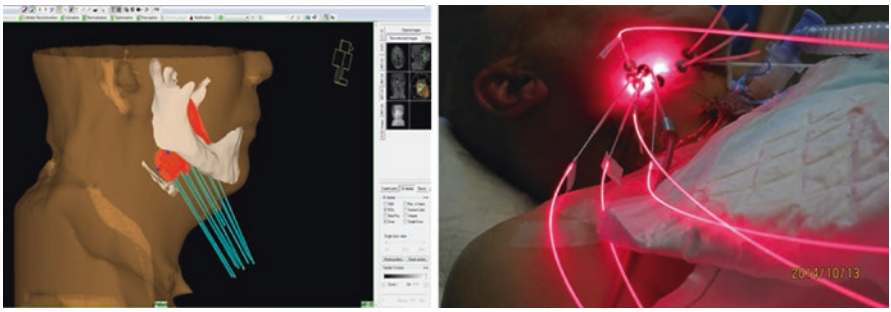

Fig. 5 At the left a sagittal 3D view of an iPDT pre-treatment plan, in white the segmented mandible and thyroid, in red the effective dose covering the tumor volume, green the delineated tumor volume (not visible), in blue the simulated transparent catheters containing the cylindrical diffusers. In red the total effective light dose. On the right a clinical picture of an iPDT treatment in progress. Five laser channels are used simultaneously. Each catheter contains a cylindrical diffuser with a pre-determined length and corresponding output power

In the operation theater the transparent brachytherapy catheters are placed according to the simulated treatment plan. Thereafter, their actual positions will be verified by a CT scan after waking up the patient or per-operative using an intra-operative Cone Beam CT. Catheter positions can be modified if needed and subsequently the catheters will be loaded using fiber optic cylindrical light diffusers to deliver a total dose of 30 J/cm diffuser length (J/cm) at a power output of 100 mW/cm for 300 s/diffuser. Large volumes of tumor can be destroyed in sites that are inaccessible to surgery or where re-irradiation and or surgery would cause unacceptable damage to vital adjacent structures with loss of functionality. Large vessels and nerves have shown to be unaffected by the PDT response. However, tumor blood vessel ingrowth should always be checked.

iPDT can be an option in the management of locally recurrent base of tongue cancer after (chemo-) radiation treatment.

Twenty patients with previously irradiated locally recurrent base of tongue cancers who were not candidates for salvage surgery or reirradiation or refused these therapies were subjects in this study [12]. The study showed that iPDT could be conducted in all patients without short-term complications. At 6 months, nine patients had complete response with four patients still free of disease at 46–80 months. Long-term complications included pharyngeal-cutaneous fistula in six patients, serious bleeding in one patient, and cutaneous metastasis in two patients. These initial results are encouraging, but there is room for improvement to control the destructive potential of iPDT through planning and monitoring tools.

Intra Luminal Illumination

For certain areas, like the nasopharynx or the paranasal sinuses, controlled and reproducible introduction of a light source is difficult. These geometries are complex and difficult to access thus making it complicated to asses' accurate information on the actual source position relative to the target area resulting in large variations in delivered light dose thereby compromising a controlled and reproducible light delivery and thus clinical response.

For the nasopharynx a special flexible silicone nasopharynx applicator was developed for an equal and stabile illumination of the difficult geometry in this area [13]. Critical risk structures (e.g. the soft palate and nasal cavity) can be protected by the shielding properties of this tool. The patented applicator can be inserted through the mouth and fixed in the nasopharyngeal cavity (see Fig. 6). For the illumination of the par nasal sinuses, research on 3D light dosimetry planning, the use of a navigation guided source positioning and source fixation by an positioning arm is currently ongoing (see Fig. 6).

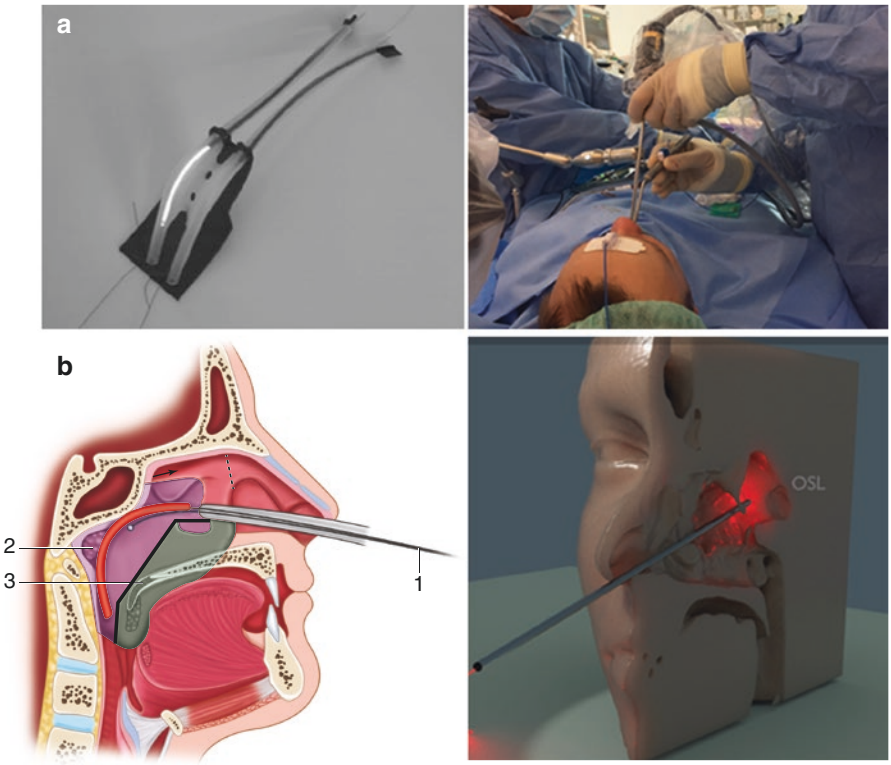

Fig. 6 At the left (**a**) Basic shape of the NPC PDT applicator device and (**b**) the position of the light application device with respect to the anatomical location. For the PDT treatments, cylindrical diffusers (1) that radially emits homogeneously over a 30, 40, 50 or 60 mm length are used. The NPC target area (2) is localized along the outer curve of the applicator. The thick black line represents the black silicon patch intended to shield light from critical healthy areas like the soft palate (3). At the right an example of an intra-luminal PDT treatment of a recurrent paranasal sinus tumor located at the base of skull. Prior the intra-luminal PDT the optimal source location is determined and simulated (lower right). The source is maneuvered to, and locked at this optimal location by means of EM navigation (upper right)

The Role of PDT in End Stage Incurable Head and Neck Cancer

The temporary approval of FOSCAN® by The European Medicines Agency (EMEA) in 2001 was obtained based on the results of the Pivotal 08b study of D'Cruz et al. on mTHPC-mediated photodynamic therapy in patients with advanced, incurable head and neck cancer: a multicenter study of 128 patients [5].

In this study an objective confirmed (according to WHO criteria) response rate of 38% was reported with an objective clinical benefit in 56% and a complete response (CR) rate of 16%. Sub group analysis even showed an objective confirmed response rate of 54% and a CR rate 30%, when tumors with a depth of infiltration <10 mm were completely illuminated.

The temporary registration was obtained pending the results of a confirmatory study. This was a multi-center, open-label, single-arm study of Foscan-photodynamic therapy in the palliative treatment of patients with advanced squamous cell carcinoma of the head and neck who had failed prior therapies, were unsuitable for curative therapy with radiotherapy, surgery or systemic chemotherapy, had a depth of infiltration of the tumor of <10 mm and were suitable for complete illumination [9]. The results of this confirmatory study showed an objective complete response of 49%. Based on these results a definitive approval was obtained for Foscan for the European market.

PDT and the Immune System

Most cancer therapies are immunosuppressive. Nowadays, research in the treatment of cancer is highly focused on the immune system. Researchers all over the world try to discover why tumor cells escape the normal immune reaction. It seems that the tumor creates a microenvironment wherein a normally expected immune reaction is suppressed, and so the tumor can survive and grow. Programmed cell death protein 1 (PD-1) is a cell surface receptor that plays an important role in down-regulating the immune system and promoting self-tolerance by suppressing T cell inflammatory activity. When it binds to its ligand PD-L1 or PD-L2 the T-cell is inactivated. PD1 and their ligands are found repeatedly in the microenvironment of multiple tumors and related to survival. T cell checkpoint blockade using anti-PD-1 antibodies is currently the most promising therapy in patients with recurrent or metastatic head and neck squamous cell carcinoma and may result in a significant clinical benefit. Anti-PD-1 treatment has resulted in an increase of 1-year overall survival rate in patients with recurrent or metastatic head and neck squamous cell carcinoma from 17% to 36% when compared to standard of care chemotherapy in patients with platinum-refractory disease [14]. Although, this is a very promising increase of survival, still there is ample room for improvement and an urgent need to enhance their efficacy.

PDT is known to stimulate the immune system [14–16]. It induces the formation of reactive oxygen species and subsequent tumor cell death through their direct cytotoxic effects. Apart from damage done directly to tumor cells and their vasculature, PDT is able to induce an anti-tumor immune response in a process called immunogenic cell death [17, 18]. Therefore, PDT is highly interesting as treatment modality to be combined with immunotherapy for patients with head and neck cancer. A combination to enhance immune checkpoint inhibition efficacy through photodynamic therapy-induced immunomodulation seems obvious.

Conclusions and Future Perspectives

PDT is an additional powerful tool in the treatment of head and neck cancer. It can especially be helpful in patients where other options are exhausted. Nowadays PDT can be applied as surface, interstitial and intraluminal illumination. Collaboration with radiotherapy departments has led to very potential treatment tools and should be continued (similarity between brachytherapy and iPDT and the nasopharynx applicator). Combination of PDT and immunotherapy can become a rewarding research line.

Conflict of Interest Part of this work was sponsored by Biolitec, Dutch Cancer Foundation (KWF) and STW.

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Part IV
Oropharynx Cancer

HPV Assessment in Oropharynx Cancer: What is the Gold Standard?

Panagiota Economopoulou, Ioannis Kotsantis, and Amanda Psyrri

Introduction

Head and Neck Squamous Cell Carcinoma (HNSCC) is the sixth most common malignancy worldwide, with an annual global incidence of more than 500,000 new cases and a death toll of approximately 300,000 patients per year [1]. Historically, HNSCC has been associated with known risk factors such as tobacco and alcohol. A causative association between infection with high risk human papillomaviruses (HPV) and oral squamous cell cancer was first described by Syrjanen et al. in 1983, who found histopathological features consistent with cervical HPV infection (morphological signs and HPV antigens) in biopsy specimens [2]. Since that time, HPV has emerged as an established etiologic factor in particular in oropharyngeal squamous cell carcinoma (OSCC) [3], with serology-based data clearly demonstrating that infection precedes cancer occurrence [4]. Among the HPV types involved in head and neck carcinogenesis, HPV16 is by far the most common, with a prevalence over 90% in OSCC, followed by HPV18 (3%) [5]. HPV-associated oropharyngeal tumors represent a distinct biological and clinical entity, have a distinct mutation landscape, and are characterized by markedly improved survival [6, 7].

It is now believed that HPV accounts for the majority of cases of OSCC in many developed countries [8]. Indeed, approximately 45–90% of newly diagnosed OSCC is HPV-related, which represents almost twice the incidence recorded during the late 1990s [9]. The gradual shift in disease etiology to that of a predominantly HPV-positive clinical and molecular entity has direct impact on the current clinical presentation of OSCC patients in the caregiver's office. For these reasons, the evaluating physician must be thoroughly educated on the current trends in the evaluation of the patient suspected of having HPV-positive (HPV+) OSCC. As future treatment strategies might become dependent on HPV status, it is of major importance to have

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diagnostic tests available that can reliably select OSCC tumors that are caused by HPV. In this context, National Comprehensive Cancer Network (NCCN) guidelines have incorporated HPV testing as part of the pathologic evaluation of a primary OSCC or a cervical metastatic SCC with an unknown primary site. Many methodical approaches are available for determining HPV status, which aim at the identification of a biologically relevant, e.g. transforming HPV infection; however, there is presently no consensus on the best methodology. In this chapter, we review the role of HPV in OSCC, molecular mechanisms implicated in HPV oncogenesis and available methodologies for evaluating HPV status and discuss the importance of assessing HPV status as a clinically relevant biomarker in OSCC.

HPV Life Cycle

HPV is a non-enveloped, double-stranded DNA virus encoding a total of 8–9 proteins in approximately 8000 base pairs, with the ability to infect cutaneous or mucosal tissues. The viral genome is organized into three regions based on their location and functional properties. The early (E) region encodes proteins regulating viral transcription (E2), viral DNA replication (E1, E2), cell proliferation (E5, E6, E7), and viral particle release (E4) [10]. E6 and E7 oncogenes also encode proteins associated with malignant lesions that are capable of immortalizing primary human keratinocytes; E6 and E7 continuous expression is critical in maintaining the cancer phenotype in infected cells [11]. The late (L) region encodes for two structural viral capsid proteins (L1 and L2). Finally, the long control (LCR) or non-coding region (NCR) regulates gene expression and replication [10].

HPV gene expression is complex, involving a synchronization of transcription, mRNA stability, splicing, and polyadenylation with keratinocyte differentiation and distinct phases of the viral life cycle [12]. The life cycle of HPV is directly related to the cellular differentiation program of the host cell. In the initial phase of the HPV life cycle, basal keratinocytes are infected by the virus that has permeated the above epidermal barrier through microwounds [13]. At least for the high-risk HPVs, it seems necessary for efficient establishment of infection that they infect actively dividing basal, or stem, epithelial cells. Infection of the basal cell is followed by an initial phase of viral genome amplification; subsequently, the viral genome is maintained as an extrachromosomal circular element, referred to as episome, at a low copy number [14, 15]. HPV uses the host cell replication machinery to initiate viral DNA replication. In the case of high risk HPVs, oncoproteins E6 and E7 promote cell-cycle progression and viral DNA replication in differentiated keratinocytes [16]. Epithelial cells differentiate as they move towards the surface epithelium, and HPV DNA replicates in a high copy number in differentiated cells near the epithelial surface. Viral protein E6 is required for episomal genome maintenance; viral protein E7 forces the infected cell to re-enter S-phase; therefore, HPV takes advantage of the active replication machinery to expand its viral genome [17]. Both E7 and E6 interact with several tumor suppression proteins in order to create an

environment suitable for viral genome replication. The integration of HPV DNA into the host genome, disrupts the expression of the main viral transcription/replication factor E2, the transcriptional repressor of E6 and E7 viral oncogenes [10]. The E6 protein binds and induces the degradation of the p53 tumor suppressor protein via an ubiquitin-mediated process, while the HPV-E7 protein binds cullin-RING E3 ubiquitin ligase complex and ubiquitinates the retinoblastoma (Rb) tumor suppressor protein and related proteins [18]. The p53 and pRb tumor suppressor pathways are dormant but active in cancer cells due to the continuous expression of E6 and E7 oncogenes [19]. Degradation of Rb induces expression of $p16^{INK4A}$, which is the hallmark of HPV-positive OSCC [7, 20]. pRb is a negative regulator of p16 protein at the transcriptional level [21] and low pRb levels lead to subsequent p16 upregulation. HPV-associated cancers contain high p16 protein levels [22]. The completion of the HPV life cycle involves the exit from the cell cycle and the expression of late viral proteins L1 and L2 to enable packing of the viral genome [23].

Oral HPV Infection

Epidemiology HPVs are a group of more than 150 related viruses which are known to cause approximately 5% of all human cancers by infecting keratinocytes in the skin and mucosa [24, 25]. There are two major HPV subtypes: high-risk types (e.g., HPV16 and 18) which cause anogenital carcinomas and OSCC, and low-risk types (e.g., HPV6 and 11) which cause genital warts and recurrent respiratory papilloma. Although most HPV infections are typically cleared by the host's immune system within 1–2 years, some individuals harbor persistent high-risk HPV which puts them at risk of HPV-related diseases, including cancer [12]. Contrary to genital HPV infection, epidemiology and natural history of oral HPV infection have not been well established. Data from a recent cohort study that was designed to evaluate incidence and clearance of oral HPV infections in men, demonstrated similar patterns of clearance with genital HPV infections [26]. In addition, a study that assessed the incidence of oral and cervical HPV infection in a 6 month interval, showed a significantly lower incidence of oral infections compared to cervical at baseline, but similar persistence of detected infections after 6 months [27].

It has been postulated that at any given time approximately 7% of the population has an oral/oropharyngeal infection [28]. This incidence is substantially lower than HPV genital infection. A recent study that included 5579 men and women aged 14–69 years and was conducted in the US as part of the National Health and Nutrition Examination Survey (NHANES) between 2009 and 2010 showed that the prevalence of HPV infection in that population was 6.9%. In addition, HPV prevalence was two- to threefold higher in men compared to women and followed a bimodal pattern, with a first peak incidence occurring at 30–34 years of age and a second peak incidence at 60–64 years [29]. Furthermore, the prevalence of high risk HPV types was 3.7% compared to 3.1% of low-risk types; prevalence of HPV16

was 1%. Of note, a recent meta-analysis of 18 individual studies that detected oral HPV DNA in a cancer-free US population, reported a lower overall incidence of oral HPV infection [30]. In Europe, prevalence of oral HPV infection has not been evaluated in large studies.

Transmission Anogenital areas harbor HPV which is transmitted to the head and neck area mainly through oro-genital contact [31]. Oral sexual partners have increased likelihood of carrying oral HPV (p-trend = 0.03; OR 3.88) [32]. The prevalence of oral HPV infection increases significantly (OR 5.20) with a higher number of oro-genital sex partners [33]. Recent evidence also supports non-sexual transmission of oral HPV; persistent maternal oral infection has been reported to correlate with the presence of oral HPV in the mucosa of newborns [34]. Furthermore, data from retrospective studies with small number of patients show that HPV infection is associated with increased number of deep (French) kissing partners, even in individuals who had never had oral sex [35, 36]. Prospective studies are warranted to further assess the incidence of oral HPV infection in relation to each type of sexual behavior.

Causative Role in OSCC Genomic DNA of oncogenic HPV is detected in approximately 26% of OSCCs worldwide. There is strong epidemiologic and molecular pathology evidence of a causative role of HPV in OSCC. Firstly, single case-control studies that included OSCC patients have demonstrated a clear correlation with sexual behavior and HPV infection [37, 38]. In one study, HPV-16 DNA was detected in 72% of tumor specimens; however, only 37% of patients had HPV DNA detected in their oral rinses [38]. Of note, detection of HPV DNA in tumor specimens does not represent direct proof of a causative association between HPV and OSCC. On the contrary, an etiological link could be confirmed if molecular techniques showed that the presence of HPV virus is required for malignant transformation of epithelial cells. Indeed, experimental studies have demonstrated that in HPV+ OSCC cell lines, continuous expression of E6/E7 oncogenic viral proteins is essential for the cells to maintain their malignant phenotype. In addition, repression of viral proteins E6 and E7 reactivate the apoptotic machinery by restoring p53 and Rb tumor suppressor pathways [6].

It is important to emphasize that studies of HPV in OSCC are complicated by etiological heterogeneity; therefore, it is unclear whether HPV infection is etiologically involved in all HPV-DNA positive OSCCs. HPV is omnipresent in humans and only a small fraction of people infected with high-risk HPVs will eventually develop cancer often decades after the original infection. The etiology of OSCC is often multifactorial. It is possible that tobacco-related OSCC undergo superinfection with high-risk HPVs, thus generating tumors containing tobacco-related molecular changes (i.e. low p16 protein expression, p53 mutations) and high-risk HPV DNA genome. In this case, HPV+ OSCCs have more dismal prognosis than those occurring in nonsmokers [7, 39]. Indeed, in a retrospective analysis of the association between tumor HPV status and survival among patients with stage III or IV OSCC who were enrolled in a randomized trial, Ang et al. showed that heavy

smokers with HPV-related OSCC and advanced N stage disease have a 3-year OS of 70% compared to 90% of light smokers with HPV+ OSCC [39].

HPV-related OSCC: Epidemiology and Clinical Characteristics

Epidemiology An estimated 85,000 cases of OSCC occur worldwide annually, of which at least a quarter are HPV-related [40, 41]. In the last decades, a decrease in the incidence of HNSCC from non-oropharyngeal sites has been observed following primary prevention strategies to reduce tobacco smoking [8]. However, in economically developed countries, the incidence of OSCC did not show such a decline, broadly mirroring the increasing burden of HPV-related OSCC [42]. Overall, globally, there was a 26.6% increase in incidence of OSCC from 1975 through 2012. In the USA, the incidence of HPV-negative (HPV-) OSCC declined by 50% between 1988 and 2004, but the incidence of HPV+ OSCC increased by 225%. [8]. Thus, HPV-driven OSCC is a major health epidemic in the western world; it has been speculated that if current incidence trends persist, by year 2020 the incidence of HPV-associated OSCC will be greater than that of cervical cancer and that in 20 years HPV+ OSCCs will represent the majority of head and neck cancers [32].

Interestingly, epidemiologic trends in HPV prevalence vary in relation to geography and socioeconomic status [43]. Studies in Scandinavia have shown that incidence rates of HPV-associated OSCC have been rising by 3.5–5% per year, with the number of cases expected to double within a decade in this region [44]. In the US, HPV-related OSCC increased at a rate of 2.5% per year [45]. Recently published data show that the population attributable fraction (PAF) of HPV-associated OSCC is estimated to be high (40-60%) in North America, Northwestern Europe, East Europe, South Korea, Japan and Australia and low (13–24%) in South Europe, China and India [46]. This is partly explained by the fact that western countries have a higher percentage of people engaged in oral sex (e.g. 78% in Boston) and higher percentage of people with multiple sexual partners [47].

Clinical Characteristics HPV+ OSCC represents a distinct disease entity. Typically, patients with HPV-related OSCC are men, white and non-Hispanic and present at a younger age (median 57 vs. 64 years for HPV+ vs. HPV− OSCC respectively). In addition, they have no or little tobacco exposure, a higher socioeconomic status, are more likely exposed to marijuana and tend to have a higher lifetime number of oral and/or vaginal sex partners [48, 49]. Furthermore, HPV+ tumors are more likely to present at an early T stage with extensive lymph node (LN) involvement and tend to be poorly differentiated (with basaloid features) [49].

Despite a more aggressive clinical presentation, the prognosis for HPV+ OSCC appears to be better than that for HPV− OSCC, particularly in locally advanced disease. An analysis of 493 HNSCC patients with LN metastases demonstrated that

LNs from HPV+ tumors were larger and more likely to be cystic; however, they were characterized by improved loco-regional control and were more likely to resolve following treatment in comparison to LNs of HPV− patients [50]. HPV-associated OSCCs have a significant survival advantage over their HPV− counterparts, with a risk of death consistently less than 60% of that of HPV− tumors [39]. Interestingly, HPV+ OSCC patients with a history of smoking have a more dismal prognosis than non-smokers, indicating the impact of tobacco use as a modulating factor in OSCC [51]. Of note, the rate of distant metastasis is similar between HPV+ and HPV− OSCC patients. Given the excellent loco-regional control of HPV+ patients, the presence of distant metastases remains as the major cause of death in these patients [52].

HPV Type Distribution HPV16 accounts for over 90% of HPV+ OSCCs and few are caused by other HPV oncogenic types [28]. Recently, Bratman et al. examined HPV genotypes in 515 HNSCCs via RNA-sequencing data from The Cancer Genome Atlas (TCGA) and found the presence of HPV transcripts in 14% of the HNSCCs, of which 84% were HPV16, and the other 16% were other subtypes (HPV33, HPV35, HPV56) [53]. However, HPV18, which has been shown to be responsible for approximately 3% of HNSCCs worldwide, was not identified in this cohort. Similarly, Goodman et al. reported the prevalence of HPV subtypes in a cohort of 529 OSCC samples, detecting HPV16 in 61% of the OSCCs, and other high risk HPV subtypes in 11% of the cases, including HPV33, HPV18, HPV35, HPV31, HPV52, HPV39, and HPV45 [54].

Molecular Phenotype of HPV-Associated OSCC

Data from the recent comprehensive epigenetic and genomic landscape studies revealed that in both HPV+ and HPV− HNSCC, p53 and pRb pathways are frequently altered; however, the mechanism of inactivation is different. Inactivating p53 mutations are rarely seen in HPV+ tumors (7.1%) compared with HPV− tumors (48.3%) [55]. As described above, the E6 and E7 viral proteins functionally inactivate p53 and pRb in HPV+ HNSCC [18]. E6 binds to p53 and targets it for degradation, whereas E7 binds and promotes degradation of pRb tumor suppressor protein. Importantly, p53 and pRb molecular pathways together regulate all critical biologic functions required for normal cellular homeostasis. The pRb signaling pathway is directly regulated by the p16 tumor suppressor protein; p16 also indirectly regulates the p53 pathway. Hyperphosphorylation of pRb causes the release of active, free E2F, which, in turn, activates several genes that control DNA synthesis. p16 binds and inhibits cyclin-dependent kinases (CDK) 4 and 6, thereby triggering cell-cycle arrest. Mutation or deletion of p16 or methylation of its promoter, which are common in HNSCC, lead to loss of p16 function. This is considered biologically equivalent to loss of pRb function. Furthermore, loss of pRb function following HPV infection leads to upregulation of p16 via a feedback mechanism [56]. On the other

hand, the majority of HPV− tumors have p53 mutations, widespread copy-number alterations, and promoter hyper-methylation and mutation of Cyclin-Dependent kinase Inhibitor 2A (CDKN2A) gene, which encodes p16INK4a protein, leading to a loss of p16 expression [57]. According to Campbell et al., SCCs harbor 3q, 5p, and other recurrent chromosomal copy-number alterations (CNAs), DNA mutations, and/or aberrant methylation of genes and microRNAs, which are correlated with the expression of multi-gene programs linked to squamous cell stemness, epithelial-to-mesenchymal differentiation, growth, genomic integrity, oxidative damage, death, and inflammation [58]. Low-CNA SCCs tended to be HPV+ and display hyper-methylation with repression of TET1 demethylase and FANCF, previously linked to predisposition to SCC, or harbor mutations affecting CASP8, RAS-MAPK pathways.

Several studies have shown considerable differences in the gene expression profiles that are specifically associated with HPV+ and HPV− HNSCC, supporting the notion that HPV+ HNSCC has a specific transcription profile depending partly on E6 and E7 expression [18]. In a hallmark study, Weinberger et al. showed that p16 protein status determined by immunohistochemistry (IHC) is a reasonable surrogate marker for a biologically and clinically meaningful HPV infection in OSCC [7]. The authors sought to determine the prevalence of biologically relevant human HPV in a cohort of 79 OSCCs. They hypothesized that p16 overexpression would define HPV-associated tumors with favorable prognosis. HPV-16 DNA viral load was determined by Real-Time Polymerase Chain Reaction (RT-PCR). Furthermore, the authors constructed a tissue array composed of these tumors and studied expression of p53, pRb and p16 proteins using a quantitative in situ method of protein analysis (AQUA). Tumor specimens were classified into three tumor classes with distinct molecular and clinical features based on HPV16 DNA presence and p16 expression status: class I, HPV−, p16 low; class II, HPV+, p16 low; and class III, HPV+, p16 high. Overall survival (OS) in class III was significantly higher compared to the other two classes (79% vs. 20% and 18%, respectively, $p = 0.0095$). Similarly, disease-free survival (DFS) for the same class was improved (75% vs. 15% and 13% respectively, $p = 0.0025$). The 5-year local recurrence was 14% in Class III versus 45% and 74% in the two other classes, respectively ($p = 0.03$). Importantly, only patients in class III had significantly lower p53 and pRb expression ($p = 0.017$ and 0.001, respectively). The prognostic value of the 3-class model was confirmed in a multivariable survival analysis [7]. Thus, the authors managed to define the molecular profile of HPV+ OSCC with favorable prognosis, namely HPV+, p16 high (class III), which is the only one that fits the cervical carcinogenesis model.

HPV+ and HPV− OSCC are characterized by distinct gene expression patterns, including mutations, amplifications and deletions. For example, HPV+ OSCCs frequently exhibit TNF receptor associated factor 3(TRAF3) loss, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) activating mutations, and E2F1 amplification; while HPV− OSCCs commonly harbor 11q amplifications, and mutations in Caspase-8 (CASP8) and HRAS [59]. Indeed, HPV-driven OSCC frequently harbors activating mutations and amplifications of the oncogene PIK3CA. Interestingly, although AKT is largely regarded as the dominant mediator

of oncogenic PI3K signaling in most cancers [60], it is still questionable whether this also occurs in HPV-driven HNSCC. A recent study, which assessed the expression of 137 total and phosphorylated proteins by reverse-phase protein array in 29 HPV+ and 13 HPV− prospectively collected OSCCs, revealed that HPV+ OSCC with activating PIK3CA mutations did not show increased phosphorylation of AKT, but increased mammalian target of rapamycin (mTOR) activity [61]. Similarly, in a mouse model of oral carcinogenesis, overexpression of PIK3CA was found to trigger tumor invasion and metastasis, but this was not accompanied with AKT activation. On the contrary, signaling was mediated through 3-phosphoinositidedependent protein kinase 1 (PDK1), and PI3K–PDK1 signaling was found to interact with transforming growth factor-β (TGFβ)–SMAD3 signaling during tumor progression [62].

Slebos and colleagues attempted to compare gene expression profiles of HPV+ and HPV− tumors. Thirty-six HNSCC tumors were analyzed using Affymetrix Human 133U Plus 2.0 GeneChip. Among them, 8 were HPV+; it was demonstrated that CDKN2A was one of the most significant differentially expressed genes between HPV+ and HPV− disease, since its expression was highly upregulated in HPV+ tumors [63]. Other genes identified to have increased expression in HPV+ group were the Rb-binding protein p18, the cell division cycle 7-related protein kinase CDC7, the transcription factors TAF7L, RFC4, RPA2, TFDP2 and the cell adhesion molecule TCAM1 [63]. Another study used cDNA microarrays to compare gene expression patterns between HPV+ and HPV HNSCCs; like the previous study, p18 and TFDP2 were found to be overexpressed in HPV+ tumors [64]. However, this study failed to validate upregulation of p16INK4a in HPV+ group as its expression levels were very low. On the other hand, a third microarray analysis confirmed the overexpression of p16INK4a in the HPV+ subgroup and additionally demonstrated that the expression of multiple genes such as ZNF238, DNMT1, MCM3, AKR1C3, GRB10, TYK2 and SART3 is significantly upregulated in HPV+ HNSCC compared to HPV− HNSCC [65]. Apparently, the reported gene expression profiles differ substantially between studies, complicating data interpretation and identification of genes responsible for carcinogenesis in OSCC.

In an attempt to discover biologically distinct HNSCC subtypes with translationally relevant characteristics, several studies focused on genomic profiling. Keck et al. used expression profiling and cluster analysis in a cohort of HNSCC patients and showed that HPV+ HNSCC could be subclassified into two gene profile groups. Compared with other HNSCC subtypes, the two HPV subtypes show low expression and no copy number events for EGFR/HER ligands [66]. In a more recent RNA sequencing study, these two groups were characterized by a signature of mesenchymal and immunological response genes (named HPV-IMU) or keratinocyte differentiation and oxidative stress genes (named HPV-KRT) [67]. More detailed molecular analysis revealed that the second group had more frequently integrated HPV, lower E2/E4/E5 expression levels and a higher ratio of spliced E6 to full length E6 transcripts. Moreover, this group was found to display PIK3CA mutations and chromosome 3q gains, while HPV-IMU tumors were enriched for chromosome 16q losses. A significant survival difference between these two subgroups could not

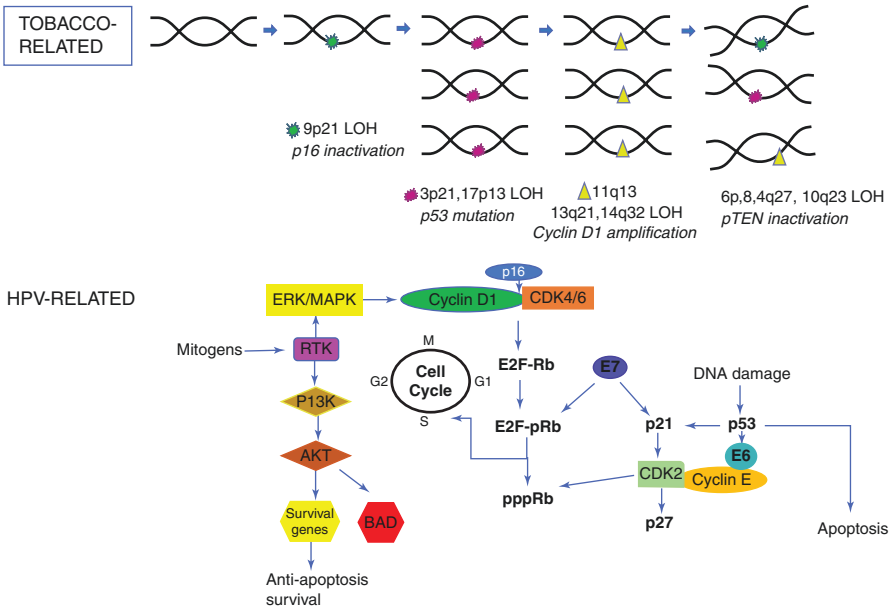

Fig. 1 Molecular pathogenesis of tobacco-related and HPV-related HNSCC

be shown, but the prognostic analysis was hampered by limited outcome data and a small sample size [67].

Molecular pathogenesis of tobacco-related and HPV-related HNSCC is illustrated in Fig. 1.

HPV Detection Assays

Given the distinctiveness of HPV-related carcinoma as a biological and clinical variant of HNSCC, the need for routine HPV testing of OSCC is compelling and urgent. The increasing incidence of HPV+ OSCC, along with the growing importance of HPV status as a versatile biomarker necessitates the establishment of HPV testing and the inclusion of HPV status as a parameter of emerging molecular staging systems. The incorporation of HPV status in the management of OSCC has prognostic and potentially therapeutic implications. Indeed, the College of American Pathologists has recently recommended routine HPV testing as part of the standard pathologic evaluation of resected OSCCs. Moreover, HPV testing has recently been included in the American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) guidelines as a standard pathological assessment for OSCC. Furthermore, NCCN guidelines recommend HPV testing for all oropharyngeal tumors. The National Cancer Institute, Cancer Therapy Evolution Program

(NCI-CTEP) suggests that HPV status must be included as stratification factor for trials including OSCC patients. In addition, the U.S Cooperative Groups and European Organization for Research and Treatment of Cancer emphasizes that HPV+ OSCC is a distinct disease entity. Despite these recommendations, UK and US head and neck cancer departments only test HPV in 79% and 67% of cases routinely, the main reasons for not testing being cost, lack of clinical relevance and time constraints. Clinically inappropriate requests are also an important issue [68].

Apparently, the value of HPV testing is not restricted to prognosis. A distinct tumor staging system has been developed for HPV+ OSCC. In addition, HPV status should be checked in patients who present with cervical lymph node metastases of unknown primary origin as an indicator of disease arising from the oropharynx. In the near future, HPV status will help guide a more individualized therapeutic approach for patients with HNSCC. Indeed, HPV+ OSCC is associated with improved response to therapy and longer lifespan [69]; thus, improving quality of life measures and avoiding late toxicity of chemotherapy and radiation are paramount objectives. Despite the numerous differences between HPV+ and HPV− OSCC, currently there is no specific algorithm, to treat HPV+ OSCC. However, there are now several clinical trials that are focused on de-intensification of treatment to avoid late toxicity of treatment. These alterations in treatment include: reducing the total dose of radiation therapy, using radiation therapy alone, and replacing conventional chemotherapy with targeted therapy [70]. There has also been a focus on the development of novel therapeutic agents that specifically target HPV-associated OPSCC, such as conventional vaccines and novel therapeutics targeting E6/E7 proteins [71]. Finally, HPV assessment may play a role in comprehensive cancer care including early cancer detection, post-treatment tumor surveillance, and more informed discussions with patients and their partners [72].

Detection strategies vary not just in design, but in their detection targets. These targets include HPV DNA, HPV RNA, viral oncoproteins, cellular proteins and HPV-specific serum antibodies [72]. In the ongoing effort to establish a consensus approach, the challenge for the oncologic community is to implement standardized HPV testing using a method that is highly accurate, technically feasible, non-invasive, economical, of low complexity and easily incorporated into routine clinical practice.

Given HPV life cycle and mechanism of entry, HPV detection strategies may look to detect: (a) HPV DNA, (b) post-integration transcription of viral E6 and/or E7 mRNA, (c) the viral oncoproteins E6 and E7, or (d) altered expression of cellular proteins such as overexpression of the p16 protein [72]. Each HPV detection strategy would be valuable and reliable if it could both recognize the presence of HPV and perceive its potential role in tumorigenesis. HPV detection methods are outlined in Table 1.

HPV DNA Detection with Polymerase Chain Reaction (PCR) Polymerase chain reaction and reverse transcriptase PCR (RT-PCR) are processes in which a signal sequence of DNA or RNA (properly, the cDNA after reverse transcription) is amplified several orders of magnitude through several rounds of denaturing at high

Table 1 HPV detection methods

Methods	Specimen	Specificity	Sensitivity	Transferability	Advantages	Disadvantages
HPV PCR	– Fresh frozen samples – FFPE – Any body fluid	Suboptimal (susceptible to viral contaminant)	Very high (<1 viral copy per host genome)	Molecular laboratory	– High sensitivity – Low specificity – Cost effective – Several commercially available primer sets – Assesses for HPV other than HPV16	– Provides no quantitative measure of viral load – No confirmation of transcriptionally active virus – Full spectrum of sequence amplification not published – No distinction between episomal and integrated DNA – More efficient in frozen tissues
Real-time PCR	– Fresh/ frozen samples – FFPE – Any body fluid	97%	92%	Molecular laboratory	– High sensitivity	– False positive and false negative products – No direct evidence of viral integration – No direct evidence of oncogene expression – Labour intensive – Time consuming
Reverse transcriptase PCR	– Fresh/ frozen samples – FFPE	High	High	Molecular laboratory	– High sensitivity – Detection of clinically significant HPV infection – Evidence of active oncogene transcription	– Technically difficult to be used in routine screening

(continued)

Table 1 (continued)

Methods	Specimen	Specificity	Sensitivity	Transferability	Advantages	Disadvantages
ISH	– Fresh samples – FFPE	100%	83%	Surgical laboratory	– High specificity – Ability to differentiate between episomal and integrated DNA – Commercially available tests	– Insufficient clinical sensitivity (83%) to be used in routine screening – Technically difficult to be used in routine screening
p16 immunostaining	– Fresh/ frozen samples – FFPE	80%	100%	Accessible to most laboratories	– High sensitivity – Easily applied to FFPE tissue – Presence of transcriptionally active virus through marker of host cell feedback mechanism	– Surrogate marker – Sensitivity less than reported for the existence of HPV positive, non-p16 overexpressing subtype – Suboptimal specificity
Combined p16 immunostaining + HPV PCR	– Fresh/ frozen samples – FFPE	96%	100%	Molecular laboratory	– Accurate analysis of HPV status – High sensitivity and specificity	– Not easily applicable to all laboratories – Not cost-effective
Combined p16 immunostaining + HPV ISH	– Fresh samples – FFPE	100%	100%	Surgical laboratory	– Accurate analysis of HPV status – High sensitivity and specificity – Ability to differentiate between episomal and integrated DNA – Transferable to surgical laboratory	– Not easily applicable to all laboratories – Not cost-effective – Technically difficult to be used in routine screening

FFPE formalin-fixed paraffin embedded, *HPV* human papilloma virus, *ISH* in situ hybridization, *PCR* polymerase chain reaction

temperature (95 °C), annealing of complimentary oligonucleotide primers at a lower temperature (55 °C), and DNA replication at an intermediate temperature (72 °C) by a heat-resistant DNA polymerase [73]. The amplification products can be visualized by electrophoresis on an agarose gel or quantified using real-time PCR. Thus, PCR amplification of HPV DNA is a target amplification technique that is capable of amplifying DNA sequences in a biological sample that contains heterogeneous cell types. PCR represents a highly-sensitive, widely-available, and cost-effective method of HPV detection. In theory, it can be used to detect as little as one copy of a DNA sequence and can be used in FFPE tissue or fresh tissue from oral biopsies, although it is more sensitive on fresh frozen tissue compared to FFPE tissue. The primer sets can be designed to target highly conserved sequences shared by multiple HPV types allowing for the simultaneous identification of a wide range of HPV types, or they can target type-specific viral DNA sequences permitting HPV genotyping [74].

The most important advantage of PCR-based methods of HPV detection is their incomparably sensitivity: these methods can detect HPV well below one viral copy genome per cell. However, standard PCR techniques have a number of disadvantages: (1) they have low specificity; (2) they do not permit the distinction between HPV that acts as a driver of malignant transformation, and transcriptionally silent virus that has no role in the process of tumorigenesis (i.e. passenger virus). The presence of latent virus leads to false positive results due to the ability of PCR to detect just a few copies of HPV DNA per cell; (3) they cannot distinguish between episomal and integrated HPV DNA; (4) they are technically complicated; (5) clinical samples are very prone to cross contamination by other specimens [75]. These limitations decrease the ability of PCR to distinguish clinically relevant HPV infection. The problem is highlighted in studies that have shown significant discordance between HPV DNA detection and the actual presence of E6/7 mRNA viral transcripts that define clinically relevant HPV infections [7].

Several PCR amplification techniques are commercially available. These PCR screening assays commonly have primers designed to amplify a region of DNA that is present in multiple HPV types (most commonly within the highly conserved L1 gene) [76]. Since most commercially available PCR kits use consensus sequences from multiple HPV subtypes, specific typing is generally not possible through PCR alone. During the last few years, many novel PCR-based HPV detection assays have been developed, including those that target other conserved regions within viral L1 or E1 regions. More recently, multiplex assays have also been described that use primers targeting different viral regions of different HPV types, rather than a conserved region [73]. Thus, differences between the different primer sets could explain the discrepancy in reports regarding HPV status in clinical samples. Furthermore, few studies have been done to directly compare the sensitivity of these primer sets in detecting HPV in OSCC [77]. Equally important for the final assay outcome is the readout system used to detect the PCR products.

HPV RNA Detection with Polymerase Chain Reaction (PCR) There are several commercially available assays for the detection of HPV by RT-PCR. These kits

target mRNA of the oncogenic E6 and E7 proteins using isothermal mRNA amplification methods such as nucleic acid sequence-based amplification (NASBA) and transcription mediated amplification (TMA) [73]. These assays utilize a reverse transcriptase to generate cDNA first, RNase H to degrade the RNA template (in case of NASBA) and a T7 RNA polymerase to produce multiple RNA copies from the cDNA. Commercially available methods detect E6/E7 mRNA in a real-time format using different molecular beacon probes for five high-risk HPV types (i.e. HPV 16, 18, 31, 33 and 45) in two assay runs to allow genotyping. Detection of viral E6/E7 mRNA by RT-PCR is now considered the gold standard for the detection of clinically significant HPV infection within tumor specimens as it detects transcriptionally active HPV; it is also the benchmark by which the sensitivity and specificity of all other detection assays are measured. The method can be applied to fresh frozen specimens, but also to FFPE samples. However, the method has the disadvantage of being time consuming and its sensitivity depends on the quality of clinical samples. In addition, many HPV (+) patients identified in the next generation sequencing study by Parfenov et al. had low expression or absence of E6/E7 expression and could be misclassified by E6/E7 mRNA detection [57].

DNA ISH ISH is the only molecular method allowing reliable detection and identification of HPV in topographical relationship to pathological lesions. Contrary to other methods, in ISH the whole HPV detection procedure occurs within the nuclei of infected cells and not in solutions. The result of the hybridization reaction is evaluated microscopically and the appearance of an appropriate precipitate within the nuclei of epithelial cells is indicative of the presence of HPV in the specimen being tested. Furthermore, the physical state of the virus can be evaluated by the presence of punctuate signals for integrated virus and diffuse signals for episomal virus [73]. Thus, this method can distinguish between episomal and integrated HPV DNA, which is very important for the assessment of a clinically relevant HPV infection.

More specifically, DNA ISH is a signal amplification technique that utilizes labeled DNA probes complementary to targeted viral DNA sequences. The DNA probes may hybridize to HPV type-specific DNA sequences, hybridize to a consensus sequence shared by multiple HPV types, or may be mixed in a single reaction to cover an extended range of HPV types (i.e. probe cocktail) [72]. Given that HPV 16 is by far the most common HPV type involved in head and neck carcinogenesis, it is expected that a type 16-specific probe will detect the majority (greater than 90%) of HPV+ oropharyngeal carcinomas. The widespread use of HPV probe cocktails (e.g. Inform® HPV-III probe cocktail, Ventana Medical systems, Tucson, AZ) now enables detection of a broader range of high risk HPV types. Many commercially available tests have demonstrated similar specificity in HPV detection of cervical specimens between DNA ISH assays containing a cocktail of probes and those containing probes of individual HPV types (HPV16); however. to our knowledge, comparisons of the commercially available tests in HPV detection of oropharyngeal lesions have not been performed [73].

Advantages of DNA ISH compared to PCR-based methods include: (1) HPV DNA ISH is effective in both formalin fixed and paraffin embedded tissues. On the contrary, PCR-based methods are more efficient when the clinical samples are available as fresh frozen tissue. (2) Adaptation of ISH to formalin-fixed and paraffin-embedded tissues, together with recent advances in DNA ISH automated instrument systems, has made this technique compatible with standard tissue processing procedures and thus widely transferrable to most surgical pathology laboratories. (3) As previously mentioned, the development of non-fluorescent chromogens allows visualization of hybridization using conventional light microscopy that, in turn, permits detection of the presence and distribution of HPV in the tissues. On the contrary, the absence of tissue in PCR-based methods does not enable to determine if viral DNA originates from cancer cells or surrounding neoplastic tissues.

On the other hand, although enhancement techniques have increased the sensitivity of DNA ISH, it is less sensitive than PCR analysis. In addition, the ISH protocol itself is not always feasible given limited sample availability and the necessity for fresh, and not frozen or paraffin embedded, tissue samples. Although commercially available HPV assays based on ISH have been validated technically, they are insufficiently clinically validated. Indeed, current ISH-based assays are considered to have insufficient clinical sensitivity to be used in routine screening [73].

RNA ISH Although the most direct evidence of HPV-related carcinogenesis is the documentation of transcriptionally active HPV in tumor cells, the detection of E6/E7 transcripts is technically challenging. The recent development of RNA ISH platforms using probes complementary to HPV E6/E7 mRNA enables direct visualization of viral transcripts in routinely processed tissues [78]. In formalin-fixed and paraffin-embedded samples of OSCCs, the sensitivity of this method has been shown to exceed that of HPV DNA ISH and match the sensitivity of p16 immunohistochemical staining [79, 80]. Advantages of RNA ISH include: (1) It is the only method that confirms the presence of integrated and transcriptionally active virus by ensuring the visualization of viral transcripts directly in tissue sections; (2) It is technically feasible and easily transferrable into the diagnostic pathology laboratory. Indeed, the availability of this method to a widely available automated staining platform promises to boost standardization across different diagnostic laboratories and enhance reproducibility among clinical trials. (3) The transcription of viral mRNA provides a natural target amplification step that may help elucidate the HPV status of complicated tumors that are p16 positive by IHC but HPV negative by DNA ISH [72]; For example, Rooper et al. used RNA ISH to detect transcriptionally active HPV in 88% of cases that were positive by p16 IHC, but negative for HPV by DNA ISH [81]. (4) It may offer useful information regarding prognosis: The presence of E6/E7 mRNA transcripts is associated with the expression of other powerful prognostic markers (p16 expression), and strongly correlates with patient outcomes [79].

P16 Immunohistochemical Staining as a Surrogate Marker of HPV– As previously mentioned, p16INK4a (p16) is a cyclin-dependent kinase inhibitor involved in the regulation of the cell cycle. P16 is commonly deployed as a surrogate

biomarker for HPV status [39, 82]. The biological rationale supporting this surrogacy originates from the fact that during the HPV life cycle, the oncoprotein E7 inactivates the Rb protein, which results in the upregulation of various cell cycle associated proteins, including p16 [13]. Immunostaining for p16 protein has recently been regarded as an alternative or complimentary procedure for HPV testing of OSCCs based on a high correlation between HPV detection and p16 overexpression in recent studies [83, 84]. P16 immunostaining has the advantage of being easy to perform on FFPE tissue, and monoclonal antibodies against p16 are commercially available [73]. The simplicity, low cost and high sensitivity of this method have prompted consideration of replacing more intensive ISH and PCR-based methods as a standalone HPV test. There are several arguments, however, against using p16 IHC as a standalone test for determination of HPV status. First, elevated p16 expression by non-viral related mechanisms has been encountered in a subgroup of HPV− HNSCCs and other tumors, such as small cell and adenoid cystic carcinoma. In surgical specimens of HNSCC, p16 IHC has been shown to be highly sensitive (approaching 100%) for high-risk HPV, but the specificity is approximately 80% [80, 84, 85]. Second, p16+/HPV− oropharyngeal squamous cell cancers are associated with dismal prognosis in some studies [86, 87]. Furthermore, p16 protein expression is not a reliable surrogate biomarker for HPV infection outside the oropharynx [88].

Successful interpretation of p16 staining requires the incorporation of histological, anatomical and clinical information [89]. Firstly, a tumor is characterized as being p16+ if there is strong (2 or 3 staining intensity) and diffuse nuclear and cytoplasmic staining of more than 70% of the malignant cells [90]. This threshold and scoring system have been validated by numerous studies and in meta-analyses [56]; however, not all studies adhere to it [91, 92]. Focal or weak staining should be supported by other forms of HPV testing. Second, interpretation of p16 staining must be informed by morphologic features of the tumor and p16 IHC staining may substitute for HPV detection in OSCCs that demonstrate the typical morphology of HPV+ HNSCC (abrupt transition between carcinoma and the adjacent surface epithelium, no histologic progression through the sequential stages of dysplasia, invade as sheets, no strong desmoplastic stromal reaction, presence of lymphoid cells, lack of significant cytoplasmic keratinization) [72]. Additional HPV testing should be performed in p16 negative (p16−) OSCCs that exhibit classic HPV-related histomorphology, and in p16+ OSCCs that do not exhibit classic HPV morphology.

As with any surrogate biomarker, there is a risk of discordance between p16 status and the actual HPV status, which can be exacerbated by a failure to use a stringent cutoff (50–70%) for percentage p16+ tumor cells. Current estimates posit that the discordance rate between p16 IHC and direct detection of HPV DNA/RNA may approach 25%, with p16+ but HPV− tumors constituting the majority of discordant cases [93]. In head to head comparisons between HPV detection methods, using HR-HPV E6/E7 mRNA expression as the gold standard for HPV status, Jordan et al. recently reported that both p16 IHC (sensitivity, 96.8%; specificity, 83.8%) and HPV16 ISH (sensitivity, 88.0%; specificity, 94.7%) showed excellent

performance in HPV detection [84]. In a series of 239 cases of OSCC, Lewis et al. reported that 187 were positive for p16 IHC and 139 were positive for HPV by ISH. Of the remaining 48, 19 were positive for HPV by PCR. However, no difference in survival was noted between p16 +, HPV− tumors and p16−, HPV+ tumors [94]. In contrast, a recent study by Thavaraj et al. using a different set of PCR primers found that only 2 out of 142 p16 positive OSCC were negative for HPV by both PCR and ISH [95]. The difference in the percentage of these p16+, HPV− tumors between the two studies may represent differences in sensitivity between HPV tests used or may reflect true differences in HPV prevalence in different populations. Indeed, similar to results reported by Chung et al. [88] regarding p16 status and biologically relevant HPV infection in non-oropharyngeal cancer cases, Harris et al. reported lack of association between p16 overexpression and HPV positivity in young patients suffering of SCC of the oral tongue [96].

HPV Testing of Cytology Specimens The sensitivity of HPV+ OSCC to chemotherapy and radiation has limited the role of surgical resection, and diagnostic tissue biopsies may not be available in a significant portion of patients with small or occult primaries. In these cases, fine needle aspirates (FNA) of metastatic OSCCs might represent a valuable substrate for HPV analysis [97]. In cytologic specimens, p16 staining is influenced by various technical and biological factors. Importantly, established cutoff values for p16 expression based on the percentage and intensity of staining in tissue samples cannot be easily applied in cytologic specimens that lack cellular integrity [98]. In FNAs of cystic LN metastases where interpretation is hampered by cell degradation, p16 staining can be weak or absent in HPV+ tumors. Indeed, the use of p16 staining as a standalone test can be risky.

The feasibility of HPV detection in cervical LN FNAs or cell blocks has been confirmed in a limited number of studies using both p16 IHC and ISH platforms. In a study by Holmes et al. p16 IHC performed on FNAs and core biopsies from metastatic HNSCC showed 98% concordance with p16 IHC and HPV DNA ISH of excisional biopsies or resections of the primary tumors [99]. Importantly, p16 IHC was scored as positive if any cytoplasmic and nuclear grading was observed, even in the presence of cellular degradation where it is difficult to quantify the percentage of positive tumor cells. Furthermore, Jalaly et al. assessed the correlation between p16 IHC performed in cell blocks from FNAs compared to surgical pathology specimens of HPV+ OSCC and demonstrated that there was 98% concordance when strong positive p16 staining of at least 15% of tumor cells in FNA cell block material was present. In addition, high-risk HPV RNA ISH showed a high correlation with p16 staining in surgical pathology specimens (96%) and FNAs (93%) [98]. On the other hand, Xu et al. performed analysis of different thresholds and demonstrated that the threshold of 10% p16 tumor cell positivity had the best overall concordance rate with surgical p16 IHC and with FNA HPV-ISH. Interestingly, applying the recommended p16 positivity threshold for surgical specimens (70%) on FNA materials resulted in low sensitivity (39%) and low negative predictive value (26%) [100]. Taken together, these findings suggest that the recommended threshold for p16 IHC in surgical specimens should not be applied to cytology specimens.

Further studies are warranted to validate these findings; for now, it would be wise to repeat the p16 IHC on a subsequent surgical specimen.

Direct transfer of cytologic samples into the liquid media minimizes specimen preparation and eliminates the need for specimen processing as cell blocks. Various liquid phase assays already in widespread use for HPV analysis of cervical cancer risk may be directly applicable to OSCC. The Hybrid Capture 2 (HC2, Digen/Qiagen, Gaithersburg, MD) HPV DNA Test is a liquid-based technique that detects HPV DNA with an in vitro nucleic acid hybridization assay coupled with signal amplification using microplate chemiluminescence for the detection of 13 high risk HPV types in cervical specimens [101]. In a limited study of 24 patients with HNSCC, the HC2 assay was found to be highly reliable in determining HPV status [102]. In this study population, there was 100% correlation between HC2 analysis of the cytologic specimens (brushings and FNAs) and DNA ISH analysis of the paired surgical resection specimens. Similar to HC2, the Cervista® HPV HR test is a liquid phase assay that is clinically validated for HPV detection in cervical cytologic specimens. In one study, this method was shown to be effective in detecting the presence of HPV in FNAs from patients with metastatic HNSCC [103]. Similarly, the Roche Cobas® HPV test is a PCR-based assay that permits distinction between HPV16, HPV18 and other HR-HPV types [72].

What is the Gold Standard? The power of p16 IHC is supported by its high sensitivity for detecting all high-risk types of HPV, but its use as a standalone test is hampered by low specificity and a false positive rate where p16 expression is driven by non-viral mechanisms. These p16+/HPV− OSCCs have been associated with less favorable survival than p16+/HPV+ cancers, suggesting that selection of patients for de-escalation clinical trials may benefit from supplementary detection assays rather than p16 staining alone [87]. DNA ISH, on the other hand, offers a high degree of specificity at the expense of suboptimal sensitivity [101]. However, direct detection of E6/E7 mRNA in fresh tissue samples via PCR is widely considered the most informative method for determining HPV status. Advantages of this method include generally high sensitivity, tumor-specific expression of the mRNA/DNA target and feasibility on formalin-fixed, paraffin-embedded tissue blocks [101]. Of note, a significant disadvantage is the potential for decreased sensitivity in lower-quality clinical samples or samples with low E6/E7 expression [73].

It is common practice to use a dual approach for HPV detection. Multimodality detection strategies look to utilize the strengths of individual assays in combination to optimize the overall reliability of HPV detection. In this context, current multimodality strategies utilize a stepwise approach that begins with p16 IHC. Weinberger et al. showed that p16 IHC+/Real-time PCR HPV16 DNA + cases were the biologically relevant HPV+ OSCC cases and those associated with the better prognosis [7]. Smeets et al. also showed that the most suitable algorithm with 100% sensitivity and specificity for determination of HPV status is p16 immunostaining followed by GP5+/6+ PCR on the p16-positive cases [85]. Therefore, the finding of a p16+ OSCC case should be followed by analysis with more rigorous HPV-specific detection assays such as HPV DNA ISH [90] and/or a PCR-based assay [85].

This approach is further supported by a recent metanalysis of 24 trials that performed p16INK4a IHC and HPV E6/E7 mRNA detection using an amplification-based method in OSCC which showed that p16INK4a IHC/HPV DNA PCR combined testing was as sensitive as either p16INK4a IHC or HPV DNA PCR alone but significantly more specific than either separate test (specificity 96% in combined testing vs. 83–84% in single testing) [104].

Although the multimodality approach may provide the most accurate analysis of HPV status, it does not represent a quick, feasible, simple and cost-effective method of HPV testing, which would be easily applicable in all laboratories. Two-pronged complex HPV detection algorithms may be most appropriate when there is no allowance for error in determining true HPV status, such as selection of patients for de-escalation therapy or therapeutic HPV vaccine trials. Interestingly, Bratman and colleagues reported that patients with head and neck squamous cell carcinoma associated with human papillomavirus (HPV) genotypes other than HPV-16 have inferior survival [53]. They propose that this finding will affect patient selection in trials of treatment deintensification, which seek to minimize long term toxic effects in patients with highly curable HPV initiated oropharyngeal cancer; p16 protein status was not available in two thirds of "HPV-other" cases and definitive conclusions cannot be drawn from this report.

HPV Status and Impact on Clinical Outcome: Prognostic and Predictive Significance

Prognostic Significance Several studies conducted in locally advanced disease have demonstrated that HPV+ OSCC is associated with better prognosis compared to HPV− OSCC [49]. A phase III study that evaluated the addition of the hypoxic cytotoxin tirapazamine to standard chemotherapy in advanced HNSCC did not meet its primary endpoint of OS [105]. However, the relationship between HPV and outcome was determined in a subanalysis of patients with OSCC [106]. Of 185 analyzed patients, 106 had p16+ tumors, whereas 79 had p16− tumors. Patients with p16+ disease had a lower T stage, a higher N stage, and a better performance status (PS) and were less likely to be current smokers. In patients from both arms, 2-year OS was superior in p16+ versus p16− tumors, and p16 status was independently associated with OS [hazard ratio (HR), 0.43; 95% confidence interval (CI) 0.20–0.93; P = 0.031]. Two-year failure-free survival was significantly improved in the p16+ subgroup, whereas the 2-year time to locoregional failure was numerically improved. The rate of distant failures appeared similar in both p16 groups despite advanced nodal status in the p16+ cohort. Thus, this study indicated p16 status as an independent prognostic factor [106].

The phase III RTOG 0129 trial assessed the role of accelerated fractionation radiation (RT) in combination with cisplatin compared to standard RT and cisplatin [39]. Despite being a negative trial, a retrospective analysis of HPV tumor status in

relation to OS demonstrated a remarkable difference in 3-year OS between HPV + and HPV− OSCCs (82.4 vs. 57.1, $p < 0.001$). Importantly, the difference was maintained after adjustment of several risk factors (age, race, tobacco use) showing a 58% reduction in the risk of death. P16 was also indicated as a strong prognostic factor. The locoregional failure rate, but not the distant failure rate, was lower for HPV+ OSCC than for HPV− OSCC. Interestingly, in this study, a separate analysis was used to stratify patients into risk categories associated with survival based on HPV status, smoking, tumor stage, and nodal stage. Most patients with HPV+ OSCC were considered to be at low risk of death, although smokers with HPV+, N2-N3 tumors were considered to be at intermediate risk. Nonsmokers with HPV−, T2-T3 tumors were also considered to be at intermediate risk, whereas all other patients with HPV− tumors were considered to be at high risk [39].

Another retrospective analysis was conducted to assess the association between HPV and clinical outcomes in the RTOG 9003 study, a 4-arm, phase III trial that compared different RT protocols [107]. In a subanalysis of 190 patients with OSCC, p16 positivity was associated with better PS, absence of anemia, T1 stage, and less tobacco use; moreover, regardless of assigned treatment, the p16+ OSCC group had superior 5-year OS and PFS compared to the p16− group [108]. Similarly, a retrospective analysis of HPV status was conducted in patients with OSCC included in the TAX 324 trial, a phase III trial that demonstrated the superiority of induction chemotherapy regimen TPF (docetaxel, cisplatin, 5-FU) as compared to PF (cisplatin, 5-FU) [109]. In this subanalysis, patients with HPV+ tumors were more likely to be nonblack, younger, and fitter and had less advanced T-stage tumors; in addition, they had significantly increased OS and PFS and experienced significantly fewer total failures and locoregional failures and slightly fewer distant failures [110]. On the other hand, the phase III DAHANCA5 trial evaluated the addition of the hypoxic radiosensitizer nimorazole to RT, which improved locoregional control (LRC) (the primary end point) compared with RT and placebo [111]. A retrospective analysis of 156 placebo-treated patients in that study was conducted to determine the relationship between HPV and outcome. Of 156 tumors tested, 35 were p16+; of those, 24 were oropharyngeal. Accordingly, a significant improvement in LRC, 5-year disease-specific survival (DSS) and 5-year OS was observed for the p16+ subgroup [112].

The relationship between HPV and clinical outcomes has been more recently evaluated in trials conducted in recurrent/metastatic (R/M) disease. First, EXTREME is a landmark phase III trial which has demonstrated that the addition of cetuximab to standard chemotherapy increased OS, PFS and overall response rate (ORR) [113]. Recently, a retrospective analysis of HPV status and outcomes of the EXTREME trial was reported [114]. Paired tissue samples were assessed for p16 expression by IHC and HPV using oligonucleotide hybridization assays. Overall, 416 of 442 patients had samples available for p16 and HPV analysis; 10% of tumors were p16+ and 5% were HPV+. Importantly, it was shown that p16 positivity was associated with longer survival than was p16 negativity in both the cetuximab and control arms, as was HPV positivity versus HPV negativity. Analysis of assessable patients with OSCC demonstrated a similar pattern [114]. In the same context, the

phase III SPECTRUM trial randomized patients to receive first-line PF with or without the anti-EGFR IgG2 monoclonal antibody panitumumab [91]. This was a negative trial, as it failed to show a statistically significant improvement in OS, which was the primary endpoint. A prespecified HPV analysis was conducted in 443 of 657 patients. Of these, 99 (22%) had p16+ tumors and 344 (78%) had p16− tumors. Interestingly, the cutoff for HPV positivity was 10% p16 expression, notably different than the usual cutoff of 70%. However, analyses using alternative cutoffs (between 10% and 70%) demonstrated consistent outcomes. An analysis for prognostic significance was restricted to the control arm because p16 status was associated with panitumumab efficacy. The p16+ subgroup had numerically improved OS than the p16− subgroup [91]. Taken together, these two studies provide substantial evidence that HPV positivity is associated with improved survival in R/M OSCC.

It is important to note that most of these analyses have limitations that pose several questions regarding their reliability, such as retrospective nature, lack of patient stratification based on demographics, different methods of HPV detection and short follow up. A further complication is the inclusion of non-OSCC tumors in these studies because p16 is unvalidated as an HPV marker at other sites [49]. Nevertheless, the existing data suggest that HPV is a prognostic factor in OSCC in both locally advanced and R/M disease. Indeed, among currently available prognostic factors for HNSCC, HPV status in OSCC is perhaps the strongest yet identified.

Predictive Significance Interestingly, HPV status has also implications in response to treatment. In a phase II study assessing the efficacy of induction chemotherapy with carboplatin and paclitaxel followed by combination RT with the same regimen in patients with stage III and IV HNSCC of the oropharynx or larynx, patients with HPV tumors had higher response rates after both induction therapy and chemoradiotherapy [115]. In this study, analysis of HPV status in relation to outcomes was prospective and confirmed the prognostic value of HPV status on outcome of patients with OSCC. On the other hand, a recent retrospective analysis of the phase III IMCL-9815 Bonner trial assessed the role of HPV status in patients with locally advanced HNSCC treated with either RT or RT + cetuximab. Interestingly, this analysis failed to show a significant correlation between HPV status and benefit from cetuximab; instead, it showed that the addition of cetuximab confers clinical benefit regardless of p16 status [116]. Similarly, a predictive analysis of the phase III DAHANCA5 trial that evaluated the addition of the hypoxic radiosensitizer nimorazole to RT demonstrated that p16+ status was not associated with treatment [117]. Moreover, a planned subanalysis of the phase III RTOG 0522 trial, which evaluated the addition of cetuximab to the combination of cisplatin and RT, investigated the predictive significance of HPV status [118]. The addition of cetuximab to the combination of cisplatin and RT did not improve PFS or OS in the overall population or the p16− OSCC subgroup (n = 86) or in the OSCC subgroup without specimens available for analysis (n = 307). A trend existed for worse outcome with the addition of cetuximab in patients with p16+ OSCC (n = 235), which might be a random event, since HPV status was not assessed in a large proportion of patients

due to lack of specimen availability. Finally, a retrospective analysis of the phase III TAX323 trial was recently performed with the objective to evaluate a possible correlation of HPV status with treatment effect associated with the addition of docetaxel to cisplatin and 5-FU chemotherapy combination. No difference in treatment effect was demonstrated between HPV+ and HPV− patients, albeit incidence of HPV positivity was too low to demonstrate a statistical evidence of predictive value in the cohort of patients analyzed [119].

Regarding R/M disease, predictive analyses of the EXTREME trial demonstrated that both p16− and p16+ cohorts that received cetuximab had longer OS and PFS than did the chemotherapy-alone subgroup [120]. This pattern remained in a combined analysis of p16 and HPV status. Thus, because the addition of cetuximab to chemotherapy improved survival regardless of tumor p16 or HPV status, these biomarkers were not predictive of treatment efficacy. Although the number of HPV+ patients in this analysis was low, the results suggested that HPV has prognostic but not predictive significance in R/M HNSCC. On the other hand, analyses of predictive significance for panitumumab efficacy in the p16+ cohort of the SPECTRUM trial did not reveal differences in OS and PFS [91]. Interestingly, in the p16− cohort, OS and PFS favored the panitumumab arm. These results suggest that p16 negativity predicts panitumumab efficacy in R/M-HNSCC. However, this information is not applicable; SPECTRUM was a negative trial and panitumumab is not approved for HNSCC. Of note, the discrepancy in predictive significance of p16 status between cetuximab and panitumumab might be partly interpreted by the fact that cetuximab-induced antibody dependent cell-mediated cytotoxicity, a mechanism of cell-mediated immune defense, increases antitumor activity against HPV+ OSCC an immunologic cancer [49]; however, this hypothesis requires clinical validation.

Concluding Remarks

A rising number of oropharyngeal squamous cell carcinomas are HPV-related. HPV-associated OSCC represents a distinct disease entity with different molecular profile. High risk HPV oncogenic E6/E7 protein expression, following viral integration, initiate a number of malignant transformation processes including bypass of cell cycle control, DNA synthesis, inhibition of apoptosis and transcriptional activation of genes that promote proliferation. Over the past decade, the role of HPV in OSCC has changed the research field; several studies have established the impact of HPV on prognosis, although limited data exist regarding its predictive significance. Indeed, the dramatic impact for staging and prognosis became manifest in the new eighth edition of the TNM staging system. Accordingly, determination of HPV status is important as it influences all aspects of patient care including prognosis, tumor staging, identification of tumor site and selection of patients most likely to benefit from certain therapeutic options. Each method currently used for HPV detection is associated with unique advantages and disadvantages. E6/E7 mRNA detection in

tissue represents the gold standard but its sensitivity depends on the quality of clinical samples and is often not feasible in FFPE. The most reliable algorithm appears to be evaluation of p16 status by IHC followed by determination of HPV DNA status in p16 positive cases.

In the ongoing effort to establish a consensus approach for HPV testing, the challenge for the oncologic community is to implement standardized HPV testing using a method that is highly accurate, technically feasible, cost effective, and readily transferrable to the diagnostic pathology laboratory. Moreover, HPV testing is particularly important because the de-intensification of current treatment protocols in HPV+ patients that are characterized by long-term prognosis is being investigated. However, candidates for deintensification should be selected very carefully; it should be noted that deintensification strategies are only appropriate in a clinical research setting. In addition, HPV-targeted approaches appear promising and may become another therapeutic option. It is hoped that these investigations will personalize and ultimately improve care for patients with HPV+ OSCC.

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A New Staging System for HPV-Related Oropharynx Cancer: Rationale, Derivation, Validation and Practical Applications

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Introduction

The TNM classification is a system to depict the anatomic extent of cancer based on assessment of three components: T—primary tumour extent; N—absence or presence and extent of regional lymph nodes; M—absence or presence of distant metastasis [1]. T, N, and M are combined into prognostically relevant stage groups. The systems was created by Dr. Pierre Denoix in the 1940's and developed over subsequent years to the point of initial adoption by the Union for International Cancer Control (UICC) for its first edition of the TNM in 1968. Subsequently the American Joint Committee on Cancer (AJCC) developed a similar classification for its first edition in 1977 and since then, both organizations have aligned for the purpose of stewarding the classification to be as close to identical as possible for their different publication formats.

The TNM classification has been widely used by clinicians, researchers, and the cancer surveillance community to facilitate patient consultation, outcome prediction,

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clinical trial design, and cancer control activities. The essential requirement of an adequate staging system includes distinctiveness (significantly worse survival with higher stage), homogeneity (relatively similar survival among subsets within each stage groups), and independent prognostic importance [2, 3]. A fundamental principle of the TNM classification is that the disease should represent a single biological entity.

Since its debut, TNM has undergone periodic updates in order to maintain its clinical relevance. The process generally incorporates sound principles and interpretation of available robust data that take into account feasibility and practicality for widespread international use. The reasons for staging 'updates' include better understanding of disease behavior that may prompt inclusion of new parameters in the classification, better assessment and/or treatment triggering T- and N-classification refinements, and recognition of a new disease process requiring a new classification. The recognition that HPV-mediated (HPV+) oropharyngeal cancer (OPC) is a new disease entity, and that the 7th edition TNM (TNM-7th) is inadequate in depicting its prognosis, required the development of a novel staging system for HPV+ OPC and unknown primary with cervical nodal metastasis (CUP) in the 8th edition TNM (TNM-8th).

The Necessity for a New Staging for HPV+ OPC

HPV+ OPC is a rapidly emerging disease entity with a generally exemplary prognosis compared to its smoking-related counterparts. However, up to the TNM-7th, HPV+ and HPV− OPC continued to share the same staging systems where any lymph node involvement is classified as advanced stage (stage III–IV). However, it is now known that the presence of lymph nodes does not necessarily reflect prognostically relevant disease extension in HPV+ OPC [4]. Studies have shown that HPV+ OPC patients often have earlier nodal involvement and about two-third of HPV+ OPC patients present with asymptomatic nodal mass with very small primaries [5]. This phenomenon is likely explained by the discontinuity of the basement membrane and the richness of intraepithelial capillaries within tonsillar crypt [6], which might facilitate tumour cell foci accessing underlying lymphatics resulting in lymph node involvement earlier in the course of tumour invasion. Clinically, a majority (>90%) of HPV+ OPC have clinical nodal involvement, even when primary tumours are small (T1–T2) [7, 8]. The earlier nodal involvement has changed the prognostic value of the traditional N-categories within the TNM classification that has traditionally relied on size, number, and side of neck affected [9, 10].

According to the TNM-7th classification, the majority of HPV+ OPC patients are classified as 'advanced stage' due to cervical nodal involvement at presentation, but evidence suggests that many appear to be cured with a 5-year overall survival (OS) rate exceeding 85% [11]. The dis-concordance of staging and prognosis presents a conundrum for clinicians and for many patients when first confronted with their cancer diagnosis. Patients risk exposure to unnecessary anxiety and confusion when informed that they have 'advanced' stage disease while in reality their prognosis often rivals that of the most curable cancers. Moreover, the advanced stage

designation (i.e. Stage IV) could perpetuate a philosophy among the oncology community that traditional intensive treatments are always needed, a concept under challenge at the present time [12–14].

Initial strategies to address these issues incorporated tumour HPV status as a stratification factor to derive a prognostic model instead of recognizing a new disease requiring potentially different treatments. However opinions have now converged on the principle that tumour HPV status is a diagnostic biomarker, rather than a prognostic factor within a homogenous entity, to detect a disease with much better prognosis compared to its HPV− OPC counterparts. A novel staging system was needed to properly depict the character and prognosis of this new disease which contrasts with smoking-related/HPV-unrelated [HPV−] OPC from which the traditional TNM classification was derived.

HPV-specific staging is necessary for several reasons: (1) Relevance to discussions with patient/family, (2) Clinical trials design and stratification since they are now addressing HPV+ and HPV− diseases separately, and (3) Practice guidelines will probably differ for both diseases in the near future. Separate classifications for HPV+ and HPV− OPC also seem applicable for both clinical care and cancer surveillance. While high HPV prevalent jurisdictions need an HPV+ OPC specific TNM, low HPV prevalence regions might be able to continue to use the traditional HPV− TNM classification at the population level since these are unlikely to impact on surveillance strategies. In addition, despite the significant survival difference between HPV+ and HPV− OPC, tumour HPV status identifies a biologically distinct disease entity underpinned by a definable and different molecular etiologic process. Therefore, a separate stage classification is needed in an anatomic region where disease would otherwise lack biological consistency and homogeneity, linked to predictable outcomes.

In response to the urgent need to properly depict the character and prognosis of this new disease, a new stage classification was introduced for HPV+ OPC in the 8th edition TNM [15, 16]. It is also the first time that separate clinical and pathological N-definitions and T–N groupings are included in the HNC classification. The clinical staging (cTNM) based on the proposal of *the International Collaboration on Oropharyngeal cancer Network* (ICON-S) group is derived from a study of 1907 HPV-positive OPC from seven institutions in four countries in Europe and North America using *Training* (a cohort from the Princess Margaret Hospital)—*Validation* (the remaining six institutions) design [7]. The pathologic stage classification (pTNM) was based on a study of 704 surgically managed cases from five cancer centers (one of which was in Europe) [17].

Derivation of cTNM and Its Clinical Implication

The derivation of 8th edition cTNM traversed three steps: a “Discovery”, a “Derivation”, and a “Validation” process (Fig. 1a). The *Discovery Study* conducted by Huang et al. [18] demonstrated the feasibility of deriving prognostically relevant anatomic stage groups using the existing 7th edition T and N classification. It also

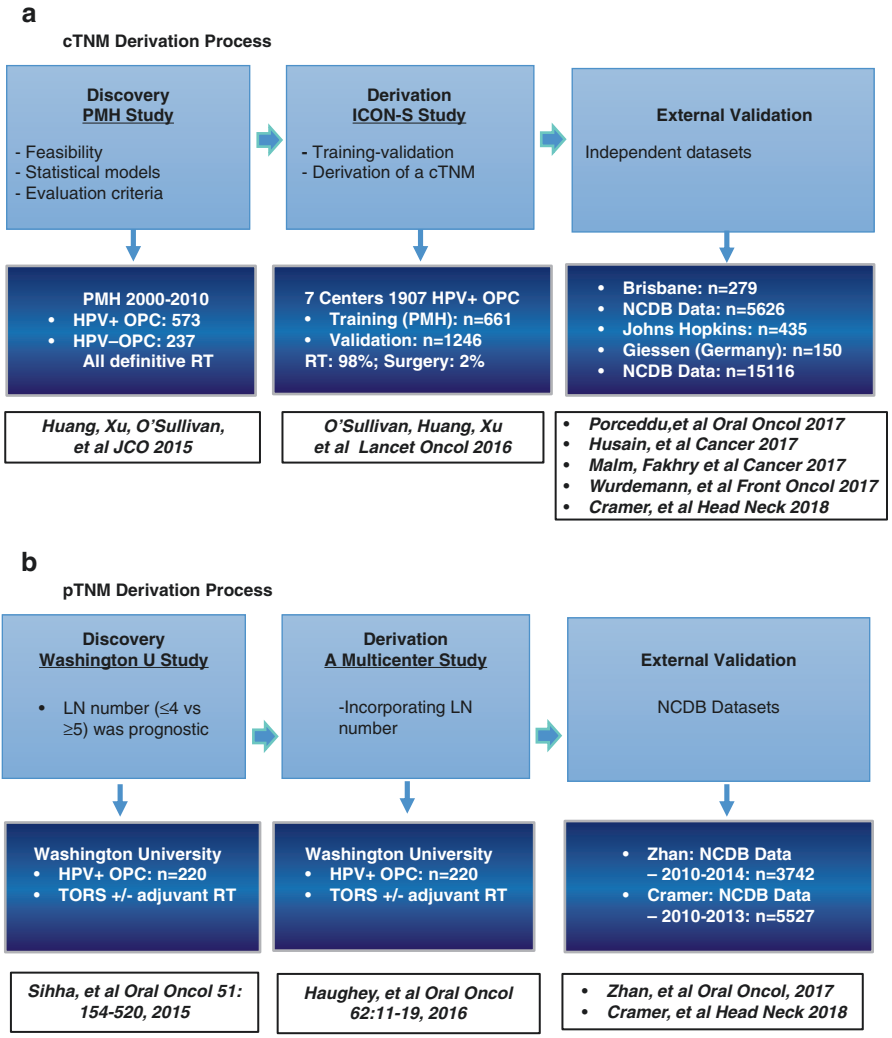

Fig. 1 Derivation of cTNM (**a**) and pTNM (**b**) for HPV+ oropharyngeal cancer

developed two statistical models to objectively derive several new staging schema and used refined evaluation criteria to select the best performing staging schema. The *Derivation Study,* conducted by O'Sullivan et al. [7] in a multi-institutional datasets comprising 1907 HPV+ OPC patients from Europe and North America, used training-validation design, and followed the same statistical modeling methods and evaluation criteria developed in the *Discovery Study*. In this way the model was regenerated de novo in new data sets without prescribing a previously generated model. The proposed cTNM has subsequently been validated in multiple independent datasets from Australia [19], United States [17, 20, 21], and Europe [22] (Table 1).

Table 1 Validations of 8th edition cTNM and pTNM

Study	Study period	Sample size	Primary Tx	5-year overall survival by 8th edition stage classification			
				I	II	III	IV (M1)
Clinical TNM classification for HPV+ OPC							
ICON-S (O'Sullivan) [7]	1998–2011	n = 1907	Sx: 2% RT: 98%	85% (n = 962)	78% (n = 564)	53% (n = 381)	NA
Australia (Porceddu) [19]	2005–2015	n = 279	Sx: – RT: 100%	94% (n = 132)	82% (n = 82)	69% (n = 65)	NA
US JHH (Malm) [23]	2005–2015	n = 435	Sx: 38% RT: 62%	92% (n = 281)	87% (n = 77)	74% (n = 72)	40% (n = 5)
US NCDB (Husain) [20]	2010–2012	n = 5626	Sx: 42% RT: 56%	90% (3-year) (n = 3631)	82% (n = 1242)	72% (n = 753)	NA
US NCDB (Cramer) [21]	2010–2013	n = 15,116	Sx: 44% RT: 54%	90% (3-year) (n = 8895)	81% (n = 3012)	68% (n = 1847)	31% (n = 320)
Germany (Wurdemann) [22]		n = 150	Sx: 69% RT: 31%	94% (n = 79)	77% (n = 31)	64% (n = 31)	25% (n = 9)
US 5 centers (Haughey) [17][a]	1985–2015	n = 704	Sx: 100% RT: –	90% (NA)	79% (NA)	70% (NA)	NA
Pathologic TNM classification for HPV+ OPC							
US 5 centers (Haughey) [17]	1985–2015	n = 704	Sx: 100% RT: –	90% (NA)	84% (NA)	48% (NA)	NA
US NCDB (Zhan) [24]	2010–2014	n = 3742	Sx: 100%	92% (4-year) (n = 3001)	81% (n = 663)	62% (n = 78)	
US NCDB (Cramer) [21]	2010–2013	n = 5527	Sx: 44% RT: 54%	92% (3-year) (n = 4793)	81% (n = 522)	73% (n = 103)	31% (n = 109)

US United States, *NCDB* National Cancer Data Base, *Tx* treatment, *ICON-S* The International Collaboration on Oropharyngeal cancer Network for Staging, *JHH* John Hopkins Hospital
[a]Regroup pT and pN according to the 8th edition cTNM staging criteria

Huang and colleagues undertook a *Discovery Study* using 810 oropharyngeal cancer patients treated almost exclusively with radiotherapy ± chemotherapy at the Princess Margaret Hospital (PMH) [18]. They showed that HPV+ patients (n = 573) did not demonstrate prognostic discrimination using the TNM-7th, which was especially apparent for Stage IVA disease [18]. The study employed two statistical models: recursive partitioning-analysis (RPA) and adjusted hazard ratios (AHR) to derive two stage classifications schema based on the existing 7th edition T and N categories for the HPV+ cohort. The RPA model creates a decision tree to split the study population into sub-populations based on hazard ratio of death for different

T-categories (T1, T2, T3,T4) and N-categories (N0, N1–N2a, N2b, N2c, N3) while adjusting for other covariates (treatment, age, and smoking). The AHR model is based on multivariable Cox model calculated HRs for risk of death (adjusted for age, smoking, and treatment) with various T–N combinations that considered minimal hazard difference, the ordinal order of T and N categories, and the sample size balance between new potential *Stage* subgroups. Both classifications were compared to the TNM-7th using five criteria [3] modified from Groome et al. [2]: (1) "Hazard Consistency" (homogeneity): to assess the similarity of OS for each T–N combination *within* each stage group, (2) "Hazard Discrimination" (distinctiveness and monotonicity): to evaluate the differences in OS *between* stage groups to assess how equally they were spaced, (3) "Explained Variance" (relative prognostic importance among covariates): to calculate the percentage of OS variation explained by the stage groupings, (4) "Likelihood Difference" (relative prognostic importance among covariates): to analyze the prognostic importance by calculating the difference of *goodness of fit* between the multivariable models with and without the stage classification, and (5) "Sample Size Balance": to examine the difference in sample sizes across stage groups. The performance of different staging schema under evaluation is ranked by calculating a relative score using the above five criteria. The highest ranked staging schema is generally picked as the proposal for use in a new or revised staging classification. The staging schema also considers simplicity and practicality.

Based on the five criteria, both RPA and AHR models yielded a valid stage classification and showed significantly improved OS prediction compared to the TNM-7th, and the RPA stage schema performed the best among the three. Therefore, the *Discovery Study* proposed the RPA-stage as the new stage classification for HPV+ OPC: stage I: T1-3N0-N2b; stage II: T1-3N2c; stage III: T4 or N3; stage IV: M1 disease. Notably, based on the RPA stage, 56% of TNM-7th stage III–IV patients would migrate to Stage I. Huang's RPA classification was subsequently validated pragmatically in an external administrative data set of the National Cancer Data base (NCDB) by Horne et al. [25] in an analysis of in 8803 HPV+ patients that included 9% treated with surgery alone.

Subsequent to Huang's *Discovery Study*, a *Derivation Study* was undertaken by the International Collaboration on Oropharyngeal cancer Network for Staging (*ICON-S)* using a multi-institutional dataset. The staging proposal derived from the ICON-S study was subsequently adopted by the UICC and AJCC for the 8th edition TNM (TNM-8th) [15, 16] to permit a more appropriate depiction of the character and prognosis of the disease [7].

The *ICON-S* study assembled 2603 OPC patients comprising 1907 HPV+ and 696 HPV− cases from seven institutions across Europe and North America. Like the original *Discovery Study* at PMH, ICON-S initially re-examined the existing TNM-7th which performed appropriately for HPV− patients with a monotonic OS reduction according to TNM-7th stage. 5-year OS for TNM-7th stage I, II, III, IVA, and IVB were: 76% (95% CI: 61–95), 68% (56–81), 53% (44–64), 45% (40–50), and 34% (25–48), respectively. However among HPV+ cases the TNM-7th fared very poorly with OS rates inseparable for stage I, II, III, and IVA ($p = 0.56$).

The 5-year OS by stage groups were: Stage I: 88% (95% CI: 74–100); II: 82% (71–95); III: 84% (79–89); IVA: 81% (79–83) but lower in stage IVB (5-year OS: 60%, 95% CI: 53–68, $p < 0.001$), essentially driven by N3 disease in this stage subset. When evaluating the performance by each T-category among all 1907 HPV+ OPC, a monotonic reduction of OS by higher T was evident, excepting between T4a and T4b where the curves were similar. Five-year OS for T1 (n = 504) were 89% (95% CI: 87–92); T2 (n = 716): 83% (80–87); T3 (n = 412): 76% (72–81), T4a (n = 231): 58% (51–65), and T4b (n = 44): 57% (44–75). In contrast, no difference in OS was found in HPV+ among N0 [n = 173, 5-year OS 80% (95% CI: 73–87)], N1–N2a [n = 416, 87% (83–90)], and N2b [n = 749, 83% (80–86)] subsets, but was significantly lower in N2c [n = 436, 74% (70–79)], and N3 [n = 133, 59% (51–69)].

As was performed in the *Discovery Study* [18], *ICON-S* used RPA and AHR models to derive stage schema from the *Training* dataset (661 HPV+ patients treated in 2000–2011 at PMH) and verified using the *Validation* dataset (1246 HPV+ patients treated in 1998–2011 in the six remaining centers). One RPA stage and two AHR stage classifications were derived. The RPA stage was in fact the same as the *Discovery Study* as was one of the two AHR stages—termed "AHR-original", but the other was a new AHR stage—termed "AHR-New". AHR-new is similar to the RPA-stage in the *Discovery Study* except that it re-classified T3N0-N2b from previous RPA stage I into an AHR-new stage II. In other words, the AHR-new classification comprised the following stage groups: stage I: T1-2N0-N2b; II: T1-2N2c or T3N0-N2c; while stage III consisted of T4 or N3. The three new stage classifications again proved to be superior to the TNM-7th, and the two AHR stage classification had identical performance in the *Training* dataset but AHR-new demonstrated the best performance in the *Validation* dataset. Because AHR-new had the best performance for OS prediction and the most practical TNM stage tabulation "grid", it was chosen as the ICON-S stage classification. Since all N1–N2b disease behaved similarly, they were classified within a single N-subcategory (N1) in the ICON-S staging; this follows the format of NPC, the other viral related pharyngeal disease, and contralateral or bilateral neck disease (formerly N2c in the 7th edition) was reclassified as N2 in a manner also similar to NPC, while N3 remained unchanged from the 7th edition. The T-categories behaved independently from T1 to T4 without apparent separation within T4 such that survival between T4a and T4b was identical. After reclassification of N categories, the eventual ICON-S stage classification was:

- ICON-S I: T1-2N0-1 (≤4 cm primary tumour without neck disease or confined to ipsilateral involvement)
- ICON-S II: T1-2N2 (≤4 cm primary tumour with bilateral or contralateral neck disease) or T3N0-2 (>4 cm primary tumour with no nodes or ≤6 cm nodes)
- ICON-S III: T4 or N3 disease
- ICON-S IV: presence of distant metastasis (M1)

The ICON-S cTNM classification was subsequently validated in several independent datasets from different institutions (Table 1) [17, 19, 20, 22, 23, 26]. The distinction in prognosis between ICON-S stage I, II, III, and IV is evident for all

Table 2 The 8th edition TNM for HPV+ oropharyngeal cancer and unknown primary

HPV+ OPC	Clinical stage			Pathologic stage		
Category	T	N	M	T	N	M
Stage I	T1, T2	N0: no regional LN	M0	T1, T2	N0: no regional LNs	M0
	T0, T1, T2	N1: ipsilateral LNs		T0, T1, T2	N1: 1–4 LNs	
Stage II	T1, T2	N2: bilateral or contralateral LNs	M0	T1, T2	N2: ≥5 LNs	M0
	T3	N0: no regional LNs	M0	T3, T4	N0: no regional LNs	M0
		N1: ipsilateral LNs			N1: 1–4 LNs	
		N2: bilateral or contralateral LNs				
Stage III	T4	Any N	M0	T3, T4	N2: ≥5 LNs	M0
	Any T	N3: >6.0 cm LN(s)	M0			M0
Stage IV	Any T	Any N	M1	Any T	Any N	M1

Stage grid for non-metastatic (M0) HPV+ OPC

	Clinical stage group (M0)					Pathologic stage group (M0)					
cT cN	T0	T1	T2	T3	T4	pT pN	T0	T1	T2	T3	T4
N0	NA	I	I	II	III	N0	NA	I	I	II	II
N1	I	I	I	II	III	N1	I	I	I	II	II
N2	I	II	II	II	III	N2	II	II	II	III	III
N3	III	III	III	III	III	N3	NA				

LN lymph node, *NA* not applicable

datasets despite the composition of cohorts (varying from 100% primary radiotherapy to 100% primary surgery). Of interest, although primarily a report of surgical management with the prime goal of deriving a post-surgical pTNM classification to accommodate the emerging strategy of endoscopic surgery, one report showed that the ICON-S classification (8th edition clinical TNM) had exemplary performance for surgically treated patients [17]. This underlines the utility of the clinical TNM classification which should be applicable to the disease overall, irrespective of the management undertaken. This is an important prerequisite, since all patients must be staged at the time of initial diagnosis before any treatment is undertaken.

ICON-S was subsequently adopted for the TNM-8th edition (Table 2) because it classifies HPV+ OPC patients into prognostically relevant groups. It is anticipated that the 8th edition TNM will facilitate patient counselling, cancer surveillance, translational research and optimize clinical trials design and outcome reporting. Notably, the 8th edition HPV+ cTNM reflects prognosis with current treatment and such patients will have received intensified treatment. Whether the excellent outcome of stage I disease can be retained with less intensified treatment remains to be evaluated in clinical trials and, in the meantime, it seems pre-mature to change treatment based on cTNM stage alone without high level of evidence (e.g. robust clinical trial data). However, the new cTNM provides a framework for updating treatment guidelines for this disease when mature clinical trial data becomes available.

Derivation of Pathological-Based pTNM

With recent advances in surgical management, transoral laser microsurgery (TLM) and transoral robotic surgery (TORS) have gained popularity in the management of T1–T2 tumours with minimal or modest nodal disease. The need to guide and investigate post-surgical treatments has prompted the derivation of a unique pTNM in this disease. The derivation of pTNM also underwent three steps: "Discovery"—"Derivation"—Validation (Fig. 1b). The *Discovery Study* was conducted by Sinha et al. [27] which suggested that high metastatic lymph node (LN) number (≥5 LNs) is an independent prognostic factor in surgically treated patients. This parameter was then adopted pragmatically in the *Derivation Study* from five institutions by Haughey et al. [17]. The proposed pTNM has subsequently been validated in two studies using NCDB datasets addressing 2010–2013 [21] and 2010–2014 [24] cohorts, respectively (Table 1).

The *Discovery* Study [27] was conducted on 220 HPV+ OPC patients who underwent transoral surgery [transoral laser microsurgery (TLM) or transoral robotic surgery (TORS)] with neck dissection. Two-third (75%) were T1–T2 tumours and 83% had N0–N2b (TNM-7th) neck disease. Multivariable analysis for disease-specific survival (DSS) showed that ≥5 pathologically positive LNs was an independent predictor for DSS together with T3–4 category tumours, and positive resection margins. Neither extranodal extension (ENE) nor high N-classification (according to the 7th edition TNM) were significant for DSS. The study results challenged the existing premise that laterality and extracapsular lymph node spread were predominant risk factors in the transoral surgical case treated with post-operative adjuvant treatment.

In the subsequent *Derivation Study*, Haughey et al. [17] expanded the cohort to include 704 surgically-managed (primary resection + neck dissection ± adjuvant therapy) HPV+ OPC patients treated between 1985 and 2015 from five cancer centers in the United States and United Kingdom. The study first confirmed lack of survival discrimination between 7th edition stage I, II, III, and IV. In the univariable analysis, increased hazard ratio (HR) for risk of death was evident with higher pT (pT1 as the reference, 279 patients): pT2 (n = 290): HR = 1.77 (95% CI: 1.03–3.05); pT3 (n = 92): HR = 2.67 (1.38–5.16); and pT4 (n = 43): HR = 5.69 (2.67–11.31). However, no clear difference in risk of death by pN0 (n = 44, as the reference), pN1 [n = 91, HR = 0.89 (0.23–3.47), p = 0.87], pN2a [n = 141, HR = 1.24 (0.35–4.40), p = 0.737], pN2b [n = 337, HR = 1.89 (0.59–6.08), p = 0.285], and N3 [n = 39, HR = 2.70 (0.64–11.31), p = 0.174] were seen, although a trend towards higher HR in the N2c subset [n = 52, HR = 3.56 (1.01–12.62), p = 0.049] seemed suggestive. The univariable analysis confirmed increased risk of death with ≥5 lymph nodes [HR = 2.93 (95% CI: 0.21–0.53), p < 0.001].

Consequently, the authors proposed a new pN-classification based on number of pathologic positive LNs identified in the neck dissection specimens: <5 LNs were classified as pN1 while ≥5 LNs were pN2. A pTNM stage grouping was proposed combining pT (where pT3-4 was considered an adverse primary feature) and pN

(where ≥5 nodes was considered adverse). Cases with neither primary nor nodal adverse features were classified as pathologic Stage I (pT1-2_pN0-N1); cases with only one adverse (either primary or nodal) features were classified as pathologic stage II (pT3-4_pN0-N1 or pT1-2_pN2); and cases with both adverse features (pT3-4_pN2) were classified as pathologic stage III. In the multivariable analysis, the newly proposed pathologic stage schema showed good separation in OS (adjusted for age, cancer center, and adjuvant radiation or chemoradiation): stage I: HR = 1.59 (0.95–2.64, p = 0.077); stage II: HR = 6.83 (3.77–12.38), p < 0.001).

It is important to recognize that adjuvant radiotherapy (HR = 0.55 (0.31–0.95), p = 0.033) and chemoradiation (HR = 0.46 (0.24–0.87), p = 0.017) significantly reduced risk of death in the multivariable analysis. Notably, in the univariable analysis, presence of perineural invasion (HR = 3.33 (1.92–5.81, p < 0.001) or lymphovascular invasion (HR = 1.92 (1.14–3.23), p = 0.014) were both also associated with increased risk of death. However, these primary tumour parameters were not included in the multivariable analysis. In the case of pathologically detected extranodal extent (pENE), this finding also showed a trend towards adverse survival on univariable analysis (p = 0.06) but was not included in the multivariable, analogous to the contralateral/bilateral neck disease situation (N2c) discussed below. These pENE findings also seem relevant to the general discussion of pENE that follows.

The proposed pTNM for HPV+ was adopted by UICC/AJCC in the 8th edition TNM [15, 16] (Table 2). The 8th edition pTNM was validated by Zhan et al. [24] utilizing 3742 patients using registry data from National Cancer Data Base (NCDB), for patients managed with surgery from 2010 to 2014 [24]. They found excellent hazard discrimination and prognostication among Stage I (n = 140), II (n = 248), and III (n = 711), and IV (n = 2643) by the 8th edition pTNM classification. On a cautionary note, however, Zhan and colleagues also noted that 41% of surgical cases had pENE and there was a significantly inferior OS in this large patient sample [4-year OS: 85% vs. 92% with vs. without ENE, p < 0.001]. When stratified by pN classification, ENE retained a significant negative impact on OS for pN1 (4-year OS: 87% vs. 92% for with vs. without pENE, p = 0.004) and pN2 (4-year OS: 77% vs. 88%, p = 0.061).

It is conceivable, that the pTNM classification will provide an opportunity to record pathological adverse features and risk factors to aid refining post-surgical risk stratification, and potentially guide post-surgical management in surgically managed HPV+ OPC. It should facilitate refinement of clinical trial risk-stratification with the caveat that some aspects continue to merit close study in the future including the presence and degree of extra-nodal extension, and the degree of intensity of treatment that should be used in the presence of such adverse features.

Determination of Tumour HPV Status in OPC and CUP

With the introduction of a new stage classification for HPV+ OPC and CUP, it is important to determine tumour HPV status for the purposes of staging and potentially treatment selection. AJCC and UICC have recommended determining tumour

HPV status in either primary tumour or cervical lymph nodes for all OPC and CUP. Several issues need consideration when implementing tumour HPV testing methods in routine clinical practice: (1) Adequate sensitivity and specificity; (2) Good test/re-test reliability; (3) Short turn-around time; (4) Well-validated; (5) Widely available; (6) Cost-effective. Several HPV testing methods have been investigated from detection of HPV E6/E7 DNA, mRNA, to downstream oncoprotein p16 expression. Each testing method has advantages and disadvantages (Table 3). Testing viral E6 or E7 mRNA is generally considered the "gold standard" for HPV detection because it provides definitive evidence of viral integration. However, it requires technical expertise and should optimally be performed on fresh frozen tissue. Thus, it has yet to be adopted into routine clinical practice [28]. Several methods have been used to detect HPV DNA. However, while these are often HPV subtype specific, they are unable to differentiate HPV as having "driver" status (responsible for the tumour carcinogenesis process) vs. "passenger" status (co-existing HPV infection, but without tumour causation). Currently, p16

Table 3 Advantages and disadvantages of commonly used HPV testing methods

Type of tumour sample	Tumour markers	Comments
Tumour tissue blocks (Formalin-fixed paraffin-embedded)	p16 by IHC staining	• Commonly used as a surrogate marker of HPV-driven OPC • High sensitivity but modest specificity • Less costly, easy to conduct • Some rare tumour histologies, such as neuroendocrine tumour, can also cause p16 overexpression
	HPV DNA (E6 and E7) by ISH	• High sensitivity • Unable to determine the role of HPV in tumour as a "driver" vs. "passenger" (the presence of HPV DNA in tumour may not always indicate that the tumour is driven by HPV) • HPV subtype specific • Reduced sensitivity when low viral loads
	HPV DNA (E6 and E7) by PCR	• Very high sensitivity • Risk of contamination • Unable to determine the role of HPV in tumour as a "driver" vs. "passenger"
	HPV RNA (E6 and E7 mRNA)	• Generally considered the gold standard for HPV detection • High sensitivity and specificity: definitive evidence of viral integration • Risk of RNA degradation (fresh or frozen material is preferred) • Not yet ready for routine clinical use
Cell blocks from fine needle aspiration (FNA)	p16 by IHC staining	• Requires enough tumour cells • Need to be fixed by Formalin rather than alcohol • Less reliable than p16 staining on FFPE

IHC immunohistochemistry staining, *ISH* in-situ hybridization, *PCR* polymerase chain reaction

immunohistochemistry (IHC) staining is recommended as a first-line or single test for HPV+ OPC because of high sensitivity with acceptable specificity, agnosticism to HPV subtype, relatively ease of interpretation, lower cost, and wide-spread availability [29–32]. It is generally considered a good surrogate marker for OPC with high positive predictive value, but is less reliable for HPV+ non-OPC. A recent meta-analysis [30] showed that p16 IHC is highly sensitive but moderately specific to diagnose HPV-transformed OPC when used as a single test. Notably, various p16 staining positivity criteria have been used in the literature. To ensure the high specificity of p16 testing for HPV+ OPC, stringent criteria should be followed when reporting p16 staining positivity that take account of staining intensity (strong intensity), proportion (≥70% tumour cells), and pattern/location (diffused pattern, in both nuclear and cytoplasma). The recently published HPV testing guideline from the College of American Pathologists (CAP) [33] defines p16 positive as ≥70% nuclear and cytoplasmic staining in OPC. If <70% nuclear and cytoplasmic staining or weak intensity are present, it should be classified as an HPV− tumour. For CUP involving cervical level II or III LNs, p16 IHC should be used as first line HPV testing ± HPV-specific testing. Cytoplasmic and nuclear staining alone yielding p16 ≥ 70% is sufficient for level II or III lymph nodes with non-keratinizing morphology. Otherwise, p16 staining should be followed by HPV-specific testing to determine HPV status for CUP. A suggested testing algorithm is depicted in Fig. 2.

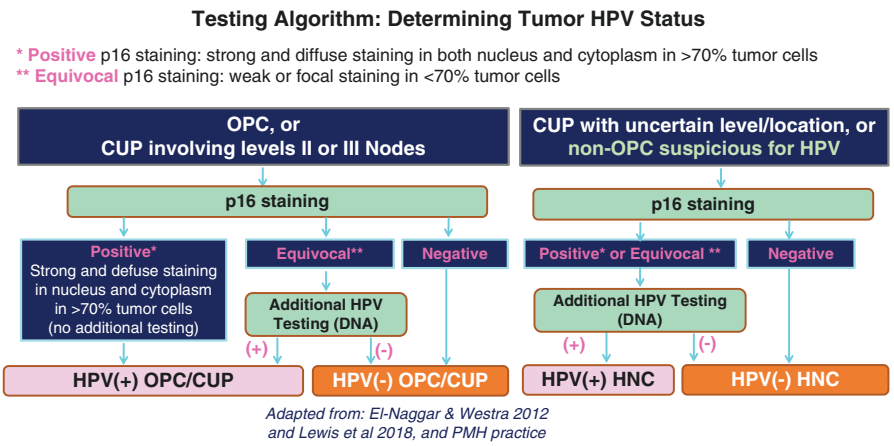

Fig. 2 Suggested HPV testing algorithm for oropharyngeal cancer OPC and unknown primary with cervical nodal metastasis (Consistent with the Guideline of the College of American Pathologist Guideline [33] and reflecting expert recommendation [29] and current clinical practice at the Princess Margaret Cancer Centre). * Positive p16 staining refers to strong and diffuse staining in both nucleus and cytoplasm in >70% tumor cells; ** Equivocal p16 staining refers to weak or focal staining in <70% tumor cells. Additional HPV testing refers to directly test the presence of high-risk HPV subtypes (e.g. E6/E7 DNA) (refers to Table 3). *OPC* oropharyngeal cancer, *CUP* carcinoma with unknown primary, *HPV* human papillomavirus, *HPV*(+) HPV-related, *HPV*(−) HPV unrelated

Relevance of TNM 8th Edition in Clinical Management

A new cTNM and pTNM HPV+ OPC is now included in the 8th edition TNM. Since many HPV+ OPC present as "unknown primary" (CUP), the 8th edition mandates testing EBV and HPV for these presentations. For p16+ or HPV+ CUP, T0 will be assigned while the cN and pN conventions use the HPV+ OPC regional node classification (Table 2). Notably, the definition of cT and pT are the same, while cN and pN uses different definitions. The difference might reflect the applicable patient population, the derivation process, and the clinical application time points, which is reflected in the respective Derivation studies for cTNM by O'Sullivan et al. [16] and pTNM by Haughey et al. [17] (Table 4). Interestingly, the pTNM *Derivation Study* [17] also showed a good survival discrimination when regrouping pT and pN according to the 8th edition cTNM criteria [15], i.e. considering nodal side (unilateral vs. bilateral/contralateral), and size (≤6 vs. >6 cm) without inclusion of lymph node number. The 5-year OS for stage I, II, and III following cTNM were 90% (95% CI 87–93), 79% (71–87), and 70% (57–83), respectively. Notably, while only 52 (7%) N2c cases were present in the pTNM *Derivation Study,* an increased risk of death was still significant (HR = 3.56, 95% CI: 1.01–12.62, p = 0.049) in the univariate analysis but was not included in the multivariate analysis, possibly related to the small subset with insufficient power in the model; this contrast with the ICON-S situation where 436 cases (23%) had N2c disease and underlines the difference in selection between cohorts chosen for surgical management compared to non-surgical approaches. From the standpoint of disease behavior, it seems intuitive to consider that contralateral nodal involvement from a lateralized primary (e.g. tonsil and lateral base of tongue tumour) represents a more extensive disease state,

Table 4 Comparison of characteristics of cTNM and pTNM derivation studies

	cTNM (O'Sullivan, Lancet Oncol 2016) [7]	pTNM (Haughey, Oral Oncol 2017) [17]
Study cohort	All M0 disease	Resectable disease only
Study period	7 centers, treated in 1998–2011	5 centers, treated in 1985–2015
HPV/p16 testing rate	50–81%	Unknown
Sample size	N = 1907	N = 704
7th T and N category	cT and cN • cT3: 412 (22%); cT4: 275 (14%) • cN2c: 436 (23%); cN3: 7%	pT and pN • pT3: 92 (13%); pT4: 43 (6%) • pN2c: 52 (7%); N3: 39 (6%)
Methodology	• Training-validation • Using models and evaluation criteria from the "Discovery Study"	• Pooled dataset • Adoption of "LN number" parameter directly from the "Discovery Study"
Time point	Assigned at the initial diagnosis	After resection of both primary and neck nodes
Application	For multiple purposes	For risk stratification

implying more “advanced” stage than disease confined to the ipsilateral neck and could also influence a straightforward count of number of lymph nodes as a prognostic factor.

The new TNM classification for the HPV+ OPC reflects prognosis under current treatment paradigms where many patients were treated with intensified treatment. Consequently there remains concern about whether the excellent prognosis of HPV+ OPC would remain in all cases if intensity was reduced. Thus, practitioners must be mindful that the TNM classification is not a guideline for treatment but provides a framework for clinical research and treatment decision-making that must continue to acknowledge other important tumour factors (e.g. presence of extranodal extension), treatment factors (e.g. resection margin status, radiotherapy dose, mode of systemic treatment) and patient factors (e.g. age, performance status, and potentially a quantified assessment of smoking history). The only safe path to modify treatment will be through the results of properly designed clinical trials.

Future Direction

The 8th edition TNM is the first step to properly stratify patient prognosis. Many questions remain. For example, can treatment of stage I disease be de-intensified? Should other adverse features (e.g. ENE) be included in subsequent editions of pTNM, or even in the clinical TNM classification in the event that medical imaging becomes more reliable at detecting these features in the future? Would improved tumour HPV testing accuracy (e.g. E6/E7 mAb) be beneficial? Finally, would the new staging classification be useful for other HPV+ head and neck cancer sites beyond the oropharynx? Clinicians and researchers need to work collectively to understand the natural course of disease behavior, and compile robust data with appropriate analyses to further refine the TNM classification.

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Should We De-escalate Treatment for HPV Positive Oropharyngeal Head and Neck Cancer?

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Rationale

The incidence of HPV oropharyngeal cancer has been increasing rapidly over the past two decades, especially in the western world [1–3]. Over the past two decades, concurrent chemoradiotherapy has become the standard of care for oropharyngeal cancer in most countries. It is, however, associated with significant and sometimes life-threatening acute and late toxicities [4, 5]. The significantly better prognosis of HPV positive disease, compared to HPV negative disease [6], has raised the possibility of reducing the intensity of treatment (also known as de-escalation) to reduce the incidence of toxicity, especially in the long-term and in particular in the younger patients with HPV positive disease, who will live for a long time with the burden of toxicity [7].

There are many potential ways of reducing the intensity of treatment. Simply put, these fall into the following categories: reducing the intensity of chemotherapy or using an alternative agent; reducing the intensity of radiotherapy; substituting surgery for chemotherapy; or eliminating chemotherapy completely. All these are viable options, with preliminary data that would support their evaluation in an experimental research setting. To date, however, none of these treatments have level-one evidence to support their use in the routine clinical setting. Therefore, it must be stressed that the treatments mentioned below should only be used within the setting of a clinical trial.

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Reducing or Substituting Chemotherapy

Cisplatin chemotherapy has been shown to double acute serious complication rates when given concomitantly with radiotherapy [4, 5] and has also been shown to increase the long-term sequelae, which is cumulative [4]. Cetuximab, an EGFR inhibitor, was shown to significantly improve overall survival (median survival 48 vs 29.3 months; hazards ratio 0.74, p = 0.03) when given with radiotherapy compared to radiotherapy alone [8]. The effect was especially marked for oropharyngeal cancer. The trial also reported similar toxicity rates between the two arms, except for dermatological toxicity. Cetuximab is therefore considered by some to be a less toxic alternative to radiation sensitisation by cisplatin. The criticism of such an approach is that there appears to be an inverse relationship between HPV positivity and EGFR status before start of treatment [9]. Therefore, an EGFR inhibitor may not be as effective as chemotherapy. However, as radiotherapy induces EGFR expression in head and neck cancers, resulting in resistance to radiotherapy, an EGFR-inhibitor may help overcome this acquired resistance to radiotherapy. Three randomised trials are currently comparing cetuximab and radiotherapy compared with cisplatin and radiotherapy. The De-escalate (UK) trial recruits low risk patients only, with toxicity as the primary outcome. The RTOG 1016 (USA) (NCT01302834) trial includes both low and intermediate risk oropharyngeal cancer patients, and is reporting on survival as the primary outcome. The TROG 12.01 trial (Australia) (NCT01855451) is examining toxicity during treatment with weekly cetuximab or cisplatin in the low risk patients.

Reducing (Chemo)radiotherapy Dose

The dose of radiotherapy has been shown to be associated with the acute and long-term toxicity, especially dysphagia and xerostomia. The rate of dysphagia increases with every 10 Gy delivered above 55 Gy given to the pharyngeal constrictors [10]. In addition, the volume of pharyngeal constrictors is directly associated with stricture and feeding tube dependence and stricture formation [11]. By reducing the dose of radiotherapy, investigators aim to reduce toxicity, but maintain tumour control. The ECOG 1308 trial (NCT01084083) [12] attempts to answer this question by reducing the dose of radiotherapy by using induction chemotherapy (paclitaxel, cisplatin and cetuximab) to identify those who are chemo/radiosensitive, who are then given a lower dose of radiotherapy (54 Gy) compared to standard dose if there is no response. Similarly, the Quarterback study (NCT01706939) by Mount Sinai Hospital (USA) delivers 3 cycles of docetaxel, platinum and 5-fluoro-uracil. This is followed by cetuximab, carboplatin and 56 Gy radiotherapy in responders, or carboplatin alone and 70 Gy radiotherapy if no response. More recently the results of the OPTIMA trial have been published, which show excellent control rates with significantly reduced radiotherapy doses. In this trial, induction chemotherapy is again

used to stratify patient response in HPV positive patients. Depending on their baseline risk profile and the extent of response to induction chemotherapy, patients received radiotherapy alone at 50 Gy, or chemoradiotherapy to 45 Gy. All poor responders received chemoradiotherapy to 75 Gy [13]. All the studies above have, however, been criticised for the fact that the overall chemotherapy dose used is increased to compensate for the reduction in radiotherapy dose.

In the post-operative setting, there is good evidence that adjuvant treatment post operatively is highly protective [14, 15]. Some studies have shown that there may be no difference in overall survival between patients who receive post-operative radiotherapy alone and those who receive post-operative chemoradiotherapy. However, there is an important caveat to these studies—these were subgroup analyses of retrospective very small sets of data. Similarly, some research has demonstrated that extracapsular spread (especially true for minimal extracapsular spread of <1 mm) may not have as much significance in HPV positive patients who are treated with post-operative adjuvant therapy as in HPV negative patients [14, 15]. However, these studies have yet to be validated prospectively in large studies. The ECOG 3311 study (NCT01898494) looks to reduce radiotherapy dose for those patients who have intermediate histopathological features following surgical resection. Low and intermediate risk HPV positive patients with perineural/vascular invasion, close margins or multiple involved nodes on post-operative histology are randomised to a radiotherapy dose of 50 Gy compared to the standard 60Gy.

Substituting Surgery for Chemotherapy

The advent of transoral laser and robotic surgery provides the opportunity to eliminate chemotherapy and possibly also radiotherapy from the treatment of some oropharyngeal cancer patients. This strategy is most likely to succeed in those patients who are least likely to require post-operative chemo radiotherapy—i.e. those with small primary tumours and no obvious or early nodal disease. Conversely, this group of patients is also the one most likely to be treated with radiotherapy alone, without chemotherapy. There is currently little prospective data on the merits of these alternative strategies, due to the difficulty in undertaking head to head randomised trials between surgical and non-surgical treatments [16]. The EORTC "Best-of" trial (NCT02984410) will compare radiotherapy to those receiving surgical resection in patients with early HPV positive (T1-T2N0) disease.

Eliminating Chemotherapy

Data from Sweden has demonstrated that patients with HPV positive disease show better outcomes when treated with radiotherapy alone compared to patients with HPV negative disease [17]. This preliminary data supports the use of radiotherapy

alone in very low risk HPV disease. A critique of this approach has been that whilst HPV positive patients show better outcomes than HPV negative patients, T4 and N3 HPV positive disease show increased risk of distant metastasis, especially when treated with radiotherapy alone [18]. Furthermore, the outcomes of HPV stage IV disease when treated with radiotherapy alone appear to be worse than patients with similar or higher stage disease treated with concomitant chemoradiotherapy [19]. In that same study, however, lower stage patients who were non-smokers showed similar survival rates whether they were treated with radiotherapy or chemoradiotherapy. Based on this information, the NRG HN-002 phase 2 trial (NCT02254278) was established, in which patients with very low risk HPV positive disease (excluding smokers, T4 and N3 disease) are treated with radiotherapy only at a dose of 60 Gy in 25 fractions. This trial is due to report in 2019. The Phase II PATHOS trial (NCT02215265) is examining whether to eliminate chemotherapy and reduce radiotherapy dose (from 60 to 50 Gy) in patients with high risk histopathological features (involved margins, extracapsular spread) post-surgical resection. It is examining whether it can demonstrate differences in swallowing outcomes, and if demonstrated, would then roll on to a Phase III trial with survival as a primary outcome.

Patient Preference

Brotherston et al. [20] demonstrated that patients were supportive of de-escalation of treatment only if there was no or little likelihood of a reduction in the efficacy of killing the tumour. Given a choice, patients would rather reduce chemotherapy then radiotherapy.

Escalation of Treatment for Intermediate Risk HPV

Whilst there has been a lot of interest in the de-escalation of HPV positive OPC, one should remember that the survival outcomes of patients within the intermediate risk OPC group (heavy smokers, or those with T4 or N3 disease) are relatively poor (approximately 70% at 3 years). As a result, some have advocated escalation of treatment for the intermediate risk patients. In the UK, the Phase III CompARE study examines different forms of treatment escalation for patients with intermediate and high risk OPC, including: dose escalated radiotherapy with concomitant cisplatin chemotherapy; the addition of transoral surgery to chemoradiotherapy; and the use of adjuvant PD—L1 durvalumab adjuvant therapy following chemoradiotherapy, compared to standard concomitant chemoradiotherapy [21].

Table 1 Summary of clinical trials investigating de-escalating therapy for patients with HPV positive tumours

Method of de-escalation	Primary (chemo)radiotherapy	Post-surgical resection
Substitute chemotherapy	De-escalate RTOG 1016 TROG 12.01	
Reduce radiotherapy	ECOG 1308 Mount Sinai OPTIMA	ECOG-3311
Substitute surgery for radiotherapy	Best of	Best of
Eliminate chemotherapy	NRG HN-002	PATHOS

Conclusions

In the coming 5 years, many of the seminal trials for HPV positive disease will be reported. These are likely to provide us with valuable insights into the future treatment of HPV disease. One of the challenges will be to decide between the different options that will become available. Until then, clinicians should not alter their management of oropharyngeal cancer, unless within the context of a clinical vial (Table 1).

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Is There a New Role for Surgery in Oropharynx Cancer?

Yann Litzistorf and Christian Simon

Introduction

Head and neck surgery has evolved in the last two decades from consisting of mostly open ablative procedures for head and neck cancers to a spectrum of treatment modalities comprising of minimally invasive techniques, open ablative approaches for advanced cases, and complex reconstructive procedures [1, 2]. Head and neck surgery in our days comes with a significant reduction of secondary morbidities through approaches that avoid unnecessary access trauma [3] and also with the possibility to recover function by reconstructing critical parts of the oral cavity, pharynx and larynx [4]. These new techniques can be exploited in the treatment of oropharyngeal cancers, a tumor entity shifting more and more towards HPV positive disease [5]. As it has been well established in numerous epidemiological studies, this tumor entity has a much better prognosis than its HPV negative counterpart [6]. Thus, the challenges of treating these patients are becoming more complex, since besides the aim of controlling the disease the aim of providing the most optimal functional recovery becomes more pertinent, given the long-lasting survival of these patients.

Surgery plays different rolls depending on the stage of the oropharyngeal cancer. In the following chapter we will explore the role of modern head and neck surgery based on the stage of the oropharyngeal cancer. We will also address HPV positive disease and discuss eventual new surgical options for recurrent disease.

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Modern Minimally-Invasive and Reconstructive Surgical Techniques

Modern head and neck surgery has evolved in the last decades and provides now a large spectrum of minimally invasive and reconstructive techniques. Based on negative experiences with open surgical approaches that were plagued with a high rate of severe complications, new surgical approaches were developed that helped to decrease treatment-related morbidity by direct exposure of the cancer, thus avoiding access trauma, which was found responsible for many complications [7].

There are three types of minimally invasive approaches to the oropharynx. The most traditional is the conventional type, which is typically used for the resection of lesions localized in the tonsil area or in the region of the soft palate and posterior pharyngeal wall [8]. This is a surgical technique that is typically undertaken with a headlight and electrical instruments and is very similar to the regular tonsillectomy as we do it for children. The second type of minimally invasive surgery for the oropharynx is called transoral laser microsurgery (TLM). This technique makes use of the CO2-laser and special laryngoscopes, in order to properly expose the lesion. It is a two-hand technique. The downside of this technique is the exclusively straight vision and the frequent necessity of changing the position of the laryngoscope. Other than that, the philosophy of the surgical procedure is based on the concept to remove the tumor in multiple pieces. Cutting through the tumor under microscopic visualization allows for excellent control of the deep surgical margin, which is based on the remarkably different carbonization of tumor tissue in comparison to normal tissue [9]. TLM is frequently used nowadays and constitutes a highly cost-effective surgical treatment modality for the oropharynx [10]. The third type of minimally invasive surgical approach is making use of novel surgical and robotic systems. The da Vinci® surgical system is currently the most widely used. The da Vinci surgical system allows for working with endoscopes of different visual angles (0°, 30°) and thus provides means to resect with higher precision. The system consists of a console for the surgeon, from where the operative instruments are controlled, and the patient card to which the endoscope and the surgical instruments are attached to. This technique is named transoral robotic surgery (TORS) [11]. Other technologies have recently entered the market. Most important to mention is the system from Medrobotics®, whose technology is based on a flexible endoscopic approach [12, 13].

Minimally invasive approaches to the oropharynx provide favorable functional recovery, in particular swallowing recovery. A review of the literature comprising of nine retrospective studies all reporting on functional swallowing outcome 1 year after finishing treatment shows that in five of nine studies a complete recovery of swallowing was achieved despite various percentages of adjuvant treatment with radiation therapy or chemoradiation. Although in four out of nine studies such a recovery of swallowing was not complete, a more in-depth analysis demonstrated that based on data from patient-reported outcomes the difference after treatment in comparison to the status prior to treatment was clinically not significant [14]. This highlights the good performance of these minimally invasive techniques.

Reconstructive head and neck surgery has also dramatically evolved within the last two decades. With the introduction of free microvascular flaps, it became possible to transfer tissue from nearly all sites of the body with a broad variety of tissue compositions to the oropharynx [15]. Typical free flaps used in contemporary head and neck reconstruction are the radial forearm free flap, the antero-lateral thigh flap, the fibula free flap, the iliac crest, the scapula tip flap, the lateral arm flap, and others [15]. While two decades ago still plagued with postoperative complications such as venous thrombosis and arterial embolism, the rates of flap failures have significantly decreased to be now in the range of only 5% [16]. In particular resurfacing of the lateral pharyngeal wall and the reconstruction of the soft palate is an indication for a free flap. Bony reconstructions of the mandible in case of frank infiltration is nowadays done with high precision using either free fibulas or iliac crests preceded by three-dimensional planning [17]. As a result, operative time has greatly been decreased, occlusion and swallowing after the intervention is nearly normal. Even dental implants are possible [18].

Early Stage OPSCCs (T1,2 N0; 7th AJCC Classification)

Early stage oropharyngeal cancer can often be treated with a single modality, either definitive radiotherapy (RT) or surgery. In a meta-analysis based on retrospectives studies comparing RT with transoral minimally invasive surgery (TOS) for early stage OPSCC, Morisod and Simon reported that TOS provides similar oncologic outcome as RT, especially regarding the 5-year DSS rates, suggesting TOS to be equally effective in terms of tumor control [19]. Considering a disease specific survival rate of about 90% at 5 years patients with early-stage disease have so to speak the time to either experience the sequelae of late toxicity or develop secondary primary malignancies (SPM). The projected incidence of SPMs after primary oropharyngeal cancers varies based on the presence or absence of an HPV-phenotype. In non-HPV-phenotype cancer patients this incidence surpasses the 25% mark already after 96 months. The incidence of SPM's in HPV-phenotype cancer patients is as expected much lower. However, it reaches the 10% mark at 108 months [20]. An explanation for the relatively high incidence of SPM's in the HPV-phenotype cancer population becomes evident, if analyzing the patient characteristics of Ang's study on the different survival of HPV positive versus HPV negative oropharyngeal cancer patients based on RTOG 0129. Looking at the HPV positive cohort approximately 65% of patients were either former or current smokers [6]. In a study on efficacy of chemoradiation for advanced stage oropharyngeal cancer in Spain for HPV positive versus HPV negative patients the percentage of patients smoking more than 20 cigarettes per day was as high as 80%, and this in both groups [21].

Treatment to the primary site might very well influence treatment options for SPMs. As demonstrated for laryngeal cancers, previous radiation therapy impacts negatively on laryngectomy free-disease free survival. This is largely the consequence of the inability to perform organ-preservation surgical approaches in the

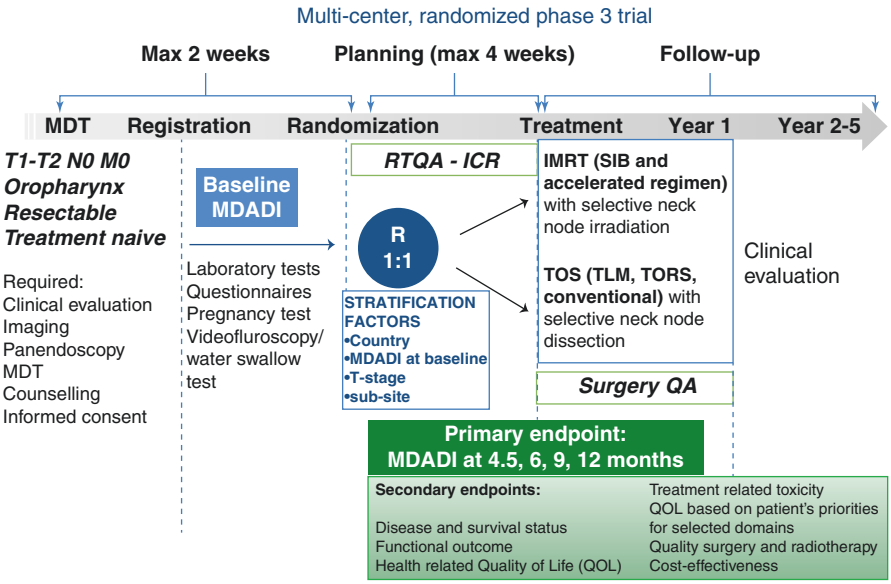

Fig. 1 Design of EORTC 1420 "Best-of"

radiated field. Patients were thus prone to have a total laryngectomy more so after previous radiation therapy than after previous surgery [22]. Similar may hold true for early stage oropharyngeal cancers. As a consequence, it seems logical to rather choose a surgical approach for a young oropharyngeal cancer patient with an early lesion.

Unfortunately, comparative data on radiation therapy such as intensity modified radiation therapy (IMRT) versus TOS for early stage oropharyngeal cancer are lacking. EORTC 1420 is a randomized phase 3 three trial comparing the patient reported swallowing outcome during the first year after treatment with either modality (ASCO 2018, Abstract TPS6098) (Fig. 1). Data from this trial will provide level-one evidence as to which of these treatments provide better functional outcome and should be chosen as primary treatment modality for this disease in the future.

Advanced Stage OPCs (T3, 4a, and/or N+; 7th AJCC Classification)

The majority of data concerned with treatment-related morbidity of surgery for oropharyngeal cancers is derived from studies in advanced stage (7th AJCC) oropharyngeal cancer patients. In a review of 51 studies with approximately 6400 patients in 2002, Parsons et al. stated that surgery provides similar oncological outcome than radiation based treatment, but with a significantly higher rate of severe complications [7]. Of note, surgical retrospective studies consisted of series,

where exclusively open approaches where used and typically regional flap reconstructions. He reported on a weighted-average severe complication rate for base-of-tongue resections across the studies of 32% and a fatal complication rate of 3.5%. Radiation based treatment compared with this was much less prone to severe complications (3.8%). Based on these findings there was an incentive to develop minimally invasive approaches. In parallel, radiation-based treatments became the treatment modality of choice for advanced stage oropharyngeal cancer. As alluded to above, surgical techniques have remarkably evolved and nowadays, even if open approaches are undertaken, reconstructive procedures such as free flaps can help the wound healing process, functional recovery, and esthetic outcome. Broome et al. reported recently only 14.7% of severe complications in a cohort of 217 surgeries *with free flap reconstruction*, which certainly compares favorably to the 32% severe complication rate with older surgical techniques. The flap loss rate was only 5.5% [3].

Considering that surgical treatment for advanced stage oropharyngeal cancer seems to become less and less morbid, surgery as primary modality may eventually become an option in the future, if properly supported by level-1 evidence data. In this context adjuvant treatment, which is inevitable in this patient population, shifts in the foreground.

The surgical resection of a head and neck cancer allows for the identification of pathological risk factors. A different risk stratification for adjuvant multimodality treatment is therefore used, as compared to primary chemoradiation, which is based on clinical risk factors.

Two prospective randomized trials both published in 2004 provided us with the current guidelines for the adjuvant treatment of pathologically high-risk head and neck cancers after surgery [23, 24]. A follow-up combined analysis of the two trials revealed that the two risk factors extracapsular extension (ECE) and positive margins are associated with an overall survival benefit, if treated in the adjuvant setting with chemoradiation [25].

New regimens are under investigation such as the postoperative combination of RT with cisplatin and cetuximab versus RT with docetaxel and cetuximab (RTOG 0234) for histological high-risk patients. 40% of the patients included in the study had an oropharyngeal cancer, but 73% of them were HPV-positive. Of note, in comparison with RTOG 9501 that provided level-1A evidence for the efficacy of postoperative chemoradiation, both new adjuvant treatment combinations performed significantly better in terms of overall survival [26]. Based on this trial a phase 3 study (RTOG 1216) (NCT01810913) is currently recruiting that uses two experimental arms consisting of RT (60 Gy) combined with docetaxel and RT (60 Gy) combined with docetaxel and cetuximab versus standard of care, which is RT with cisplatin.

The new adjuvant treatment strategies are promising, given that the experimental treatment arm consisting of RT, docetaxel and cetuximab produced excellent 2 years overall survival rates of 80%, which compares even favorably with the HPV negative population in RTOG 0129 [6]. In the latter study, the 2 years overall survival (OS) rate was found to be 65%.

Future trials that will examine the role of immunotherapy in the adjuvant setting of surgically resected high-risk head and neck cancers will need to take these new combinations into consideration.

Also, for pathologically intermediate risk head and neck cancers a phase 3 post-operative trial is currently ongoing. RTOG 0920 (NCT00956007) allows patients with resected oral cavity, oropharynx, and larynx cancer, T2-4a N0-2 or T1N1-2, to be randomized to either RT alone (60 Gy) or RT (60 Gy) plus cetuximab, if one of the following intermediate risk factors is found in the pathology specimen: perineural invasion, lympho-vascular infiltration, N2-3 (no ECE), T3-4a, close (<5 mm) margins (no positive margins), T2 oral cavity cancer with depth of invasion (DOI) >5 mm. The estimated study completion year is 2026.

HPV Positive OPCs

HPV-positive tumors are in general more radiosensitive, have better overall survival and progression-free survival rates independent of treatment modality [6, 27]. Management of this specific entity must be focused on the reduction of morbidity without affecting the oncological outcomes. According to Huang et al. de-escalation might be feasible for a particular low risk population, with a tobacco exposure less than 20 pack years, T1,2N0-N2 stage (7th AJCC). This population has a good 5-year overall survival rate of 83%, but they still experience significant morbidity [28]. For this subgroup treated with standard-of-care RT or CRT, Goepfert et al. reported that 12 months after treatment, the swallowing function was "adequate" (mean MDADI score 78) but still remained significantly below the baseline (mean MDADI 92) with more than 10 points. This difference signifies the "minimal clinically important difference" (MCID) [29]. Transoral surgery might be an alternative for this condition. Typically, patients can be treated with surgery only for T1,2N0-1 (7th AJCC), if no additional risk factors are identified. Recent data even suggest that for up to N2c-disease surgery-only may provide excellent tumor control (ASCO 2018, Abstract #6003). However, for patients with ≥ N2a-disease adjuvant RT is current practice. Surgery-only provides according to currently available retrospective data full recovery of swallowing already after 6 months [30]. If combined with RT it might be superior compared to CRT as demonstrated in a prospective non-randomized single institutional trial on 40 patients [31].

De-escalation strategies are easier to implement and study in the surgical patient population given the availability of a pathological specimen and consecutive possibility for a pathological risk-stratification. Current concepts of de-escalation in the above defined patient population consist of a reduction of radiation dose for pathologically intermediate-risk OPCs, an omission of chemotherapy for patients with pathological high-risk features, and a reduction of adjuvant RT-volumes via sparing either the R0-resected tumor bed or in case of unilaterality of the disease the non-affected neck.

A reduction of adjuvant radiation dose from 60 to 50 Gy for intermediate-risk HPV-positive OPSCCs is currently tested in a phase II trial (ECOG 3311). The endpoint is progression free survival (PFS) at 2 years in combination with various functional recovery, toxicity, and quality of life endpoints [32]. An omission of chemotherapy for pathological high-risk OPSCC patients (positive margins in the specimen, ECE) is currently investigated in a British phase II trial (PATHOS, NCT 02215265). The primary endpoint is the MDADI-score at 1 year after treatment. This trial is planned to proceed into a phase III non-inferiority trial with OS as the primary endpoint. Retrospective data suggest that sparing the tumor bed after transoral R0-resections [33] or uni-lateral neck irradiation for strictly uni-lateral disease [34], does not reduce tumor control rates, but improves functional outcome demonstrated by a reduction long-term tracheostomies and use of percutaneous endoscopic gastrostomy feeding tubes (PEGs), thus being hypothesis-generating for future surgical de-escalation trials in this patient population.

Recurrent OPCs

Recurrent oropharyngeal cancers can be categorized into loco-regional resectable, loco-regional non-resectable, and metastatic disease. 5-Years OS rates for the first two categories have been approximately 18% and patients had to be chosen wisely for salvage procedures, given poor functional recovery in combination with a guarded prognosis. With the rise of HPV-positive disease this has changed. Recurrent OPCs operated before 2000 had an OS at 5 years of no more than 18% whereas after 2000 this increased to up to 51% [35]. To some degree this may be driven by the technical improvements of surgery, but for the most part this is the consequence of an ever-increasing incidence of HPV-positive disease [5]. Considering p16-positivity as a surrogate marker for HPV-positivity an analysis on recurrent p16-positive OPCs demonstrated a significant survival improvement for loco-regional progressive, metastatic, recurrent-resected and non-resected patients [36]. In this analysis patients with recurrent p16-positive OPCs that underwent surgical salvage had a 2-year overall survival rate of 80%. In this context and also considering novel surgical techniques helping to improve functional recovery, surgery becomes again meaningful as a treatment for recurrent oropharyngeal malignant disease.

Conclusion

As of now surgery for the treatment of oropharyngeal cancer should be considered for early stage disease (7th AJCC classification) independent of the HPV-status and can be considered to be equivalent to radiation therapy in terms of tumor control. Which one of the treatments will provide better functional recovery, will be

answered by ongoing phase III prospective trials comparing novel minimally-invasive techniques with IMRT. Standard of care will change in the future accordingly. Patients at high risk for secondary primaries and particularly young patients should currently be rather considered for surgery.

The standard of care for advanced stage oropharyngeal cancers (7th AJCC classification) remains concurrent chemoradiation. However, for stage I HPV-positive disease (8th AJCC classification) primary surgical treatment followed by risk-stratified adjuvant treatment using one of the available minimally invasive techniques is an alternative. Currently ongoing de-escalation trials exploiting either surgery or radiation therapy as the primary treatment modality will inform in the future as to which approach will become the standard of care.

To determine the value of primary surgical treatment for more advanced HPV-negative disease requires careful investigation in prospective trials that are testing novel, more efficacious adjuvant treatment strategies.

Patients with HPV-positive recurrent operable disease should be treated aggressively given their rather favorable prognosis.

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Can We Expect Less Toxicities with Newer Forms of Radiotherapy?

Volker Budach and Alexander Thieme

Introduction

Squamous Cell Carcinomas of the Head and Neck (SCCHNs) comprise the largest subgroup (>90%) of all head and neck cancers and can be differentiated in Human Papillomavirus (HPV) postive or negative tumors, which both have a distinct prognosis. However, all head and neck tumors share a common anatomy which is particularly difficult to treat due to the proximity of the treatment site to critical organ structures. Cosmetic and functional deficits of surgical treatment for SCCHN have led to a reliance on organ preservation provided by radiation therapy (RTx). The major objective of external beam RTx has always been to deliver the prescribed dose to the cancer target while minimizing radiation exposure to the surrounding normal tissue and organs at risk (OARs) which are subject to radiation injury. Dryness of the mouth (xerostomia) and long lasting dysphagia, sometimes with feeding tube dependency for many years, are common side effects of RTx to the head and neck region. The vast majority of patients today are treated with photon based RTx. Due to the physical properties of this radiation type most of the energy is deposited along the entry path of the beam and it has an infinite range in patient tissue. Despite this unfavorable energy deposition profile, modelling the dose distribution to effectively spare normal tissue structures has been a key factor to reduce side effects for photon based RTx. Technological advances in conformal radiation techniques like 3-Dimensional Conformal RTx (3DCRT), Tomotherapy, Intensity-Modulated Radiation Therapy (IMRT), Volumetric Arc Therapy (VMAT) and Stereotactic RTx (SRT) and Radiosurgery (SRS) in conjunction with computed tomography (CT)-based treatment planning were major steps forward to an improved therapeutic index by limiting toxicities and increasing locoregional control (LRC). Since the nature of photon radiation cannot be changed, all

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aforementioned treatment modalities have in common that a higher number of beams is utilized in comparison with classical 2D radiation treatment. This leads to a reduced volume of normal tissue receiving higher radiation doses and a redistribution of the integral dose to a larger volume of healthy tissue receiving lower doses. This development required more precise daily positioning controls of patients and was paralleled by major improvements in image-guided radiotherapy (IGRT) techniques like Kilovoltage or Cone Beam CT-imaging.

Furthermore, proton therapy is a promising technology for SCCHN treatment which exploits the physical properties of protons to offer even further reductions in radiation exposure of healthy tissue and RTx related side effects experienced by patients.

Advances in External Beam Radiation Techniques

Conventional RTx

Conventional RTx had its advent with two-dimensional radiotherapy (2DRT). Simple setups with single beams from 1 to 4 directions and planning based on plain (2D) x-ray images were its characteristics. Large volumes of healthy tissue were irradiated to high doses causing significant acute and late morbidities like mucositis, xerostomia, dysphagia and fibrosis. With introduction of Computed Axial Tomography (CAT)-scans in the 1970s the tumor and OARs could be visualized for the first time, which prompted the development of a new kind of three-dimensional conformal radiotherapy (3DCRT). With 3DCRT radiation field setups could be substantially improved to better fit the size and shape of the tumor using multi-leaf collimators (MLCs), which in parallel led to a significant reduction of the irradiated volume of surrounding normal tissues compared with 2DRT. Still, there was room for improvement, because radiation fields used for 3DCRT were rather simple and delivered a uniform dose from a limited number of incident beams.

IMRT

Further advances in technology led to IMRT which enabled to generate highly complex radiation volumes from multiple angled radiation fields. In contrast to 3DCRT this technique uses MLCs with continuous movement during beam delivery and features intensity modulation by variable exposure times of leaf pairs. IMRT allows for more precise sparing of OARs and also enables target volumes with an inhomogeneous dose distribution. With IMRT a “simultaneous integrated boost” can be delivered, which uses different levels of doses for different target regions. Volumetric modulated arc therapy (VMAT) is an advanced type of IMRT, where the MLCs move dynamically as the gantry of the linear accelerator (LINAC) rotates around

the patient. Instead of choosing specific angles for treatment, VMAT may utilize principally a 360° coplanar arrangement for beam delivery while steadily adjusting to the tumor shape.

Proton Therapy

Proton therapy (PT) might lead to additional benefits for a selected group of patients with SCCHN. The profiles of energy deposition between protons and photons are entirely different and protons are in favor for cancer treatment. Protons release most of their energy in a characteristic peak, the so-called Bragg Peak. Beyond this peak there is a steep dose fall-off which results in a finite range of protons in patient tissue. Also the entry portion of the beam path receives a less integral dose than with photons. Both physical properties facilitate an improved OARs sparing in proton based RTx. PT was first introduced as passive scattering beam technique which uses scattering devices to broaden the proton beam and a range-modulation device to create a spread-out Bragg peak. The scanning beam technique is of a more recent date and uses magnets to deflect the proton beam to deliver the radiation dose to the target volume layer by layer with protons of different energies. With the scanning beam technique a higher conformality to the target volume can be achieved using inverse planning methods. Inverse planning can be done either with single field optimization (SFO) or multifield optimization (MFO). MFO has more degrees of freedom and is generally more conformal than SFO. Intensity-modulated proton therapy (IMPT) refers to proton therapy taking advantage of MFO. Substantial reductions of the integral dose in healthy tissue may afford new proton RTx schemes with decreased treatment related toxicities and/or increased tumor control by dose escalation.

Take Home Message for Advances in External Beam Radiation techniques

- Modern radiation techniques allow sparing of healthy tissue while retaining tumor efficacy and thus afford a further improvement of the therapeutic index of RTx by minimizing the incidence of adverse events.

Adverse Events Assessment

Grading Scales for Adverse Events

RTx is a major treatment option for locally advanced SCCHNs which may be used as sole treatment or as part of a multimodality approach. Aggressive treatments with curative intent may include surgery, RTx, chemotherapy (CTx) and immunotherapy or a combination thereof. Often, each of the involved treatments and their

combinations are associated with characteristic acute and late side effects which for themselves represent an important clinical outcome. Acute side effects occur during the treatment or within a 90 days period since the start of treatment, whereas late effects evolve over time and can occur many years later. Acute effects are generally reversible; however, they may endanger the oncological outcome due to inability or noncompliance of the patient to complete the entire course of the planned treatment. On the other hand, once late side effects emerge, they usually persist and are consequently an important factor to substantially reduce the patients' quality of life (QoL). The ability to grade acute and late side effects according to their severity is crucial and led to the development of sophisticated grading scales such as the RTOG/EORTC Radiation Toxicity Grading [1], LENT-SOMA [2] and CTCAE [3].

CTCAE stands for Common Terminology Criteria for Adverse Events and has been developed by the US National Cancer Institute (NCI). Many efforts have been devoted to the CTCAE grading system whose version 5.0 has been published and became effective in April 2018. CTCAE incorporates acute and late morbidities, delivers exact definitions about each adverse event (AE) and associates the severity of the AE to grades reaching from 1 (mild symptoms), 2 (moderate), 3 (severe), 4 (life-threatening) to 5 (patient died related to AE). For each AE item clinical parameters are provided to precisely define the severity grading and make the assessment observer-independent. CTCAE consists of more than 800 defined AEs and their grading which delivers the definitions necessary to comprehensively describe side effects in trials and routine clinical practice. Table 1 contains the CTCAE v5.0 definitions for the assessment and grading of xerostomia. AEs can be grouped into three general categories: (1) laboratory abnormalities (e.g., neutropenia), (2) observable events (e.g., radiation dermatitis) and (3) symptomatic AEs (e.g., nausea). In many trials it is still current practice that all of these three categories are investigator-reported. Particularly for the category of investigator-reported symptomatic AEs some authors have reported a lack of reliability [4] and systematic under-reporting [5–8]. Furthermore, the current workflow to assess AEs in clinical trials usually involve a cascade of data transfer between multiple health care professionals which is prone to the loss of valuable information [9].

Table 1 Exemplarily AE assessment and grading of xerostomia with CTCAE v5.0

Adverse event		Dry mouth
Definition	A disorder characterized by reduced salivary flow in the oral cavity	
Grade	1	Symptomatic (e.g., dry or thick saliva) without significant dietary alteration; unstimulated saliva flow >0.2 ml/min
	2	Moderate symptoms; oral intake alterations (e.g., copious water, other lubricants, diet limited to purees and/or soft, moist foods); unstimulated saliva 0.1–0.2 ml/min
	3	Inability to adequately aliment orally; tube feeding or TPN indicated; unstimulated saliva <0.1 ml/min
	4	Not applicable
	5	Not applicable

Patient-reported outcomes (PROs) may offer a solution to the problems faced and facilitate that patients directly enter AEs into the research database without the need of additional layers. A complementary grading system called PRO-CTCAE has been created by the NCI and has been published in its first version with a library consisting of 124 items which characterize severity, frequency and activity interference of symptomatic AEs [9]. In a recent phase III trial comparing web-based PROs and classical scheduled follow-up, an improved overall survival (OS) could be demonstrated for patients with lung cancer due to earlier relapse detection [10].

Comparison of Toxicity Profiles

Oncological therapy is inherently associated with AEs and a complete, accurate and examiner-independent documentation at regular intervals is essential for clinical trials. Toxicity information is necessary to weigh the costs in terms of AEs and benefits in regard to OS and tumor control of oncological treatments. While outcome by the means of OS can clearly be defined with a binary variable (dead vs. alive), toxicities may present a variegated picture. Different types of AEs may occur sequentially or simultaneously characterized by severity, frequency, duration and even relevancy for the patient. In the course of a clinical trial AEs are collected at protocol-specific time intervals. A vast amount of data is generated in this step which contains in-depth information about the toxicity profile of the oncological treatment in question. While AE grading systems like CTCAE are very well capable of capturing the type of AE and its severity, the resulting toxicity profiles need to be summarized and the information condensed to allow comparisons of different treatment regimens. Traditionally, the following methods have been used: maximum toxicity over time (max-time) and maximum grade among events (max-grade). The max-time method summarizes a toxicity profile by use of the highest grade of each AE type in a given time interval. Especially with higher-toxicity multimodality approaches a patient might encounter several episodes of AEs with different severity (e.g., episodes of nausea with each cycle of CTx). With the max-time method, information regarding lower-grade AEs and the number of episodes are lost. Mahoney et al. [11] concluded from the AE datasets of 26 North Central Cancer Treatment Group trials that 72% of the AE data would be omitted with the max-time method. The max-grade method summarizes the maximum grade across multiple types of AEs and calculates an incidence rate. If a patient experiences several high-grade AEs of different type (e.g., neutropenia and radiation dermatitis), this information is concealed by the max-grade method. With an increasing number of AEs per patient max-time as well as max-grade methods progressively exclude AE data and underestimate toxicity. For this reason a new scoring system called TAME to summarize AE data was introduced by Trotti et al. [12] which accounts for the multiplicity and time dimensions of AEs. The datasets comprising 5 RTOG head and neck cancer trials were evaluated and compared by use of the max-grade method and TAME. The max-grade method excluded 29–70% of high-grade AEs with a

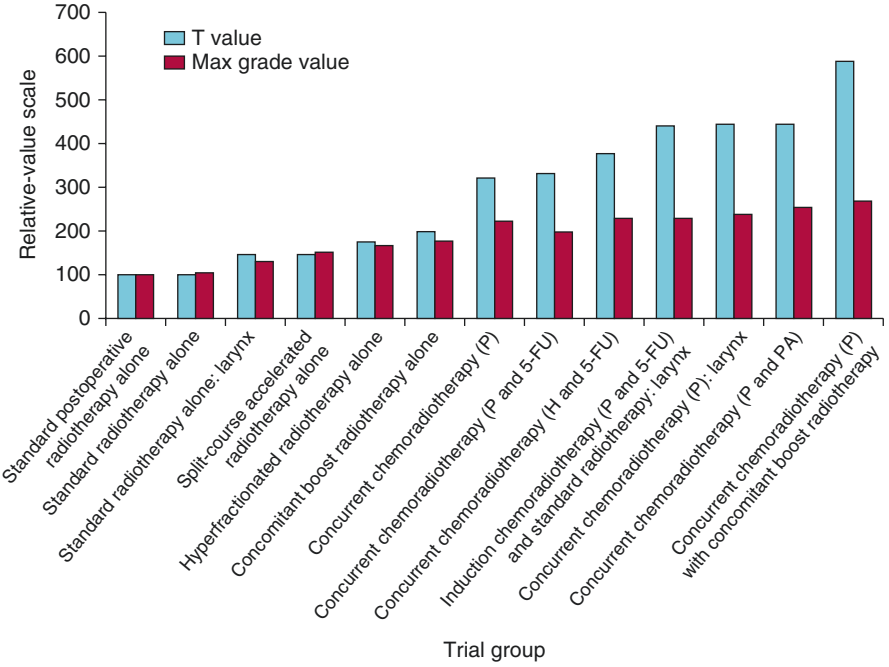


Fig. 1 Acute toxicity datasets of 13 treatment regimens summarized by max-grade method and TAME T-value relative to standard postoperative RTx. Abbreviations: *P* platinum, *H* hydroxyurea, *5-FU* fluorouracil, *PA* paclitaxel [12]

tendency for increased bias towards higher-toxicity treatment regimens. TAME calculates 3 different scores to characterize toxicity: the T-value represents the average number of grade 3 and 4 acute AEs; the A-value the average number of grade 3 and 4 late AEs; and the M-value the risk of death related to cancer treatment. For 13 treatment regimens with prospectively collected acute toxicity data of 2300 patients relative max-grade and T-values in relation to postoperative RTx were calculated (Fig. 1). T-values showed up to a 590% increase in the toxicity burden compared with up to a 270% increase with the max-grade method. Addition of CTx in larynx preservation was associated with an increase of 300% in the T-value and 75–82% increase with the max-grade method. TAME scores suggest a far higher toxicity profile than classical methods.

Take Home Message for Adverse Events Assessment

- Comprehensive grading systems, most notably *CTCAE*, have been developed to assess AEs in the setting of clinical routine as well as complex RTx trials involving multimodal treatment.
- *Patient-reported outcome* can be used to improve symptomatic AE assessment and to better incorporate the patient's perspective on side effects.

- Summarizing AE data can be challenging because a large portion of the in-depth information of toxicity profiles can be concealed. If treatment regimens are compared, it must be taken into consideration that toxicity might be underestimated, especially if classical approaches (e.g., max-time or max-grade methods) are utilized to condense AE data. New scoring systems like *TAME* need to be further elaborated.

Toxicity of IMRT vs. Conventional RTx

LCR and OS of locally advanced SCCHN could be significantly improved by intensification of RTx either using altered fractionation schedules [13] or concurrent CTx [14]. However, these treatment schedules have led to increased toxicities [12, 14, 15]. Modern radiation techniques like IMRT or VMAT allow dose-escalated radiotherapies accompanied by highly conformal dose distributions to planning target volumes (PTVs) and a steep dose fall-off outside these volumes allowing decent sparing of healthy tissues. In order to enable normal tissue sparing critical structures need to be delineated in the planning CT and the optimized RTx plan must comply with dose constraints specific for each organ. A variety of OARs in the head and neck region including the salivary glands, swallowing structures, larynx, spinal cord, brainstem and oral cavity must be considered supplemented by additional structures like inner ears, optic nerves, chiasm and temporal lobes for nasopharyngeal cancer. From today's perspective accurate delineation of critical structures for SCCHN already represents a challenge, nonetheless further delineation of anatomical substructures can be expected in the future [16, 17].

A selection of prospective trials comparing acute and late toxicities of conventional RTx (2DRT or 3DCRT) with IMRT in the management of SCCHN can be found in Table 2. In the following we will focus on side effects of xerostomia, dysphagia, mucositis, fatigue and impairment of voice.

Xerostomia

Irradiation of the head and neck region can cause radiation injury of the parotid and submandibular glands resulting in hypofunction and dry mouth. There is an inverse relationship between xerostomia and QoL. Xerostomia has been defined by Eisbruch et al. as the reduction of salivary flow below 25% of the pre-therapeutic value [18]. Since the advent of IMRT efforts were made to reduce xerostomia by parotid sparing.

In a phase III trial by Nutting et al. [19], patients (n = 94) with stage I-IV oropharyngeal and hypopharyngeal SCCHN were randomized either to 2DRT or IMRT. Fractionation schemes included 5 × 2.17 Gy up to 65 Gy for primary and

Table 2 Selection of prospective (randomized and non-randomized) trials comparing toxicity and QoL of conventional RTx with IMRT

Author/ year/ reference	Study type	No. patients	Sites/no. patients NP/ OP/OC/HP/L	Stage (UICC)	Fractio-nation schedule CRT/ IMRT	OTT (weeks) CRT/ IMRT	Total dose CRT/ IMRT	CRT	CTX	Time of Measure-ment (w = week, m = month, yr = year)	Conclusions
Chen et al./2012/ [40]	Non-Rand.	155	13/61/43/–/– (HP + L = 21)	I–IV	NS/NS (SIB)	NS/NS	NS/NS	3D	Conc	Post-RTx: 2 w, 4 w, then every 2–3 m till 1st yr, every 6 m till 3rd yr, every 12 m thereafter	The early QoL improvements associated with IMRT not only are maintained but apparently become more magnified over time
Nutting et al./2011/ [19]	Rand	94	–/80/–/14/–	I–IV	Primary: 2.17 Gy/2.17 Gy Adjuvant: 2 Gy/2 Gy	6/6	Primary: 65 Gy/65 Gy Adjuvant: 60 Gy/60 Gy	2D	Neo	Baseline, post-RTx: 2 w, 3 m, 6 m, 1 yr, 18 m, 2 yr	IMRT significantly reduces the incidence of xerostomia and leads to recovery of salvia secretion and improvements of QoL
Fang et al./2008/ [41]	Non-Rand	203	203/–/–/–/–	I–IV	1.8 Gy/1.8 Gy	7.2–8.4/7.2–8.4	64.8 Gy–75.6 Gy/ 64.8 Gy–75.6 Gy	3D	Conc	Baseline, during RTx, post-RTx 3 m, 1 yr, 2 yr	Potential advantage of IMRT might occur in QoL outcome during the recovery phase of acute toxicity

Jabbari et al./2005/ [42]	Non-Rand	40	–/17/10/4/9	III–IV	NS/NS	NS/NS	63–76.8 Gy/60–78 Gy	NS	Yes	Baseline, post-RTx: 1 m, 3 m, 6 m, 12 m, 18 m, 24 m	Xerostomia and QoL improved over time (≥6 m post-RTx) with IMRT and not CRT
Pow et al./2006/ [43]	Rand	45	45/–/–/–/–	II	2 Gy/2 Gy	6.8/7	68 Gy (+10 Gy boost)/68–72 Gy	2D	No	Baseline, post-RTx: 2 m, 6 m, 12 m	IMRT was significantly better in terms of parotid sparing and improved QoL
Vergeer et al./2009/ [34]	Non-Rand	241	8/80/29/29/90	I–IV	Primary: 2 Gy/2 Gy (SIB) Adjuvant: 2 Gy/2 Gy (SIB)	Primary: 5.8–7/5.8–7 Adjuvant: 5.6–6.6/5.6–6.6	Primary: 70 Gy/70 Gy Adjuvant: 56–66 Gy/56–66 Gy	3D	Conc	Baseline, 6 w post-RTx, then every 6 m	IMRT results in significant reduction of investigator- and patient-reported xerostomia. Significant improvement of HRQoL
Gupta et al./2012/ [23]	Rand	60	–/32/–/17/11	I–IV	2 Gy/2.2 Gy (SIB)	7/6	70 Gy/66 Gy	3D	Conc	4–6 w post-RTx, 3–4 m till 2nd yr, 6 m thereafter	IMRT reduces incidence and severity of xerostomia
Kam et al./2007/ [24]	Rand	56	56/–/–/–/–	I–II	2 Gy/2Gy	6.6/6.6	66 Gy + brachy-therapy 18 Gy/66 Gy + brachy-therapy 12 Gy	2D	No	Baseline, post-RTx: 6 w, 6 m, 1 yr	IMRT results in less severe delayed xerostomia. No significant difference in PROs

(continued)

Table 2 (continued)

Author/ year/ reference	Study type	No. patients	Sites/no. patients NP/ OP/OC/HP/L	Stage (UICC)	Fractio-nation schedule CRT/ IMRT	OTT (weeks) CRT/ IMRT	Total dose CRT/ IMRT	CRT	CTX	Time of Measure-ment (w = week, m = month, yr = year)	Conclusions
Ghosh-Laskar et al./2015/ [20]	Rand	59	–/25/3/19/12	I–IV	2 Gy/2.2 Gy (SIB)	7/6	70 Gy/66 Gy	3D	Conc	8 w post-RTx, then every 3 m till 2nd yr, every 6 m till 5th yr	IMRT reduces incidence of acute and late ≥2 grade xerostomia
Peng et al./2012/ [25]	Rand	616	616/–/–/–/–	I–IV	2 Gy/2.12 Gy	7–7.4/6.6	70–74 Gy (+ boost + brachy-therapy)/70 (+ brachy-therapy)	2D	Neo/ Conc/ Adj	4 w post-RTx, every 3 m till 2nd yr, thereafter every 6 m	IMRT had improved local-recurrence free survival and lower incidence of acute and late toxicities e.g. xerostomia, hearing loss, temporal lobe neuropathy
Braam et al. /2006/ [44]	Non-Rand	56	–/56/–/–/–	I–IV	Primary: 2 Gy/2.3 Gy Adjuvant: 2 Gy/ NS	Primary: 7/6 Adjuvant: 5–7/NS	Primary: 70 Gy/69 Gy Adjuvant: 50–70 Gy/NS	Mostly 2D	No	Baseline, post-RTx: 6 w, 6 m	IMRT significantly reduces the number of parotid flow complications

Abel et al./2017/ [45]	Non-Rand	207	–/138/39/11/–	I–IV	NS/1.7 Gy bid or 2 Gy	NS/3.8–5.7	NS/64.6 Gy[a] or 68 Gy[a]	3D	Neo	Baseline, post-RTx: 1 m, 2 m, 3 m, 6 m, 12 m	IMRT has improved long-term QoL but might cause more acute side effects
Total	Non-Rand: 6 Rand: 6	1832	941/489/124/ 94/122					2D: 5 3D: 6 NS: 1	Yes: 9 No: 3		12/12 trials see favorable effects for IMRT

[a]Combination of IMRT and 3DCRT has been used

Abbreviations: *Rand* randomized, *Non-Rand* non-randomized, *NP* nasopharynx, *OP* oropharynx, *OC* oral cavity, *HP* hypopharynx, *L* larynx, *UICC* union for international cancer control, *CRT* conventional RTx, *IMRT* intensity-modulated RTx, *bid* two fractions per day, *OTT* overall treatment time, *CTx* chemotherapy regime, *Conc* concurrent, *Neo* neoadjuvant, *Adj* adjuvant, *2D* 2D radiation therapy, *3D* 3D conformal RTx, *NS* not specified, *QoL* quality of life

5 × 2 Gy up to 60 Gy for postoperative RTx in both arms. Neoadjuvant CTx was administered to 40–43% of the patients. Mean dose to contralateral parotid was significantly lower in the IMRT-arm (25.4 Gy vs. 61 Gy; $p < 0.0001$). At the first follow-up after 3 months post-RTx patient-reported grade 2 or worse xerostomia was assessed for 87% of the patients in the 2DRT-arm versus 76% in the IMRT-arm (Fig. 2). In subsequent visits symptoms significantly improved in the IMRT-arm; at 12 months the rate of patients with grade 2 or worse xerostomia dropped to 38% in the IMRT-arm while only slightly improving to 74% in the 2DRT-arm ($p = 0.0027$). Similar findings could be made for investigator-reported RTOG scaling of xerostomia (Fig. 2). Additionally, an objective parameter of salivary flow was measured. At 12 months post-RTx an unstimulated salivary flow from the contralateral parotid could be measured in 47% of the patients in the IMRT-arm and 0% in the 2DRT-arm. At the same time there was also a significant difference in the stimulated salivary flow in favor for the IMRT-arm. A strong correlation between salivary flow and xerostomia symptoms was observed.

Ghosh-Laskar et al. [20] randomized 56 patients with stage I–IV SCCHN to equally sized arms comparing conventional 3DCRT with IMRT. Patients in the 3DCRT-arm were treated with 5 × 2.0 Gy up to 70 Gy and in the IMRT-arm with a simultaneous integrated boost (SIB) with 5 × 2.2 Gy up to 66 Gy. Patients received concurrent CTx with a weekly cisplatin regime (30 mg/m^2) in both arms. Sparing of parotid glands in the IMRT-arm resulted in lower mean doses of the ipsilateral (40.5 Gy vs. 56.7 Gy; $p < 0.001$) and contralateral parotid (34.1 Gy vs. 48.2 Gy; $p < 0.001$). The author correlated this with the salivary scintigraphy data at 8 weeks post-RTx which can be used as an objective measure for the parotid function [21]. The post-sialogue ejection fraction of the ipsilateral parotid was significantly higher in the IMRT-arm compared with the 3DCRT-arm (32.9% vs. 18.2%; $p = 0.019$). The rate of patients with grade 2 or worse xerostomia was significantly lower in the IMRT-arm at 8 weeks post-RTx (24% vs. 53%; $p = 0.024$) and subsequent visits. Furthermore, long-term weight loss in the 3DCRT-arm was significantly higher (50% vs. 21%; $p = 0.038$).

Marta et al. [22] conducted a meta-analysis comparing IMRT with 2DRT and 3DCRT. The data of four prospective phase III randomized trials [19, 23–25] was combined to substantiate the effect of radiation techniques on xerostomia. A significant overall benefit of IMRT could be identified with a hazard ratio (HR) of 0.76 (95% CI: [0.66, 0.87]; $p < 0.0001$) (Fig. 3).

In summary, a consistent picture can be drawn from all aforementioned studies: IMRT significantly reduced the dose to the contralateral parotid gland with less post-RTx salivary flow decline and less xerostomia as assessed by investigator and patient-reported AEs and QoL scales.

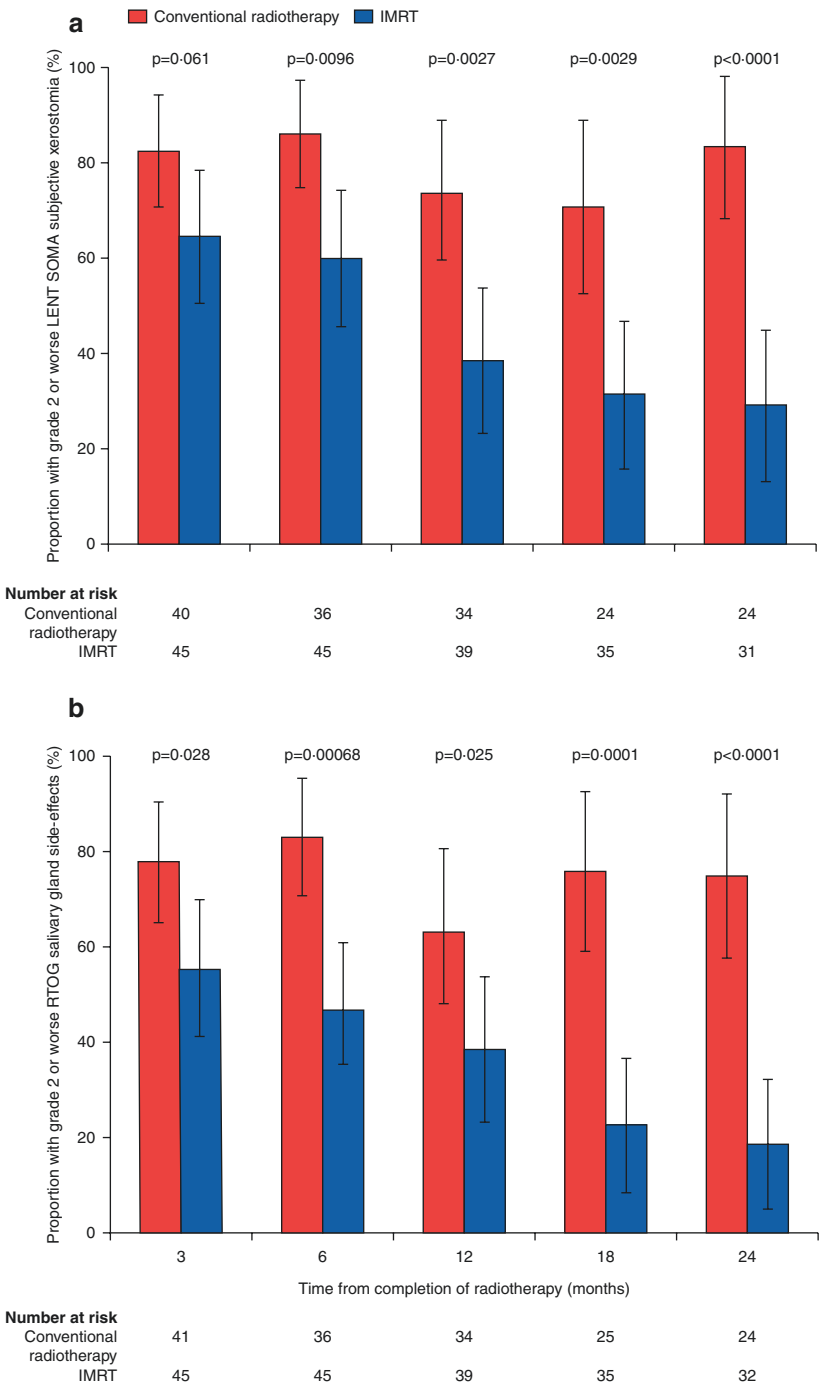


Fig. 2 Temporal evolvement of xerostomia 3 to 24 months post RTx. (**a**) patient-reported grade 2 or worse xerostomia (LENT-SOMA), (**b**) investigator-reported grade 2 or worse (RTOG). Over time there is an increasing gap in favor of IMRT [19]

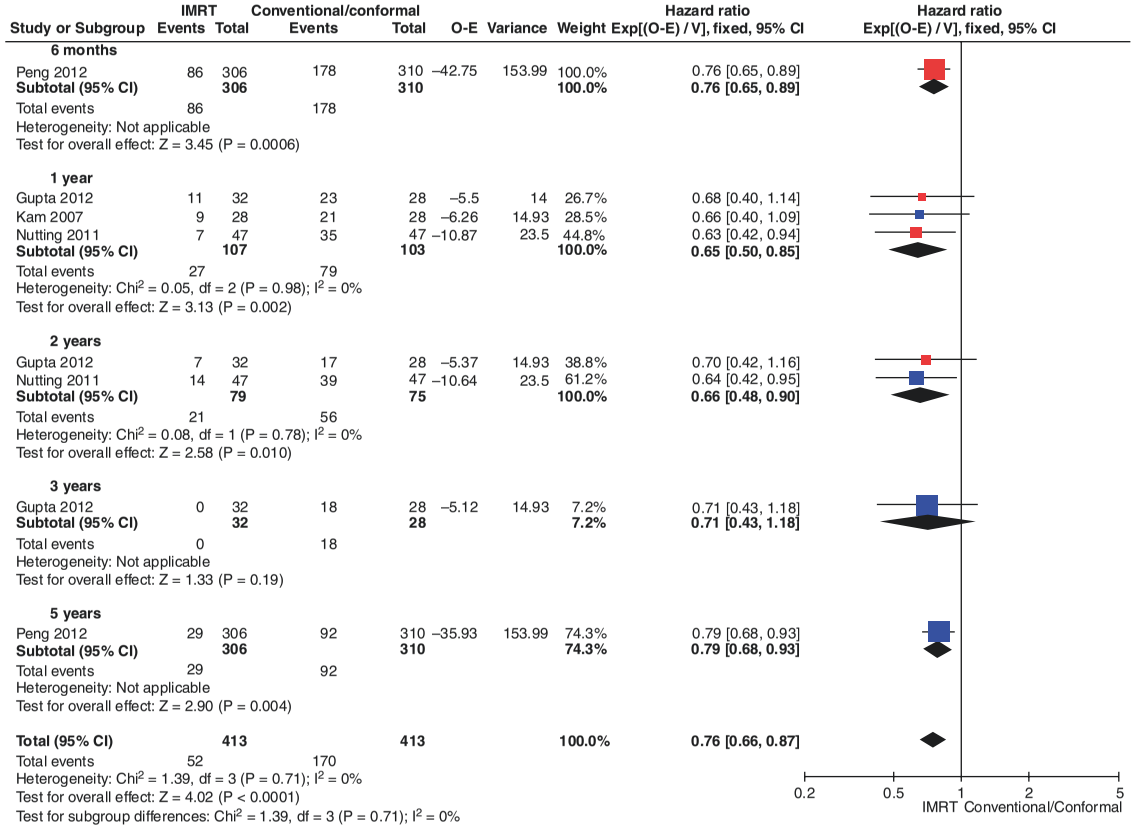


Fig. 3 Meta-analysis of xerostomia grade 2 or worse comparing 2DRT/3DCRT with IMRT. A significant overall benefit of IMRT was found: HR = 0.76 (95% CI: [0.66, 0.87]; $p < 0.0001$) [22]

Dysphagia

Dysphagia is a common side effect following RTx of the head and neck region. Dysphagia is rated as an important AE with one of the highest priorities for patients [26, 27]. Anatomical structures associated with RTx induced dysphagia are the pharyngeal constrictor muscles (PCM) and glottic and supraglottic larynx (GSL) [28, 29]. Retrospective studies suggest a dose-effect relationship with these structures [30]. Nutting et al. [19] reported dysphagia grade 3 or worse at 12 months post-RTx in 5% of the patients in the 3DCRT-arm and 9% in the IMRT-arm which did not implement sparing of swallowing structures. In a study of Maurer et al. [31] at 12 months post-RTx 15% of the patients experience grade 3 or worse dysphagia. Feng et al. [32] prospectively evaluated this topic in a non-comparative study of 73 patients with stage III-IV oropharyngeal cancer. IMRT was implemented to specifically spare the swallowing structures PCM, GSL and esophagus. Average mean doses and standard deviations delivered to these structures were 58 Gy ± 8 Gy for PCM, 48 Gy ± 14 Gy GSL and 34 Gy ± 13 Gy for the esophagus. The following instruments were used to assess dysphagia before and after RTx: patient-reported Swallowing and Eating Domain scores of the Head and Neck Quality of Life questionnaire, CTCAE and Swallowing Performance scale utilizing videofluoroscopy. Despite having no control group, the authors could conclude that long-term investigator, patient-reported and objective measures worsened only mildly in comparison with pre-therapeutic scores. A phase III randomized multicenter trial called DARS (ISRCTN25458988) has been initiated which investigates the benefits of IMRT based swallow sparing strategies and will provide further insight into this topic [33].

Mucositis

Mucositis is an acute toxicity which results from the irradiation of mucosal tissue located in the oral cavity. It is one of the main limiting factors of chemoradiation (CRTx) for locally advanced SCCHN. Incidence of mucositis increases if RTx is combined with CTx, particularly with the pyrimidine-analog 5-fluorouracil. Mucositis can be a direct effect of treatment which interferes with the turnover of epithelial cells or an indirect effect of the invasion of bacterial and fungal species due to a compromised immune status of the patient. Pain resulting from mucositis frequently leads to less oral intake compromising the nutrition status of the patient often resulting in weight loss. In a randomized controlled trial by Gupta et al. [23], 60 patients with stage I-IV SCCHN received concurrent CRTx with a total dose of 70 Gy in 35 fractions in the 3DCRT-arm and 66 Gy in 30 fractions in the IMRT-arm. Grade 2 mucositis was observed in 22 of 28 patients (78.5%) in the 3DCRT-arm and 23 of 32 patients (71%) in the IMRT-arm. This difference was even greater for grade 3 mucositis affecting 4 of 28 patients (14.5%) in the 3DCRT-arm compared with 2 of 32 (6%) in the IMRT-arm, however the overall effect was statistically not

significant in this cohort ($p = 0.2$). A prospective non-randomized trial conducted by Vergeer et al. [34] found a significant difference in grade 3 mucositis from weeks 3 to 7 after start of treatment in favor of IMRT (p values ranging from 0.0001 to 0.031). The authors attribute this finding to the reduced dose per fraction to elective target volumes since a simultaneous integrated boost concept was used. The authors also noted that the primary objective of this trial was parotid sparing with evaluation of effects on xerostomia. Another explanation could be that preservation of the salivary function has protective effects which also tend to decrease the incidence of mucositis and oral infections.

Fatigue

Fatigue is a multi-factorial symptom which can be related to the treatment (surgery, CTx and RTx), lower oral energy intake, disease, co-morbidities and other patient factors. CTCAE describes fatigue as a general weakness which at grade 2 or worse is not relieved by rest and affects the activities of daily living. An unexpected finding of the phase III parotid sparing trial by Nutting et al. [19] was an increased fatigue of patients in the IMRT-arm. Fatigue symptoms 12 months after treatment were present in 55 of 89 patients (74%) in the IMRT-arm vs. 18 of 44 patients (41%) in the 3DCRT-arm which was statistically significant ($p = 0.0015$). In a secondary analysis of the data by Gulliford et al. [35] a dosimetric explanation for this observation was attempted. Due to IMRT-typical redistribution of the integral dose, higher mean and maximum doses in the posterior fossa, brainstem and cerebellum could be observed compared with 3DCRT. There was a statistically significant association with the mean and maximum dose of these structures with grade 2 or worse acute fatigue ($p \leq 0.01$). Powell et al. [36] also found associations between radiation of CNS structures and fatigue in a retrospective analysis comprising 40 patients receiving CRTx (39/40) for nasopharyngeal cancer. 60% of the patients experience fatigue grade 2 or worse during or following CRTx. Mean and near maximum doses (D2%) of the pituitary gland and basal ganglia were associated with acute fatigue symptoms during RTx ($p < 0.01$). Mean doses of the cerebellum correlated with late fatigue symptoms ($p < 0.01$). Prospective studies investigating the dose to these anatomical structures are necessary to further support these findings and eventually take some preventive measures by establishing new dose constraints for tolerance.

Impairment of Voice

Destruction caused by head and neck tumors and cancer treatment can adversely impact voice and speech outcomes. Depending on the site of the tumor, articulation and speech can be affected by oral cavity tumors (e.g. due to restrictions in the mobility of the tongue) or quality of voice by laryngeal tumors. Impairment of voice

quality related to CRTx may be associated with degraded vocal cord movement and incomplete closure, dryness of the laryngeal mucosa, muscle atrophy, fibrosis, hyperemia and erythema [37]. Symptoms experienced by patients include vocal efforts, audible or excessive breathing and hoarseness [38]. Currently, there are no data from randomized controlled trials concerning the impact of conventional RTx and IMRT on voice outcomes. In a study by Kraaijega et al. [39] voice and speech of 22 patients were assessed more than 10 years after concomitant CRTx. The patients are long-time survivors of a randomized trial conducted between 1999 and 2004 and were treated for stage IV oral cavity, oropharynx or hypopharynx SCCHN. They received normo-fractionated 70 Gy with concurrent cisplatin in three cycles of 100 mg/m^2 or four cycles of high-dose (150 mg/m^2) intra-arterial injections. Of these 22 patients, 10 (45%) had been treated with IMRT and 12 (55%) with conventional RTx. Voice outcomes were assessed by speech language pathologists (SLPs), automatic computerized evaluation and PROs. As assessed by two SLPs patients treated with IMRT showed better scores for perceptual speech intelligibility (median score 873 vs. 616; $p = 0.006$). For automatic evaluation, no significant association with the RTx technique could be found. However, PROs showed significantly better scores for the Voice Handicap Index and Speech Handicap Index ($p = 0.021$), further supporting IMRT over conventional RTx.

Take Home Message for Toxicity of IMRT vs. Conventional RTx

- *Xerostomia*: IMRT with parotid sparing has a superior toxicity profile compared with conventional RTx.
- *Dysphagia*: So far no randomized trials have shown a benefit of IMRT. IMRT could be beneficial, if additional OARs are contoured and specifically optimized for sparing of swallowing structures. Results from the DARS trial are awaited with great interest.
- *Mucositis and Impairment of Voice*: Studies showed that IMRT was beneficial, but currently there are no randomized controlled trials to support these results.
- *Fatigue*: Retrospective studies showed a higher prevalence in patients treated with IMRT which could be explained by a redistribution of the integral dose to the CNS.

Potential Benefits of Proton Therapy (PT)

Due to favorable physical properties and dose distribution, PT has the potential to deliver less toxic RTx to patients with SCCHN. However, up to date randomized controlled trials investigating a difference in toxicity of protons compared with photons have not been conducted. PT is associated with high installation and operating costs. There is an ongoing debate whether benefits of PT justify the investments. On the other hand randomized controlled trials can only be performed if sufficient capacity to treat patients with PT is available which leads to a causality dilemma.

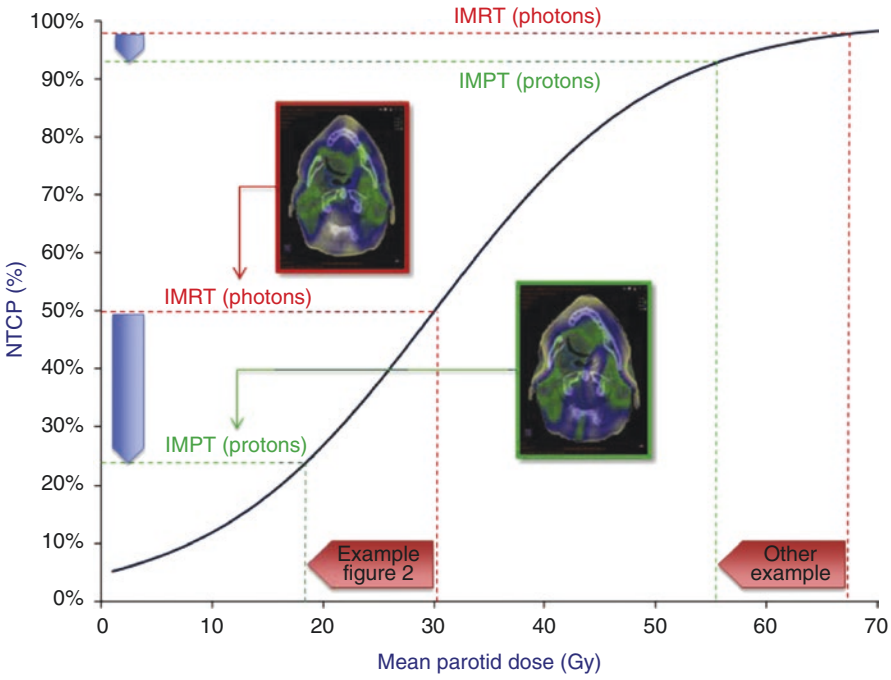


Fig. 4 Translation of dosimetric parameters from a ISPC-study to changes in NTCP probability for xerostomia. A reduction of the mean parotid dose from 30.1 Gy to 18.4 Gy leads to a reduction of NTCP probability for xerostomia from 50% to 24% (lower blue arrow). The same absolute reduction results only in minimal changes for the NTCP probability from 98% to 94% if the initial mean dose is higher (upper blue arrow) [46]

Langendijk et al. [46] describe a model-based approach for effective proton vs. photon RTx comparisons. The model-based approach consists of two phases. The purpose of the first phase is to select patients who will most likely benefit from PT. It consists of 3 steps: (1) development and validation of Normal Tissue Complication Probability (NTCP) models with the data of patients treated with photons, (2) in silico planning comparative studies (ISPC) comparing photon vs. proton RTx plans and (3) estimation of the reduction of side effects by combining the dosimetric results of ISPC with NTCP-models (Fig. 4). Using this methodical pattern patients can be identified who are eligible to receive PT (Fig. 5). The second phase of the model-based approach consists of the clinical validation of the selected group by either sequential prospective observational cohort studies with historical comparison or by randomized controlled trials.

Van der Laan et al. [48] presented a ISPC study for 25 patients with oropharyngeal and hypo-pharyngeal cancer for reduction of swallowing dysfunction. Swallowing OARs (SW-OARs) were the superior pharyngeal constrictor muscle and the supraglottic larynx. Standard IMRT (ST-IMRT) was compared with SW-OAR optimized photon (SW-IMRT) and proton (SW-IMPT) treatment plans.

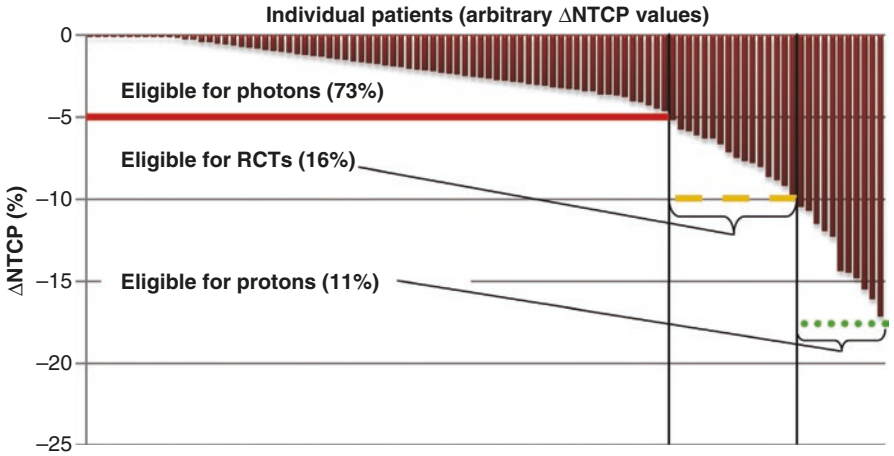


Fig. 5 Difference in NTCP (ΔNTCP) for individual proton and photon plans. Patients with a <5% ΔNTCP should continue to be treated with photons, with a 5–10% ΔNTCP should be evaluated in randomized controlled trials and with >10% ΔNTCP should receive protons [47]

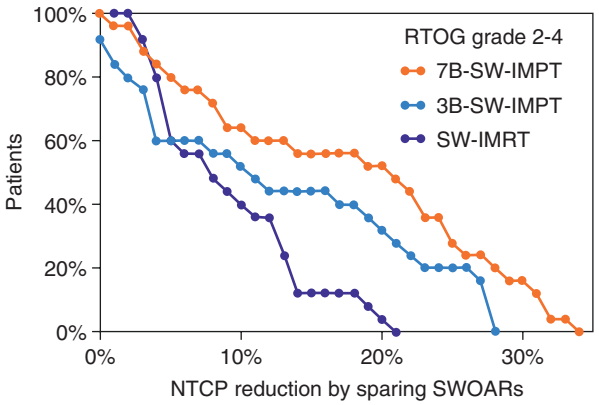


Fig. 6 Potential reductions of NTCP for swallowing dysfunction by swallowing OARs optimized IMRT (SW-IMRT) and IMPT with 3 and 7 beams (3B-SW-IMPT/7B-SW-IMPT) [48]

Dosimetric results were combined with a NTCP model to predict investigator-reported RTOG grade 2–4 swallowing dysfunction [49]. On average the probability of swallowing dysfunction could be reduced by 8.8% with SW-IMRT and 17.2% with SW-IMPT utilizing 7 beams. Cumulative plots for potential reductions are shown in Fig. 6.

Cheng et al. [50] describes the prototype of an online platform for proton decision support (PRODECIS) which compares photon and proton RTx plans in measures of dosimetry, predicted toxicity and cost-effectiveness. The decision support system can be used by a website to upload comparative photon and proton RTx plans and to enter clinical relevant parameters necessary for NTCP calculation. The system was evaluated with the dataset of van der Laan comprising 25 patients with

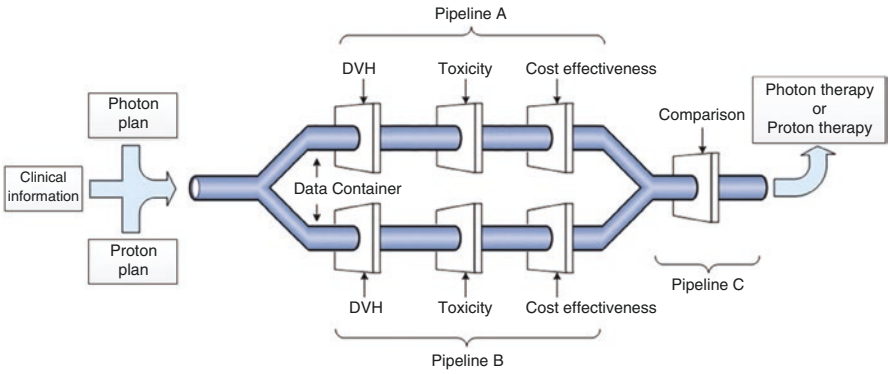


Fig. 7 Working pipeline of the proton decision support (PRODECIS) system comparing dosimetry, predicted toxicity and cost-effectiveness [50]

SWOAR optimized IMRT and IMPT RTx plans [48]. Cost-effectiveness was calculated as cost per quality-adjusted life-year (QALY) with a limit of 80.000€ per additional QALY. PT was found to be cost-effective in 8 of 23 patients (34.7%). The working pipeline of the system is shown in Fig. 7.

Take Home Message for Potential Benefits of Proton Therapy

- *Protons* have favorable physical properties for RTx which could translate into less toxicity in SCCHN treatment.
- *Evidence* from randomized controlled trials for reduced toxicity of PT is still *missing*.
- *A model-based approach* which incorporates comparative PT treatment planning and NTCP modelling is promising to select patients who benefit most likely from PT.
- However, this model has to be clinically validated and might ultimately replace some randomized controlled trials.

Treatment De-Intensification Strategies for Human Papillomavirus (HPV)-Positive Oropharyngeal Squamous-Cell Carcinoma (OPSCC)

There is a growing body of evidence that HPV-positive OPSCC is a distinct biological entity [51, 52] with a higher response rate to CRTx and a significantly better prognosis independent of nodal status, age and tumor stage compared with tobacco- and alcohol-associated SCCHN [53–57]. In a study conducted by Ang et al. [53] patients with HPV positivity had a 58% reduction in the risk of death and a 3-year OS rate of >90%. In a meta-analysis Mehanna et al. [58] concluded that the

incidence of HPV-associated OPSCC increased from 40.5% between 2000 and 2004 to 72.2% between 2005 and 2009 ($p < 0.001$). Patients with this favorable prognosis are generally younger [59] and therefore would maximally profit if the toxicity profile of the SCCHN treatment could be reduced without impairment of treatment efficacy. Recent trials use different strategies for treatment de-intensification: dose de-escalation; substitution or elimination of concurrent CTx; and minimally invasive procedures.

Dose De-Escalation

Several prospective phase II and III trials are currently being conducted which pursue dose de-escalation strategies (Table 3). Chera et al. [60] published preliminary results from the NCT01530997 study comprising 43 patients with stage I–III, HPV-positive OPSCC and minimal smoking history. In 40 patients (86%) a pathologic complete response (pCR) could be achieved by CRTx with a de-intensified radiation dose of 60 Gy (instead of the typical 70 Gy) and 6 cycles of concurrent low-dose cisplatin (30 mg/m^2 weekly). Marur et al. [61] published first outcome results of the ECOG1308 trial which implemented a study design incorporating induction CTx response. Inclusion criteria were HPV-positive OPSCC with stages I-III and a smoking history of less than 10 pack-years. Patients (n = 80) were treated with a

Table 3 Current studies evaluating dose de-escalation for HPV-associated SCCHNs [62]

Trial	Phase	N	Inclusion criteria	Treatment
Radiotherapy de-intensification trials				
NRG HN-002 (NCT02254278)	II	296	T1–2, N1–2b, or T3, N0–2b disease and <10 PY HPV-positive OPC	Reduced-dose IMRT (60 Gy) with/without weekly cisplatin
NCT01530997	II	40	T1–3, N0–2c HPV-positive OPSCC if <10 PY or >5 years of abstinence	IMRT (54–60 Gy) with weekly cisplatin (30 mg/m^2)
ECOG 1308 (NCT01084083)	II	80	Resectable stages IIIA/IIIB and IVA/IVB HPV-positive OPSCC (p16- high or HPV-16 ISH positive)	IC, then response-adapted RT (54 or 66–70 Gy) with cetuximab
The Quarterback trial (NCT01706939)	III	365	Stage III/IV (M0) HPV-associated OPSCC/ unknown primary/ nasopharynx. Excludes active smokers/>20 PY	IC with TPF: patients with CR/ PR randomly assigned 2:1 to carboplatin with RT (56 versus 70 Gy) per week. Non-responders receive standard RT

Abbreviations: *p16* p16INK4A expression associated with HPV, *ISH* in situ hybridization, *OPC* oropharyngeal cancer, *OPSCC* oropharyngeal squamous cell carcinoma, *IC* induction chemotherapy, *TPF* docetaxel, cisplatin and fluorouracil, *CR* complete remission, *PR* partial remission, *PY* pack year, *RT* radiotherapy

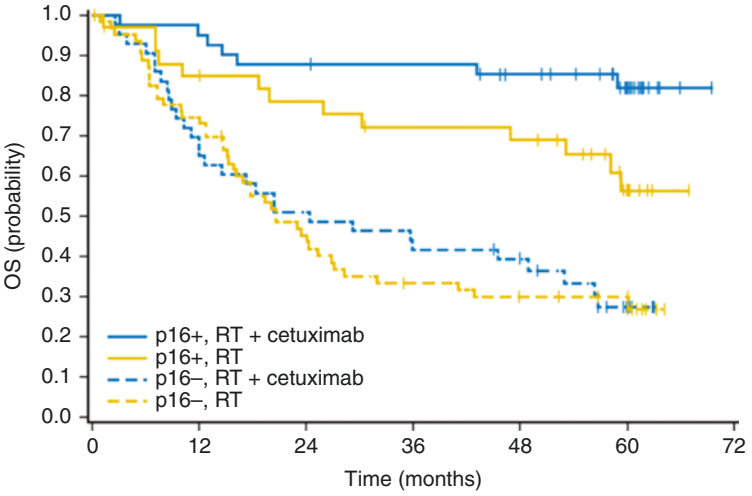
combination of cisplatin, paclitaxel and cetuximab as induction CTx and a radiation dose depending on the response to induction CTx. Patients with a clinical complete response (cCR) were defined as low-risk receiving 54 Gy. All other patients were considered high-risk and were treated with a radiation dose of 69.3 Gy. In both situations the radiotherapy was combined with cetuximab (250 mg/m^2 weekly during RTx). A cCR to induction CTx could be reached in 71% and a 1-year progression free survival (PFS) of 97% and 87% were observed in the low-risk group and high-risk group, respectively. Investigator-reported grade 4 toxicities were less than 5% in both cohorts.

Conventional CTx Substitution with Cetuximab

Cetuximab is a monoclonal antibody that specifically blocks the epidermal growth factor receptor (EGFR) and inhibits signaling pathways that promote cell growth. Up to 90% of SCCHNs express high levels of EGFR. Over expression of EGFR and TGF-α are associated with poor prognosis and radioresistance [63]. In a study of Bonner et al. (2006, 2010) [64, 65] patients (n = 213) with locally advanced SCCHN (stage III–IV) were treated with high-dose RTx with or without cetuximab weekly. RTx treatment regimens consisted of 70–72 Gy once-daily fractions and 72–76.8 Gy twice-daily fractions. The median OS in the cetuximab-arm was 49 months and 29.3 months with RTx alone (p = 0.03). Also the median duration of LCR was prolonged in the cetuximab-arm with 24.4 months vs. 14.9 months (p = 0.005). A further analysis of the data by Rosenthal et al. [66] indicated that the p16 status was strongly prognostic for patients with oropharyngeal cancer in this study and that the addition of cetuximab to RTx improved clinical outcomes regardless of p16/HPV status versus RTx alone. However, the interpretation of the data has led to discussions, as in particular the results in the p16 positive cohort was felt to be promising. The 3-year OS rate in that cohort was 82.1% in the cetuximab-arm vs. 70.4% in the RTx alone arm (Fig. 8) while LRC was 81.5% in the cetuximab arm versus 64.8% in the RTx alone arm. For this reason, the substitution of conventional CTx with cetuximab is evaluated in a series of prospective trials (Table 4).

De-Intensification of Adjuvant CRTx

In the setting of post-operative HPV-positive SCCHN several strategies are combined. New surgical techniques no longer require mandibulotomy to enable resection of oropharyngeal cancer. Instead, miniaturized instruments are utilized for transoral robotic surgery (TORS) to resect tumors through the mouth, significantly



No. of patients at risk

p16+, RT + cetuximab	41	39	36	35	31	17	0
p16+, RT	34	28	25	22	21	10	0
p16–, RT + cetuximab	43	29	22	18	15	6	0
p16–, RT	64	47	27	19	16	13	0

Fig. 8 Kaplan-Meier plots for OS regarding patients with locally advanced SCCHNs in relation to their HPV-status and high-dose RTx with or without cetuximab [66]. Rosenthal et al, J Clin Oncol 2016, 34/12, 1300-1208, reprinted with permission©

Table 4 Selection of phase III trials evaluating substitution of conventional CTx with cetuximab for HPV-positive SCCHN [62]

Trial	Phase	N	Inclusion criteria	Treatment
Chemotherapy de-intensification trails				
RTOG 1016 (NCT01302834)	III	706	T1–2, N2a–3, or T3–4, any N, HPV-positive OPSCC	Cetuximab versus high-dose cisplatin concurrent with accelerated IMRT (70 Gy in 6 weeks)
De-ESCALaTF HPV (NCT01874171)	III	304	Stage III–IVA HPV-positive OPSCC (T3N0-T4N0, T1N1–T4N3). Excludes > N2b, >10 PY	Cetuximab versus high-dose cisplatin concurrent with RT (70 Gy)
TROG 12.01 (NCT01855451)	III	200	Stage III (excluding T1–2, N1) or IV (excluding T4, N3, or M1) HPV-positive OPSCC if ≤10 PY. If >10 PY, only N0–2a	Cetuximab versus weekly cisplatin concurrent with RT (70 Gy) once per week

Abbreviations: *OPSCC* oropharyngeal squamous cell carcinoma, *PY* pack year, *RT* radiotherapy

Table 5 Selection of prospective trials evaluating modern surgery techniques with de-intensified (C)RTx regimens [62]

Trial	Phase	N	Inclusion criteria	Treatment
De-intensification of surgery/adjuvant therapy				
ECOG 3311 (NCT01898494)	II	377	Resectable stage III–IVB pl6-positive OPSCC	TORS then risk-adapted post-operative treatment (observation/50 versus 60/66 Gy with weekly platinum)
PATHOS trial (NCT02215265)	II/III	242	Resectable T1–T3, N0–2b HPV-positive OPSCC. Excludes active smokers with N2b disease	TORS then re-adapted post-operative treatment (observation/50 versus 60 Gy/60 Gy with or without weekly cisplatin)
ADEPT (NCT01687413)	III	500	Transoral resected p16-positive OPSCC (R0 margin), T1–4a, pN positive with ECE	Post-operative adjuvant 60-Gy RT with or without weekly cisplatin
NCT01932697	II	40	P16-positive OPSCC (R0 margin), stage I–IVB. Excludes ≥10 PY or smoking within 5 years	Surgery followed by hyperfractionated IMRT (36 Gy/20 fractions BID) + weekly docetaxel

Abbreviations: *TORS* transoral robotic surgery, *p16* p16INK4A expression associated with HPV, *ECE* extracapsular extension, *OPSCC* oropharyngeal squamous cell carcinoma, *PY* pack year, *BID* bi-daily, *RT* radiotherapy

reducing severe complications and mortality of interventions. A selection of trials combining modern surgery techniques with dose de-escalation and elimination of (C)RTx can be found in Table 5.

Take Home Message for Treatment De-Intensification Strategies

- *HPV-positive OPSCC* is a distinct entity with better treatment response and superior prognosis compared with tobacco- and alcohol-associated SCCHN.
- *De-intensified treatments* aim to reduce toxicity while still maintaining oncological efficacy.
- *Strategies for de-intensification* include dose de-escalation, substitution or elimination of CTx and minimally invasive procedures and are currently being evaluated in several prospective trials.
- Further *evidence is needed* before any type of treatment de-intensification can be implemented in clinical routine.

Future Perspectives of Radioimmunotherapy

DNA instability and mutations of multiple genes in tumor cells lead to production of altered proteins which, in principle, can be recognized by the adaptive immune system. Under selection pressure tumor cells develop subtle escape mechanisms to conceal themselves from the immune system. One known pathway is the downregulation of T-cells through inhibitory T-cell receptors PD-1 and CTLA-4. Monoclonal antibodies have been developed which target PD-1 (e.g., Nivolumab) and CTLA-4 (e.g., Ipilimumab) receptors and block the interaction with their ligands. Targeted immunotherapy can counteract the T-cell inactivation caused by tumors which downregulate immune response. SCCHNs feature a high rate of PD-L1 expression and genetic instability leading to neoantigen presentation [67]. There is a known interaction between PD-L1 and tumor-infiltrating lymphocytes [68]. Both HPV-positive and -negative SCCHNs belong to the 10 most immune-infiltrated tumors [69]. All these properties of SCCHNs are in favor of immunotherapy utilizing PD-1 and CTLA-4 checkpoint inhibition. Ferris et al. [70] and Chow et al. [71] demonstrated efficacy of regimens incorporating PD1 inhibition without RTx in patients with recurrent or metastatic SCCHNs. It is known that RTx leads to further immune modulatory effects, e.g., antigen presentation and upregulation of PD-L1, which forms the rational basis to combine immunotherapy with radiation [72]. A selection of trials combining RTx with immunotherapy for SCCHN treatment is presented in Table 6. Optimization in terms of the sequence of modalities, timing of treatment and fractionation schemes still needs to be evaluated to harness highest synergies between both modalities. While immunotherapies themselves may lead to typical adverse events related to hyperactivation of the immune system, e.g., rash, diarrhea, colitis, arthralgia and endocrinopathies (Fig. 9) [73, 74], they also might enable de-intensification of RTx regimens and to improve the toxicity profile of future treatments.

Take Home Message for Radioimmunotherapy

- SCCHN feature *genetic instability*, a high PDL-1 expression and immune-infiltration in favor of immunotherapy.
- Immunotherapies utilizing *checkpoint inhibitors* have proven to be effective in metastatic and recurrent SCCHN.
- Due to further *immune modulatory effects of RTx*, a combination of both modalities appears to be promising and may facilitate favorable changes of the toxicity profile of future treatment regimens.

Table 6 Selection of trials combining RTx with immunotherapy for SCCHN treatment [75]

Immune checkpoint inhibitor	Trial NCT/name	*N* of pts	Phase	Setting	Arms	Primary endpoint
Pembrolizumab	KEYNOTE-012	224	I	Solid tumors (including a HNSCC cohort)	Pembrolizumab 10 mg/kg q 2 weeks	ORR, safety
Pembrolizumab	NCT02586207	39	Ib	Stage III–IV (non metastatic) HNSCC	Pembrolizumab + weekly cisplatin + RT	AEs
Pembrolizumab	NCT02609503	29	II	Stage III–IV (non metastatic) HNSCC	Pembrolizumab + IMRT	PFS at 20 weeks
Pembrolizumab	RT0G3504	185	I/II	Advanced OPC	IMRT + cetuximab (HPV+) or cisplatin (HPV) + nivolumab	PFS
Pembrolizumab	NCT02289209	48	II	Locoregional relapse/second primary	Reirradiation + pembrolizumab	PFS
Pembrolizumab	KEYNOTE-05 5	150	II	Platinum and cetuximab refractory HNSCC	Pembrolizumab	ORR, AEs
Pembrolizumab	KEYNOTE -04 8	750	III	R/M HNSCC, first line, >6 months from curative therapy	Pembrolizumab vs. pembrolizumab + platinum/5-FU vs. cetuximab + platinum/5-FU	PFS
Pembrolizumab	KEYNOTE-04 0	446	III	Platinum refractory HNSCC	Pembrolizumab vs. cetuximab, methotrexate, or docetaxel	PFS, OS
Nivolumab	CHECKMATE 141	360	III	Platinum refractory HNSCC (progression or relapse <6 months of last platinum dose)	Nivolumab vs. cetuximab or methotrexate or docetaxel	OS at 28 months
Nivolumab	NCT02 253992	200	I	Multiple tumors, including HNSCC	Nivolumab + urelumab	ORR, safety
Nivolumab	NCT02 426892	28	II	HPV-16+ advanced solid tumors including OPC	Nivolumab + ISA-101	ORR at 11 weeks
Durvalumab	NCT02 291055	66	I/II	R/M cervical or HNSCC, ≤3 lines of therapy	ADXS11-001 vs. Durvalumab vs. ADXS11-001 + durvalumab	PFS at 2 years, AEs

Immune checkpoint inhibitor	Trial NCT/name	*N* of pts	Phase	Setting	Arms	Primary endpoint
Durvalumab	NCT01 693562	1038	I/II	Advanced solid tumors including HNSCC	Durvalumab	ORR, AEs
Durvalumab	HAWK	112	II	Platinum refractory HNSCC	Durvalumab in PD-L1+	ORR, AEs
Avelumab	NCT02 554812	147	Ib/II	Advanced solid tumors (HNSCC cohort)	Avelumab + PF-0 508256 6	ORR, AEs
Durvalumab + tremelimumab	CONDOR	240	II	Platinum refractory R/M HNSCC PD-L1 negative	Durvalumab vs. tremelimumab vs. durvalumab + tremelimumab	ORR
Durvalumab + tremelimumab	EAGLE	720	III	Platinum refractory R/M HNSCC <6 months from therapy	Durvalumab vs. durvalumab + tremelimumab vs. standard of care	PFS, OS

Abbreviations: *OPC* oropharyngeal cancer, *R/M* recurrent/metastatic, *HNSCC* head and neck squamous cell carcinoma, *ORR* overall response rate, *RT* radiotherapy

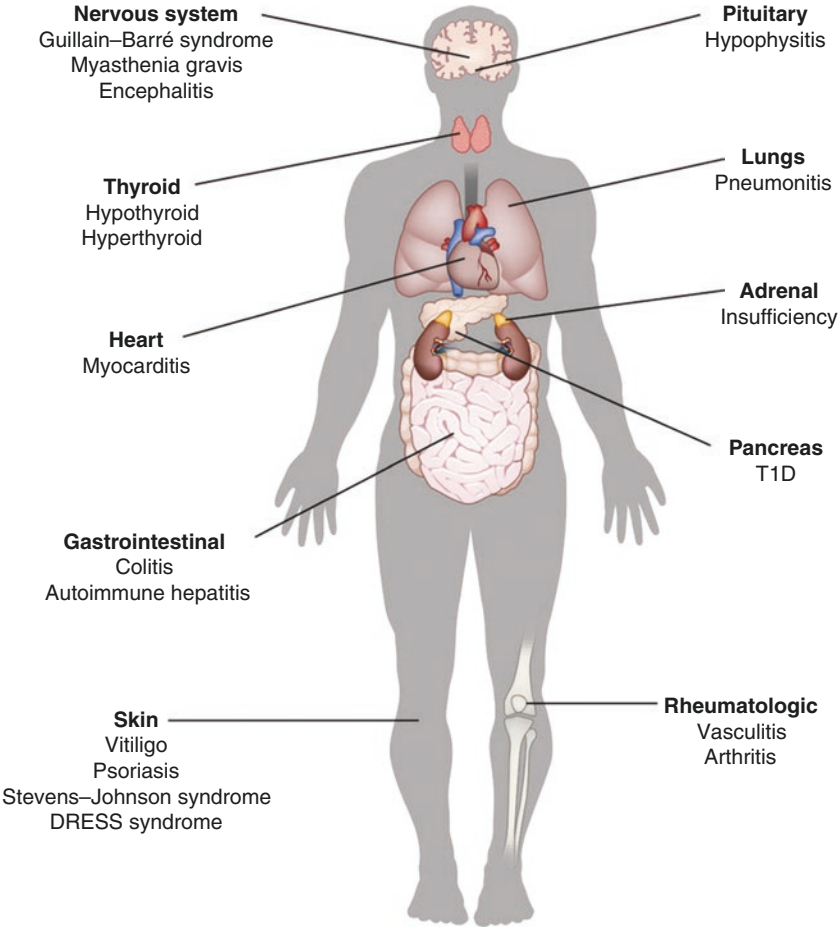


Fig. 9 Typical adverse events of immunotherapies related to hyperactivation of the immune system [74]

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Part V
Miscellaneous Topics

What Is the Optimal Larynx Preservation Approach and Who Are the Candidates?

Jean Louis Lefebvre

Since the beginning of the twentieth century two major options were available for the treatment of laryngeal and hypopharyngeal squamous cell carcinomas: definitive irradiation or laryngectomy with or without postoperative irradiation. The respective indications varied according to institutional policies.

In the past, there were no concerns about the function of the larynx in patients with early disease, as both partial laryngectomy and irradiation did not compromise laryngeal function. However, the contrary was true for patients with locoregionally advanced disease, who required total laryngectomy. Total laryngectomy was able to control most of these diseases but at the price of notable sequelae compromising the quality of life (loss of a normal voice and permanent tracheostomy). Radical irradiation was also able to control these diseases but sometimes with post irradiation sequelae (fibrosis for example) that could compromise the larynx function and the salvage surgery when required. The published series of surgery and of irradiation were difficult to compare. Actually these series did not consider similar populations (resectable diseases in operable patients in the surgical series vs less selected tumours and populations in the radiation ones). In addition all were retrospectives series.

Unfortunately there was no consensus for initiating what should have been the first larynx preservation randomized trial comparing radical surgery vs definitive irradiation in similar groups of advanced larynx cancer patients. This information is missing forever.

All along the century, surgical research aimed at extending the indications of partial laryngectomies as well as at improving the quality of voice rehabilitation (in particular with the use of trachea-oesophageal puncture and insertion of a voice prosthesis). In parallel the radiotherapy research aimed to improve the disease control in particular with different modalities of altered fractionation. These researches did not notably change the picture and the debate remained open.

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For long there were no efficient chemotherapy regimens that could be integrated in the armamentarium of treatment with curative intent for head and neck cancers. Chemotherapy was mainly used for palliation. In the 1980s the Wayne Sate University published its experience of induction chemotherapy using cisplatin and 5-fluorouracil in previously untreated patients with head and neck cancers. In a series of 35 patients treated with three cycles of induction chemotherapy with cisplatin and 5-fluorouracil (the so-called PF protocol) they observed a reduction of at least 50% of the local disease in 94% of the patients and a complete clinical disappearance of the disease in 63% of the patients [1]. Despite it concerned a small series of patients these results showed that induction chemotherapy potentially could be used in protocols with curative intent. They also published their results in 60 patients treated by induction cisplatin-based chemotherapy showing that in the 42 patients who had demonstrated a tumour response over 50%, 97% of them were controlled by a subsequent irradiation. On the contrary only 6% of the 18 patients with a tumour reduction below 50% were controlled by a subsequent irradiation [2]. These results generated the concept of a possible selection role of induction chemotherapy that could be used in the frame of advanced larynx cancers treatment pending there was no deleterious impact on disease control and survival. In that respect, it is worth mentioning that the Chicago team published a prospective study in which they concluded that by far the priorities for head and neck patients are "being cured of the disease" and "living long" and patients are more willing than non-patients to undergo aggressive treatments and endure acute distress in the interest of these potential long-term gains [3]. On these bases the clinical research on larynx preservation started when the best definition of "larynx preservation" still had to be defined.

The Validation Trials

The concept of these prospective randomized larynx preservation trials was to compare in suitable previously untreated HNSCC patients total laryngectomy with postoperative irradiation as a control arm with a control arm an experimental arm i.e. induction PF followed in responders by irradiation and salvage surgery if required or by a total laryngectomy with postoperative irradiation in other patients. The goal of this research was to assess the safety of the concept and the primary endpoint was overall survival. Each cycle of chemotherapy consisted of cisplatin 100 mg/m^2 on day 1 followed by 5-fluorouracil 1000 mg/m^2/day during 5 days and was delivered every 3 weeks. Definitive irradiation was delivered at a dose of 70 Gy and postoperative irradiation at a dose of 60 Gy. "Responders" to chemotherapy were defined as patients with a tumour regression of at least 50%.

The Veterans Administration Larynx Cancer Study Group (VALCSG) Trial [4]

In the United States, the department of VALCSG conducted such a trial in 332 laryngeal cancer patients. Of those, 166 were randomly enrolled in the control arm and 166 in the experimental arm, which consisted of two cycles of PF followed in responders by a third cycle and irradiation or surgery and postoperative irradiation in non-responders. Overall survival was the primary endpoint. At a median follow-up of 33 months, the 2-year survival was 68% in both treatment arms (95% Confidence Interval [CI]: 60–75% in the surgery arm vs 60–76% in the chemotherapy arm, P = 0.9846) and the larynx was preserved in 64% of the patients in the experimental arm. In the chemotherapy arm, salvage laryngectomies were indicated significantly more often in patients with stage IV disease than in those with stage III (p = 0.048) and the same was true for those with T4 diseases versus those with T3 disease (P = 0.001). Of note distant metastases were observed less frequent in the chemotherapy arm.

The European Organization for Research an Treatment of Cancer (EORTC) 24891 Trial [5, 6]

In Europe, the EORTC Head and Neck Cooperative Group conducted a similar trial in patients with advanced hypopharyngeal and lateral epilarynx tumours requiring a total laryngectomy. In this EORTC 24891 trial, 194 previously untreated patients were enrolled. A partial response (PR) after two or three cycles of chemotherapy was required to receive radiation therapy. Chemotherapy consisted of 100 mg/m^2 cisplatin given intravenously over a 1-h period followed by fluorouracil 1000/m^2/day given as a 120-h infusion over 5 days (total dose 5000 mg/m^2). The primary endpoint was overall survival in terms of non-inferiority in the experimental arm with a hazard ratio (HR) $\leq$1.43. In the first evaluation the median duration of survival was 25 months in the immediate-surgery arm and 44 months in the induction-chemotherapy arm and, since the observed hazard ratio was 0.86 (log-rank test, P = 0.006), which was significantly less than 1.43, the two treatments were judged to be equivalent. The 3- and 5-year estimates of retaining a functional larynx in patients treated in the induction-chemotherapy arm were 42% (95% CI: 31–53%) and 35% (95% CI: 22–48%), respectively. In addition there were fewer distant metastases in the chemotherapy arm.

These results were confirmed by long-term evaluation. At a median follow-up of 10.5 years, the 5-year and 10-year overall survival rates were respectively 32.6% (95% CI: 23.0–42.1%) and 13.8% (95% CI: 6.1–21.6%) in the surgery arm vs

38.0% (95% CI: 28.4–47.6%) and 13.1% (95% CI: 5.6–20.6%) in the chemotherapy arm. In 37 patients still alive at 5 years in the chemotherapy arm, 22 (59.5%) had retained a normal larynx.

Conclusions of Theses Two Trials

These two trials showed that the concept was validated both for laryngeal and hypopharyngeal cancers, larynx preservation could be obtained in around two thirds of the patients without compromising survival or disease control. This clinical research therefore continued with larynx preservation (under various definitions) as the primary endpoint.

The Concept of Larynx Preservation

There are different ways to consider larynx preservation. The simplest one is to define it by the only one parameter: larynx in place (i.e. no laryngectomy), whatever the local control and the function. A more comprehensive one is to consider both the organ and its function: no laryngectomy, no long-term tracheotomy, and no long-term feeding tube, which implies also that local control is obtained. As survival is an important issue, it may also be integrated: either laryngectomy-free survival, or, more detailed, survival with a functional larynx in place. A group of experts has worked on the best definition of larynx preservation taking into account all parameters participating to the real benefit for the patients. They elaborated the "laryngoesophageal dysfunction-free survival" that combined as events: death, local failure, salvage laryngectomy, and tracheotomy or feeding tube at 2 years or later [7, 8]. Of course when evaluating a report on larynx preservation, it is of the upmost importance to consider which definition has been used before comparing the results with other reports. Rosenthal has applied these various definitions to the same database; the curves were impressively different [9].

Trial with Concomitant Administration of Chemotherapy and Radiation Therapy

The EORTC 24954 Trial for Alternating Chemo-Irradiation [10, 11]

The EORTC Head and Neck and Radiotherapy Oncology Cooperative Groups designed a randomized trial in order to assess whether more cycles of chemotherapy could improve both the survival and the larynx preservation rate. The EORTC 24954

trial compared two different schedules for delivering the chemotherapy cycles and the irradiation: a sequential schedule like in the previous trial versus an alternating one as described by Merlano et al. [12]. The sequential arm consisted of two cycles of PF with the same doses and administration as in the 24891 trial. After 2 cycles responders received two additional cycles of PF and were then treated with irradiation at a dose of 70 Gy. The non-responders were treated by total laryngectomy and postoperative irradiation. In the alternating arm, patients received on weeks 1, 4, 7, and 10 a cycle of chemotherapy consisting of cisplatin at a dose of 20 mg/m^2 per day on days 1–5 (for a total dose of 100 mg/m^2) and 5-fluorouracil by bolus infusion at a dose of 200 mg/m^2 per day on days 1–5 (for a total of 1000 mg/m^2). During the three two-week intervals patients were treated by irradiation at a dose of 20 Gy per course for a total of 60 Gy. As a result, the total doses of 5-fluorouracil and of irradiation were lower in the alternating arm. A total of 450 patients were enrolled in this trial (224 to the sequential arm and 226 to the alternating arm).

For the first evaluation the median follow-up was 6.5 years. Survival with a functional larynx was similar in the sequential and alternating arms (hazard ratio of death and/or event = 0.85, (95% CI: 0.68–1.06), as were median overall survival (4.4 and 5.1 years, respectively) and median progression-free interval (3.0 and 3.1 years, respectively). Grade 3 or 4 mucositis occurred in 64 (32%) of the 200 patients in the sequential arm who received radiotherapy and in 47 (21%) of the 220 patients in the alternating arm. Late severe oedema and/or fibrosis was observed in 32 (16%) patients in the sequential arm and in 25 (11%) in the alternating arm.

For the long-term evaluation, the median follow-up was 10.2 years. Ten-year survival with a functional larynx (primary end-point) and overall survival were similar in the sequential and alternating arms (18.7% and 33.6% versus 18.3% and 31.6% respectively). Late toxicity was also similar even if there was a trend for higher larynx preservation and better laryngeal function in the alternating arm. However due to the organizational difficulties when delivering such an alternating schedule in daily practice, it is rarely used.

The Radiation Therapy Oncology Group (RTOG) 91-11 Trial for Concurrent Chemo-Irradiation [13, 14]

A large meta-analysis [15] had demonstrated that concurrent radiotherapy plus cisplatin (100/m^2 on days 1, 22 and 43 of the radiotherapy) achieved a significantly higher survival benefit when compared with induction cisplatin fluorouracil.

The RTOG and the Head and Neck Intergroup in the US conducted a three-arm randomized trial comparing the standard alternative to total laryngectomy validated by previous trials (induction chemotherapy with cisplatin plus fluorouracil followed by radiotherapy) vs radiotherapy with concurrent cisplatin vs radiotherapy alone in 547 previously untreated patients with locally advanced larynx cancer. Laryngectomy-free survival was the primary endpoint while larynx preservation (larynx in place) and survival were secondary endpoints.

In the first report no difference was found in acute toxicity during the radiotherapy between the induction chemotherapy and the radiotherapy alone arm. There were fewer distant metastases in the two arms with chemotherapy when compared with radiotherapy alone, but only the difference between the concurrent and the radiotherapy alone arm was significant. Regarding the 2-year and the 5-year estimates for laryngectomy-free survival, these were respectively 59% and 43% in the induction arm, 66% and 45% in the concurrent arm, and 53% and 38% in the radiotherapy alone arm. The difference was not significant between the induction and the concurrent arms. The 2-year and 5-year overall survival did not differ significantly according to the treatment arm. The rate of larynx preservation at a median follow-up of 3.8 years was significantly higher in the concurrent arm (84%) when compared with the induction arm (72%, $P = 0.005$) or with the radiotherapy alone arm (67%, $P < 0.001$).

The long-term analysis with a median follow-up of 10.8 years in surviving patients confirmed that the two chemotherapy arms significantly improved laryngectomy-free survival compared with radiotherapy alone without significant difference between these two arms. Overall survival did not differ significantly between the treatment arms, although there was a trend for a higher survival in the induction arm. The difference favouring the concurrent arm with regards to the larynx preservation persisted at 10 years 67.5% (95% CI: 60.4–74.6%) in the induction arm, 81.7% (95% CI: 75.9–87.6%) in the concurrent arm, and 63.8% (95% CI: 56.5–71.1%) in the radiotherapy alone arm. There was no significant difference in late toxicity between the three arms. However the rate of deaths not related to the study cancer was significantly higher in the concurrent arm compared with the induction one (69.8% vs 52.8% respectively at 10 years, $P = 0.03$).

Trials Integrating Docetaxel or Cetuximab

Two large randomized trials [16, 17] had shown that adding docetaxel to cisplatin fluorouracil (the so-called TPF regimen) before irradiation (or chemoradiation) resulted in a significantly higher survival compared to that observed with the duplet regimen (PF).

Another randomized trial [18] had shown that adding cetuximab to irradiation resulted in a significantly higher survival and loco-regional control over irradiation alone.

The Groupe Oncologie Radiotherapie Tete Et Cou (GORTEC) 2000-01 Trial with Docetaxel [19, 20]

In France, the GORTEC conducted a two-arm randomized trial in 220 patients with a locoregionally advanced laryngeal or hypopharyngeal cancer eligible for a total laryngectomy. The aim of the trial was to assess if adding docetaxel to induction PF

could improve larynx preservation. The patients were randomized between 2 induction arms: an experimental one starting with TPF (docetaxel at 75 mg/m^2 on day 1, cisplatin at 75 mg/m^2 on day 1, and 5-fluorouracil at a dose of 750 mg/m^2 by 120-h continuous infusion over 5 days) compared with the classical PF one (cisplatin 100 mg/m^2 on day 1 and 5-fluorouracil given at a dose of 1000 mg/m^2 by 120-h continuous infusion over 5 days). Three cycles at a 3-week interval were planned in the two arms and responders were treated by irradiation while non-responders had total laryngectomy and postoperative irradiation. Larynx preservation was defined as a larynx in place without tumour, tracheostomy or feeding tube. Larynx preservation was the primary endpoint; overall survival and progression-free survival were secondary endpoints. 220 patients were enrolled, of whom 213 were eligible (110 in the TPF arm and 103 in the PF arm).

The first evaluation revealed different chemotherapy-induced toxicities with more alopecia, neutropenia in the TPF arm and more stomatitis, thrombocytopenia and creatinine elevation in the PF arm. In the TPF arm 69 patients (62.7%) could receive the complete treatment without delay or dose reduction versus 33 patients (32%) in the PF arm. The response rates were 80% with TPF arm and 59.2% with PF (P = 0.002). As a result, larynx preservation was offered to 78.8% of patients in the TPF arm versus 55.3% in the PF arm. With a median follow-up of 36 months, the 3-year actuarial larynx preservation rate was 70.3% in the TPF arm versus 57.5% in the PF arm (P = 0.002). However, there were no significant differences in terms of survival.

The long-term evaluation confirmed the initial results. The 5-year and 10-year larynx preservation rates were 74.0% (95% CI: 64–82%) versus 58.1% (95% CI: 47–68%) and 70.3% (95% CI: 58–80%) versus 46.5% (95% CI: 31–63%, P = 0.01) with TPF and PF, respectively. There was no significant difference in 5-year and 10-year overall survival, or disease-free survival. Importantly there were fewer grade 3–4 late toxicities in the TPF arm (9.3%) than in the PF arm (17.1%, P = 0.038).

Of note, in this trial it was left to institutional policies to deliver either radiotherapy alone or concurrent chemoradiotherapy in responders. In the TPF arm 17 patients and in the PF arm 9 patients received concurrent chemo-radiation (with either cisplatin or carboplatin plus fluorouracil). The impact of these chemoradiation protocols on the overall results is unknown.

The GORTEC "TREMPLIN Trial" with Docetaxel and Cetuximab [21]

Assuming that induction chemotherapy and concurrent chemotherapy could be complementary, there was a trend to combine induction chemotherapy and subsequent chemoradiotherapy in locally advanced head and neck cancers. A similar approach was tested in the larynx preservation setting. Anticipating an overall toxicity that could compromise the larynx function, and taking into account the results of the radiotherapy plus cetuximab trial [18], the GORTEC conducted a randomized phase II study to assess what could be the best post-induction protocol.

Patients with larynx or hypopharynx cancer justifying a total laryngectomy were eligible for that study. Patients received 3 cycles of TPF and responders were randomized between radiation plus cisplatin (100 mg/m^2 on day 1, 22 and 43 of irradiation) and radiation plus cetuximab (a loading dose of 400 mg/m^2 and 250 mg/m^2 per week during irradiation). The primary endpoint was larynx preservation (no residual disease justifying immediate salvage laryngectomy) 3 months after the end of treatment. The secondary endpoints were larynx function preservation and overall survival 18 months after the end of treatment.

Of the 153 enrolled patients, 116 were randomized (60 in the cisplatin arm, and 56 in the cetuximab arm). Substantial acute toxicity was observed in both arms, in particular in-field skin toxicity in the cetuximab arm and renal, haematological, and performance status alteration in the cisplatin arm. Limiting acute toxicity led to protocol modification in more patients in the cisplatin arm than in the cetuximab arm (71% and 43% vs 71%, respectively). Except for grade 1 renal toxicity (mainly in patients who had received in total 6 cycles of cisplatin in the chemo-radiation arm), late toxicity did not differ significantly between both arms. At last examination, there were fewer local recurrences in the cisplatin arm (8 patients) compared with 12 patients in the cetuximab arm, but successful salvage surgery could be performed only in the cetuximab arm.

There was no significant difference in larynx preservation at 3 months, being 95% (95% CI: 86–98%) in the cisplatin arm versus 93% (95% CI: 83–97%) in the cetuximab arm. There was no obvious difference in secondary endpoints at 18 months as well. The larynx function preservation was 87% (95% CI: 76–93%) in the cisplatin arm versus 82% (95% CI: 70–90%) in the cetuximab arm. The overall survival was 92% in the cisplatin arm (95% CI: 82–96%) and 89% (95% CI: 79–95%). At a median follow-up of 36 months overall survival was 75% (95% CI: 62–85%) and 73% (95% CI: 60–84%) in the cisplatin arm and cetuximab arm, respectively.

As the composite end-point of laryngoesophageal dysfunction-free survival had been described after the trial was initiated and had been published at the time of the trial evaluation, this end-point was tested in retrospect. Two years after the end of treatment there was no significant difference in that end-point: 79% (95% CI: 67–89%) with cisplatin versus 72% (95% CI: 65–89%) with cetuximab.

Of importance, the comparison of larynx preservation rates with previous trials must be taken with caution as in the TREMPLIN trial they related to the population selected after induction chemotherapy (i.e., 75% of the overall population).

The conclusion was that there was no signal that one arm was superior over the other one, and none appeared to be superior to induction TPF followed by irradiation alone when taking into consideration results of other trials (such as the GORTEC 2000-01 trial).

The German "Delos II Trial" with Docetaxel and Cetuximab [22]

The German Larynx Organ preservation Study group (DeLOS) conducted another randomized phase II study assessing the place of cetuximab in larynx preservation for patients with larynx or hypopharynx cancer. The initial trial design was to

compare induction TPF followed by irradiation with TPF plus cetuximab (E) followed by irradiation plus cetuximab. Due to 4 treatment-related deaths among the first 64 patients, the protocol was amended and fluorouracil was omitted from induction chemotherapy in both arms. There were no further treatment-related deaths thereafter. The evaluation was made after one cycle and responders continued the protocol while non-responders went to laryngectomy. The primary objective was a 2-year functional laryngectomy-free survival (fLFS) above 35%.

Of the 180 patients randomized in the trial, 173 fulfilled Intent To Treat (ITT) criteria. At final examination, the objective response rates in the arm without cetuximab were 79.1% in patients who had received PF, and 94.7% in patients who had received TP. In the arm with cetuximab they were 80% in patients who had received TPFE, and 94.9% in patients with TPE, 94.9% (i.e. similar to TPF). The primary objective was similarly met in both arms: 44.7% in the arm without cetuximab and 46.6% in the cetuximab arm (OR: 0.9268, 95% CI: 0.5094–1.6863). There was no difference in 2-year overall survival: 68.2% in the arm without cetuximab, and 69.3% in the cetuximab arm (OR: 0.9508, 95% CI: 0.4997–1.8091).

Conclusions

Considering these results, it must be underscored that, to date, only two protocols have been validated: induction TPF followed by irradiation alone (GORTEC 2000-01) and irradiation with concurrent cisplatin (RTOG 91-11). To translate these trials in daily practice it is important to strictly follow the study protocols with respect to initial work-up and eligibility criteria, chemotherapy protocols, prophylaxis/management of treatment-induced toxicity, response to treatment evaluation, as well as schedule and tools for post-treatment follow-up.

The majority of patients enrolled in these trials received conventional irradiation. The new radiotherapy technologies (such as IMRT) have reduced the radiotherapy side effects in particular at the level of pharyngeal constrictors muscles. This must be taken into consideration in future trials.

The decision of enrolling a patient in a larynx preservation protocol must be taken by a multidisciplinary tumour board. Patients eligible for a larynx preservation strategy today are patients with advanced larynx and hypopharynx cancers who are not eligible for partial surgery. Overall, T4 diseases and tumours extending to the post-cricoid area are not eligible for larynx preservation. Of note in hypopharynx cancer, only protocols based on induction chemotherapy have been evaluated, there are no data with concurrent chemoradiotherapy for this primary site.

The composite end-point of laryngoesophageal dysfunction free survival has been approved by a group of experts and should be used in further studies.

As the RTOG 91-11 trial was initiated before the TPF induction regimen has proved to be superior to the PF one, there is a need to compare the RTOG and the GORTEC trial. The on-going French phase III trial (GORTEC 2014-03-SALTORL) is comparing induction TPF followed by irradiation in responders vs concurrent cisplatin-based chemoradiotherapy.

Disclosure

JL Lefebvre has been member of the advisory boards of Sanofi-Aventis (docetaxel) and Merck Serono (cetuximab).

JL Lefebvre has been lecturer for Sanofi-Aventis and Merck Serono and is still lecturer for Merck.

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Is the Approach to Patients with Unknown Primary Tumor any Different in 2018?

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Introduction

The presence of cervical lymph node metastasis with no conclusive evidence of a primary tumor despite full clinical work-up (clinical, radiological, and surgical) is defined as a cancer of unknown primary site (CUP) [1]. This entity classically accounted for 1.5–9% of all head and neck cancers [2, 3], but the rate may be decreasing to no more than 3% due to improvements in clinical detection [4]. Subsequent manifestation of the primary lesion occurs in an extremely wide range of patients (1.4–54%), clearly reflecting the different degrees of accuracy of the clinico-pathologic work-up and the impact of radiotherapy on mucosal sites potentially harboring the primary lesion.

Notably, squamous cell carcinoma (SCC) accounts for 53–77% of CUP to neck nodes [5, 6]. Histologies other than SCC should also be considered (i.e., adenocarcinoma, undifferentiated carcinoma, lymphoma, melanoma, papillary thyroid carcinoma, and central nervous system tumors). Adenocarcinoma represents a minority of CUP cases, typically presents in the levels IV–VA, and is suggestive of a primary site below the clavicles [7, 8]. Cutaneous SCC should be considered as a possible primary site in regions with a high prevalence of skin cancer: the estimated nodal metastatic rate is around 5% for cutaneous primaries of head and neck region [9].

In the last decade, a changing but important trend in the epidemiology of SCC CUP has been observed, which parallels what is evident on a larger scale for SCC of the oropharynx in relation to the human papillomavirus (HPV)-status. In the past, the typical patient was male, 55–65 years old, with a history of chronic tobacco and/or alcohol use. More recently, clinicians have observed the gradual rise of a different type of patient: younger, less commonly heavy smoker and/or alcohol abuser, with

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HPV-related disease [10]. In view of the peculiarity of HPV-related lesions of the oropharynx to arise from the reticulated crypt epithelium of the tonsils and base of the tongue and have a slow growth, their identification can be extremely challenging.

In 2015, Boscolo-Rizzo et al. [11] performed a systematic review of the literature to identify studies testing HPV and/or p16 status in tissue samples from patients with CUP. Based on the analysis of 18 articles published in the period 2007–2015, they found overall median prevalences of HPV-DNA positivity, p16 positivity, and positivity for both HPV markers in 37.0%, 48.5%, and 36.0% of patients, respectively [11]. Subsequently, many other papers have confirmed the increasing rate of HPV-positivity in CUP patients and shown that they have a better prognosis compared to those who are HPV-negative [12–14]. Due to the rarity of CUP, data from the literature are based on retrospective studies mostly analyzing small cohorts of patients treated with variable schedules. The only prospective study, which was planned by the EORTC (22205) to answer questions such as the need for bilateral neck and mucosal irradiation, was prematurely closed after 2 years for lack of accrual. However, in recent years the interest towards CUP has been growing as a consequence of refinements in diagnostic procedures and better understanding of the natural history of the disease. The publication of guideline recommendations by the National Comprehensive Cancer Network (NCCN) [15] in the US and an official guideline endorsed by the specialty associations involved in the care of head and neck cancer patients in United Kingdom [16] make it easier to follow a diagnostic and treatment algorithm in a disease in which there is no level I evidence [17]. Finally, the recent introduction by AJCC and UICC committees of a specific classification for CUP, which differentiates virus-related (HPV and Ebstein-Barr virus [EBV]) from non-related forms, provides clinicians with an official tool to stratify patients and compare results (Table 1) [18].

Table 1 Eighth edition of TNM classification for HPV/p16 positive and EBV positive CUP [18]

	HPV/p16 positive
Clinical classification	*cN1*: unilateral lymph node(s), all ≤ 6 cm *cN2*: contralateral or bilateral lymph node(s), all ≤ 6 cm *cN3*: lymph node(s) > 6 cm
Pathological classification	*pN1*: metastasis in 1–4 lymph node(s) *pN2*: metastasis in ≥ 5 lymph nodes
	EBV positive
Clinical classification	*cN1*: unilateral lymph node(s), and/or unilateral or bilateral metastasis in retropharyngeal lymph nodes, all ≤ 6 cm, above the caudal border of cricoid cartilage *cN2*: bilateral lymph node(s), all ≤ 6 cm, above the caudal border of cricoid cartilage *cN3*: lymph node(s) > 6 cm and/or extension below the caudal border of cricoid cartilage *Notes* Midline nodes are considered ipsilateral nodes
Pathological classification	pN categories correspond to cN categories

The present chapter reviews the current knowledge of CUP with the intent to define what is the ideal clinical, radiologic, and surgical work-up and available therapeutic guidelines in 2018.

Clinical Presentation

The typical clinical presentation is a cervical mass (94–100% of patients), corresponding to a single or multiple lymph nodes, eventually associated with pain (9%), weight loss (7%), or dysphagia (3.6%) [6, 19]. Although patients typically report a recent appearance of a mass in the neck, in HPV-related forms the lesion can have an indolent growth over a period of months. The lump is typically located at level II, followed by level III, with bilateral involvement reported in less than 10% [5, 6, 19–22]. The clinical N stage at presentation is usually N2a, N2b, and N2c (according to the former classification), with a median nodal size of 3.5–5 cm [5, 19–22].

Clinical History and Work-Up

Ideally, patients with a suspicious metastatic neck node should be assessed according to a specific algorithm and treated in tertiary care centers (Fig. 1). In daily practice, they are not infrequently referred for treatment after an incomplete and sometimes inappropriate work-up, which leads to delayed diagnosis (Fig. 2). Office-based evaluation should start with accurate analysis of clinical history of the patient addressing not only present signs and symptoms, but also other relevant elements: (1) risk factors (e.g. tobacco or alcohol use); (2) previous history of malignancy; and (3) prior resection, destruction, or regression of cutaneous lesions [15]. A complete examination of the head and neck, paying attention to possible skin lesions especially in geographic areas with a high incidence of cutaneous cancer, and upper aerodigestive tract (UADT) fibroscopy with direct visualization of the nasopharynx, oropharynx, larynx, and hypopharynx are the first steps required. The position of the mass in the neck provides clues to specifically focus on selected areas to identify the primary tumor (Table 2): levels I–III are more often involved in case of oral cavity or lip cancer; levels II–IV in case of oro/hypopharyngeal, laryngeal, and thyroid cancer; level V in case of nasopharyngeal cancer; intraparotid lymph nodes in case of skin or salivary gland tumors. Of utmost importance, 50% of masses limited to level IV and/or supraclavicular fossa derive from primary tumors arising below the clavicle (i.e. lung, breast, gastrointestinal tract, kidney and ovary), and are mainly adenocarcinomas [7]. If no mucosal lesions of the UADT are detected, fine-needle aspiration cytology (FNAC) under ultrasonographic guidance should be performed to confirm the clinical suspicion and, based on the results, guide the next steps of work-up.

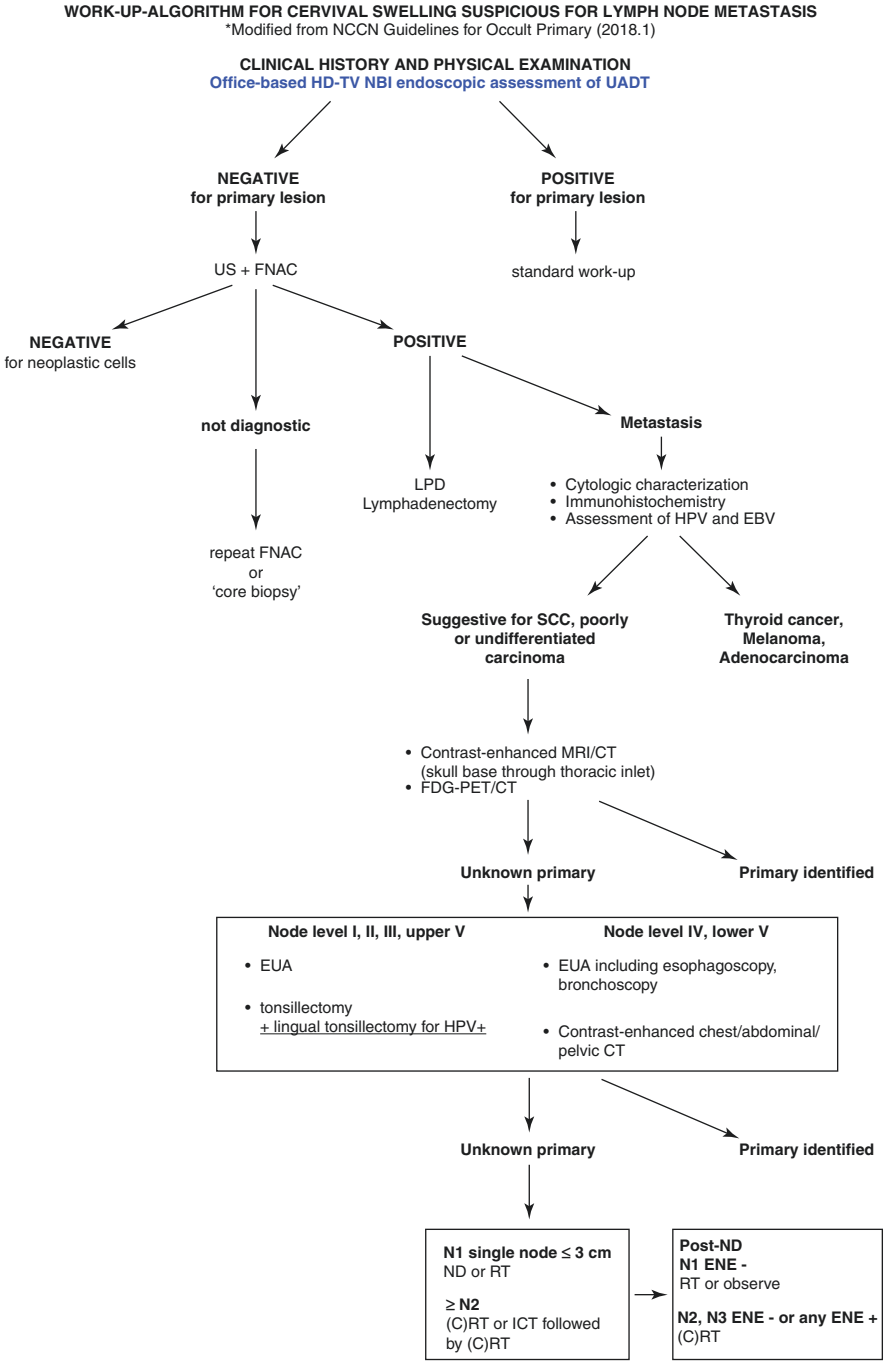

Fig. 1 Algorithm for CUP modified from NCCN guidelines [15]

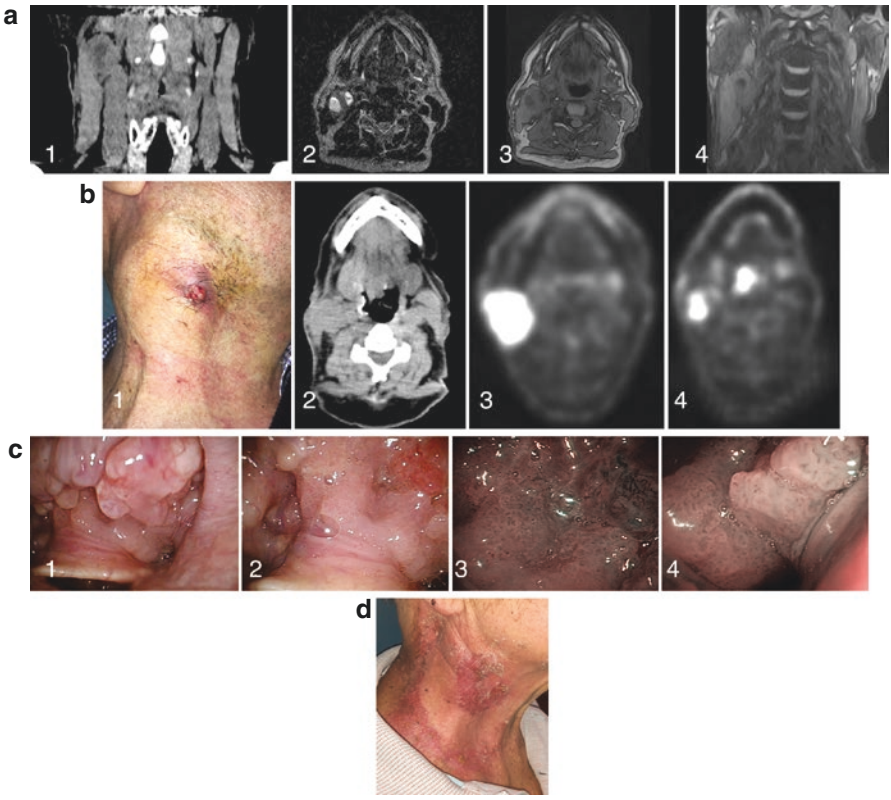

Fig. 2 Right neck swelling lasting two years in a 63-year-old man, former smoker (until 20 years before presentation). At another institute, after conventional white-light (WL) UADT endoscopy (negative) and imaging (neck CT, MRI), he received a diagnosis of branchial cleft cyst. (**a**) After more than 1 year, for increasing in size of neck swelling with initial skin ulceration, he underwent FNAC, which was diagnostic for SCC G3 (p16/HPV/EBV status was not assessed). A radiological re-staging was performed, which included contrast-enhanced CT of the neck-chest and total body PET. Both examinations detected a suspicious lesion in the right base of the tongue. (**b**) The patient was referred to our department. Office-based high-definition Narrow Band Imaging (HD-NBI) UADT endoscopy confirmed the presence of a wide tongue base neoplasm. A biopsy of the lesion was positive for HPV16-related SCC G3. The final clinical staging was cT2N1M0, stage I. (**c**) The multidisciplinary board proposed a RT-CHT treatment, that was accepted by the patient (For abbreviations see the text in the manuscript). (**a**) CT without contrast medium in the coronal plane [1], MRI T2- and T1-sequences without gadolinium in the axial plane [2, 3], MRI T1-sequence in the coronal plane [4]. The exams showed a cystic-necrotic nodal conglomerate strictly adherent to the sternocleidomastoid muscle, without any suspicious primary lesion. (**b**) after more than 1 year a skin ulceration developed [1], contrast-enhanced CT in the axial plane [2], PET-scan [3, 4]. Imaging showed an increase in size of the nodal mass with suspicious infiltration of the sternocleidomastoid muscle, skin, parotid gland, and submandibular gland associated with internal jugular vein compression. PET-scan also showed a pathologic uptake in the ipsilateral base of tongue and epiglottis. (**c**) endoscopic findings (WL and HD-NBI). Under WL, irregular mucosal surface of the lingual surface of the epiglottis, bulging of the right base of tongue; under NBI, thick dark spots and winding vessels. (**d**) external view of the neck after RT-CHT. No further signs of skin ulceration and complete regression of the right neck swelling

Table 2 Common patterns of lymph node metastasis in head and neck [8]

Nodal group	Primary tumor sites
Level Ia (submental)	Anterior oral cavity, lower lip
Level Ib (submandibular)	Oral cavity, anterior nasal cavity, submandibular gland, mid facial face skin
Level II (upper jugular)	Oropharynx, oral cavity, nasopharynx, nasal cavity, larynx, hypopharynx
Level III (mid jugular)	Oropharynx, oral cavity, nasopharynx, larynx, hypopharynx
Level IV (lower jugular)	Oropharynx, larynx, hypopharynx, upper esophagus, thyroid
Level V (posterior triangle)	Nasopharynx, posterior scalp skin, thyroid
Level VI (anterior compartment)	Thyroid, larynx, hypopharynx, upper esophagus
Supraclavicular	Non-head and neck, thyroid
Retropharyngeal	Nasopharynx, posterior pharynx
Parotid	Lateral/upper facial and scalp skin, parotid gland

Cytology and Associated Exams

FNAC is an efficient, minimally invasive, and cost-effective diagnostic method with negligible risk of seeding tumor cells along the needle track [23]. The accuracy of sampling is enhanced when performed under the guidance of ultrasonography (US), avoiding areas of necrotic or cystic degeneration. Diagnosis is usually established with routine cytologic staining, supplemented by immunohistochemistry, polymerase chain reaction (PCR), and in situ hybridization (ISH) for oncogenic virus detection, which has a sensitivity of 83–97% and a specificity of 91–100% for metastatic lesions [23, 24]. In case of an uncertain or non-diagnostic report of FNAC, the examination should be repeated or, as an alternative, core biopsy should be performed. Open biopsy is indicated when a lymphoproliferative disorder is suspected or diagnosis is not achieved with FNAC and core biopsy. In the latter situation, NCCN guidelines suggest that the patient should be prepared for neck dissection [15]. FNAC positivity for HPV [25–29] or EBV helps the clinician to focus the attention on the oropharynx or nasopharynx, respectively, in looking for the primary lesion. However, the rare possibility of an HPV- or EBV-positive cancer in other head and neck sites should not be neglected.

The diagnosis of HPV-related cancer on FNAC specimens can be challenging. First, the basaloid appearance of cells can overlap with those of several other head and neck neoplasms [30]. Moreover, FNAC classification as non-keratinizing or keratinizing SCC does not always correlate with histology [31]. Second, p16 expression, which is still recommended in several guidelines as a surrogate marker for HPV positivity, has clear limitations: p16 overexpression can occur in other malignancies [32, 33]; p16 overexpression is seen in up to 31% of cutaneous SCCs, making this test alone insufficient in differentiating mucosal from cutaneous primary sites [34]; at immunohistochemistry, staining is generally more focal and patchy, and a cut-off for determining positivity has not been established [8];

50% of benign branchial cysts are often p16 positive [35]. A threshold of 10% applied to FNAC p16 staining gives a reported sensitivity and specificity of 94% and 75% [36].

Consequently, HPV DNA or RNA detection studies are currently recommended to accurately identify "true" HPV-related cancers. HPV-DNA can be detected by PCR analysis or ISH: both these techniques are often used in combination with p16 immunohistochemistry. PCR analysis has the advantage of high sensitivity but, if used alone, can also detect clinically insignificant infections, particularly if used outside of the context of a non-keratinizing SCC of the oropharynx. ISH has lower sensitivity, especially in cases of HPV-related SCC with a low viral load, but is highly specific, with the advantage of directly visualizing the presence of signal in the malignant cells [28, 31, 37]. ISH for RNA is also highly specific and has the added advantage of detecting transcriptionally active HPV, which might be considered the "gold standard" for high-risk-HPV detection [38].

EBV plays a central role in the etiology of nasopharyngeal carcinoma (NPC), which can present as CUP. Aspirates are typically cellular and contain clusters of malignant epithelioid cells in a background of lymphocytes correlating to histologic features [39]. Association of EBV with NPC is much stronger with the non-keratinizing NPC variant, which can be further subdivided into differentiated and undifferentiated forms [39]. EBV association with non-keratinizing NPC approaches 100% when a sensitive method such as ISH for EBV-encoded RNA (EBER) is used. Cytologically, non-keratinizing NPC can appear identical to HPV-associated SCC [39]. Therefore, the initial evaluation of a non-keratinizing carcinoma of unknown primary in a cervical lymph node, particularly those that appear undifferentiated, should ideally include testing for both EBV and HR-HPV [39]. In cytological samples, detection of EBV by ISH should be performed using an EBER RNA probe: EBV ISH can be successfully performed in tissues from formalin-fixed paraffin-embedded cell blocks or even on cytospin preparations [26, 40, 41]. Alternatively, DNA- or RNA-based PCR can be used [42].

Cystic lymph node metastases from SCC of Waldeyer's ring are reported in 20–61.3% of cases [43]: Goldenberg et al. found that 87% of cystic metastatic nodes were HPV positive [43, 44], but also other head and neck cancer types (such as EBV-related NPC) and papillary thyroid carcinoma may produce cystic lesions [45]. In case of cystic metastases, a false-negative rate of 42% with FNAC has been reported [46] with a sensitivity of 33–50% [47]. It may be difficult to make a definite cytological diagnosis due to limited cellularity (e.g. cyst contents only), cellular degeneration, and the morphological overlap that exists between branchial cleft cyst and metastatic cystic head and neck squamous cell carcinoma [48]. The differential diagnosis can also include other benign conditions such as abscess and tuberculosis [42, 43, 45]. Cystic metastases of HNSCC often harbor HR-HPV and, in turn, overexpress p16. Caution is warranted, however, in interpreting p16 immunohistochemistry alone since focal strong p16 immunoreactivity can be seen in the superficial lining cells of branchial cleft cyst [49, 50]. The detection of HPV, EBV, and thyroglobulin within FNA samples may facilitate pathological diagnosis of malignant cystic lymphadenopathy and detection of occult primary tumors [45, 51].

Office-Based Conventional and Biological Endoscopy

Conventional office-based transoral oropharyngoscopy and transnasal pharyngolaryngoscopy is the first-level examination to detect primary lesions in case of CUP. With the progressive development of bioendoscopic techniques, the use of white-light (WL) should be integrated with high-definition Narrow Band Imaging (HD-NBI) assessment. NBI combined with magnifying endoscopy enhances the visualization of any superficial microvascular variation associated with early neoplastic changes (neoangiogenesis). The most accurate comparison of WL versus NBI office-based UADT and esophageal panendoscopy has been performed in a prospective randomized controlled multicenter trial [52], which evaluated 320 high-risk patients for superficial head neck cancer and esophageal SCC. The sensitivity of WL versus NBI was 7.7% versus 100% with a specificity of 95.5% versus 78.6%, respectively. The positive and negative predictive values for WL versus NBI alone were 50% versus 83.3% and 63.6% versus 100%, respectively [52]. A systematic review by Cosway et al. [53] specifically analyzed the diagnostic performance of NBI in identifying of the primary lesion in CUP. The sensitivity and specificity for NBI were 74% (range, 58–87%) and 86% (range, 76–93%), respectively. The authors recommended initial assessment with WL on the downward pass of the nasoendoscope, and any lesion should be assessed with both WL and a switch to NBI. This should be followed by NBI examination on the upward pass of the endoscope, with any lesion identified further assessed in WL mode [53]. A recent single center study on office-based NBI for SCC CUP confirmed these favorable results in terms of sensitivity, specificity, positive, negative predictive values, and accuracy (91%, 95%, 91%, 95%, and 90%, respectively), increasing the detection rate by 34.5% [54]. Based on these data, NBI examination of UADT in the office should be adopted in all tertiary referral centers. However, none of the most recent guidelines mention the role of office-based NBI-guided endoscopy in the management of unknown primary [16, 17].

Morphologic and Functional Imaging

Cross-sectional imaging of the neck such as contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) has demonstrably changed the detection of small primary cancers. Their detection potential is in the range of 9.3–23%, rising to 60% when suspicious radiologic findings direct subsequent endoscopic biopsies [4]. The availability of new MRI sequences has contributed to increased accuracy of this technique in the identification of the primary lesion. A recent study by Yoo et al. [55] compared the utility of contrast-enhanced three-dimensional (3D) T1-weighted high-resolution isotropic volume examination (THRIVE) MRI sequence, spin-echo (SE) T1-weighted MRI sequence, and CT in 73 patients with neck metastasis and undetected primary tumor at physical and

endoscopic examination. A gadolinium-enhanced MRI was performed with a 3-tesla system and contrast-enhanced CT using a protocol for the neck with at least contiguous 3-mm scans of the neck. The maximum detection rate of 73% was obtained with contrast-enhanced 3D THRIVE sequences, which had a higher sensitivity and accuracy for detecting primary tumors compared to contrast-enhanced SE T1-weighted sequences and contrast-enhanced CT [55]. The introduction of metabolic imaging techniques such as 18F-FDG-PET and subsequently 18F-FDG-PET/CT has provided the clinician with new tools to localize the primary tumor in patients with CUP. Thanks to these techniques, pooled data from a meta-analysis and systematic review showed a 37% increase in the discovery of small primaries with negative clinical and endoscopic examination [56]. Keller et al. [57] compared the diagnostic validity of the two techniques in CUP of the head and neck, and showed that 18F-FDG-PET/CT had a significantly higher overall detection rate than 18F-FDG-PET alone (55.2% vs 30.8%). Similarly, 18F-FDG-PET/CT has shown superior diagnostic performance in detecting the primary lesion compared to contrast-enhanced CT or combined contrast-enhanced CT and MRI [58]. In a meta-analysis of seven studies (246 patients) [59] focusing exclusively on 18F-FDG-PET-CT, the detection rate varied from 28% to 79%. This extremely wide range is clearly related to the high variability in the type, timing, and accuracy of the examinations included in the work-up. The pooled sensitivity and specificity were 0.97 and 0.68, respectively, with the data confirming that 18F-FDG-PET is associated with a high number of false-positive, which commonly correspond to an inflammatory uptake of Waldeyer's ring. This finding, together with the inability of 18F-FDG-PET-CT to identify very small lesions located in the base of tongue or tonsil that are typically seen to be responsible for HPV-related neck metastasis, have mitigated the initial enthusiasm on the accuracy of the examination to identify the primary lesion [60]. However, the ability of 18F-FDG PET-CT to diagnose distant metastasis as well as second tumors and serve as a baseline study for future comparisons in patients undergoing concurrent radiotherapy (RT)-chemotherapy (CHT) should not be neglected.

Endoscopy Under Anesthesia and Diagnostic Surgical Procedures

According to the literature, conventional examination under anesthesia (EUA) identifies the primary tumor in 16–26% of cases [61, 62]. None of these studies included HD-NBI in the work-up. In the latest NCCN guidelines, when office-based endoscopy and radiologic examination (MRI/CT and FDG-PET/CT) are negative, it is indicated to perform EUA [15]. When metastatic nodes involve level IV or lower level V, the guidelines recommend EUA including direct laryngoscopy, esophagoscopy, and bronchoscopy. On the other hand, for level I, II, III, or upper level V nodes, EUA using appropriate straight and angled telescopes, palpation and

inspection of the oral cavity and oropharynx, biopsy of areas of clinical concern and tonsillectomy, eventually associated with lingual tonsillectomy are recommended [15–17, 62]. The European Society for Medical Oncology guidelines recommend esophagogastroduodenoscopy to identify the primary site only if gastrointestinal symptoms are present or suspicion of a primary in the upper gastrointestinal tract is high based on patient's symptoms, history (e.g., alcohol abuse), or pathological laboratory or radiological parameters [63]. Bronchoscopy is recommended when there is an abnormality of the lung on chest imaging.

Performing blind biopsies from the nasopharynx, palatine tonsil, tongue base, and piriform sinus was a common practice in the past [64, 65]. Nowadays, most authors concur that it is not necessary to perform random biopsies of the nasopharynx or piriform sinus if these areas are radiologically and clinically negative. Tanzler et al. [65] reviewed the principal papers analyzing biopsy results by subsite, and mentioned that there was not a single positive random biopsy in the nasopharynx or piriform sinus. By contrast, the rate of a positive finding in the ipsilateral palatine tonsil and tongue base was high, ranging from 11% to 88% and 8% to 18%, respectively. Furthermore, Karni et al. [66] showed that transoral laser microsurgery (TLM) with targeted biopsies identified 94% of primary oropharyngeal cancers compared with only 25% with standard EUA.

Tonsillectomy is superior to deep tonsil biopsy in identifying an occult primary (39% vs 13% for the ipsilateral side) [67, 65]. Contralateral tonsillectomy identifies the primary tumor in 15–25% of cases. Although it is a controversial issue, performing bilateral tonsillectomy can be a reasonable option, given the minimal additional morbidity of the procedure [67, 68].

According to the official UK guidelines, the role of tongue base mucosectomy by TLM or transoral robotic surgery (TORS), with or without PET–CT or HPV positivity, needs prospective evaluation. The suggestion is to limit its use to centers where facilities and expertise are present [16]. Recent case series of CUP reported high rates of detection ranging from 86% to 94% using TLM and TORS [64, 69–73]. The optimal extent of TORS for identification of CUP is not known: the NCCN guidelines offer the option of performing a lingual tonsillectomy while not specifying whether this should be performed unilaterally or bilaterally [15, 17]. Interesting clues are provided by the study conducted by Geltzeiler et al. [69] on a group of 50 patients, where the overwhelming majority had an HPV-related neck node metastasis. By lingual mucosectomy and tonsillectomy, they identified the primary lesion in 37 (74%) patients, with a more frequent location in the base of the tongue than in palatine tonsil (86% vs 14%) [69]. Interestingly, bilateral tongue base resection identified the primary tumor more often than unilateral (80% vs 68%), and negative margins could be obtained in 19 (51%) patients [69]. This means that approximately half of patients should still receive adjuvant RT-CHT on the pharynx, thus minimizing the chance that lingual tonsillectomy decreases morbidity [74]. Another argument for not performing bilateral lingual and palatine tonsillectomy simultaneously can be based on the theoretical risk of pharyngeal stenosis after surgery, which may be compounded by the use of adjuvant RT or RT-CHT [75].

Therapeutic Options and Prognosis

In view of the rarity of the disease, current recommendations of treatment for CUP are not based on prospective or randomized studies. NCCN guidelines [15] allow various treatment options for each nodal status, which can be modulated taking into account the patient's comorbidities and the need and extent of irradiation to the mucosal sites potentially harboring the primary lesion. In this respect, HPV and EBV positivity have a relevant role in guiding the identification of mucosal sites at highest risk. Basically, therapeutic options include neck dissection (ND) alone, RT with or without concurrent CHT, followed by ND in case of PET positivity in the neck thereafter, and a combination of ND and RT with or without concurrent CHT [76]. The aim of treatment is to eradicate nodal disease as well as the hidden focus of the potential index cancer, without adding undue morbidity.

In 2016, Liu et al. [77] published the results of a meta-analysis performed on 33 studies with the intent to compare the therapeutic efficacy of different treatment regimens and provide a higher level of evidence for optimizing the RT approach in CUP. The conclusion was that surgery combined with RT to bilateral neck and potential primary sites of the tumor might be the preferred treatment option, since it was associated with improvement in local and regional control [77]. Only 2 studies included in the meta-analysis [78, 79] analyzed the difference in terms of toxicity between unilateral and bilateral neck irradiation, and found that in the latter case there was an increased risk of severe acute toxicity and xerostomia. However, the conclusions of the meta-analysis were affected by some flaws. The studies were collected over a longtime interval, so that the results of RT might have been influenced by changes in RT technology; the additional value on outcomes of CHT was not analyzed; and no quality of life issues could be addressed due to lack of information. These limitations prompted Dharmawardana et al. [80] to plan a further systematic review which is still ongoing.

Similarly to what is generally observed for advanced head and neck cancers, the current trend in the management of CUP is to try to minimize the use of three modalities of treatment (surgery, RT, CHT). In line with this principle, a preference for RT-CHT followed by ND only in patients who do not achieve complete clinical or metabolic disease response is clearly seen for advanced stage neck disease. On the other hand, the role of ND has been viewed with renewed interest in early stage neck disease (pN1 and early pN2a without extracapsular nodal extension [ENE]). This approach has the advantage to accurately stage the neck, using histopathological findings to guide the indications for RT and to detect unexpected findings (i.e. ENE), which call for adjuvant RT-CHT. In fact, it has been estimated that approximately one third of patients with N1 presentation are up-staged as a result of ND [17, 81]. An argument against the use of surgery in favor of RT is the possibility to treat, at the same time, the neck and the mucosal sites that are at risk of harboring the primary lesion.

Classical issues on the treatment of CUP which are still open to discussion are: (1) the extent of irradiation fields (unilateral or bilateral neck? mucosal sites at risk

of harboring the primary lesion?) (2) the type of ND to be performed. However, in the last decade the debate in the literature has focused more on HPV status, in view of its well-demonstrated impact on survival outcomes.

Extent of Irradiation Fields and Type of RT

A study on a large number of patients [19] and review articles [82] published in the early 2000s suggested that bilateral irradiation could lead to a lower rate of loco-regional recurrences. Although not all subsequent papers confirmed the presence of an advantage in loco-regional control [21], the earlier mentioned meta-analysis, reported by Liu et al. [15], showed that significantly less neck recurrence or emergence of a primary tumor were noted in patients receiving bilateral irradiation, with a trend toward increased 5-year overall survival (OS) and disease-free survival (DFS) [77]. In the setting of primary treatment, NCCN guidelines recommend to deliver 66–70 Gy to the primary nodal disease levels, 45–50 Gy to the lower-risk neck sites, and 60–66 Gy to the mucosal risk sites. Treatment volume should be determined by the nodal levels involved, tumor size, and HPV/EBV status [15]. In the attempt to balance disease control and side-effects of treatment, bilateral neck irradiation probably should be reserved to patients with advanced N-stage or with a high likelihood of having the primary lesion located along the midline (base of the tongue or nasopharynx), as typically suggested by positivity for HPV or EBV virus. Retropharyngeal nodes are commonly included in the target volume. However, a study from the University of Toronto of 68 patients specifically addressing the issue of retropharyngeal lymph node involvement in CUP came to the conclusion that patients with p16 positive CUP and limited lateral neck nodal disease (N1–N2a) without radiographic evidence of ENE may be candidates for prospective studies evaluating avoidance of prophylactic treatment to the retropharyngeal region [83].

Another crucial issue in managing CUP is the need to include in the irradiation field the mucosa of the pharyngo-laryngeal axis. This was common practice in the past. However, the observation that (1) the larynx and hypopharynx are rarely the site of late appearance of a mucosal primary, (2) patients with CUP are now more frequently non-smokers with HPV-positive disease, and (3) the inclusion of the larynx and hypopharynx in the field of irradiation substantially increases toxicity [84] has favored the exclusion of the laryngo-hypopharyngeal area [85]. The widespread increasing occurrence of HPV-related CUP is leading to modify the principles which guide the decision to irradiate the nasopharynx and oropharynx. Although EBV-related cancer rarely occurs in non-nasopharyngeal head and neck sites, EBV positivity of FNAC is highly suggestive for the presence of a microscopic carcinoma in the nasopharynx, so that pharyngeal mucosa and the neck should be treated accordingly. In HPV-positive patients the likelihood that the primary is located in the oropharynx is extremely high so that irradiation of the oropharynx is strongly recommended. Mourad et al. [86], in a single-institution retrospective study of 68 patients with CUP, who were not tested for HPV/EBV status and who underwent conservative mucosal-sparing RT targeting only the oropharynx and bilateral neck,

observed an actuarial 3-year LC of 98.5% and the emergence of a primary in 1 (1.5%) patient only. These data might suggest that even in SCC CUP patients who are HPV-negative/EBV negative RT to mucosal sites could be limited to the oropharynx, when all other investigations have excluded primary lesions elsewhere in the head and neck area [86].

Intensity-modulated RT (IMRT), which has progressively been applied during the last 20 years, has contributed to reduce early and late toxicity also in the management of CUP without jeopardizing survival outcomes [87, 88]. Chen et al. [87] retrospectively analyzed 51 patients with CUP treated by RT. Twenty-four (47%) patients received conventional RT, and 27 (53%) were treated with IMRT. The proportion of patients receiving concurrent CHT during RT was similar in the two groups. There were no significant differences in OS, loco-regional control (LRC), and disease-specific survival (DSS) related to the RT technique. However, the incidence of severe xerostomia in the late setting was 58% and 11% among patients treated with conventional RT and IMRT, respectively ($p < 0.001$). The percentages of patients who were gastrostomy-tube dependent at 6 months after treatment were 42% and 11%, respectively ($p < 0.001$). Madani et al. [88] compared the results of IMRT on putative mucosal and bilateral nodal sites in 23 patients with CUP with those observed in a group of 18 patients undergoing conventional RT. Compared with conventional RT, the incidence of grade 3 acute dysphagia was significantly lower in the IMRT group (4.5% vs. 50%, $p = 0.003$). By 6 months, grade 3 xerostomia was detected in 11.8% patients in the IMRT group vs. 53.4% in the control group ($p = 0.03$). No long-term grade 3 dysphagia or skin fibrosis was observed after IMRT, while these late complications occurred in the same number of patients after conventional RT (26.7%, $p = 0.01$). The 2-year OS and distant metastasis free probability after IMRT did not differ significantly from those of conventional RT (74.8% vs. 61.1% and 76.3% vs. 68.4%, respectively). A recent paper from MD Anderson Cancer Center [84] reviewed a series of 260 patients with CUP treated with IMRT, which principally targeted both sides of the neck, nasopharynx, and oropharynx. They reported 5-year OS, regional control (RC), and distant metastasis free survival of 84%, 91%, and 94%, respectively. The analysis of toxicity revealed that over 40% of patients required gastrostomy tube use for up to 6 months, and 7% patients were diagnosed with chronic radiation-associated dysphagia. These results highlight how difficult it is to balance control of the disease with treatment-related toxicities.

Recently, attention has been also focused on the use of more limited-field radiation techniques, particularly in the setting of HPV/16-positivity. The assumption that most HPV-positive CUPs originate within the oropharynx is supported by recent TORS series that reported primary detection rates exceeding 80% in HPV-positive patients [1, 89, 69]. In particular, Chen et al. [90] showed that excellent rates of LRC and progression-free survival (PFS) were obtained with the use of limited RT fields targeting the ipsilateral oropharynx and cervical neck. The fact that oropharynx-tailored ipsilateral irradiation resulted in a dramatic improvement in swallowing function is likely translated into gains in quality of life. The actuarial 2-year estimates of LRC, PFS, and OS were 91%, 87%, and 92%, respectively [90]. Also Tiong et al. proposed unilateral irradiation specifically for p16/HPV-positive CUPs after observing no contralateral failure in this group of patients [91].

The Role of Surgery and Type of ND

Surgical approaches for primary identification are becoming well-established procedures. Different authors have accurately evaluated their diagnostic potential with the aim to progressively refine their role in the work-up of CUP.

On the other hand, the role of ND both in single modality therapy and in multimodal treatment approaches has not been clearly understood. In general, as mentioned earlier, ND has the advantage to accurately stage the neck using histopathologic findings to guide the indications for RT and to detect unexpected findings (i.e., ENE), which call for adjuvant RT-CHT. In fact, it has been estimated that approximately one-third of the CUP patients with N1 presentation are up-staged as a result of ND [17, 81]. However, there are no trials comparing the efficacy of ND ± RT(CHT) vs. RT(CHT) ± ND in either early or advanced CUP. Furthermore, the majority of retrospective studies are burdened by substantial limitations: significant risk of selection bias, low number of patients, and lack of an adequate distinction between HPV-related and not HPV-related tumors.

In case of limited neck disease (N1 and N2a without ENE), Iganej et al. [92] demonstrated that surgery alone was an effective treatment for CUP, resulting in an 81% regional control. Mizuta et al. [93] further reinforced this concept by showing satisfying disease control with ND alone and emphasizing the efficacy of salvage treatment in patients who developed regional recurrence (20% in total, all in the ipsilateral neck). In this view, postoperative RT could be omitted in patients with N2a as well as N1 disease if pathological ENE is not confirmed.

When all stages of neck involvement are considered, the results are more controversial. Wallace et al. compared the regional control rates of patients who received RT alone with those who were treated by ND + adjuvant RT or RT + planned ND. They found that patients undergoing both RT and ND obtained significantly better RC compared to patients undergoing RT alone [94]. In general, a combined modality is strongly favored in case of advanced stage neck disease. However, based on existing evidence, it is not apparent what the optimal therapy should be: surgery with postoperative RT ± CHT or RT ± CHT followed by ND only in patients who do not achieve complete clinical or metabolic (PET) response.

Amsbaugh et al. [95] reported on 66 patients affected by CUP, more than 50% of whom with intermediate-advanced neck disease (N2b, N2c, N3): 37 received ND followed by adjuvant RT±CHT and 29 primary RT ± CHT. The first group had improved local (96.7% vs. 54.1%, $p = 0.003$) and LRC (82.2% vs. 46.4%, $p = 0.068$), but OS was the same among the two cohorts ($p = 0.641$). Given these discrepancies and considering the low number of patients, it is difficult to clearly favor a surgical or non-surgical approach based on these data.

Interestingly, in the multicenter analysis by Mizuta et al. [93] the treatment options were not a significant factor concerning neck control, especially considering patients with advanced disease (N2b–3). This has also been confirmed by Aslani et al. [20], reporting on the results of 61 CUPs treated by surgical or non-surgical modalities with no difference in OS or local relapse-free survival. However, it

should be noted that patients in this series were treated with older techniques (including cobalt RT in some cases) and mostly with large bilateral fields. A study by Zhou et al. [14], showed similar results using more recent RT techniques (3D conformal or intensity-modulated RT). In fact, 5-year OS and DFS for RT-based and surgery-based treatments were comparable (OS 73% vs 68%, respectively; DFS 65% vs 64%, respectively).

A recent analysis on a large series of patients (N = 260) with CUP treated at MD Anderson Cancer Center [84] helps to refine the therapeutic choice. In particular, there was no statistically significant difference in survival between patients who did or did not undergo ND (in both primary or secondary setting). A subgroup analysis of patients who received RT ± CHT without evidence of gross lymph node disease (including patients who underwent previous NDs or excisional biopsies), compared with those who had residual nodal disease, revealed an improved survival rate in the former group (5-year OS rate: 92% vs. 82%, p = 0.02). However, this variable was not significant in multivariate analysis (p = 0.45).

Considering the advantages of current radiation techniques, it is possible to state that surgery- and RT-based regimens lead to similar survival outcomes. Therapy should be chosen on a case by case basis, by carefully assessing patient characteristics and balancing the expected impact of each different approach. This is especially true when considering advanced disease. In particular, surgery may be a useful first-line approach for patients with significant comorbidities contraindicating platinum-based CHT. These patients may be effectively managed by ND followed by adjuvant RT.

The decision regarding the type of ND should be individualized and based on the extent of nodal disease. The United Kingdom National Multidisciplinary Guidelines [16] suggest performing a modified radical ND, preserving the ipsilateral sternocleidomastoid muscle, internal jugular vein, and accessory nerve in the vast majority of cases. However, it is also stated that, in early-intermediate disease, selective ND can be used with similar efficacy to modified-radical ND [96]. In fact, selective ND is widely accepted treatment choice as all five levels of the neck are rarely simultaneously at risk in this setting. Generally, even in the case of extensive neck involvement, sublevel IA dissection may be omitted [4]. However, even concerning this issue there are no randomized data to support the choice between selective or modified-radical ND.

Prognostic Factors: The Dominant Role of HPV Status

By applying a prognostic algorithm based on clinico-pathologic subgroup, performance status, and the absence of leucocytosis, Petrakis et al. [97] proved that patients suffering from CUP of the head and neck represent the most favorable group among other localizations, with 5-year OS and LRC rates which are currently in the range of 59.4–84% [98, 84] and 65–74% [13, 14] for OS and DFS, respectively.

Many factors are well-known to have a negative impact on the prognosis of CUP of the neck: older age [99, 100, 13], advanced N stage [100, 84, 98], ENE [98], N3 [13, 101], non-squamous cell carcinoma [98], multiple level positive nodes [101], and positive nodes in the lower neck [101].

However, the specific literature of the last decade has drawn the attention on the relevant role that p16/HPV status has on the prognosis of CUP (Table 3). Apart from 3 studies [102–104], the overwhelming majority of recent papers have demonstrated that p16/HPV positivity has a favorable impact on different survival outcomes. In the study of Kamal et al. [84], in which p16/HPV status was known in 113/260 patients and positivity was assessed based on the concordance of both tests, positive patients had improved rates of OS, neck control, and distant metastases-free survival at 5 years, (91%, 94%, and 95%, respectively).

Inspite of a clear separation of CUP patients in two groups with distinctive prognostic features, there is at present not enough scientific evidence to modify the treatment of HPV-positive patients by de-escalating RT, CHT, or both modalities, similar to what is advised for oropharyngeal cancer, which has a much higher incidence. To provide high-level evidence that de-escalation can be as effective as standard treatment, several studies are ongoing in patients with oropharyngeal cancer. However, the rarity of CUP prevents planning randomized prospective studies of this specific disease. With the intent to bypass this limitation, some authors [105, 1] have evaluated if survival outcome measures in HPV-positive oropharyngeal cancer parallel those of HPV-positive CUP. Hosni et al. [105] retrospectively compared treatment outcomes in 2 groups: 54 patients with T1-category base of tongue carcinoma (50 [92.6%] p16-positive) treated with definitive IMRT, and 61 patients with CUP (38 [62.3%] p16-positive) who received definitive (42 [68.9%]) or postoperative (19 [31.1%]) IMRT. There were no significant differences in LC, RC, DC, DSS, and OS between the BOT carcinoma and CUP site groups stratified by p16 status. Ross et al. [1] performed a matched-pair analysis to compare outcomes between T0N1-3M0 HPV+ CUP and T1-2N1-3M0 HPV+ oropharyngeal cancer (OPC). Of 298 patients with T1-2N1-3 OPC, 48 were matched to 48 HPV+ CUP patients (32 with confirmed and 16 imputed HPV status). There were no significant differences between the CUP and OPC groups for 3-year DFS (89% vs. 85%, $p = 0.44$), and 3-year OS (91% vs. 91%, $p = 0.11$), respectively.

Obviously the evidence provided by these 2 studies is very limited in view of the small numbers of patients under analysis. Matched-pair analyses on large groups of patients are needed to definitively confirm that the differences in terms of survival outcomes between HPV-positive and negative OPC patients are similar to those between HPV-positive and negative CUP patients. This would allow to apply the results of ongoing de-escalation trials on OPC to CUP.

Table 3 Prognosis of CUP of the head and neck in the 'HPV era'

Authors	No of pts	Results of UA	Results of MA	Survival
Vent et al. [32]	47	*Better prognosis*: p16 positive	NP	*5-year OS* *(p = 0.045)* • *p16-positive*: 69% • p16-negative: 33% *5-year DFS (p = NS)* • *p16-positive*: 89% • p16-negative: 77%
Keller et al. [106]	39	*Better prognosis*: • p16 positive • Younger age • Higher grade *Worse prognosis*: • Macroscopic extracapsular extension	NP	*5-year OS* *(p < 0.001)* • *p16-positive*: 92% • p16-negative: 30% *5-year DSS* *(p = 0.09)* • *p16-positive*: 92% • p16-negative: 60%
Dixon et al. [12]	73	*Better prognosis*: • Gender (males) • p16 positive *Worse prognosis*: • N2c–N3	*Better prognosis*: • p16 positive *Worse prognosis*: • N2c–N3	*3-year OS (p = 0.1)* • *p16-positive*: 87% • p16-negative: 74% *3-year DFS* *(p = 0.007)* • *p16-positive*: 79% • p16-negative: 56%
Axelsson et al. [13]	68	*Better prognosis:* • p16 positive *Worse prognosis:* • Advanced age • N3 • Neck dissection alone	*Better prognosis:* • p16 positive *Worse prognosis:* • Advanced age • N3-stage	*10-year OS* *(p < 0.001)* • *p16-positive*: 82% • p16 negative: 39%
Sivars et al. [29]	88	*Better prognosis:* • HPV-DNA positive • p16 positive • High $CD8^+$ tumor-infiltrating lymphocytes	*Better prognosis:* • HPV-DNA positive • p16 positive *Worse prognosis:* • Advanced age • Smoking history	*3-year OS* *(p = 0.028)* • *HPV-DNA positive:* 91.7% • HPV-DNA negative: 42.9% *3-year DFS* *(p = 0.045)* • *HPV-DNA positive:* 100% • HPV-DNA negative: 66.7%

(continued)

Table 3 (continued)

Authors	No of pts	Results of UA	Results of MA	Survival
Zhou et al. [14]	75	*Better prognosis:* • p16 positive *Worse prognosis:* • Advanced age • Smoking history • N2c–N3	*Better prognosis:* • p16 positive *Worse prognosis:* • Advanced age • Smoking history • N2c–N3	*5-year OS (p < 0.001)* • *p16-positive*: 96% • p16-negative: 33% *5-year DFS (p = 0.001)* • *p16-positive*: 90% • p16-negative: 33%

DFS disease free survival, *DSS* disease-specific survival, *MA* multivariate analysis, *N* N category, *NP* not performed, *NS* not significant, *OS* overall survival, *pts* patients

Conclusions

In spite of geographical variations, the occurrence of HPV-related CUP is constantly rising. Several studies have clearly shown that HPV-positive patients have a better prognosis compared to HPV-negative. The standard application of a thorough work-up including morphologic and metabolic imaging as well as, especially in HPV-related lesions, surgical procedures (tonsillectomy, mucosectomy of the base of the tongue) has consistently reduced the number of "true" CUP. Recent data indicate that the population of CUP patients include two main broad categories (HPV-positive and HPV-negative), which differ in terms of natural history and prognosis and probably require a different treatment. In the years ahead, the scientific community has the challenge to provide evidence that treatment in HPV-positive CUP can be de-escaleted.

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Cytotoxic Chemotherapy and Targeted Therapy in Nasopharyngeal Cancer

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Introduction

Nasopharyngeal carcinoma (NPC) has distinct epidemiologic, biologic and clinical characteristics that separate it from other head and neck cancers. Approximately 86,500 cases were diagnosed globally in 2012, with a unique geographical and ethnic distribution [1]. In the United States and Western Europe, the incidence is 0.5–2 of cases per 100,000 [2]. In Southern China, where it is endemic, the incidence is as high as 25 cases per 100,000. About 71% of the new cases diagnosed in 2012 were in East and Southeast Asia. The incidence is also two- to threefold higher in males than in females with peak age of occurrence between 50 and 60 years [1]. Though the incidence rates between Asian, North American, and Nordic countries vary, each region documented a decrease in the annual age-standardized incidence rates of NPC (from −0.9 to −5.4%) and in the age-standardized mortality rates (from −0.9 to −6.5%) over the past several decades [3].

NPC is categorized into several histologic subtypes according to the World Health Organization (WHO). Type I consists of differentiated tumors with surface keratin; Type II is non-keratinizing differentiated tumors, and Type III is non-keratinizing undifferentiated tumors. Type III is most common in endemic areas and is strongly associated with Epstein-Barr virus (EBV) infection [4]. In the most recent WHO classification schema released in 2005, types II and III have been combined to form the non-keratinizing group, and basaloid squamous cell carcinoma was added [5].

EBV has been extensively studied as a primary etiologic factor in the pathogenesis of NPC. It is being used clinically for disease screening, monitoring, as a predictive biomarker, and as a potential targeted therapy in advanced disease. Human

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papillomavirus (HPV) has also been linked to NPC, specifically with non-endemic disease in the Caucasian population [6, 7]. Studies have shown that EBV- and HPV-associated disease are almost always mutually exclusive [8, 9]. Non-endemic patients with HPV-associated tumors have better local control and overall survival, and patients with EBV-associated tumors more commonly have distant metastatic involvement [10]. Non-viral NPC has worse overall outcome compared to viral-associated disease. A recent 2018 study of endemic NPC patients showed that HPV-associated disease had improved locoregional control and overall survival compared to EBV-associated disease [8].

Other environmental risk factors include exposure to the cooking of salt-cured food, consumption of salted fish and fermented food, and use of Chinese herbal medicines. The risk of NPC may be influenced by genetic factors as well. Genome sequencing has shown susceptibility within the MHC region of chromosome 6p21 which codes for human leukocyte antigen (HLA) and non-HLA genes [11–13]. Genes involved with DNA repair, cell cycle checkpoint regulation, and cell adhesion and migration have also been linked.

Over the past decades, both incidence and mortality from NPC have fallen [3]. The exact reason for the decline in incidence is unclear, but lifestyle modification including decreased intake of salted fish in endemic areas has likely contributed. Improvements in treatment outcomes are likely a result of population screening, enhanced imaging methods, and advances in radiation and systemic therapy. This chapter will review the current strategies in NPC treatment, with a focus on systemic chemotherapy, and will explore novel therapy that is actively being developed.

Treatment in Localized Disease

The goal of treatment in localized, non-metastatic NPC is cure. This is typically achieved with single modality (i.e. RT alone) or bimodality therapy which depends on disease stage. Early stage disease (stage I, i.e. T1N0) has excellent outcomes with radiotherapy (RT) alone. Intermediate (stage II) and locally advanced disease routinely requires a more intensive treatment approach with combinations of RT and chemotherapy. Due to the deep anatomical location of the nasopharynx and its proximity to vital neuro-vasculature, surgery is used as initial or salvage treatment in highly selected cases based upon the availability of a skilled surgeon [14]. Neck dissection may be performed after initial treatment for residual nodal disease or neck recurrence.

Radiotherapy

Due to the high radiosensitivity of NPC RT is the mainstay of treatment. Intensity-modulated radiation therapy (IMRT) has become a standard approach since it allows for tumoricidal doses of radiation to be administered while minimizing exposure to

adjacent normal tissue. IMRT has improved locoregional control and overall survival in NPC while decreasing the incidence of late toxicity compared to a conventional two-dimensional technique [15, 16]. Modifying the fraction size and treatment time has not shown specific benefits in treatment outcomes [17, 18]. RT alone has yielded very high locoregional control and a 90% overall survival rate in patients with early stage (stage I) NPC [19].

Chemotherapy

Systemic chemotherapy is utilized in multiple settings to treat NPC. Chemotherapy can be integrated into a multimodality regimen either at the same time with RT as concurrent chemoradiotherapy (CCRT), after RT as adjuvant chemotherapy (AC), or before the start of RT as induction chemotherapy (IC).

Concurrent Chemoradiotherapy

Since the Intergroup trial by Al-Sarraf et al. (INT-0099) established the superiority of CCRT (using concurrent cisplatin and radiation therapy followed by adjuvant cisplatin and 5-fluorouracil) in treating locally advanced NPC, a large number of trials have investigated the optimal role of chemotherapy [20] (Table 1). In stage II NPC, Chen et al. compared RT alone to CCRT with weekly cisplatin (30 mg/m^2). This study included patients with WHO types II–III NPC stages T1-2N1M0 or T2N0M0 with parapharyngeal extension; 13% of all patients had AJCC 7th edition stage N2, i.e., stage III disease. The addition of cisplatin resulted in a statistically significant improvement in overall survival (OS) rate (5-year OS of 94.5% versus

Table 1 Phase III randomized trials of CCRT vs RT in NPC

Study	Year	Stage	N	Regimen	5-year PFS (%)	5-year OS (%)
Lin [59]	2003	III–IV (AJCC 4th edition)	284	RT alone RT + cisplatin/5FU	53 72 P = 0.001	54 72 P = 0.002
Chan [60, 61]	2002, 2005	Ho's N2/N3 or node ≥4 cm (NI)	350	RT alone RT + cisplatin weekly (40 mg/m^2)	52 62 P = 0.076	59 72 P = 0.048
Zhang and Wu [25, 26]	2005, 2013	III–IV (AJCC 5th edition)	115	RT alone RT + oxaliplatin	63 74 P = 0.02 (DMFS)	60 73 P = 0.03
Chen [21]	2011	II (Chinese 1992)	230	RT alone RT + cisplatin weekly (30 mg/m^2)	83.9 94.8 P = 0.007	85.5 94.5 P = 0.007

PFS progression-free survival, *OS* overall survival, *5FU* 5-fluorouracil; Chen et al. inclusion criteria: T2 but only with parapharyngeal extension (AJCC stage III (i.e. N2) were 13%)

85.5%, HR 0.30, 95% CI 0.12–0.76, P = 0.007) with the difference being attributed primarily to an improvement in distant metastasis-free survival (at 5 years, 94.8% versus 83.9% [HR = 0.27, 95% CI 0.10–0.74, P = 0.007]) [21].

For locally advanced stages, a combined modality approach including CCRT (with or without additional chemotherapy as either IC or AC) is considered standard of care. Various combination regimens have been studied, but the widely accepted standard chemotherapy agent is cisplatin in either 3-weekly or weekly schedules (100 mg/m^2 on days 1, 22, and 43 or 40 mg/m^2 weekly during RT), with a suggested minimum cumulative cisplatin dose for optimal efficacy 200 mg/m^2 [22]. Three-weekly versus weekly cisplatin schedules in CCRT have been compared in recent randomized trials that did not show a significant difference in overall survival or failure-free survival between the two regimens [23, 24]. However, the toxicity profiles of these two approaches may differ as seen in the phase III study by Liang et al., where weekly cisplatin was associated with increased rates of grade 3–4 leukopenia (27% versus 16%, P = 0.002) and thrombocytopenia (5% versus 1%, P = 0.015) compared to three-weekly dosing. There were no significant differences in other acute toxicities [22].

Alternative agents that have been studied in CCRT include oxaliplatin and carboplatin. CCRT with oxaliplatin resulted in a significant improvement in overall survival when compared to RT alone (2-year OS 100% versus 77%, P = 0.01 and 5 year OS 73.2% versus 60.2%, P = 0.028) [25, 26]. A non-inferiority study comparing CCRT with carboplatin to CCRT with cisplatin showed a similar 3-year overall survival rate (79.2% versus 77.7%, P = 0.99; HR 0.83, 95% CI: 0.63–1.01) [27]. Carboplatin was better tolerated with less renal toxicity, leucopenia, and anemia compared to cisplatin. There was, however, an increased incidence of thrombocytopenia. Carboplatin is an option for patients who are elderly, have borderline performance status, or decreased renal function.

Adjuvant Chemotherapy

Controversy continues to surround the role of AC and IC. AC typically consists of cisplatin and 5-fluorouracil given every 4 weeks for 3 cycles after the completion of CCRT as was done in the US INT-0099 [20]. Several trials have shown a benefit in overall survival when comparing CCRT plus AC to RT alone (Table 2). However, AC is generally poorly tolerated. The major question is whether AC adds further benefit to standard CCRT. In a large multicenter study by Chen et al., 508 patients with locally advanced non-metastatic disease (AJCC 5th edition stages III–IVB, excluding T3-4N0) were followed after randomization to CCRT plus AC with cisplatin and 5-fluorouracil versus CCRT alone. There was no improvement in failure-free survival with CCRT plus AC versus CCRT alone (75% versus 71%, HR 0.88, 95% CI 0.64–1.22) [28, 29]. The MAC-NPC meta-analysis showed an overall survival benefit in both the CCRT group (HR 0.80, 95% CI 0.7–0.93) and CCRT plus

Table 2 Selected phase III randomized trials of CCRT plus AC vs RT alone, and IC plus CCRT vs CCRT alone in NPC

Study	Year	Stage	N	Regimen	5-year PFS (%)	5-year OS (%)
Al-Sarraf [20]	1998, 2001	III–IV (AJCC 4th edition)	147	RT alone RT + cisplatin (3-week) followed by cisplatin + 5FU	29 58 P = 0.001	37 67 P = 0.001
Wee [62]	2005	III–IV (AJCC 5th edition)	221	RT alone RT + cisplatin (3-week) followed by cisplatin + 5FU	53 72 P = 0.009 (3-year)	65 80 P = 0.061 (3-year)
Chen [28]	2012	III–IV (AJCC 6th edition)	251	RT + cisplatin (weekly) RT + cisplatin (weekly) followed by cisplatin + 5FU	84 86 P = 0.13 (2-year)	92 94 P = 0.32 (2-year)
Chen [63]	2013	III–IVb (AJCC 5th edition)	316	RT alone RT + cisplatin (weekly) followed by cisplatin + 5FU	57 68 P = 0.015	62 72 P = 0.043
Lee [64]	2010	III–IVb (AJCC 5th edition)	348	RT alone RT + cisplatin (3-week) followed by cisplatin + 5FU	53 62 P = 0.035	64 68 P = 0.22
Cao [36]	2017	III–IVb	476	RT plus cisplatin Cisplatin, 5FU followed by RT plus cisplatin	82 74 P = 0.028 (3-year)	88.2 88.5 P = 0.815 (3-year)
Sun [35]	2016	III–IVb	476	RT plus cisplatin Docetaxel, cisplatin, 5FU followed by RT plus cisplatin	72 80 P = 0.034 (3-year)	

RT radiation therapy, *CCRT* concurrent chemoradiotherapy, *AC* adjuvant chemotherapy, *IC* induction chemotherapy, *5FU* 5-fluorouracil, *PFS* progression-free survival, *OS* overall survival

AC group (HR 0.65, 95% CI 0.56–0.76), with an overall survival benefit in patients receiving any form of chemotherapy with radiation (HR 0.79, 95% CI 0.73–0.86) [30]. Similarly, a 2017 individual patient data network meta-analysis, which included the trials in the MAC-NPC meta-analysis, found the highest probability of overall survival benefit in patients receiving CCRT plus AC when compared to RT alone (HR 0.65, 95% CI 0.56–0.75) [31]. There was also a significant benefit in CCRT versus RT alone (HR 0.77, 95% CI 0.64–0.92), but there was no benefit when comparing the CCRT plus AC group to CCRT alone (HR 0.85, 95% CI 0.68–1.05). Therefore, the use of CCRT plus AC is supported by phase III data and meta-analyses, primarily based on comparisons with RT alone. However, the added benefit of AC to CCRT has not been demonstrated yet.

The Impact of EBV DNA on the Use of Adjuvant Chemotherapy

In patients with baseline detectable EBV DNA levels prior to starting treatment, there is mounting evidence that detectable post treatment levels lead to an increased likelihood of recurrence and are a poor prognostic factor [32]. Recent studies have aimed to incorporate EBV levels to guide treatment. In a phase III study, the Hong Kong NPC study group evaluated the potential role of adjuvant chemotherapy in AJCC 6th edition stages IIB-IVB NPC patients with detectable EBV DNA PCR 6–8 weeks following RT or CCRT [33]. One hundred and four patients were randomized to receive 6 cycles of cisplatin and gemcitabine or routine follow up without chemotherapy. There was no significant difference in relapse-free (HR 0.92, 95% CI 0.51–1.66) or overall survival (HR 1.09, 95% CI 0.57–2.11) between the two arms. However, we note that the sample size of this study may not have been large enough to look at small survival differences with the use of AC. The survival outcome of the entire cohort (N = 789) confirmed that patients with detectable post-RT EBV DNA had a significantly worse prognosis with regard to relapse-free survival (at 5 years, 49.2% in EBV DNA+ vs 79.6% EBV DNA−; HR 3.36, 95% CI 2.58–4.38; $P < 0.0001$) and overall survival (at 5 years, 60.2% in EBV DNA+ vs 87.5% EBV DNA−; HR 3.19, 95% CI 2.38–4.27; $P < 0.0001$). The NRG is currently conducting a randomized study investigating AC in AJCC 7th edition stages II–IVB NPC based on post-treatment EBV DNA levels [NRG HN001, NCT02135042]. EBV DNA negative patients are randomly assigned to AC with cisplatin and 5-fluorouracil or no AC. EBV DNA positive patients are randomly assigned to receive AC with either cisplatin-5-fluorouracil or paclitaxel-gemcitabine.

Induction Chemotherapy

The role of IC prior to CCRT or RT is also controversial. In a phase II randomized study, Hui et al. investigated induction cisplatin (75 mg/m^2) and docetaxel (75 mg/m^2) every 3 weeks for 2 cycles prior to CCRT (IMRT with cisplatin 40 mg/m^2 weekly) versus CCRT alone and found improved progression free survival (88.2% versus 59.5%, HR = 0.49; 95% CI, 0.20–1.19; P = 0.12) and overall survival (94.1% versus 67.7% HR = 0.24; 95% CI, 0.078–0.73; P = 0.012) in the IC group at 3 years [34]. The results of phase III studies, however, have been inconsistent. In both MAC-NPC and a 2017 individual network meta-analysis, there was no benefit of induction chemotherapy on overall survival [30, 31]. However, two recent phase III studies have supported the use of induction chemotherapy. A multicenter study by Sun et al. compared CCRT (IMRT + cisplatin 100 mg/m^2 every 3 weeks × 3 cycles) with and without IC using cisplatin (60 mg/m^2 day 1), 5-fluorouracil (600 mg/m^2/day days 1–5) and docetaxel (60 mg/m^2 day 1) (TPF) for 3 cycles every 3 weeks in NPC patients with AJCC 7th edition stages III–IVB disease (T3-4N0 patients excluded) [35]. The IC plus CCRT group had a significant improvement in failure-free survival (80 versus 72%, HR 0.68, 95% CI 0.48–0.97; P = 0.034) and overall survival (92 versus 86%, HR 0.59, 95% CI 0.36–0.95; P = 0.029) at 3 years.

Moreover, Cao et al. investigated IC with cisplatin (80 mg/m^2 on day 1) and 5-fluorouracil (800 mg/m^2/day days 1–5) every 3 weeks for 2 cycles with CCRT versus CCRT alone in patients with AJCC 7th edition stages III–IVB (T3N0-1 patients excluded) [36]. Although IC plus CCRT resulted in an improved failure-free survival, there was no statistically significant difference seen in 3-year overall survival (88.2% versus 88.5%, P = 0.815) or in locoregional relapse-free survival (94.3% versus 90.8%, P = 0.430). The differences among patient selection and treatment regimens has led to wide variability in study outcomes. Although IC followed by CCRT has emerged as a potentially promising treatment option, additional clinical trials with this approach are warranted.

Toxicities

Common acute side effects of RT include mucositis, xerostomia, dysphagia and dermatitis. Symptoms usually improve following cessation of therapy but late toxicities are not infrequent. Hypothyroidism may occur as a result of neck irradiation so thyroid stimulating hormone should be routinely monitored [37]. Chemotherapy can exacerbate those acute toxicities as well as cause neuropathy, ototoxicity, nephrotoxicity and hematologic complications [38, 39]. Hearing loss and xerostomia are related to the cumulative cisplatin dose [40]. The incidence and severity of late toxicities are important since there are many long-term survivors after treatment.

Therapy of Recurrent or Metastatic Disease (RM)

Approximately 30% of localized NPC will relapse. Local recurrences can be salvaged with RT or surgery. Patients who initially present with distant metastasis may require locoregional treatment in addition to systemic therapy.

For patients with distant metastasis, platinum-containing doublets are standard first-line therapy. Multiple combination regimens have been studied, however cisplatin and gemcitabine has been established as the preferred regimen. A phase III study compared cisplatin and gemcitabine to cisplatin and 5-fluorouracil in the first-line treatment of RM NPC and found significant improvement in progression-free survival (median 7 months versus 5.6 months, HR 0.55 [95% CI 0.44–0.68]; p < 0.0001) and overall survival (median 29.1 months versus 20.9 months, HR 0.62 [95% CI 0.45–0.84]; P = 0.0025) with cisplatin and gemcitabine versus cisplatin and 5-fluorouracil [41]. There was, however, a significant increase in hematologic toxicities but a decrease in mucosal inflammation with the cisplatin and gemcitabine regimen. Alternative doublets are listed in Table 3. Carboplatin is an option for patients who cannot tolerate cisplatin.

There has been no benefit seen in triplet platinum containing regimens [42]. Alternative single agent options include docetaxel, paclitaxel, 5-fluorouracil, methotrexate, gemcitabine, and capecitabine. These second-line agents are usually considered in patients who are refractory to platinum-based regimens.

Table 3 First line platinum-based doublets in RM NPC

Author	Year	N	Regimen	Response rate (%)	Complete response (%)	Median overall survival (months)
Au [65]	1994	24	Cisplatin + 5FU	66	13	11
Stein [66]	1996	18	Cisplatin + ifosfamide	59	15	Not reported
Yeo [67]	1996	42	Carboplatin + 5FU	38	17	12.1
Yeo [68]	1998	27	Carboplatin + paclitaxel	59	11	13.9
Tan [69]	1999	32	Carboplatin + paclitaxel	75	3	12
McCarthy [70]	2002	9	Cisplatin + docetaxel	22	0	76% (1 year)
Chua [71]	2005	19	Cisplatin + docetaxel	63	6	12.4
Ngan [72]	2002	44	Cisplatin + gemcitabine	73	20	15
Ma [73]	2002	14	Cisplatin + gemcitabine	64	14	68% (1 year)
Wang [74]	2006	39	Gemcitabine + vinorelbine	36	3	11.9
Ma [75]	2009	40	Gemcitabine + oxaliplatin	56	0	19.6

Targeted Therapy

In platinum refractory disease, inhibition of epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) has been evaluated in phase II studies (Table 4).

Cetuximab, an anti-EGFR monoclonal antibody, when used in combination with carboplatin in heavily pretreated patients with RM NPC showed a 12% response rate with 48% of patients having stable disease [43]. EGFR tyrosine kinase inhibitors gefitinib and erlotinib, however, have shown minimal activity as monotherapy [44–46].

Sorafenib is a small molecule inhibitor of multiple tyrosine kinases including VEGFR. It has been studied as monotherapy in RM NPC showing modest activity and in combination with cisplatin and 5-fluorouracil demonstrating activity but a significant rate of bleeding events [47, 48]. More studies of combinations of chemotherapy plus anti-angiogenic agents are of potential interest. Sunitinib, another multikinase small molecule inhibitor that targets VEGFR showed clinical activity, however, with an increased risk of bleeding [49]. VEGF inhibitors that have shown promise are pazopanib and axitinib. In 33 patients, pazopanib showed a response rate of 55% including 2 partial responses and 16 with stable disease [50]. Axitinib was studied in 37 heavily pretreated RM NPC patients with clinical benefit seen in 78% of patients (1 confirmed partial response, 6 unconfirmed partial response, 22 stable disease, 1 year OS = 45.4%) with rare bleeding events [51]. Further studies are needed to evaluate the inhibition of the EGFR and VEGFR pathways in RM NPC. A phase II randomized trial of carboplatin and paclitaxel with or without bevacizumab in patients with RM NPC is ongoing (NCT02250599).

Table 4 Targeted therapy for RM NPC

Author	Setting	Phase	N	Regimen	OR (%)	Median PFS or TTP (months)	Median OS (months)
Chua [44]	3rd line or beyond	II	19	Gefitinib	0	4	16
Ma [45]	2nd line or beyond	II	16	Gefitinib	0	2.7	12
You [46]	2nd line	II	20	Erlotinib after 6 cycles of GP	0	6.9	Not reached
Chan [43]	2nd line	II	60	Cetuximab + carboplatin	11.7	2.7	7.8
Elser [47]	2nd line	II	27	Sorafenib	3.7	3.2	7.7
Xue [48]	1st line	II	54	Sorafenib + cisplatin + 5FU	77.8	7.2	11.8
Hui [49]	2nd line or beyond	II	13	Sunitinib	10	3.5	10.5
Lim [50]	2nd line or beyond	II	33	Pazopanib	6.1	4.4	10.8
Hui [51]	2nd line or beyond	II	40	Axitinib	18.9	5	10.4

OR overall response, *PFS* progression free survival, *TTP* time to tumor progression, *OS* overall survival, *GP* gemcitabine plus platinum-based chemotherapy, *5FU* 5-fluorouracil

Immunotherapy

Programmed death ligand 1 (PD-L1) expression has been detected in a high percentage of NPC patients and is associated with worse outcomes [52]. Antibodies against PD-1 and PD-L1 are being studied as a potential treatment option (Table 5). In the KEYNOTE-028 study, 27 heavily pretreated patients with RM NPC who had PD-L1 positive tumors, defined as membranous staining on ≥1% or more tumor cells, received pembrolizumab, a PD-1 inhibitor, every 2 weeks. A decrease in target lesions was observed in 67% of patients. A partial response was seen in 7 patients (26%) and stable disease in 14 patients; the 1-year OS was 63% and the 1-year PFS 34% [53]. In a phase II study of another PD-1 inhibitor, nivolumab, 44 RM NPC patients were enrolled regardless of PD-L1 tumor status. Complete response was seen in 1 patient and partial response in 8 patients for an objective response rate of 20.5% with a trend for a higher response rate in PD-L1 positive tumors. The 1-year

Table 5 Summary results of immune checkpoint inhibitor trials in RM NPC

Author	N	Agent	Population included	Response rate (%)	1-year overall survival (%)
Hsu [53]	27	Pembrolizumab	PD-L1 positive Prior platinum therapy	26	63
Ma [54]	44	Nivolumab	PD-L1 positive and negative Prior platinum therapy	20	59
Delord [55]	24	Nivolumab	PD-L1 positive and negative Prior platinum therapy	21	74 (6-month)

OS was 59% and the 1-year PFS 19% [54]. The phase I/II CheckMate 358 trial reported results in 24 patients with RM NPC treated with nivolumab. A partial response was observed in 5 patients (21%) and stable disease in 6 patients (25%); the 6-month OS rate was 74% [55]. Ongoing trials including a phase III trial of pembrolizumab versus standard second-line therapy (KEYNOTE-122, NCT02611960) will continue to evaluate the efficacy and safety of immune checkpoint inhibitors in RM NPC.

EBV-directed therapy through adoptive T-cell immunity has also been studied as a potential treatment. In NPC cells, a restricted set of less immunogenic viral antigens is expressed, mainly EBNA1, and latent membrane protein (LMP) 1 and 2. EBNA1 is a dominant target for CD4+ T cells, while LMP1 and LMP2 are targets for CD8+ T cells, with LMP1 being poorly immunogenic. Vaccines have been developed to target these antigens, including Modified Vaccinia Ankara expressing an EBNA1 and LMP2 fusion (MVA-EL). MVA-EL has been tested in two parallel phase I clinical trials in Hong Kong and the United Kingdom with results showing safe and immunogenic responses across a diverse ethnic patient base [56, 57]. MVA-EL continues to be tested in phase Ib and II trials. In a phase II trial, 35 patients with RM NPC received adoptive EBV cytotoxic T cell (CTL) transfer with first line chemotherapy with a response rate of 71%, 3 complete responses, and 22 partial responses. Median OS was 29.0 months [58]. A phase III of autologous EBV-specific CTLs as first line therapy with 330 RM NPCs patients is ongoing [NCT02578641]. Although early in development, immunotherapy and other targeted therapies are promising and may become standard treatment options for RM NPC in the future (Table 6).

In conclusion, advances in the treatment of NPC have contributed to improved outcomes over the past few decades. In localized disease, IMRT paired with CCRT has improved disease control and survival. AC or IC added to CCRT has shown controversial results in randomized trials, however, they may contribute to better survival outcomes and are being frequently utilized in more advanced stage disease. In patients with detectable post-treatment EBV DNA levels, AC is being actively studied as a potential treatment option. In RM NPC, platinum-containing doublets continue to be first-line therapy. There are exciting developments in targeted-, immune-, and EBV-directed therapy, which may alter the standard treatment of NPC in the near future.

Table 6 Ongoing trials in RM NPC

Agent	Category	Phase	N	NCT number
Pembrolizumab	PD-1 Immune checkpoint inhibitor	III	230	NCT0261196
PDR001	PD-1 Immune checkpoint inhibitor	IIR	114	NCT02605967
Avelumab	PD-1 Immune checkpoint inhibitor	II	39	NCT02875613
Ipilimumab and nivolumab	PD-1 inhibitor plus CTLA-4 Immune checkpoint inhibitor	II	35	NCT03097939
Cisplatin and nivolumab	Platinum chemotherapy plus PD-1 Immune checkpoint inhibitor	II	40	NCT03267498
Recombinant EBV vaccine	Vaccine	II	25	NCT01094405
Autologous EBV-specific cytotoxic T lymphocytes	Adoptive T cell therapy	III	330	NCT02578641
Bevacizumab (carboplatin/paclitaxel ± bevacizumab)	Angiogenesis	IIR	80	NCT02250599

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Are There Alternative Chemotherapy Regimens for EXTREME in Recurrent/Metastatic Squamous Cell Carcinoma of the Head and Neck (R/M-SCCHN)?

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Introduction

Patients with recurrent and/or metastatic (R/M) squamous cell cancer of head and neck (SCCHN) who are not amenable to radiation therapy or surgery have a poor prognosis with a median survival of 10–12 months. In this palliative situation, the objective of treatment is to slow down tumor progression, to prolong life as well as to reduce the tumor size and cancer related symptoms (pain, swallowing problems, speaking difficulties) while maintaining the quality of life.

For patients who are fit to receive a chemotherapy combination as a first-line systemic therapy, a platinum-based chemotherapy in combination with cetuximab, an anti-epidermal growth factor receptor (EGFR) monoclonal antibody (mAb), is recommended [1, 2]. Platinum-5FU (PF)-cetuximab regimen is the only chemotherapy combination which has provided an improvement in overall survival (OS) in a phase III study named EXTREME [3] in this setting.

Promising new taxane based chemotherapy regimens, such as TPEx (docetaxel-cisplatin-cetuximab) or CPE (carboplatin-paclitaxel-cetuximab), could be an alternative to the EXTREME regimen [4]. Results from the large European randomized study "TPExtreme" comparing EXTREME to TPEx are eagerly awaited [5].

Immunotherapy with checkpoint inhibitors (CPIs) has emerged in the treatment of patients with R/M SCCHN. Nivolumab and pembrolizumab (both anti-PD-1 monoclonal antibodies) increase the overall survival (OS) of R/M SCCHN patients who progressed on/after platinum [6–8], and have been approved for this indication by the Food and Drug Administration (FDA). Although ongoing studies explore combinations of CPIs with chemotherapy, one important question has come up: should the backbone for such immuno-chemotherapy combinations remain the PF combination?

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The aim of this review is to discuss alternative chemotherapy regimens for EXTREME in the first-line recurrent/metastatic disease setting. Targeted therapies, Immuno-oncology (IO) agents, and second-line treatment will not be discussed in this chapter.

EXTREME: The Current Gold-Standard for First-Line Systemic Treatment of Patients with R/M-SCCHN

The cisplatin/infusional fluorouracil (PF) regimen has been the standard first-line treatment for 25 years, as it demonstrated the best overall response rate (ORR) compared to methotrexate (MTX), 5-fluorouracil (5-FU) or cisplatin (CDDP) alone, despite any clear benefit in overall survival [9–11]. Ten years ago, the phase 3 EXTREME trial showed that adding the anti-EGFR cetuximab to cisplatin/carboplatin and 5-FU, followed by single agent cetuximab maintenance treatment, significantly improved OS by nearly 3 months (from 7.4 to 10.1 months), and progression-free survival (PFS) by 2.3 months (from 3.3 to 5.6 months) compared with chemotherapy alone [3]. Median PFS from the start of the maintenance treatment was 3 months. Moreover, ORR increased, cisplatin-based chemotherapy plus cetuximab leads to 38.9% (vs 23% with chemotherapy alone); and carboplatin-based chemotherapy plus cetuximab leads to 30.4% response (versus 15% with chemotherapy alone). In both occasions the increase was significant (with cis $p = 0.0035$; with carbo $p = 0.0267$). In terms of tolerance, the safety profile of the EXTREME regimen was consistent with that expected for the administered agents, including cardiac events associated with 5-FU (7% grade ≥3 cardiac), anorexia, and skin reactions, but with an increase in the number of sepsis (4% vs <1%). Skin adverse effects related to cetuximab were evaluated as tolerable. Indeed, the EXTREME regimen became the gold-standard of care in this setting, as supported by international guidelines [1]. For elderly FIT patients, the ELAN FIT trial is currently evaluating the benefit of the EXTREME regimen with carboplatin [12].

The EXTREME regimen is composed of ≤6 cycles of chemotherapy. Cetuximab is delivered weekly during the chemotherapy phase, then is delivered alone as a maintenance treatment until progressive disease (PD) [3]. It has been suggested that the maintenance part of the regimen is an important part of the EXTREME regimen, with the potential to yield additional tumor responses [3, 13]; however, there is no randomized study to substantiate this.

Results of the EXTREME study have been confirmed in real life. The DIRECT (EMR 62202-556) study was a phase IV, observational study to evaluate the feasibility and tolerability of the EXTREME regimen in the real-world setting [14]. Contrary to the EXTREME study, during the maintenance phase of the DIRECT study, cetuximab could be given once every two weeks. The DIRECT study outcomes (PFS, OS, safety profile) were similar to those observed in the cetuximab-containing arm of the EXTREME study (4.5 vs 5.6 months for PFS and 9.4 vs 10.1 months for OS, respectively). Nearly 50% of patients in the DIRECT study

were able to complete the combination phase and thus continued with cetuximab as a single agent in the maintenance phase. Most of patients received 4 cycles of chemotherapy (5 cycles in EXTREME trial) and the median duration of maintenance phase was the same, 3 months. In terms of tolerance, the majority of treatment interruptions and dose reductions occurred in the combination therapy phase, related to hematological, cardiovascular and renal toxicities due to the chemotherapeutic agents, cisplatin, carboplatin and 5-FU. Maintenance therapy with weekly cetuximab or cetuximab every 2 weeks was tolerable and feasible and required minimal treatment delays or dose reductions. Other prospective observational studies [15] also confirmed the feasibility and results of the EXTREME regimen in the real world.

Searching for Alternative Chemotherapy Regimens for EXTREME in R/M SCCHN

Obviously, the main objective is to look for a more active regimen in the first-line setting, as the median survival of R/M SCCHN patients treated with the EXTREME regimen still remains limited to 10–12 months and ORR less than 40%. Even if there are some long survivors [13], median PFS from the start of the maintenance treatment is of only 3 months. Improving the efficacy of the maintenance treatment is still a challenge.

Ongoing studies exploring combinations with CPI raise the need to define the best backbone chemotherapy [6–8]. The usefulness of using a taxane instead of 5-FU as a component of a platinum-based regimen remains an open question.

There are several limitations to the use of EXTREME in every day clinical practice. Only 12% of patients in the cetuximab arm of the EXTREME study had an ECOG PS of ≥2 [3], and most of the patients needed to be fit to tolerate this chemotherapy regimen. The DIRECT study showed that most of patients were able to receive only 4 cycles of chemotherapy instead of planned 6 cycles. 14% of 157 patients could no receive 5-FU and 22% stopped it due to toxicities (cardiovascular or GI adverse effects). 13.5% of the patients switched from cisplatin to carboplatin [14]. Patients with dihydropyrimidine dehydrogenase (DPD) deficiency are at high risk of severe 5-FU–associated toxicity and in such patients the use of 5-FU is contraindicated [16]. These data make it clear that physicians would like to avoid the use of 5-FU and would prefer delivering another drug with a more favorable safety profile as a component of first-line chemotherapy regimen for R/M SCCHN patients.

Finally, according to ESMO guidelines [1], unfit R/M SCCHN patients for platinum based combination but able to receive more than a best supportive care (BSC), should be treated with methotrexate or cetuximab monotherapy. As an exemple, for unfit elderly R/M SCCHN patients, the randomized ELAN UNFIT trial [17] is currently comparing methotrexate and cetuximab. However with these treatments, expected ORR are less than 13% and OS less than 6 months. Looking for a more active regimen for these unfit patients is still an unmet need.

Alternative Chemotherapy Regimens for EXTREME: New Combinations Without Taxane

Since the approval of the EXTREME regimen, the following studies looked at improving the efficacy and/or reducing the toxicity of the systemic therapy.

Several new regimens have been evaluated, either replacing 5-FU by pemetrexed [18], cetuximab by panitumumab [19], or adding another agent such as cilengitide or IMO-2055 [20, 21]. All randomized studies testing these new combinations failed to demonstrate any improvement in efficacy and no rationale to further develop these combinations for treatment of R/M SCCHN.

Focusing on the research for a more efficient treatment for fit R/M SCCHN patients, there is a need to consider molecular mechanisms and to select drugs with the potential to synergize with each other.

Alternative Chemotherapy Regimens for EXTREME: New Combinations with a Taxane

There are several reasons to replace the 5-FU with a taxane (docetaxel or paclitaxel):

- Such a regimen could be administered to patients with contraindications to 5-FU, and could avoid cardiovascular adverse events (angina, rhythm disturbances and ischemic events), or DPD deficiency related toxicities, as previously mentioned. In addition, cetuximab plus a taxane may be an alternative treatment for patients who are unfit to receive EXTREME regimen [22].
- This regimen seems easier to deliver, in one day, thereby avoiding a 4-day continuous infusion with pumps in hospitalisation or at home, and reduces the risk at complications.
- There are pre-clinical data suggesting a potential immunogenic and proapoptotic synergy between cetuximab and taxane [23, 24]. Immunostimulatory effects of cetuximab and taxane agents may cooperate towards and increase tumor cell killing [25].

First Trials Comparing Taxane-Platinum to PF

Taxanes/platinum combinations emerged in the treatment of R/M SCCHN patients in 2005. The ECOG trail E1395 showed no difference in OS between PF and paclitaxel-cisplatin regimen [26].

Trials Testing a Taxane-Platin-Cetuximab Chemotherapy Regimen

Taxane-platin-cetuximab regimens have shown promising results in several phase 2 studies [4, 27–29] (Table 1). However, there are many differences between these

Table 1 Trials with taxane + platinum + cetuximab regimens in first-line R/M SCCHN

Trial	N	Regimen	ORR (%)	PFS (months)	OS (months)
GORTEC 2008-03 (TPEx) [4]	54	Docetaxel + cisplatin (75 mg/m^2 q3w) 4 cycles + weekly cetuximab (400 mg/m^2 then 250 mg/m^2) + G-CSF Maintenance: cetuximab 500 mg/m^2 q2w	44.4	6.2	14
CSPOR-HN02 [27]	45	Paclitaxel (100 mg/m^2 on days 1, 8) + carboplatin (AUC 2.5 on days 1, 8, q3w, up to 6 cycles) + cetuximab (400 mg/m^2 then 250 mg/m^2 qw) Maintenance: cetuximab 250 mg/m^2 qw	40	5.2	14.7
NCT01830556 (CET-MET) [29]	85	Paclitaxel (175 mg/m^2 qw) + carboplatin (AUC 5) + cetuximab (400 mg/m^2 then 250 mg/m^2 qw) Maintenance: cetuximab 500 mg/m^2 q2w vs Cisplatin/carboplatin (100 mg/m^2 q3w/AUC 5, respectively) + 5-FU (1000 mg/m^2/24 h for 96 h continuous infusion) + cetuximab (400 mg/m^2 then 250 mg/m2 qw) Maintenance: cetuximab 500 mg/m^2 q2w	51.2 vs 47.6	6.5 vs 4.37 HR = 0.65 (95% CI : 0.41–1.03) p = 0.064	10.2 vs 8.4 HR = 0.71, 95% CI: 0.43–1.16 p = 0.166
CET-INT (B490) [26]	201	Cisplatin (100 mg/m^2 q3w) + cetuximab (400 mg/m^2 then 250 mg/m^2 qw) Maintenance: cetuximab 250 mg/m^2 qw (n = 100) vs Paclitaxel (175 mg/m^2 q3w) + cisplatin (100 mg/m^2 q3w) + cetuximab (400 mg/m^2 then 250 mg/m^2 qw) Maintenance: cetuximab 250 mg/m^2 qw (n = 91)	41.8 vs 51.7	6 vs 7	13 vs 11 HR = 0.77, 95% CI: 0.53–1.11 p = 0.117 by log-rank

trials, not only for the population enrolled and taxane used (docetaxel or paclitaxel), but also for doses and schedule of administration of drugs.

The GORTEC 2008-03 TPEx trial suggested that this combination may yield an improved ORR and OS compared with the EXTREME regimen [4]. This phase 2 study evaluated a new chemotherapy regimen, based on experience obtained with the docetaxel-cisplatin (TP) combination in lung cancer and the TPF combination in SCCHN. Only 4 cycles of TP (75 mg/m^2 for each drug q3w) with G-CSF support in combination with weekly cetuximab (termed TPEx) were delivered every three weeks as first-line treatment in 54 R/M SCCHN fit patients. Cetuximab was administered at a dose of 500 mg/m^2 every 2 weeks during the maintenance phase, as commonly used in GI cancer.

The Japanese phase 2 CSPOR-HN02 study assessed the efficacy and safety of up to 6 cycles of the paclitaxel-carboplatin-cetuximab combination in 45 R/M SCCHN patients, administered on the outpatient clinic [27]. Overall response rate, the primary end point, was 40%. Median overall survival was 14.7 months and progression-free survival was 5.2 months. Grade 3/4 adverse events included neutropenia (68%), skin reaction (15%), fatigue (9%) and febrile neutropenia (9%). A potentially treatment-related death occurred in one patient with intestinal pneumonia.

Two randomized phase 2 trials evaluating paclitaxel-containing regimens have been conducted: the *CET-INT/B490 trial* showed that cetuximab plus cisplatin was not inferior to paclitaxel-cisplatin-cetuximab in 191 RM-SCCHN patients [28]; the *CET-MET trial* showed non-inferiority (PFS, OS) with less toxicity (≥ grade 3 adverse events [AE] 40% vs 60%, p = 0.034) of a paclitaxel-carboplatin-cetuximab combination versus the EXTREME regimen in 85 R/M-SCCHN patients. Both arm in the latter study used the two-weekly dose regimen of cetuximab for maintenance [29].

Several characteristics of the patients enrolled in these trials differed from those of the EXTREME study [3, 4, 27–29]. Both the GORTEC study (70.4%) and the CSPOR-HN02 (82.2%) enrolled more metastatic patients compared to the EXTREME study (47%) and enrolled only patients with an ECOG PS of 0 or 1.

Main efficacy results of these studies are summarized in Table 1. All four studies met their primary endpoints. Results suggest an improvement in efficacy, with an increase in ORR with this modified regimen. For patients treated with TPEx, half of the evaluable patients obtained an objective response which was reached already after only 2 cycles for most of patients, and 70% of patients could start a maintenance treatment with cetuximab. The median OS was 14 months including long survivors [30]. In real life, the same results could be observed [31], and this was encouraging to start the TPExtreme trial. Regarding paclitaxel-platinum containing regimens, the ORR with these regimens seem to be improved compared to standard PF regimen, with a range from 40.0% to 51.9%, while OS seem at least non inferior compared to standard first-line treatment, with a range of median OS of 10.2–14.7 months.

Ongoing studies are trying to confirm these results and to explore new combinations. Results from TPExtreme, a large European randomized trial comparing the

EXTREME regimen versus the TPEx regimen in 540 R/M SCCHN patients are awaited in 2019. The main endpoint is OS, and the trial includes HPV status, quality of life (QOL) assessment and socio-economic ancillary studies [5]. The ongoing American phase 2 CACTUX trial evaluates in R/M SCCHN patients, the combination of nab-paclitaxel (100 mg/m^2 qw), cisplatin (75 mg/m^2 q3 or carboplatin (AUC 5) and cetuximab up to 6 cycles followed with a maintenance combining weekly cetuximab and nab-paclitaxel; The planned interim analysis for 32 patients has provided encouraging results (ORR: 50%, PFS: 6.3 months, OS: 18.4 months) [32].

Safety profiles of taxanes/platinum combinations plus cetuximab are different from that of EXTREME regimen. The most common grade 3/4 AEs include neutropenia, fatigue, alopecia and skin toxicities (including acneiform rash). To reduce the risk of developing febrile neutropenia and sepsis, G-CSF support is mandatory with the TPEx regimen and steroids are required to reduce allergic reactions, when administering taxanes [33, 34]. Neurotoxicity can be another problem with the use of paclitaxel [35].

Other Taxane-Platinum Based Combinations

A large randomized study compared four arms with different combinations including either PF or TP with or without bevacizumab. The study did not meet its main endpoint, with more bleedings in bevacizumab arm. However, the taxane-containing combination provided the best efficacy results [36]. The randomized phase 2 PARTNER trial demonstrated an improvement in PFS but no advantage in OS when panitumumab was added to TP compared to the TP regimen alone [37]. This trial also showed that G-CSF support was mandatory to avoid febrile neutropenia and sepsis with the TP regimen.

Finally, encouraging results from a phase 2 randomized trial comparing erlotinib plus TP to placebo plus TP have been reported at ASCO meeting in 2017 [38]. An increase in ORR (56% vs 44%, NS), PFS (6.1 vs 4.4 months, $p = 0.026$) and OS (17.7 vs 13.7 months, HR = 0.67, 95% confidence interval 0.43–1.04, $p = 0.07$) was observed in the experimental arm compared to the placebo arm, respectively. However, there was no comparison to the standard EXTREME regimen in this trial.

Taxane-Cetuximab Regimen

The combination of a taxane plus cetuximab has shown efficacy as a treatment for R/M SCCHN patients unsuitable for cisplatin or carboplatin, due to poor ECOG PS, absolute or relative contraindications or a recurrence with a very short interval after a platinum based chemo-radiotherapy. Only a few studies have been conducted. The earlier mentioned Spanish prospective study, reported by Hitt et al. evaluated the

weekly paclitaxel/cetuximab combination as first-line treatment in 46 patients: They reported an ORR of 54%, with 22% complete responses and a disease control rate of 80% [22]. Other retrospective studies have also shown that docetaxel/cetuximab regimens may result in promising disease control rates and prolonged survival in this setting [39, 40]. The safety profile of these combination appears to be manageable.

Conclusion

New taxane based chemotherapy regimens may be an alternative for the gold-standard EXTREME regimen either for fit or unfit R/M SCCHN patients. Available data suggest that taxane, platinum and cetuximab is a powerful combination for patients in first-line R/M SCCHN. Indeed, results of completed TPExtreme trial are eagerly awaited.

There is an urgent need to explore these new combinations with emerging immunotherapies in the first-line setting [41].

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New Data on Systemic Therapy of Salivary Gland Tumors

Salvatore Alfieri and Lisa Licitra

Introduction

Salivary Gland Cancers (SGCs) include more than 20 histotypes with different pathological features and clinical behavior. By natural history, epidemiological features and outcome, two groups can be clinically identified: adenoid cystic carcinoma (ACC) and non-ACC.

Adenoid-Cystic Carcinoma (ACC)

The historical chemo- and radio-refractory nature of this group led to study further druggable targets. Unfortunately, these are still lacking. The low rate of DNA mutations (0.3 mutations/megabase) in this cancer group may be accountable for that [1]. ACC is typically considered a silent tumor. The most recurrent gene alteration of ACCs (50–60% of cases) is the translocation of the MYB oncogene [t(6:9) MYB-NFIB translocation]. *MYB-NFIB* regulates genes involved in cell cycle control, DNA replication/repair and RNA processing. The *MYB-NFIB* fusion is regulated through AKT-dependent signaling induced by *IGF1R* over-expression and it is downregulated upon *IGF1R*-inhibition. The MYB-NFIB-induced transcriptional

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program affects critical oncogenic mediators that are normally controlled by *MYC* and is reversed by pharmacological inhibition of *IGF1R*. This is very intriguing in the hypothesis of a tailored approach [2]. To date, there are no drugs active on *MYB* even if two trials (NCT00002592 and NCT00780052, not published yet) tested *MYB* antisense oligonucleotides in hematologic malignancies.

In the last few years, many targeted therapies have been studied in this setting. *KIT* and *EGFR* are frequently overexpressed but rarely mutated or amplified. Imatinib was tested but failed to show meaningful activity [3–8]. Indeed, the lack of KIT somatic mutations in ACCs could justify its poor results. All anti-EGFR tested family members (lapatinib, gefitinib, cetuximab and trastuzumab) failed to detect any responses in ACCs [1]. Dasatinib, a small molecule of SRC-family protein tyrosine kinase inhibitors (TKIs), showed a low response rate (2.5%) in cKIT-expressing ACC and disease control in half of the cases [9]. Dovitinib is an anti fibroblast growth factor receptor-1 (FGFR-1) TKI. This receptor is commonly highly-expressed and activated in ACCs. Despite the biological premises, dovitinib showed a very modest response rate (4.5%) [10, 11].

The unsatisfactory results of tailored agents have led to focusing on new compounds characterized by a different mechanism of action. Literature evidences [1] suggest a linkage between the vascular endothelial growth factor (VEGF) and a worse outcome in SGCs, supporting the use of antiangiogenic drugs in ACCs. However, first and second-generation TKIs with antiangiogenic properties, such as sunitinib, sorafenib and axitinib, have been studied in patients with ACC, with poor results: overall response rates (ORR) were 0%, 11%, 9% respectively [1]. In addition, similar disappointing results have been reported also for nintedanib, regorafenib and pazopanib [1]. In the trial with axitinib, the tumor samples of two responding patients harbored both MYB/NFIB fusions as well as the 4q12 amplification. This latter increases the gene copy number for three molecular targets of axitinib: *PDGFR-A (platelet-derived growth factor receptor)*, *KDR* (*VEGFR2*) and *KIT*. This evidence supports the hypothesis of oncogenic dependence upon PDGFR-A/KDR/KIT signaling and susceptibility to axitinib by a subset of ACC.

Many other targeted agents (e.g. bortezomib, everolimus, nelfinavir, MK-2206 and vorinostat) have been studied in ACCs [1]. Among them, vorinostat, a histone deacetylase (HDAC) inhibitor, has to be cited for its activity in 7% of cases and its role in upregulating PD-1 or PD-L1 expression (targets of the most recent immune-modulators), offering the rational to design a trial (NCT02538510) combining vorinostat and pembrolizumab in advanced SGCs (ACCs included). This study has been presented at the ASCO meeting 2018 [12] showing a lower rate of responses in SGCs, especially in ACC (ORR: 16%, with only 1 out of 4 partial responses (PR) in ACC) compared to what was reported in head and neck squamous cell carcinoma (8 PR out of 25 patients = 36%).

At the same meeting, interesting results from two phase II trials (one from Italy and one from USA in 26 and 32 patients, respectively) using lenvatinib in ACC patients were presented [13, 14]. Lenvatinib is a multi-tyrosine kinase third generation inhibitor with potent antiangiogenic action, approved for first-line treatment of radioactive, iodine-refractory, progressive, metastatic differentiated thyroid

carcinoma patients. In both trials [13, 14], lenvatinib showed similar activity and PR ranged from 12% in the Italian study [14] to 15.6% [13] in the USA trial, thus resulting as the highest activity obtained by an antiangiogenic drug in this context. The USA trial included fewer patients with loco-regional recurrence, which can usually impair the patients' outcome, and two patients with breast ACC who in general have a better prognosis compared to those arising from salivary and lacrimal glands. Similar disease control rate and acceptable safety profile were observed in both trials. Tumor reduction rate (size reduction = 20% at least) was 25% and 27%, respectively considering the USA [13] and Italian trial [14]. In brief, lenvatinib (alone or in combination with new agents) deserve further evaluation in the ACC population. Predictive biomarkers of response still need to be identified.

Another pursued strategy was to combine target agents and chemotherapy such as cisplatin + imatinib (vs imatinib), doxorubicin + bortezomib (vs bortezomib), cetuximab + platinum-based chemo-radiotherapy/chemotherapy which resulted in promising ORR. However, the benefit was merely given by the addition of traditional chemotherapies suggesting that the activity of targeted agents when used alone was absent or very low [1].

Immunotherapy, the newest therapeutic tool in medical oncology, is currently being tested in this setting. PD-L1 expression is very low in ACC (about 2%). A retrospective analysis of 21 patients did not show any PD-L1 expression on tumor cells. On the other hand, 60% of primary tumor samples and 72% of distant metastases demonstrated a PD-L2 expression. Intratumoral lymphocytic infiltration was present in 42% of patients both in primary and metastatic lesions. In this context, PD-L1 expression has been reported in 86% and 80% of immune cells in primary and distant metastases, respectively. The presence of lymphocyte intratumoral infiltration correlated with the expression of some genes, such as SyK ($p = 0.04$), IL2RB ($p = 0.02$) and TGFbeta ($p = 0.02$). From these data, the use of anti PD-1 seems to be more rational than anti-PD-L1 [15]. Pembrolizumab, a PD-1 inhibitor, is currently under evaluation in several malignant diseases, including SGCs. In a phase 1b trial Keynote-028, the use of pembrolizumab did not lead to any response in ACC cases [16].

Non-Adenoid Cystic Carcinoma (Non-ACC)

Among the non-ACC, two subgroups can be hypothetically identified. In the first one, some subtypes [e.g. acinic cell carcinoma (Aci-CC), polymorphous low-grade adenocarcinoma (PLG-ADC), mammary analog secretory carcinoma (MASC), and epithelial-myoepithelial (epi-myoepi) carcinomas] have generally indolent clinical evolution compared to other more rapidly growing and more aggressive subtypes in the non-ACC cohort, such as mucoepidermoid carcinoma (MEC), salivary duct carcinoma (SDC), adenocarcinoma (ADC), not otherwise specified (NOS) carcinoma, and carcinoma ex pleomorphic adenoma (Ca-ex-PA)]. These two clinically different subgroups can also be distinguished from a genetic point of view. Ross et al. [17]

demonstrated on 623 samples of SGCs that Aci-CC, PLG-ADC, MASC and epimyoepi carcinoma are characterized for instance by a lower (2.1 mutations/tumor) tumor mutational burden (TMB) and a lower TP53 mutation rate (<20%) than the more aggressive histotypes (including MEC, SDC, ADC, NOS, Ca-ex-PA) whose TMB and TP53 mutation rates were much higher (TMB: 4.3 mutations/tumor and TP53 mutation rate >40%, respectively). The most frequent genetic alterations in the non-ACC group include those regarding ERB-B2 (HER2) and PI3KCA pathways. Furthermore, it is known that TMB plays an important role in conditioning the immune system response to the immune checkpoint inhibitors (ICIs) [18]. In some high-grade SGCs, an up-regulation of PD-L1, the main target for most commonly used ICIs, was identified (e.g. 36% of PD-L1 expression in Ca-ex-PA, 30% in SDC, 8% in ADC) [19]. These evidences could support future and more tailored investigation of immunotherapy in SGCs (e.g tumor with higher TMB and PD-L1 expression). The previously cited phase 1b trial, Keynote-028, [16] tested Pembrolizumab, in 26 SGCs (ACC and non-ACC). Among non-ACC, three PRs were reported, two in ADC and one in high-grade serous carcinoma (none in the ACC subgroup as mentioned above). Stable disease (SD) was registered in 12 cases (46.2%). Drug-related adverse events (DRAEs) were very common (84.6%): diarrhea, reduced appetite, pruritus and fatigue were the most frequent DRAEs. Three patients (11.5%) experienced G3–G5 DRAEs with one case evolved in toxic death (lung inflammation). The phase II study, Keynote-158 (NCT02628067), is currently ongoing exploring the pembrolizumab activity in this specific cancer setting. Several trials testing the activity of a single agent or the combination of multiple immunemodulators are ongoing. The efficacy of nivolumab (anti-PD1 agent) alone (NCT031332038) or combined with ipilimumab (NCT03146650) is under investigation in two trials. The evaluation of PD-L1 expression is not mandatory for inclusion in both trials, and all SGC patients with metastatic disease, progressive within the last six months, are eligibile. As mentioned above, the activity of pembrolizumab plus vorinostat has already been evaluated in another trial (NCT02538510) with 3 out of 4 PR of SGCs in the non-ACC group [12].

In the last decade, SGCs have been field of investigation for multiple new targeted agents.

Androgen receptor (AR) is the most studied target in this context. SDC is characterized by AR expression in 75–99% of cases while it is also reported in about 21–33% of ADC, being an hallmark of these two histotypes [20].

Therefore, activity of Androgen Deprivation Therapy (ADT) has largely been investigated in more recent years. From 2011 to 2017, only few retrospective analyses were reported [21–23]. Despite their small patients' dataset, all of them confirmed a such activity of ADT in AR-expressing SGCs, both using bicalutamide alone or combined with Luteinizing Hormone Releasing Hormone (LHRH) analogue as combined androgen blockade (CAB) strategy. As expected, ORR was higher with CAB (up to 64.7%) [23] than with bicalutamide (ORR: 20%) [21] or LHRH analogue (ORR: 25%) [22] alone.

The results obtained by CAB have been recently confirmed in a phase II trial prospectively conducted in 36 patients [24]. ORR was 41.7% including 4 complete

remissions (CR), with a median PFS of 8.8 months (range 6.3–12.3 months) and a median OS of 30.5 months (range 16.8–not reached). The lower ORR, as compared to what has been reported with CAB in the retrospective analysis from Locati et al. [23], could potentially be explained by the enrollment of 6 patients with an AR expression in less than 70% of the cells, hypothesizing in this group of patients a lower activity of CAB.

At present, ADT can be considered as standard only in second-line of recurrent and/or metastatic AR-positive SGCs.

A prospective randomized trial, held by the European Organization for Research and Treatment of Cancer (EORTC), is currently ongoing to demonstrate the efficacy and safety of ADT versus chemotherapy as first-line therapy for recurrent and/or metastatic, AR-positive SGCs (NCT01969578).

Following prostate cancer (PCa)-based treatment approaches, abiraterone, an inhibitor of androgen synthesis (1000 mg daily plus prednisone 5 mg q12 h), is currently under investigation in AR-expressing SGCs, failing first-line ADT. In fact, abiraterone has been approved as a second-line treatment in hormone-resistant PCa patients. Recently, two patients with recurrent/metastatic adenocarcinoma, NOS, progressive to ADT and responding to abiraterone therapy have been reported [25]. Starting from this evidence, a phase II trial was designed to test the activity of abiraterone in a larger cohort of patients with hormone-resistant, AR-expressing, SGCs (NCT02867852). Another phase II trial with enzalutamide is currently recruiting in the same setting (NCT02749903). Recently, Cappelletti et al. [26] found circulating tumor cells (CTCs) in the blood of an AR-positive SDC patient treated with abiraterone and they suggested to further investigate the role of liquid biopsy and detection of CTCs in the management of SDC patients. The authors demonstrated also the opportunity to detect in this way the AR-v7 expression, which is also recognized in PCa patients as the most important resistance mechanism to abiraterone. This could open the way to an easier and practical manner to study the hormone-resistance mechanisms in AR-expressing SGCs

Another extensively studied target in SGCs is HER2 (ERB-B2) which is overexpressed in about 44% and amplified in about 35–50% of the SDC [27]. Trastuzumab, an anti-HER2 monoclonal antibody, is approved for HER2-positive breast cancer and HER2-positive, metastatic, stomach or gastroesophageal junction adenocarcinoma. This monoclocal antibody, administered at an initial dose of 4 mg/kg followed a weekly 2 mg/kg dose schedule, was investigated in a phase II trial in 14 patients with SGCs. HER2 was overexpressed only in 10 out of 14 cases and trial was closed early due to low rate of HER-2 overexpression in the enrolled patients; only one long-lasting partial response was described in a case of MEC with HER2 overexpression, and no data on HER2 amplification status was reported [28]. Other anecdotal responses using trastuzumab as single-agent in HER2-overexpressing SGCs have been reported, confirming its low activity in this setting [1]. Moreover, according to breast cancer model, a double (trastuzumab and pertuzumab) and a sequential (trastuzumab and pertuzumab followed by trastuzumab emtansine-TDM1 at disease progression) HER2 blockade strategy was tested in two case reports suggesting a possible benefit of a potentiated HER2 targeting [29].

Recently, the activity of other targeted drugs, such as entrectinib [30] and crizotinib [31], cabozantinib and regorafenib [1] was reported in the presence of specific genetic, fusion- or duplication-based, alterations. Examples of this are *ETV-NTRK3* for entrectinib and crizotinib, *NCOA4-RET* for cabozantinib, and BRAF kinase domain duplication for regorafenib. In particular, there is an increased interest for NTRK-inhibitors in ETV6-NTRK3 rearranged SGCs, typically seen in the MASC subgroup [30], for which this genetic fusion represents the disease hallmark.

In general, antiangiogenic drugs have some activity in non-ACC tumors. Unlike results obtained in ACC, sorafenib [32], tested in 18 subjects with recurrent/metastatic non-ACC, showed an ORR of 22%. Partial remission was reported in one SDC, one ADC, one NOS, one high-grade MEC and in one poorly differentiated carcinoma. The antiangiogenic activity seems to be the main mechanism of action, supported by the lack of correlation between the activity of sorafenib and the expression of its targets as well as the overexpression of PDGFR-β in the stromal component of responding cases. However, results with more potent antiangiogenic compounds, such as pazopanib, were not satisfactory [33]. In this phase II trial including 20 non-ACC patients, only one PR (6%) was observed with 6-m PFS >40% [33].

In this scenario, research efforts are still needed to ameloriate the treatment armamentarium available for this heterogenous and complex group of cancers. This ambitious aim may be met only starting from a better knowledge of the baseline ACC and non-ACC biology and resistance mechanisms to therapeutics agents used till now.

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Treatment of Elderly Patients with Head and Neck Cancer

Petr Szturz and Jan B. Vermorken

Introduction

In 1981, when the first conference addressing the topics of cancer in the elderly was held by the US National Institute of Aging, the history of geriatric oncology began to unfold. Following this event, several literature reviews pointed out the apparent underrepresentation of older individuals in clinical trials. As a result, studies funded by the US National Cancer Institute no more considered advanced age automatically as an exclusion criterion, and new trials focusing specifically on the elderly population were initiated. It has become clear that despite being more vulnerable to complications of cytotoxic chemotherapy, senior persons may derive the same benefit as their younger counterparts if biological and not chronological age is taken into account [1]. In fact, about half of patients over 70 years of age can be treated with standard oncologic approaches, while the other half will require more extensive measures [2–5]. Still, for numerous reasons including disqualifying medical conditions, logistical issues, long-established institutional practices, and personal physicians' attitudes, the needs of elderly individuals remain largely unmet. They have been underrepresented in prospective trials, undertreated in routine practices, and refrained from a proper geriatric assessment. The former remains to be a continuing issue despite the fact that their willingness to participate in clinical research does not seem to pose a barrier [6]. Here, the ensuing lack of evidence-based data hampers effective implementation of novel drugs and development of clinical practice guidelines.

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Focusing mainly on systemic therapy, this chapter details cancer care in the elderly with squamous cell carcinoma of the head and neck (SCCHN), both in the locoregionally advanced and the recurrent and/or metastatic settings. It sets out to briefly review the answers to the following two fundamental questions: "How to select an appropriate approach to an elderly person?" and "What is the current state of clinical evidence in these patients?" Usually amenable to single-modality surgery or radiotherapy, the early disease setting will not be addressed here.

Cancer and Ageing

As documented in many epidemiological studies, there is a marked association between tumour development and ageing. Advanced age is indeed the major risk factor for cancer, which in turn represents the second most common cause of death for persons 65 years and over in Europe [7, 8]. In accordance with demographic projections clearly showing the steadily growing number of elderly people, the global cancer burden will nearly double in the near future. By 2030, up to 22 million new cases (12 million in those 65 years or older) and 13 million cancer deaths (8.4 million in those 65 years or older) are to be expected worldwide each year. Of note, these figures exclude non-melanoma skin cancers, which are frequent and generally well curable [9]. However, the biological landscape of malignant transformation in older adults is far from being straightforward. Besides the dominant role of somatic mutations accumulating over lifetime, other age-related processes promote but also hinder tumorigenesis. Vascular ageing and a decline in circulating levels of various hormones probably counteract neoplastic progression, while it may be fostered by chronic low-grade inflammation and an increased fraction of senescent cells [7]. Interestingly, cancer incidence and mortality were reported to decrease or plateau in the oldest population (over 90 years) owing partly to the selection of less vulnerable individuals [10].

With an annual incidence reaching almost 700,000 cases worldwide, SCCHN follows the same epidemiologic trends as outlined above [11]. According to the 2010 cancer incidence projections for the United States, 54% of malignant head and neck cancer cases occurred in patients 65 years and older. By 2030, the proportion is expected to rise to 66% [12]. Considering that at present the median age at diagnosis of laryngeal carcinoma is 65 years, and of oral cavity and pharynx cancers 63 years, such estimates are certainly understandable [13]. Although major risk factors for SCCHN in the elderly are still tobacco and alcohol consumption, their prevalence is lower than in an unselected population (40% versus 70%) underscoring age alone as an important risk factor. Compared to younger patients, older age groups have a higher ratio of female cases and are more likely to have primary tumours located in the oral cavity and larynx but less in the hypopharynx. Metastatic spread to the regional lymph nodes and human papillomavirus-associated (HPV) oropharyngeal cancer also appear to be less frequent in the elderly [14]. More importantly, however, there is an increase in non-cancer-related mortality responsible for about one third of deaths within the first 5 years after SCCHN diagnosis in senior patients [15].

Chronological Versus Biological Age

But how to define old age? This is one of the key questions; unfortunately, no universally accepted criteria exist that would facilitate clinical decision-making. The elderly are usually classified into young old (65–75 years), old old (76–85) and oldest old (>85) [1]. This categorisation has been adopted by the National Institute on Aging and the National Institutes of Health, whereas most clinical studies use the age of 70 (or even 75) as a cut-off defining the elderly [16]. In fact, the latter cut-off point may better capture the reality in terms of biological alterations occurring with advancing age, because aging is associated with a progressive loss of functional reserve of multiple organ systems, increased prevalence of chronic diseases, enhanced susceptibility to stress, and fluctuations in social support and economic resources [1]. These age-related changes occur at different rates in different individuals, and we already begin to recognize and actively pursue ways to delay them. Owing to the progress in medical care and improvements in the quality of our everyday life, senior people nowadays are distinct from their ancestors' generations. In 2011, the first wave of the Baby Boom generation, born after the Second World War between 1946 and 1964, reached the pension age of 65. The so-called Boomers demand more involvement and competence in their health care, seek social engagement and healthy lifestyle, continue to have physical and intellectual activity, and use the Internet and modern information technologies [17]. Interestingly, the positive impact of a more active and healthier lifestyle in elderly people on the development of dementia has recently been reported in the United States [18].

Thus, it has become clear that chronological age does not sufficiently correlate with biological parameters and provides only limited information for personalized management. In the elderly, progressively declining organ functions and associated metabolic changes are responsible for higher prevalence of comorbidities and deterioration in cognition, functional and nutritional status, and psychological state. For this reason, biological age represents a more suitable parameter to express the heterogeneity of the geriatric population. Such diversity is reflected by individual life expectancy, functional reserve, and even the risk of treatment side effects [19]. In clinical practice, the crucial step is to distinguish a fit-old individual, who will likely withstand a radical treatment with curative intent, from a frail-old patient, who will probably not tolerate such approach. So, coming back to the topic of chronological age, is there any point in using landmarks for defining the elderly? Actually, there is. It should instigate us to evaluate the patients for their biological age by applying geriatric assessment as will be discussed later in the text [20].

Despite these arguments, many physicians concerned about excessive toxicity still tend to use chronological age as a sole discriminator and opt for non-standard or less aggressive therapies in otherwise healthy elderly persons [21]. Retrospective data indicate that only half of these patients are managed according to institutional policies [22–24]. The resulting suboptimal treatment has been hypothesized as one of the reasons for shorter survival. In oral cavity and pharynx cancers, Surveillance, Epidemiology, and End Results (SEER) data from 2007 to 2013 revealed 5-year

overall survival of 56% and 69% for older (≥65 years) and younger patients, respectively [13]. Further factors contributing to such a difference in the outcomes include serious age-related comorbidities and individual patient decisions to avoid receiving full-dose regimens [25]. This is in line with the results of a long-term prospective observational study of 266 individuals showing that chronological age has no independent prognostic value as opposed to comorbidities and non-standard treatment [26].

One possible solution of how to address the complexity in delivering patient care at an individual level is a team approach in treatment planning represented by multidisciplinary tumour boards. These meetings should offer a collaborative review of each case with a special attention to disease factors (site, stage, biology, and risk factors for locoregional or distant relapse), patient factors (age, sex, performance and nutritional status, comorbid conditions, oral health, life-style habits, and socio-economic background), treatment options, and patient preferences. A geriatrician is not always available, so to tailor cancer care for older patients, practicing oncologists should familiarize themselves with some of the assessment tools described below.

Geriatric Evaluation in Oncology

Although often used as traditional oncology measures, performance status scores alone (e.g. Karnofsky or Eastern Cooperative Oncology Group [ECOG]) do not convey sufficiently accurate information about functional status, comorbidities, and physiological reserves. However, these characteristics are crucial to differentiating between fit and frail persons of the same chronological age. Functional status evaluated by a geriatrician comprises an assessment of the patient's ability to complete activities of daily living (ADLs) such as bathing, dressing, feeding oneself, maintaining continence, and transferring from a bed or chair without assistance and instrumental activities of daily living (IADLs) like doing housework, using transportation, shopping, and taking medications. Both ECOG and functional status assessed by IADL predict postoperative morbidity, toxicity to chemotherapy, and survival [19].

Comorbidities are defined as additional concurrent diseases unrelated to cancer. They should be evaluated independently from functional status, because in a large prospective study, the relationship between these two variables was found to be low or absent [27]. Due to worsening pulmonary functions with reduced vital capacities and gas exchange, weaker cardiac output, decreasing renal blood flow, and changes in hepatic metabolism, the prevalence of comorbid conditions increases with growing age [4]. About 60% of SCCHN patients suffer from at least one co-existing illness and this percentage is estimated to approach 75% in the population over 70 years old [28, 29]. Among various comorbidity scores, Charlson Comorbidity Scale and Adult Comorbidity Evaluation 27 (ACE-27) were shown to have independent prognostic value for overall survival in retrospective analyses of SCCHN cases with primary or recurrent disease [30, 31]. In oropharyngeal squamous cell

carcinoma, the inclusion of a comorbidity score measured by ACE-27 led to a further refinement of a prognostic model described earlier by Ang et al. In the new model, the ensuing factors were involved: age, gender, tumour and nodal stages, pack-years of tobacco smoking, alcohol consumption measured by unit years, comorbidity, and HPV status. As expected, HPV status was the principal determinant of overall survival, while the second place was reserved for comorbidity and nodal stage in HPV-positive and -negative subgroups, respectively [32, 33].

In addition to functional status and comorbidities, further factors linked to survival include cognition, nutritional status, social support, and psychological state (depression) [19]. In an outpatient oncology setting, the following health issues and their prevalence were reported in older cancer patients: comorbidity (>90%, severe in 30–40%), IADL dependence (50–60%), nutritional compromise (30–50%), depression (20–40%), cognitive impairment (25–35%), ADL dependence (about 20%), and ECOG ≥2 (about 20%) [34]. Moreover, with prevalence reaching up to almost 50%, falls and problems with balance and/or walking are significantly more frequent in some elderly cancer survivors compared with the pre-diagnosis period. These difficulties are associated with poor quality of life, dependence in ADLs, increased mortality, and higher costs of health care [35].

To address the complexity of geriatric assessment, certain scales and tools were developed for use in clinical practice.

Comprehensive Geriatric Assessment

Comprehensive geriatric assessment (CGA) was introduced by geriatricians to estimate overall health status of an individual, detect unknown deficits, predict survival, and anticipate on adverse effects of chemotherapy. It includes validated tests for evaluation of functional status, comorbid conditions, cognition, nutritional status, social support, psychological state, and polypharmacy [14, 36] (Table 1). Information about life expectancy may help guide treatment decisions. A CGA can predict morbidity and mortality not only in the general geriatric population but also in elderly patients with cancer, where it was shown to modify the initially proposed treatment plan in as much as 49% of patients [2, 3, 19]. This multidimensional interdisciplinary process is thus both a diagnostic and therapeutic tool aiming at improving quality of life, compliance to therapy, and overall survival. With a notable remark that results from randomized trials are available mostly for non-malignant diseases, a CGA has been recommended by the European Society for Medical Oncology (ESMO), the National Comprehensive Cancer Network (NCCN), the European Organisation for Research and Treatment of Cancer (EORTC), and the International Society of Geriatric Oncology (SIOG) [37–40]. The first randomized controlled study of a CGA in elderly SCCHN patients is the EGeSOR trial currently recruiting participants in France. In the experimental group, GCAs are performed by geriatricians at predefined time points. The primary endpoint is a composite of death, ADL,

Table 1 Components of comprehensive geriatric assessment and how to measure them, adapted from [14, 36]

Assessment of functioning	Social assessment
Definition: ability to live independently at home and in the community, physical performance (mobility, balance, fall risk) Measurement: ADLs, IADLs, history of falls, timed up and go, short physical performance battery, handgrip testing	Definition: adequate social support to undergo treatment Measurement: needs assessment of financial capabilities, transportation, and caregiver status; Medical Outcomes Survey Social Support
Medical assessment	Psychological assessment
Comorbidity and medication Measurement: Charlson comorbidity scale, adult comorbidity evaluation 27, cumulative illness rating scale-geriatrics, comorbidity count and severity, medication count, Beers criteria[a] *Nutritional status* Measurement: mini-nutritional assessment, weight loss, body mass index	*Cognition* Measurement: mini-mental status examination, blessed orientation memory scale, short portable mental status questionnaire, montreal cognitive assessment *Depression and anxiety* Measurement: geriatric depression scale, hospital anxiety and depression scale

[a]Beers criteria for potentially inappropriate medication use in older adults
ADLs activities of daily living, *IADLs* instrumental activities of daily living

and weight loss ≥10%. The investigators expect at least a 10% decrease in the primary endpoint to be achieved by the intervention [25].

Notwithstanding its importance, a CGA is rarely performed in oncology practices. It is time-consuming, not necessary for all patients, and requires skilled professionals. Consequently, a two-step approach has been developed furnishing clinicians with geriatric screening tools to decide: (1) which patients will need a full assessment, (2) who will benefit from a specific examination, and (3) in which cases no further testing is required.

Geriatric Screening Tools

Several geriatric screening tests have been used in oncology including the G8, Flemish version of the Triage Risk Screening Tool (fTRST), Groningen Frailty Indicator, Vulnerable Elders Survey-13 (VES-13), and abbreviated Comprehensive Geriatric Assessment. The G8 and fTRST were prospectively validated in a non-interventional, multicentre study (Tables 2 and 3). Both instruments demonstrated high sensitivity and moderate negative predictive value to identify patients with a geriatric risk profile. Moreover, they were prognostic for overall survival, especially the G8 [41]. In a recent update of the SIOG recommendations, a systematic review of 44 studies on the use of 17 different screening tools was reported. The G8 proved to be more or at least equally sensitive compared to other tests. Although the screening tools should not replace a full assessment, the authors concluded that a busy practice setting entitles the physicians to use them for triage decisions prior to a CGA [42].

Table 2 G8 screening questionnaire in elderly patients [41]

Items	Score
1. Has food intake declined over the past 3 months due to loss of appetite, digestive problems, chewing, or swallowing difficulties?	0 = Severe reduction in food intake 1 = Moderate reduction in food intake 2 = Normal food intake
2. Weight loss during the last 3 months	0 = Weight loss more than 3 kg 1 = Does not know 2 = Weight loss between 1 and 3 kg 3 = No weight loss
3. Mobility	0 = Bed or chair bound 1 = Able to get out of bed/chair but does not go out 2 = Goes out
4. Neuropsychological problems	0 = Severe dementia or depression 1 = Mild dementia or depression 2 = No psychological problems
5. Body mass index (BMI) = weight in kg/height in m^2	0 = BMI < 19 1 = 19 ≤ BMI < 21 2 = 21 ≤ BMI < 23 3 = BMI ≥ 23
6. Takes more than 3 medications per day	0 = Yes 1 = No
7. In comparison with other people of the same age, how does the patient consider his/her health status?	0.0 = Not as good 0.5 = Does not know 1.0 = As good 2.0 = Better
8. Age	0 = Over 85 years 1 = 80–85 years 2 = Under 80 years
Total score	0–17 (abnormal if ≤14)

Table 3 Flemish version of the triage risk screening tool [41]

	Score	
Items	Yes	No
1. Presence of cognitive impairment (disorientation, diagnosis of dementia, or delirium)	2	0
2. Lives alone or no caregiver available, willing, or able	1	0
3. Difficulty with walking or transfers or fall(s) in the past 6 months	1	0
4. Hospitalized in the last 3 months	1	0
5. Polypharmacy: 5 medications	1	0
Total score	0–6	

Abnormal if ≥2 within the geriatric population and ≥1 within the oncologic population

Stratifying elderly head and neck cancer patients according to the VES-13 test into frail, vulnerable, and fit cohorts, Perri et al. proposed possible approaches for their management. Frail (VES-13 score = 3) and vulnerable (score = 1–2) groups should undergo a CGA, while standard therapy is advised for the remaining patients. Importantly, physicians should respect physiological changes in the elderly

concerning drug metabolism as well as limited bone marrow reserve reflected in guidelines for growth factor prophylaxis. Where indicated, a CGA tailors planned interventions, so that frail persons receive best supportive care only, whereas patients designated as vulnerable are treated with anticancer modalities. However, in the latter category, doses are often reduced, drugs substituted, and regimens switched in order to prevent excessive toxicity [43].

Frailty

Given the wide range of available anti-cancer approaches, the ultimate goal of geriatric assessment is to select which elderly patients are fit enough to receive such a treatment. However, it seems more practical to define the opposite quality, i.e. frailty, declaring at the same time that those who are not frail are candidates for a systemic and/or locoregional treatment with or without individualized modifications. Frailty can be regarded as a physiologic phenotype highly vulnerable to impaired homeostasis after an exposure to minimal stress. The negative health-related outcomes are reflected by physical disability, high-risk of falls, hospitalization, and mortality [44]. In the 65 years and over population, prevalence of frailty may reach almost 60%, increasing with age. Frailty also appears to occur more frequently in women and in those with poorer self-assessed health, more comorbidities, lower education, and lower income [5, 44]. Being considered a clinical syndrome, its wide symptomatology includes weight loss, fatigue, decreased muscle mass, gait disturbance, mild changes in cognition, and social withdrawal [45]. However, clinically silent frailty with no symptoms may be present in apparently healthy people, where it evolves into an overt form under destabilizing conditions. In 2001, Fried and colleagues defined frailty based on the presence of at least three of the following five criteria: muscle weakness, poor endurance, weight loss, low physical activity, and slow gait speed [44]. This concept was later validated in a cohort of 4735 participants enrolled in the Cardiovascular Health Study, which was a population-based, longitudinal trial aimed at finding etiological factors of coronary heart disease and stroke [46]. In addition, several other validated instruments to measure frailty have been described in literature, and interested readers are advised to refer to the review article by Buckinx et al. [5].

As already emphasized, not all senior patients are frail. Many of them are actually in a very good general condition. Acknowledging the unique characteristics of every individual in the real-world setting, fitness and frailness represent the very two ends of an imaginary scale with various intermediate stages which often pose challenges to clinical judgement in routine practice. Consequently, with the aim of creating the basis for treatment decisions, various assessment tools have been established for a proper patient categorisation. This is not only relevant for senior patients, but of importance for their younger counterparts as well. The most commonly used measures involve organ functions, usually defined by hematologic, renal, and hepatic parameters, and performance status (e.g., Karnofsky or ECOG score).

Although these may be sufficient for younger patients and are routinely incorporated in clinical trial protocols, older patients require a different approach consisting of geriatric assessment and a specific trial design. In the latter respect, it is not only the inclusion criteria but also study endpoints which are at stake. In spite of an increased risk of death from non-cancer-related causes in the elderly, disease-specific survival has been rarely reported in trials dedicated to this population. Similarly, the use of clinically meaningful objectives like functional status or Patient Reported Outcomes remains limited [47].

Two Continuums of Care

We explained the differences between chronological and biological age and between fit and frail persons. We also illustrated that the boundaries are not rigid and definitions not absolute, creating space for interdisciplinary discussions, flexible reassessment, and individualized management. To visualize this perception we constructed a model of two continuums presented in Fig. 1. The vertical continuum implies a transition from chronological to biological age during the process of pretreatment evaluation. Containing all intermediate stages, the horizontal continuum

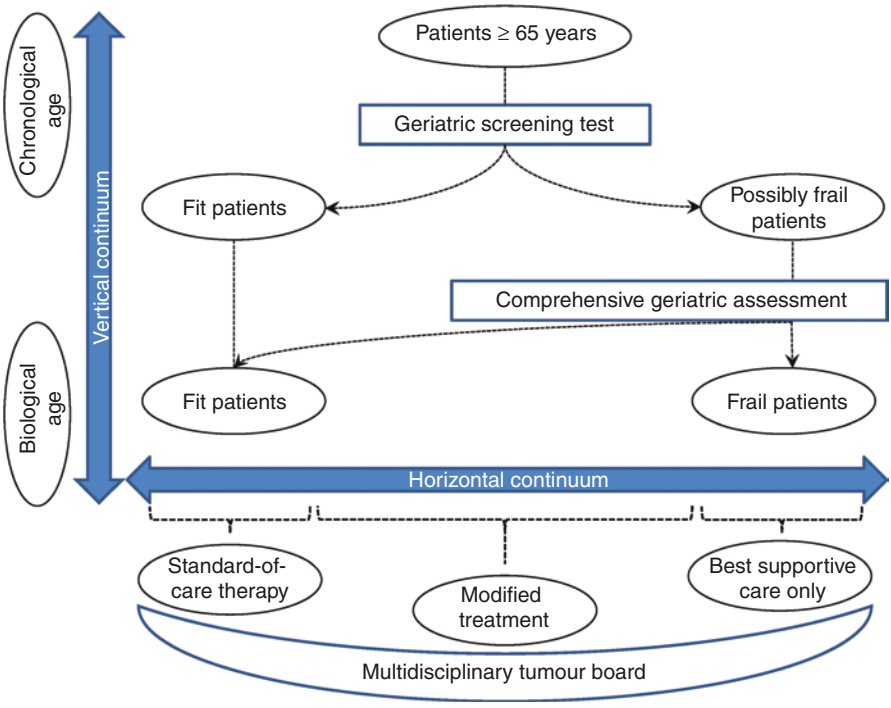

Fig. 1 Two continuums of cancer care in the elderly

leads from fitness to frailness and vice versa. The whole algorithm starts at the top, where patients whose age surpasses 64 years are indicated for some kind of geriatric testing. Performed by any professional competent in internal medicine, a basic screening helps to categorise patients by selecting those who are almost certainly fit and can proceed directly to the therapy-decision phase. In the other patients, who are possibly frail, a detailed assessment by a geriatrician is warranted, equipping the treating physician with information on the presence or absence of frailty and its severity. Based on the outcomes, a multidisciplinary tumour board provides decisive recommendations for clinical practice.

Locally Advanced Head and Neck Cancer

In this setting, according to the ESMO guidelines, there are two principal approaches with level I evidence and grade A recommendation. Either patients undergo surgery with adjuvant radiotherapy which is complemented by single-agent cisplatin in case of positive surgical margins and/or extracapsular nodal extension. Or if surgery is deemed too mutilating or the disease is unresectable or patients are not operable for medical reasons, physicians may opt for definitive platinum-based concurrent chemoradiation. Alternatively, radiotherapy with curative intent may be combined with cetuximab (level II, grade B). Organ preservation approaches with concurrent chemoradiotherapy or by induction chemotherapy followed by radiation can be considered in patients with locoregionally advanced resectable larynx and hypopharynx cancer (level II, grade A). Other procedures like sequential treatment (induction chemotherapy followed by chemo- or bioradiation) are still under evaluation [48]. Herein, only the former two standard-of-care strategies will be addressed.

Surgery

Under the condition of a careful preoperative evaluation of comorbidities and appropriate perioperative management, advanced calendar age does not seem to be an independent determinant of eligibility for limited or extensive surgical treatment. One of the first reports on the risks of major head and neck surgery dates back to the late 1970s, when McGuirt and co-workers reviewed medical records of 714 cases undergoing radical neck dissection. About one quarter of patients were over 70 years old. Major surgical complications comprised operative mortality, cutaneous fistula, carotid blowout, and haemorrhage, while minor complications were defined as wound infections, necrosis, seroma, chyle fistula, and flap elevation from hematoma formation. The incidence of both major and minor surgical complications was comparable between those aged above and below 70 years. However, medical complications, mostly of cardiovascular and pulmonary origin, were higher by 8% in the elderly cohort. Perioperative mortality rates, defined as death within 30 days of

intervention, were 7.4 and 1.4% in older and younger subjects, respectively [49]. Perioperative mortality was also addressed in a large retrospective study of 810 patients over 64 years, and reported to be 3.5% [50].

Smaller series published later by other investigators showed similar findings even in the oldest old category. Clayman et al. compared 79 patients younger than 65 years with 43 who were 80 years of age or older. Even though median overall survival was significantly lower in the older age group, it was similar to the actuarial survival of the general octogenarian population. Furthermore, despite a higher rate of preoperative comorbid conditions in the older age group, the investigators did not observe significant differences in terms of perioperative or postoperative complications between the two study groups [51]. Recently, L'Esperance et al. looked at postoperative complications and mortality in 219 octo- and nonagenarians. Independently associated with American Society of Anesthesiologists (ASA) score of 4 or greater and operating room time of 6 h or longer, serious complications within 30 days of surgery were noted in about one third of study population. About 11% of participants died within 90 days of surgery with an increased risk observed in nonagenarians, in case of a high comorbidity score measured by the ACE-27, and in the presence of preoperative dysphagia and/or a large extent of resection [52].

Although preferred by patients and treating physicians, conservative, non-destructive surgical procedures are not always feasible. Therefore, reconstructive surgery with microvascular free tissue transfer has become an integral part of aggressive surgical interventions in locally advanced SCCHN. The procedure can be used safely and effectively even in the elderly population. The higher rate of perioperative complications was reported to be more likely a result of an increased prevalence of comorbid conditions than advanced chronological age [53, 54]. Apart from comorbidities, which have indeed been identified as the main predictive factor for postoperative complications, several other factors such as type of surgery and disease stage merit attention. Among them, case volume at treatment centres has often been mentioned in relation with quality of care, albeit published data are still scarce. In a cross-sectional study by Jalisi et al., a total of 4544 elderly patients treated in 93 US hospitals were included. According to the number of performed surgical cases, the institutions were arbitrarily categorized into tertiles, i.e. high- ($\geq$50 cases), moderate (22–49), and low-volume ($\leq$21) hospitals. After performing multiple analyses, the authors concluded that high-volume academic centres showed a significantly shorter intensive care unit stay ($p = 0.0144$) and a marginally lower mortality ($p = 0.4699$) [55].

Chemoradiotherapy

High-dose three-weekly cisplatin (100 mg/m^2 on days 1, 22, and 43) given concurrently with conventionally fractionated external beam radiotherapy represents the standard of care in the postoperative setting of patients with high-risk features in the pathology specimen and in patients with locoregionally advanced SCCHN in whom

a non-surgical definitive approach is preferred. The benefit of adding cisplatin to the radiation was shown in four large phase III studies showing a significantly better locoregional control and/or overall survival [56–59]. The downside of this approach is an increase in acute toxicity, notably in mucositis, myelosuppression, and gastrointestinal side effects, and an increase in late toxicity [60]. Three of the four above mentioned studies focused on survival or locoregional control as the primary endpoint. Compared with radiotherapy alone, the absolute benefit in overall survival at 5-years ranged between 8% and 13% [56–58]. In the fourth trial, with larynx preservation as the primary end point, the 5-year overall survival was numerically worse with concurrent chemoradiotherapy (54% versus 55% versus 56% for concurrent chemoradiotherapy versus induction chemotherapy followed by radiotherapy versus radiotherapy alone, respectively). The difference between concurrent chemoradiotherapy and radiation alone became more evident with longer follow-up, but could not be attributed to larynx cancer or the treatment itself suggesting an unexplained higher incidence of competing causes of deaths in the concurrent arm which warrants further investigation [59–61].

So a question has been raised as to whether concurrent chemoradiation with high-dose cisplatin is a suitable approach for the elderly. In the four pivotal phase III studies, no restrictions were put on the upper age limit. Consequently, the recommendations are valid for the whole adult patient population. Nonetheless, in all these four trials, the median age at randomisation was in the fifth decade, and no geriatric screening or assessment of comorbid conditions or functional status were undertaken. Therefore, taking into account the substantial toxicity and only a limited long-term survival benefit (about 10% or less), many practicing physicians have been hesitating to use high-dose cisplatin during radiotherapy in the older population. This notion has been nourished by an imprecise interpretation of a large individual patient-based meta-analysis, which will be discussed later in the text.

Fortunately, considering the complexity of geriatric care and the discrepancy between chronological and biological age, some research groups made an effort to clarify the utility of chemoradiation in geronto-oncology. At present, besides numerous retrospective observations and subset analyses of prospective trials, several meta-analyses of controlled clinical trials and reports of population-based registries are available. In this chapter, we will concentrate on the latter two sources. Despite the fact that both represent the strongest currently available evidence in this field, there are several limitations to address in the first place. Registry reports are retrospective in nature, some critical details including treatment specifications are often lacking, and available patient data might not always be complete. On the other hand, many trials have been criticised for the limited representativeness of the study population and the ensuing poor generalisability of the results to the real-world practice. Furthermore, the inclusion period of some trials started more than 20 years ago or even earlier, and older people nowadays (Boomers) are different from their parents' generation (see above). Finally, oncologic care recently experienced a rapid evolution marked by refinements in treatment and supportive care protocols along with a number of new drugs on the market, which all have contributed to changing paradigms in clinical medicine.

Meta-Analyses

Combining data from 87 randomized trials performed between 1965 and 2000, a large individual patient-based meta-analysis demonstrated an absolute survival benefit of 6.5% at 5-years when adding concomitant chemotherapy to loco-regional treatment in locoregionally advanced SCCHN. However, the magnitude of the survival advantage conveyed by concomitant chemoradiotherapy was smaller in older than in younger adults. The declining effect of chemotherapy with age ($p = 0.003$, test for trend) has often been cited to contradict such treatment in those over 70. In this respect, we advocate more caution when interpreting the outcomes. Trials performed after 1994 exhibited a progressively growing proportion of non-cancer related deaths with advancing age (15% in those under 50, 39% in those over 70). This might have been a consequence of comorbidities, frailty, and a higher susceptibility to chemotherapy toxicity. Thus, a question remains as to whether a proper selection of fit patients could have had a more favourable impact on the results [62].

Second, a subset analysis of three Radiation Therapy Oncology Group (RTOG) trials (RTOG 91-11, 97-03, and 99-14), exploring different radiation and chemoradiation regimens, found that apart from advanced T-stage and larynx/hypopharynx primary site, older age is an independent risk factor for the development of severe late toxicity after concurrent chemoradiation (odds ratio 1.05 per year; $p = 0.001$) [60].

The third meta-analysis was presented only as an abstract at an international conference, and a full-text publication is still pending. It also involved three phase III RTOG trials exploring radiotherapy with or without concurrent chemotherapy, but these were different from the previous ones (RTOG 9003, 0129, and 0522). Here, patients aged 70 years or older were more likely to be female with a poorer performance status, heavier smoking history, and a negative p16 status ($p < 0.001$ for each parameter). After adjusting for covariates, elderly patients had worse overall survival (hazard ratio [HR] for death, 1.55; 95% confidence interval [CI], 1.35–1.77; $p < 0.001$), regardless of smoking history or p16 status. The relationship was more pronounced in the combined modality trials with cisplatin (RTOG 0129 and 0522), in which senior individuals experienced, in addition, significantly more grade 3–5 thrombocytopenia ($p = 0.02$), anaemia ($p = 0.03$), nephrotoxicity ($p = 0.01$), and ototoxicity (borderline significant; $p = 0.06$) than their younger counterparts, which was surprisingly not the case of severe mucositis exhibiting an opposite correlation ($p = 0.04$). In RTOG 9003, comparing two types of radiotherapy (standard versus altered fractionation) without chemotherapy, toxicities were similar by age [63].

Registries

In none of the three above mentioned meta-analyses, details on the proportion of frail and polymorbid patients were provided. It should be kept in mind that the number of older people enrolled in prospective trials has traditionally been low (8–12% in the three meta-analyses), while the frailty represents a common

phenomenon (up to almost 60%, see above). Therefore, the outcomes of such clinical investigations are not appropriate for concluding on the management in the elderly. Until age-specific prospective trials supply high-quality evidence, retrospective reviews of population-based cross-sectional registries will remain the reference source of information. We refer to five registry reports on the use of chemoradiation (versus radiation alone) in elderly patients with locoregionally advanced SCCHN. They all used the National Cancer Data Base of the US as source, from which records of Charlson-Deyo comorbidity scores could be obtained. The growing interest of healthcare professionals in this topic reflects the fact that all five papers were published in the last 2 years (Table 4) [64–68].

Amini et al. reported an overall survival gain achieved by adding chemotherapy concurrently to definitive irradiation in SCCHN patients older than 70 years. Five-year overall survival was 30.3% and 15.2% in those who received concurrent chemoradiation and radiotherapy alone, respectively. According to a recursive partitioning analysis, the survival benefit was limited to patients not older than 81 years, with low comorbidity scores, and either T1-2/N2-3 or T3-4/N0-3 disease [64]. Definitive treatment setting was also a subject of interest to researchers from the Cleveland Clinic, who confirmed an improved overall survival in those receiving concurrent chemoradiation. In a complex propensity score-adjusted multivariate model, controlled for age, insurance status, income, comorbidity, tumour site, differentiation, tumour and nodal stages, and different radiotherapy variables, the association remained statistically significant. Importantly, the authors did not find any age threshold for this correlation between 56 and 90 years, as measured by three-year overall survival gains. Additionally, an increase in the use of systemic therapy in the elderly was noticed from 64% in 2004 to 86% in 2012 [65].

Contrary to the two analyses mentioned above, which concerned elderly SCCHN patients who had been treated with definitive chemoradiotherapy, the next three retrospective analyses focused on patients who had been treated with chemoradiotherapy or radiation alone after surgery of tumours which showed high-risk features (positive surgical margins and/or extracapsular extension) on pathology review. All three analyses revealed a prolonged 3-year overall survival with the combined approach (53.8% versus 44.6%, 50.7% versus 44.4%, and 52.4% versus 43.4%, respectively), although this was less apparent on multivariate analysis in the study by Giacalone et al. [66–68]. This author group demonstrated a reduction in the risk of death with the use of chemoradiotherapy which was non-significant but could be considered as potentially important (50.7% versus 44.4%; HR, 0.88; 95% CI, 0.73–1.06; $p = 0.17$), particularly in a subgroup with a low Charlson-Deyo score (HR, 0.84; 95% CI, 0.69–1.02; $p = 0.08$). No meaningful difference was shown on propensity score matching ($p = 0.839$) [67]. Also in the adjuvant setting, as noted in two of the studies, the percentage of elderly patients that received concurrent chemoradiation increased over time [66, 67].

Table 4 Retrospective reviews of population-based cross-sectional registries exploring concurrent chemoradiotherapy in elderly patients with SCCHN

First author, year	Clinical setting	Inclusion period	Definition of elderly (years)	Proportion of elderly pts.	Elderly pts. receiving		Overall survival benefit on multivariate analysis	
					CCRT	RT	CCRT group (elderly vs. younger pts.)	Elderly group (CCRT over RT)
Amini, 2016 [64]	Definitive	1998–2011	>70	100% (4042)	2538	1504	N/A	Yes (HR, 0.63; $p < 0.001$)
Ward, 2016 [65]	Definitive	2004–2012	>70	14% (4165/30,399)	3028	818	Sustained benefit from 56–90 years	Yes (HR, 1.46; $p < 0.001$)[a]
Woody, 2017 [66]	Post-operative	2004–2012	>70	100% (445)	187	258	N/A	Yes (HR, 0.74; $p = 0.04$)
Giacalone, 2017 [67]	Post-operative	1998–2011	≥70	100% (1686)	491	1195	N/A	Maybe (HR, 0.88; $p = 0.17$)
Yoshida, 2018 [68]	Post-operative	2004–2013	≥70	100% (1199)	531	668	N/A	Yes (HR, 0.75; $p = 0.001$)

[a]Pertaining to larger cohort of 3347 patients comprising also those who received induction chemotherapy (n = 91) and where the type of systemic treatment was unclear (n = 228)

SCCHN squamous cell carcinoma of the head and neck, *pts.* patients, *CCRT* concurrent chemoradiotherapy, *RT* radiotherapy alone, *N/A* not available, *HR* hazard ratio for death

Recurrent and/or Metastatic Head and Neck Cancer

With an expected overall survival usually not exceeding 1 year, recurrent and/or metastatic SCCHN is a devastating disease qualifying most of the patients for palliative measures. At present, evidence from the literature is insufficient to draw firm conclusions regarding the management of the elderly population. In cases without distant metastases, locoregional treatment options should be considered [14]. However, only a minority of locoregional recurrences can be successfully salvaged by complete resection or irradiation [69]. As was recently reported, carefully selected cases with metachronous pulmonary metastases may also be considered for surgical intervention [70]. In the remainder, irrespective of age, treatment goals focus primarily on symptom control and improvement of quality of life. A single-drug regimen or best supportive care alone are offered to frail patients with poor functional status and comorbidities. In first line, however, fit patients may benefit from multi-drug chemotherapy with or without the targeted agent cetuximab (epidermal growth factor receptor [EGFR] inhibitor) [69].

Cytotoxic Chemotherapy

As a result of age-related changes in pharmacokinetics and pharmacodynamics, chemotherapy administration carries safety concerns in the elderly. In a combined analysis of two phase III trials conducted by ECOG (1393 and 1395), Argiris et al. compared the toxicity, response rates, and survival of elderly recurrent and/or metastatic SCCHN patients (70 years or older) with their younger counterparts. The ECOG 1393 trial randomized participants to receive a cisplatin/paclitaxel doublet at two dose levels, while treatment arms in the ECOG 1395 trial consisted of cisplatin plus either 5-fluorouracil or paclitaxel. Altogether, 53 older patients were compared with 346 younger ones. No statistical difference was observed in terms of objective response rate (28% versus 33%), median time to progression (5.25 versus 4.8 months), median overall survival (5.3 versus 8 months), or 1-year survival (26% versus 33%) between these two subgroups, respectively. However, the authors noted a significantly higher incidence of severe nephrotoxicity, diarrhoea, and thrombocytopenia in the elderly population, which was paralleled by a trend towards a higher toxic death rate (13% versus 8%). In conclusion, cisplatin-based doublets yielded comparable survival outcomes among fit elderly and younger patients, yet at the cost of increased side effects in the former group [16].

Targeted Treatment

The landmark EXTREME (Erbitux in first-line treatment of recurrent or metastatic head and neck cancer) trial found significant overall survival improvement with the platinum (cisplatin or carboplatin)/5-fluorouracil/cetuximab combination over the

chemotherapy doublet alone. It is the only approved standard first-line systemic treatment in platinum-sensitive recurrent/metastatic SCCHN today. Population aged 65 years or older made up 17% of the total number of enrolled patients (77/442) and was equally distributed between both treatment arms. Subgroup analysis of this cohort revealed that the survival benefit conferred by adding cetuximab to platinum/5-fluorouracil chemotherapy fell short of statistical significance, in contrast to younger adults and the whole intention-to-treat population. Median progression-free survival was 4.2 and 3.2 months (hazard ratio [HR], 0.65; 95% confidence interval [CI], 0.38–1.12) and median overall survival 9.1 and 7.8 months (HR, 1.07; 95% CI, 0.65–1.77), in the cetuximab and control arms of the elderly subpopulation, respectively [71].

Analogous data are available in the second-line setting. The LUX-Head & Neck 1 trial evaluated the clinical efficacy of afatinib, an irreversible human epidermal growth factor receptor (ERBB) family blocker, matched up to methotrexate in a 2:1 ratio among 483 eligible subjects (128 [27%] aged 65 or more). Although the study was sufficiently powered, no improvement in overall survival was achieved by the ERBB antagonist. However, afatinib induced a marginal but significant improvement in median progression-free survival versus methotrexate in the overall population (2.6 versus 1.7 months; HR, 0.80; 95% CI, 0.65–0.98, p = 0.030) [72]. Moreover, similar progression-free survival benefit with afatinib versus methotrexate was observed in patients 65 years or older (2.8 versus 2.3 months; HR, 0.68; 95% CI, 0.45–1.03, p = 0.061) as well as younger individuals (2.6 versus 1.6 months; HR, 0.79; 95% CI, 0.62–1.01, p = 0.052). Also objective response rates with afatinib versus methotrexate were 10.8% versus 6.7% and 10.0% versus 5.2% and disease control rates were 53.0% versus 37.8% and 47.7% versus 38.8% in older and younger patients, respectively, without an indication of excessive toxicity in the older population [73]. Currently, afatinib is recommended in the NCCN guidelines (category 2B) for patients with recurrent/metastatic who fail on platinum containing chemotherapy [74].

Immunotherapy

Immune checkpoint inhibitors emerged as a ground-breaking discovery in several areas of oncology including head and neck cancer. The mechanism of action resides in restoration of the natural anticancer potential of the host immune system. As an immunosuppressive disease, SCCHN evades immunosurveillance, i.e. recognition and elimination of malignant cells, by manipulating its own immunogenicity, producing immunosuppressive mediators, and promoting immunomodulatory cell types [75]. The process of aging is characterised by a gradual decline in immune functions, referred to as immunosenescence. Although available evidence supports an association of advancing age with decreased immunosurveillance, the tumour-promoting properties of the immune system seem to be compromised as well. Thus, the real impact of immunosenescence on cancer development remains unclear, and chronic inflammation observed in aging tissues may be more important [7].

Contrary to classical cytotoxics and targeted agents aiming therapeutically at tumour cells, immune checkpoint inhibitors are monoclonal antibodies against receptors and ligands found primarily on lymphocytes and myeloid elements. Translated into clinical practice, these new medicines have become known for their potential to induce durable responses even in heavily pre-treated patients at the cost of relatively low incidence of severe adverse events. One of the most studied is the signalling axis between programmed cell death protein-1 (PD-1) and its ligand PD-L1. In SCCHN, drug development has already moved forward to phase III protocols, both in the locoregionally advanced and the first-line recurrent and/or metastatic settings. In the second-line recurrent and/or metastatic setting, final results have been published, bringing important changes to treatment guidelines [76]. Compared with the control arm containing weekly single-agent methotrexate, docetaxel, or cetuximab, the CheckMate-141 trial demonstrated a 30% reduction in risk of death in patients assigned to the experimental arm with nivolumab, an anti-PD-1 inhibitor. Correspondingly, median overall survival rose from 5.1 months to 7.5 months. In a subgroup analysis of patients who recurred within 6 months after chemoradiation, the benefit of nivolumab over standard therapy was also observed. Based on these findings, nivolumab at an intravenous dose of 3 mg/kg every 2 weeks is the current standard in platinum-refractory recurrent and/or metastatic SCCHN. Of the 361 randomized patients, 113 (31%) were 65 years or older. In those aged 65–74, a subgroup analysis fell short of statistical significance (HR, 0.93; 95% CI, 0.56–1.54) [77]. However, in this context, it is important to mention that data from trials exploring immune checkpoint inhibitors in melanoma, non-small cell lung cancer, and renal cell carcinoma revealed that responsiveness and safety are not impaired in the elderly [78]. The tolerance to these agents was recently illustrated in a case report of a 96-year-old woman with SCCHN progressing on cetuximab, showing tumour shrinkage on durvalumab, an anti-PD-L1 blocker, with no serious treatment-related toxicity [79].

Conclusions

Increasing average life expectancy is one of the prosperity indicators, and modern societies have been deliberately undergoing profound multifactorial changes towards maximizing this outcome. However, the aging population exerts enormous strains on health infrastructure. Elderly people deserve the same quality of medical care as their younger counterparts. The situation gets more and more challenging with a widening gap between chronological and biological age driven by the advent of new generations reaching retirement, with novel drugs hitting the market, and with rapidly rising costs in oncology. Practicing physicians have to be prepared for that. However, this will not be possible without collaboration with experienced trialists and other stakeholders involved in clinical research.

To better understand the behaviour of cancer in patients at an advanced age and to offer them a high-quality evidence-based approach, we advocate a strong support

in the development and implementation of elderly-specific prospective trials instead of settling for stratifications based on age. The integration of formal geriatric assessment with co-morbidity scores should take into account a direct applicability to daily clinical practice. The institution of predictive models for chemotherapy toxicity and outcome, examination of tumour genetics, and comparative molecular genomic analysis of elderly patients versus their younger counterparts may further assist us in defining new standards of care in this population [63].

Senior persons derive benefit from intensified treatment approaches but need careful decision making and attentive follow-up. They have shown to develop effective coping strategies and maintain quality of life comparable with their younger counterparts. In fact, elderly patients report even better socioemotional functioning probably because of lower expectations, since they might have less to lose and need fewer adjustments to their lifestyle [80, 81]. Oncologists must be cautious in generalizing results from clinical research to the geriatric population. These patients have often been underrepresented in prospective studies, which were not primarily designed to integrate a population requiring a special diagnostic evaluation. A change has to be made now. We need to abandon the traditional perception of aging and focus on trials that show us how to approach patients we really encounter in our offices.

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Part VI
Thyroid Cancer

Worldwide Thyroid Cancer "Epidemic": What Is Going On?

Salvatore Vaccarella

Upwards Trends in Thyroid Cancer Incidence

Large increases in the incidence of thyroid cancer (TC) have been observed in the past two or three decades in several high-resource countries worldwide, Fig. 1 [1, 2]. Although the female-to-male ratio is approximately threefold, the temporal patterns of TC incidence trends are consistent across gender. Large geographical variations exist in the magnitude of the TC increases, even among geographically close countries and, within countries, across different regions. Rapid increases of the incidence rates were observed, for instance, in France [3], Italy [4], Australia, United States [5–7], and most notably in the Republic of Korea [8], although only mild increases were observed in countries from northern Europe and Japan [2, 9]. TC incidence is however also quickly rising in some regions in large medium income countries, such as Brazil, Turkey and China, as shown by the newly released version of the Cancer Incidence in V Continents, Volume 11 [10], Fig. 2. The most common histological TC types are papillary (representing approximately 85% of all TC), follicular (11%); medullary (3%) and anaplastic (1%), which carry also different prognosis [5]. While the prognosis for anaplastic TC is very poor (90% mortality at 5 years), survival for papillary TC is excellent (1–2% mortality at 20 years). The fast rising TC incidence trends and the differences in TC rates observed between countries, are largely attributable to the increase in small papillary carcinomas i.e., <30 mm in diameter [7] or even smaller, <10 mm, in the Republic of Korea [8]. No significant changes are observed in the incidence of the other common histological types, i.e., follicular, medullary, and anaplastic thyroid cancers.

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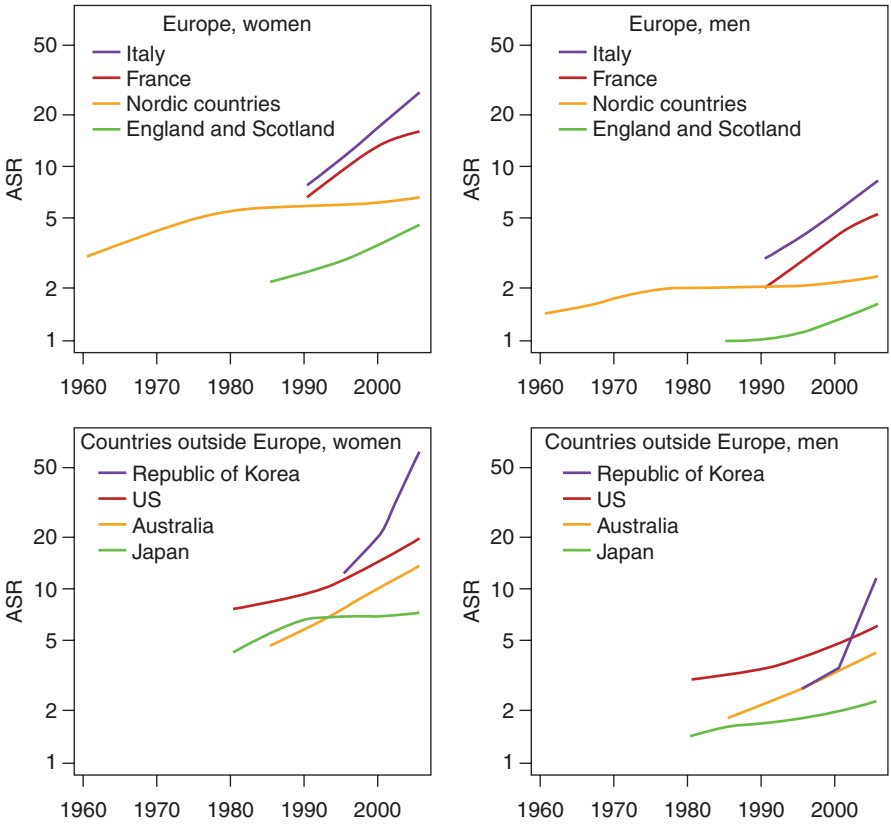

Fig. 1 Trends of age-standardised (world population) incidence rates (per 100,000) of thyroid cancer, by sex and country, age 15–79 years, up to 2007. Source: Vaccarella S, Dal Maso L, Laversanne M et al. The impact of diagnostic changes on the rise in thyroid cancer incidence: a population-based study in selected high-resource countries. Thyroid 2015;25:1127–36. Copyright and Permissions: Mary Ann Liebert, Inc. The figure shows a rapid increase of the incidence of thyroid cancer in some high-resource countries

Minor Changes in TC Mortality Trends

In contrast to the increases in incidence, TC mortality trends have been stable at very low levels or declining in the majority of the studied countries [2]. Small papillary TCs are considered to be low risk as they are very unlikely to cause morbidity or premature mortality, and this is particularly true when found in young adults women. In high-resource countries, the incidence-to-mortality ratio for TC (all carcinomas included) in women age 15–44 years is currently around 300–400, much higher than the corresponding ratio for other cancers. Even for cancers of the breast, prostate, and skin melanoma, where upward incidence trends in the past decades

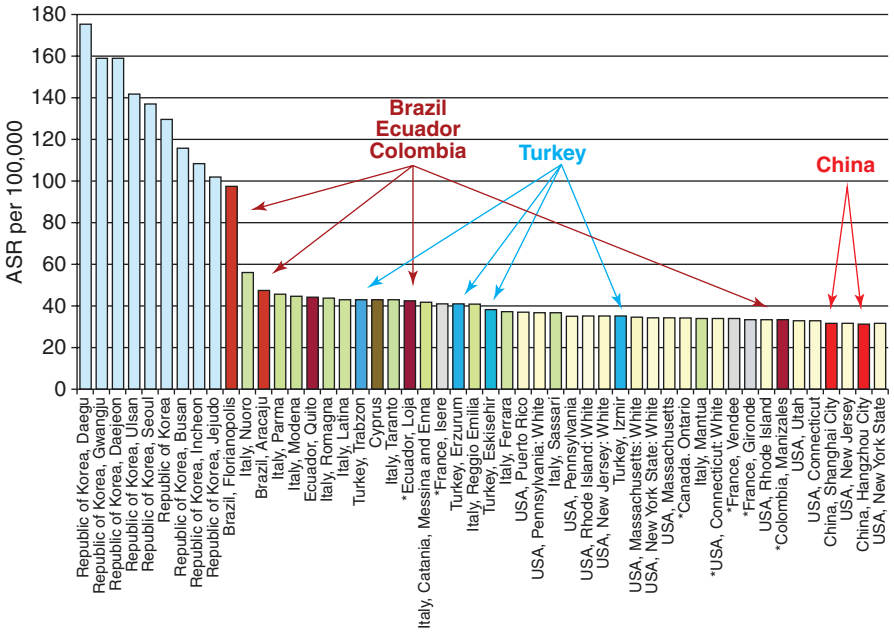

Fig. 2 Age-standardised (world population) incidence rates (per 100,000) of thyroid cancer, women age 15–79 years, 2008–2012. Source: Cancer incidence in five continents, International Agency for Research on Cancer (available at http://ci5.iarc.fr/CI5-XI). Copyright and Permissions: IARC Publications: Publications@iarc.fr. The figure ranks the first 50 high-quality cancer registries with the highest incidence of thyroid cancer. Among them, there are several registries from emerging countries

were accompanied by smaller changes in mortality rates, the gap between incidence and mortality rates has been much smaller, with incidence-to-mortality ratios for the same age group ranging between 10 and 20 [11].

Lack of Evidence of a Major Impact of Known or Unknown Risk Factors

Benign thyroid disease and ionizing radiation are established risk factors for TC. The thyroid gland is a very radio-sensitive site in children and adolescents [12]. Medical exposure to ionizing radiations increased substantially in the past decades—in the United States for instance, from 11% of total exposure in 1980 to 36% in 2006 [13]—and could therefore explain, at least partly, the rising TC trends. However, no consistent association of TC with history of x-ray procedures has been found [14]. CT scans entail much heavier radiation than x-rays and exposure in children and adolescents. However, TC trends started to increase long before the widespread use

of CT scan, which was relatively limited before year 2000, and the association with increased TC risk, although statistically significant is relatively modest in magnitude (risk ratio = 1.40, 95% CI, 1.23–1.59) [15]. Approximately 5–15% of all TC cases and up to 20% of medullary TC could be attributable to familial and genetic factors. Other factors, such as nutrition, dietary habits and microelements present in food or water, as well as hormonal and reproductive factors, could possibly be related to TC but the magnitude of the association is likely small. Overall, it is unlikely that changes in exposure of known [16–18] or unknown risk factors have occurred that could explain the remarkable upward trends and the geographical heterogeneity in TC incidence observed even between neighboring countries and regions [19]. Conversely, technological advances in detecting small thyroid lesions, access and coverage to health care, physicians' practices and increased medical surveillance [20], and the extent of intentional inspection of the thyroid gland or incidental findings are likely to have largely contributed to the current TC epidemic [1, 8, 21].

The Role of Overdiagnosis of Thyroid Cancer

Overdiagnosis is the diagnosis of thyroid tumors that, if left untreated, would not cause symptoms or death. In order for overdiagnosis to occur, three conditions must be present: (a) a vast reservoir of latent, subclinical tumors; (b) a mechanism that can identify the tumors; (c) an increased level of organized or disorganized activities of early detection [22]. Approximately 12% of 8619 thyroids from 15 autopsy studies showed occult papillary carcinoma, mainly <0.3 cm, with similar prevalence across males and females. In the United States, nearly 16% of computed tomography and magnetic resonance images show incidental thyroid nodules, of which around three quarters are <1.5 cm [5], and more patients receive a TC diagnosis after an evaluation of an incidentally found thyroid nodule than after the evaluation of a symptomatic or palpable nodule. The organization of the health systems, the number and attitude of physicians and the penetration of new diagnostic and screening practices are known to vary substantially across countries/regions and have been shown to be associated with the risk of detection of benign and malignant thyroid diseases. The average national figures on TC overdiagnosis can therefore hide marked regional inequalities, depending on the local medical practices. A study in the United States found a correlation between increased TC incidence and the use of thyroid ultrasound and fine-needle aspiration [23]. Access to health care services and health care practices, such as surveillance of the thyroid gland and scrutiny of thyroid specimens, have a major impact on the increased TC incidence [1, 8, 21, 24].

With an increasingly larger number of imaging tests performed [23, 25, 26], and with a greater propensity of health care providers to intervene for increasingly smaller findings, more thyroid cancers are uncovered.

Estimating Overdiagnosis of Thyroid Cancer

A method to obtain estimates of TC overdiagnosis at the population-level has been described [1] based on the observation of the shape of the age curves for available periods in several countries. There is a progressive distortion of the age curves across periods, Fig. 3. Incidence rates have progressively increased in middle-aged women but varied to a much lesser extent at older ages. The shape of the age curves over the years changes from roughly an exponential shape to an inverted U-shape. In the Republic of Korea, adults had voluntarily undergone thyroid ultrasound within the framework of organized screening programs for five other types of cancer and the highest participation was in women aged 50–59. Conversely, TC increases in the United States, Australia and Italy, started in the late 1980s, particularly in women below age 45 alongside the earliest introduction of ultrasonography in gynecologic and obstetric clinics, which favored the opportunistic examinations of the thyroid gland in reproductive age women. The observed shape of TC age-specific rates made in each country was compared with the expected historical shape if detection of TC had continued occurring without the use of technological diagnostic advances and with the same level of thyroid surveillance as in the past. The expected shape was obtained by using the historical age-specific rates from the Nordic countries in 1958–1967, prior the introduction of ultrasonography. Historical TC rates roughly increase exponentially with age [27], similarly to the behavior of other epithelial cancers and in agreement with the multistage model of carcinogenicity of Armitage and Doll [28]. This model implies a linear relationship between the logarithm of incidence rate and the logarithm of age and the consequent possibility to estimate the slope of this log-log relationship.

Overdiagnosis was estimated as the excess of observed compared to expected rates. The number of TC cases attributable to overdiagnosis in each studied country was estimated by multiplying the observed and expected sex-age-specific rates in areas covered by cancer registries by the corresponding national population estimates. This analysis led to the conclusion that a large fraction of TC diagnoses in high-resource countries is likely due to overdiagnosis. This fraction could be as high as 60–70%, e.g., in Italy, France, United States, Australia, and reached >80% in the Republic of Korea. These estimates corresponded to approximately ½ million overdiagnosed TC cases in 12 countries, the large majority of whom underwent total thyroidectomy and harmful treatment.

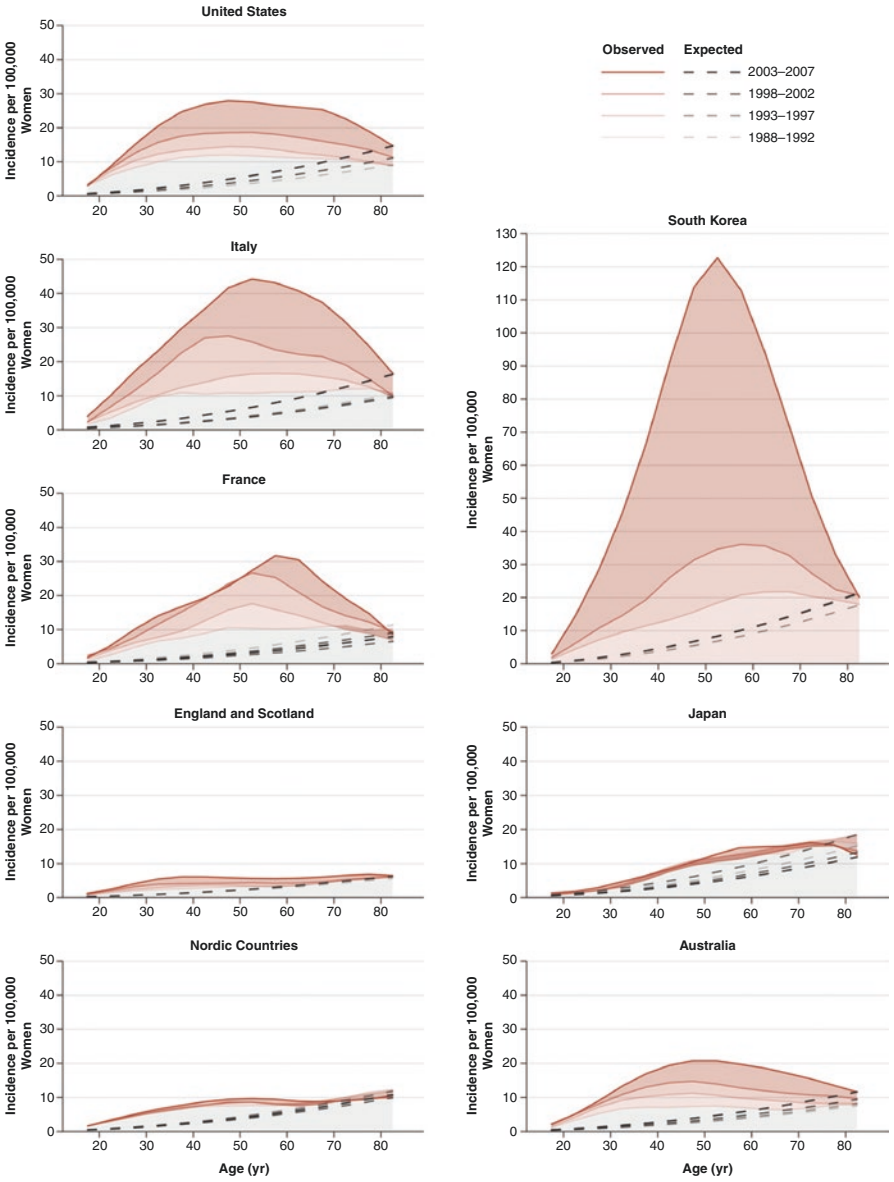

Fig. 3 Observed versus expected age-specific incidence rates (per 100,000) of thyroid cancer in women, 1988–2007. Source: 1. Vaccarella S, Franceschi S, Bray F et al. Worldwide thyroid-cancer epidemic? The increasing impact of overdiagnosis. N Engl J Med 2016;375:614–7. Copyright and Permissions: Massachusetts Medical Society. The observed rates were derived from Cancer Incidence in Five Continents, International Agency for Research on Cancer (available at http://ci5.iarc.fr/CI5I-X). The expected rates were based on the observation that before the introduction of ultrasonography and other novel diagnostic techniques, thyroid cancer incidence increased exponentially with age in all countries with available long-term data, in keeping with the multistage

Conclusions

The majority of TC patients, predominantly young/middle aged women, live full lives after diagnosis (10-year survival rate over 90%), but are likely to undergo heavy treatments and lifelong surveillance. It is important to realize and quantify the major clinical and economic burden associated with TC overdiagnosis and overtreatment. This may also change the understanding of the societal burden of TC overdiagnosis, improve the clinical practice of physicians caring for patients with TC, and inform future policy changes.

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model of carcinogenesis described by Armitage and Doll (rate proportional to age^k, where the exponent k is to be estimated from incidence data). For each 5-year period, the expected age-specific rates were obtained by hypothesizing that the disease would have retained the historical age curve described by the multistage model. Since thyroid-cancer incidence varied only minimally across periods among people 80–84 years of age, we added a constraint that sets as equal the expected and observed incidence rates for this age group. We hypothesized that the progressive departure of the observed rates from the multistage model was attributable to the increased detection of asymptomatic, nonlethal disease—that is, overdiagnosis

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Surgical Approach to Thyroid Cancer

John Cramer and Robert L. Ferris

Introduction

Surgery is the primary mode of treatment for patients with differentiated thyroid cancer (DTC). DTC includes papillary thyroid cancer (PTC) and follicular thyroid cancer (FTC) and accounts for 88% of thyroid cancer. Most patients with DTC have an indolent nature and excellent prognosis. However, a small minority of DTC behaves aggressively and requires radioiodine therapy (RAI) and thyroid hormone suppression therapy for disease control with a propensity for recurrence despite appropriate aggressive treatment. This presents a challenge to the surgeon to deintensify treatment and minimize morbidity for the large majority of patients with a favorable prognosis while tailoring more aggressive surgery, postoperative RAI and thyroid hormone suppression to patients with more aggressive tumors.

Epidemiology

In 2018 there are an estimated 53,990 cases and 2060 deaths from thyroid cancer in the United States [1]. Thyroid cancer has increased threefold in the United States between 1974 and 2013 [2]. Much of this increase is thought to be due to incidental detection of small PTC on imaging modalities [3]. Suggesting that there is a

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substantial reservoir of clinically inconsequential thyroid cancer the prevalence of thyroid nodules at autopsy is up to 50%, although only 5–10% of these are malignancy [4]. Conversely, while thyroid cancer mortality is low, there has been a smaller 1.1% per year increase in incidence-based mortality suggesting that a true increase in the occurrence of thyroid cancer may also be occurring [2]. Among those malignant thyroid nodules, PTC accounts for 85%, Hurthe-cell carcinoma for 1%, FTC for 2%, MTC for 4%, poorly differentiated for 6% and anaplastic for 1% [5].

Thyroid Cancer Genetics

Thyroid tumors develop when a single thyroidal epithelial cell or neural crest derived thyroid C cells escapes the normal mechanisms regulating cellular division. Both PTC and FTC that together make up DTC arise from the same follicular epithelial cell type. Most tumors are slow growing but a minority are aggressive. The genetics are highly dependent on the disease histology. In addition PTC and FTC express different clinical characteristics. FTC to be associated with vascular invasion, infrequent spread to regional lymph nodes and are typically RAI avid [6]. The genetic alterations may explain the diverse clinical phenotypes observed and help to direct therapy. Most genetic alterations represent somatic (acquired) mutations as most tumors are sporadic and not familial. Poorly differentiated and anaplastic thyroid carcinomas similarly arise form follicular cells but they are comparatively rare and associated with aggressive disease. Medullary thyroid cancer (MTC) arises from separate C-cells and displays distant biologic features.

PTC Genetic Alterations

Most PTC are indolent consistent with one of the lowest mutation densities of any cancers studied by whole-exome sequencing [7]. PTC typically carries genetic alterations in the mitogen-activated protein kinase (MAPK) pathway that promotes cellular division [8]. Alterations in RET and NTRK1 tyrosine kinases, or activating mutations in BRAF and RAS are successive components leading to activation of the MAPK pathway. An individual papillary thyroid tumor typically carries only one mutation in the MAPK pathway [8]. The most frequent genetic variant is BRAF (V600E) which is present in 45% of PTC [9]. BRAF V600E is associated with aggressive characteristics including lymph node metastases, extrathyroidal extension, advanced disease and progression to poorly differentiated and anaplastic thyroid cancer [10, 11]. Even in micropapillary carcinomas, the presence of BRAF V600E alteration is associated with higher rates of extrathyroidal extension and lymph node metastases [12]. RET/PTC genetic rearrangements are also associated PTC, most commonly seen as a result of radiation induced thyroid cancer. However, RET/PTC genetic rearrangements carry a favorable prognosis with a lower chance

of dedifferentiation [13]. The Cancer Genome Atlas sequencing project has dramatically expanded our knowledge of genetic alterations in DTC [14]. The project confirmed many of the mutations seen in thyroid cancer but also identified multiple less common new driver mutations such as EIF1AX [14].

Follicular Genetic Alterations

Follicular adenomas and FTC frequently overexpress c-myc, c-fos, or display mutations in HRAS, NRAS and KRAS. RAS pathway mutations display a more variable phenotype and are present in both benign and malignant processes. In combination with TIMP1, NRAS mutations are associated with malignancy and can aid in deciding surgical options [15]. RAS gene family mutations are occasionally also seen in 13% of papillary cancers, but generally seen in the encapsulated follicular variant [16]. PAX8/PPAR is a rearrangement in transcription factors regulating expression of genes controlling thyroid proliferation and thyroid hormone receptor and are associated with FTC, FVPTC and Hurthle cell thyroid cancer [16]. Hurthle-cell carcinomas are classified as a variant of FTC but are genetically separate [17]. Unlike FTC, Hurthle-cell carcinoma are frequently refractory to RAI and are characterized by extensive capsular and vascular invasion [5].

Familial Pathways

While most DTC occurs as a result of somatic mutations, 3–9% are familial. Germline mutations adjacent to the master thyroid regulators FOXE1 and NKX2-1 have been identified [18]. Cowden's disease, familial adenomatous polyposis and Werner's syndrome arise from mutations in PTEN, APC, and WRN respectively and may cause a small proportion of these familial cancers. The rapid advances in our knowledge of the genetics of thyroid cancer and the development of next generation sequencing has enabled molecular profiling of thyroid nodules and tumors that greatly advanced in recent years.

Evaluation of Thyroid Nodules

Most patients with DTC present with an asymptomatic thyroid nodule, the majority of which are benign. Only approximately 10% of thyroid nodules are malignant [19] and only a tiny fraction of those with a malignancy will ever die of their disease. A diagnostic approach with neck ultrasonography (US), judicious use of fine-needle aspiration biopsy (FNAB) and selective use of molecular testing enables a personalized evaluation to foster high-quality care and minimize unnecessary

testing. Surgeons are often on the front lines of evaluating thyroid nodules for the potential presence of malignancy and must be familiar with the current techniques available to facilitate evaluation.

Ultrasonography (US)

Neck US is key to initial nodule risk-stratification. Neck US should be performed for all nodules as it helps to determine if FNAB is necessary and is helpful in planning any surgical procedures. High-resolution (12 MHz) US probes provide excellent image quality for the thyroid and associated cervical lymph nodes as they are generally located within 4 cm from the skin. A diagnostic neck US must include examination of the thyroid (background parenchyma, nodule location, size, sonographic features) and evaluation of central and lateral neck lymph nodes [20]. Some US evaluations will not include an examination of central and lateral neck lymph nodes however this is inadequate and may miss patients with clinically overt metastatic lymphadenopathy.

CT, MRI and PET/CT

The sensitivity of neck US for extrathyroidal extension is low [10]. Thus, if there is preoperative suspicion for extrathyroidal extension based on preoperative symptoms of stridor, hemoptysis, rapid growth, dysphonia, dysphagia, nerve paralysis, recurrent cancer or pain then the workup should include routine CT and/or MRI [10]. Esophagosocpy, tracheoscopy and bronchoscopy may also be indicated based on the specific symptoms. While the risk of malignancy from an asymptomatic thyroid nodule detected by US, CT or MRI ranges from 5 to 13%, the risk of malignancy is significantly higher (55%) with nodules detected with focal uptake on PET scan [21]. Thus, PET-avid thyroid nodules warrant a more aggressive workup. In the staging of thyroid cancer, PET scans are not commonly required for DTC but may is useful in the staging of thyroid cancers at high risk of distant metastasis including Hurthle cell carcinoma, poorly differentiated carcinoma and anaplastic carcinoma.

Fine-Needle Aspiration Biopsy and Molecular Markers

FNAB offers the most definitive diagnostic information when evaluating thyroid nodules [10]. If the nodule is not easily palpable then US guidance during FNAB will improve accuracy. Reporting of cytology for FNAB should be standardized based on the 2017 updated Bethesda classification system [22]. The updated

Bethesda system adjusts the risk of malignancy after the recent recategorization of the noninvasive follicular thyroid neoplasm with papillary-like features (NIFTP) that will be discussed in greater detail later in this chapter. Results of thyroid FNA should be reported as (1) nondiagnostic or unsatisfactory, (2) benign, (3) follicular lesion of undetermined significance (FLUS)/atypia of undetermined significance (AUS), (4) follicular neoplasm or suspicious for a follicular neoplasm, (5) suspicious for malignancy, or (6) malignant. Each carries its own risk of malignancy that is classically reported as 1–4%, 0–3%, 5–15%, 15–30%, 60–75% or 97–99% respectively [23]. The risks of malignancy have been decreased after the recategorization of some cancers as NIFTP as a significant proportion of patients that were previously considered to have malignancy have now been recategorized as this benign NIFTP category. The indications for FNAB in 2015 ATA guidelines emphasize judicious use of FNAB in nodules that are potentially clinically significant to minimize overdiagnosis [10]. These now recommend FNAB of a nodule that is highly or intermediately suspicious on imaging only if the size is ≥1 cm, of a nodule that has a low suspicion if ≥1.5 cm, or a very low suspicion only if ≥2 cm [10].

Molecular Testing

As discussed previously, the results of thyroid FNA fall into one of six Bethesda categories. However the indeterminate categories (Bethesda III–V) pose a challenge and potentially expose patients to overtreatment. Meta-analysis of thyroid FNA results identified that 24% yield an indeterminate result [24]. Low-volume cytopathologists frequently have even higher frequency of indeterminate results [25]. These patients have a 5–75% rate of malignancy potentially exposing patients, many of which will ultimately have benign disease to the risks of a diagnostic thyroid lobectomy [23]. Molecular profiling of thyroid nodules is primarily aimed to personalize care by identifying patients with indeterminate thyroid nodules that truly have malignant disease.

There are two molecular testing strategies that are commonly used, mutational analysis and gene expression analysis. In these analyses genetic information can be derived from the same FNAB specimen. Initial molecular profiling testing were developed in 2007 with a set of seven genes however rapidly the sophistication of molecular profiling has increased to over one hundred genetic alterations in the current iteration of many tests [26]. Three companies ThyroSeq (CBL Path), ThyraMir (Interspace Diagnostics) and Afirma (Veracyte) now offer genetic testing of thyroid nodules. If molecular testing identifies a risk of malignancy of <5%, comparable to that of a benign thyroid FNAB, then patients can be observed and avoid a potential operation. While patients with a high risk of malignancy on molecular testing can be directed to appropriate surgical intervention.

The Afirma test uses gene expression analysis of 167-genes and a proprietary algorithm to classify nodules as benign of suspicious. The test is designed primarily

as a "rule out" test to identify nodules that do not require surgery. A negative result indicates <5% risk of malignancy, however a positive result carries a lower risk of malignancy of 40–50% [26]. A 7-gene panel was initially an alternative to Afirma and acted as a "rule-in" test. This panel has been replaced by two alternatives ThyroSeq and ThyraMir that function as both a rule-in and rule-out tests for indeterminate nodules. These tests use a combination of gene expression and genetic alterations to identify a risk of malignancy. TyroSeq is a somatic mutation panel that now uses next-generation sequencing, which in the third version has expanded to a 112-gene panel that tests for multiple different types of genetic alterations including markers of aggressive disease such as TERT and tumor protein 53. The ThyroSeq v3 allows the analysis of additional genetic alterations including copy number alterations and expands the number of genes analyzed from 56 to 112. This improves the tests accuracy with diagnosing Hurthle cell (oncocytic) lesions, identification of medullary thyroid cancer and nonthyroidal lesions that can occur in this region such as parathyroid lesions. A 10-center prospective validation study of 805 nodules using Thyroseq v3 identified a negative and positive predictive value of 97% and 64% for Bethesda III nodules and a negative and positive predictive value of 98% and 68% for Bethesda IV nodules [27]. Similar to ThyroSeq, ThyraMir is designed as both a "rule in" and "rule out" with higher risk of malignancy when positive. These tests both have a similar negative predictive value to the Afirma panel but the much-improved positive predictive value allows them to also function as a rule-in test. An important consideration with molecular testing is that the rate of malignancy for each Bethesda subgroup at an individual institution will affect the predicted sensitivity and specificity. Thus it is crucial for clinicians to understand the prevalence of malignancy for each Bethesda subgroup at one's own institution. For example a "rule out" test such as Afirma will perform better in a setting with a lower incidence of cancer and in cytologic categories with lower risks of malignancy. Conversely a "rule-in" test will perform better in settings of a higher incidence of malignancy. Thus, all three available genetic testing strategies may be applicable in settings with a lower risk of malignancy such as AUS/FLUS (Bethesda III) and follicular neoplasm/suspicious for follicular neoplasm (Bethesda IV). However, with a suspicious for malignancy (Bethesda V) lesion only a "rule-in" test such as ThyroSeq or ThyraMir should be used [28].

Both the ATA and NCCN guidelines have both embraced molecular profiling and recommend consideration of molecular diagnostic testing for indeterminate categories [10, 29]. Further, molecular testing enables a substantial number of patients to avoid unnecessary expense of diagnostic lobectomy. Analyses have indicated that molecular testing is cost-effective secondary to decreasing the number of diagnostic lobectomies and the complications associated with surgery [30]. However these conclusions are based on simulated modeling and not actual patient data. Molecular testing is expensive, thus to avoid adding costs it important to use a thoughtful approach to molecular testing in situations when it may change management in order for these gains in cost-effectiveness to be realized.

Prognostic Stratification Based on Molecular Testing

Molecular testing is primarily used to aid diagnosis of indeterminate thyroid nodules to direct decision making about observation versus diagnostic lobectomy. Some investigators propose that molecular testing may inform decisions about the extent of surgery and adjuvant therapy. The evidence basis to inform the use of molecular testing in prognostic stratification is early and emerging. There are multiple clinical decisions that prognostic information from aggressive mutations on molecular testing could inform including: Is lobectomy or total thyroidectomy sufficient for 1–4 cm DTC? Should surgery be performed for a microPTC? Should a prophylactic CND or a more comprehensive therapeutic neck dissection be performed? Should RAI be given? How closely should the patient be followed? Use of molecular testing to answer these questions is primarily the subject of research at this point in time, however is aggressively being pursued. In order to make decisions about the extent of surgery, molecular testing must provide a high positive predictive value and information on the specific genetic mutations. For example BRAF and TERT mutations have been shown to be highly predictive of the risk of extrathyroidal extension, lymph node and distant metastases, indicating a potential for molecular profiling to be used to risk-stratify patients [10]. Meta-analysis of 17,732 patients identified that TERT mutations were associated with worse disease-specific survival with a hazard ratio of 7.64 [31]. The highly significant hazard ratio with TERT mutations may be enough to change decision-making. BRAF V600E mutations were also associated with recurrence with a hazard ratio of 1.41, making-altering decision making more controversial based on this more common mutation [31]. Concomitant mutations in both BRAF and TERT display an even stronger association with aggressive features [32].

In the future molecular testing could be incorporated into staging systems, however at this time this remains a subject of research. Potentially patients identified with TERT or TP53 mutations may warrant more aggressive extent of surgery, RAI and follow up. While use of molecular testing for prognostic stratification to decide decision-making is an exciting area of investigation studies validating this approach have not been published and need to be understood before introduction into routine clinical practice. However as molecular testing rapidly evolves with improved diagnostic accuracy the ability to offer a personalized surgical therapy is expected to advance.

Integration of Molecular Testing into Evaluation of Thyroid Nodules

The typical diagnostic approach to thyroid nodules discussed above is shown in Fig. 1. This is intended to be used as a general guide. However it is also important to take into consideration patient preferences as well as the clinical and radiographic

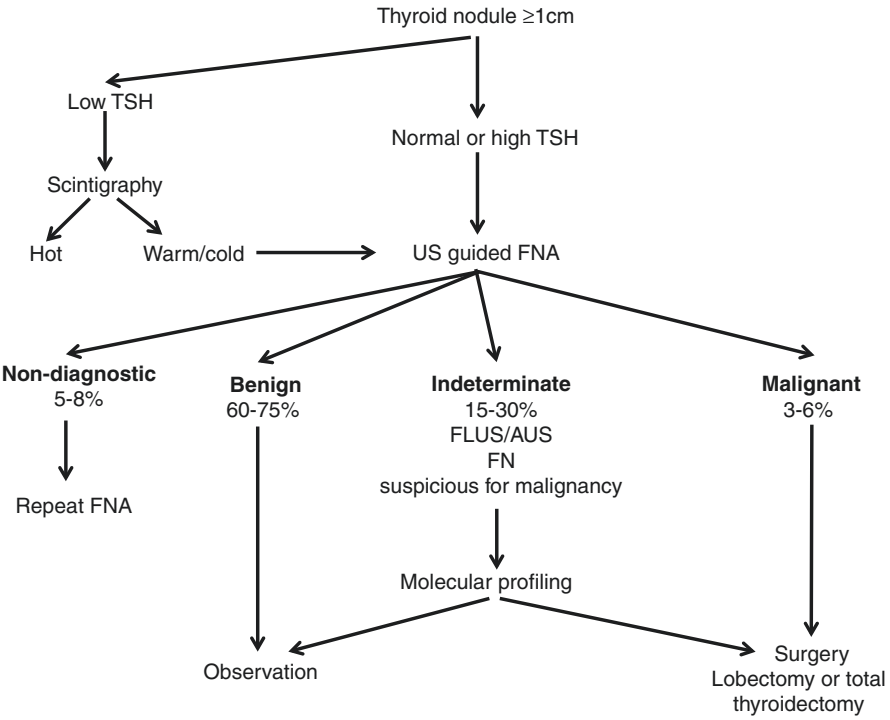

Fig. 1 Workup of thyroid nodule

characteristics of the nodule and any other nodules to direct management. For example molecular testing should only be used in a patient who is willing to undergo observation if the testing indicates that the risk of malignancy is low. If a patient is unwilling to have a nodule with a low-risk of malignancy observed then they should undergo diagnostic lobectomy and not undergo molecular testing.

Overview of Differentiated Thyroid Neoplasia

Thyroid Adenoma

Thyroid adenomas are truly benign thyroid neoplasms that often arise from thyroid follicular cells in a multinodular goiter or thyroiditis. Several histologic types have been described including follicular adenomas, oncocytic (Hurthle cell) adenoma, hyalinizing trabecular adenoma and nodular adenomatous goiter. Thyroid adenomas typically have benign FNAB results and warrant follow up imaging. Nodule growth during follow up warrants repeat FNA. If surgery is indicated the minimum surgery is recommended.

Papillary Thyroid Carcinoma (PTC)

PTC is the most common type of thyroid cancer in both adults and children. PTC is two to three times as common in women. Most radiation induced thyroid cancers are PTC. Several histologic variants of PTC have been identified including microscopic PTC (microPTC), FVPTC, diffuse sclerosing variant, oxophyilic cell variant and the aggressive variants tall cell and columnar cell. PTC <1 cm in diameter (microPTC) occur in up to 30% of the general population yet are rarely clinically consequential [5]. Thus, nodules <1 cm in size do not need to be biopsied unless there is also extrathyroidal extension, nodal metastases, or previous radiation exposure.

Noninvasive Follicular Thyroid Neoplasm with Papillary-Like Nuclear Features (NIFTP)

The follicular variant of papillary thyroid carcinoma (FVPTC) was previously recognized as a favorable form of thyroid cancer. Recently there has been recognition that there are encapsulated forms both with and without invasion. Based on new evidence demonstrating an extremely low malignant potential the encapsulated form of the FVPTC, without any evidence of invasion, was recategorized as a non-malignant NIFTP in 2016 [33]. NIFTP is best thought of as a potential precursor to the FVPTC as shown in Fig. 1 [33]. NIFTP is diagnosed based on pathological findings of an encapsulated nodule with a follicular growth pattern with cells that display nuclear features of PTC, however there is a lack of invasive characteristics, papillary structures, psammoma bodies, significant solid growth, tumor necrosis or a high mitotic rate.

NIFTP cannot be definitively diagnosed based on cytology. However the reclassification of non-invasive FVPTC as NIFTP carries significant implications for the cytological diagnosis of thyroid cancer as it decreases the risk of malignancy for all FNA specimens. In a study of 6943 thyroid FNA specimens from five institutions, 2.5% were classified as NIFTP in the surgical specimen with a preoperative FNA cytological diagnosis of non-diagnostic (1%), benign (9%), atypia of undetermined significance (31%), follicular neoplasm/suspicious for follicular neoplasm (27%), suspicious for malignancy (24%) and malignant (9%) [34]. As NIFTP is considered a non-malignant lesion this reclassification will involve a decrease in the risk of malignancy for multiple intermediate diagnostic categories including up to a 50% decrease in the risk of malignancy for Bethesda class V—Suspicious for Malignancy [35].

Although not a cancer, NIFTP typically requires surgical removal and histologic examination for diagnosis. However while requiring lobectomy, NIFTP represents an opportunity to deescalate therapy with less bilateral thyroid surgery, less TSH suppression and less RAI treatment. The challenge is identifying a potential lesion as NIFTP prior to the pathologic diagnosis to permit de-escalation. Thus the

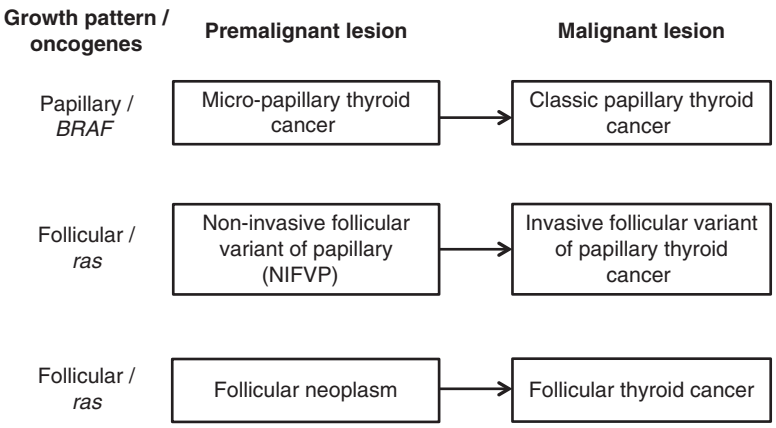

Fig. 2 Premalignant and malignant thyroid lesions

recategorization of NIFTP is one consideration among many in the trend towards greater use of thyroid lobectomy for patients with low-risk disease between 1 and 4 cm (Fig. 2).

Follicular Thyroid Carcinoma

FTC is more aggressive compared with PTC. Previous radiation exposure increases the risk of FTC, even more so than for PTC. Diagnosis of FTC requires capsular and lymphovascular invasion making this entity difficult to be diagnosed on cytology alone. The incidence of nodal metastasis of 15–20%, is lower than for PTC, however the incidence of distant metastasis, typically to the lung or bone is more common (10–15%) [36].

Hurthle Cell Carcinoma

Hurthle cell or oncocytic carcinoma is uncommon and considered a variant of FTC by the World Health Organization. Hurtle cells are large follicular epithelial cells with dense eosinophilic cytoplasm. Similar to other forms of FTC, Hurthle cell carcinoma requires capsular and vascular invasion. Unlike other forms of thyroid cancer, it occurs twice as commonly in males. Hurthle cell carcinoma is aggressive and characterized by a low incidence of lymph node metastases (6–9%) but a high incidence of distant metastases (34%) [37].

Staging

The 8th edition AJCC staging guidelines for thyroid cancer include major changes that result in downstaging of a significant number of patients. Unlike the ATA thyroid cancer staging system that is designed to predict recurrence, the AJCC staging system is designed to predict survival. The new 8th edition guidelines more accurately reflect the low risk of dying from thyroid cancer. There are several principle changes in the 8th edition AJCC system summarized in Table 1. First, the age cutoff that has been used in thyroid cancer staging for years was increased from 45 to 55 years of age. Raising the age cutoff from 45 to 55 years downstages a significant number of patients to stage I disease without increasing mortality [38]. Second lymph node metastasis and microscopic extrathyroidal extension were removed from the definition of T3 disease. Third, T3a is a new category of tumors >4 cm confined to the thyroid and T3b is a new category for tumors >4 cm with extrathyroidal extension into the strap muscles. This stresses the importance of gross

Table 1 Summary of the AJCC 7th and 8th edition staging systems for differentiated thyroid caner

Age	Stage	7th edition	8th edition
Younger	I	<45 years All patients without distant metastasis regardless of adverse features	<55 years All patients without distant metastasis regardless of adverse features
	II	<45 years Distant metastases	<55 years Distant metastases
Older	I	≥45 years ≤2 cm Confined to thyroid	≥55 years ≤4 cm Confined to thyroid
	II	≥45 years 2–4 cm tumor confined to thyroid	≥55 years ≥4 cm tumor Any size with central or lateral neck lymph node metastasis Gross extrathyrodal extension into strap muscles
	III	≥45 years ≥4 cm tumor Or minimal extrathyroidal extension Or central neck lymph node metastasis	≥55 years Gross extrathyroidal extension into subcutaneous tissue, larynx, trachea, esophagus, recurrent laryngeal nerve
	IV	≥45 years Gross extrathyroidal extension Or lateral neck lymph node metastasis Or distant metastasis	≥55 years Gross extrathyrodal extension encasing major vessels or into prevertebral fascia Distant metastasis

extrathyroidal extension while minimizing microscopic extrathyroidal extension that is only identified on the pathologic specimen. Finally, patients with distant metastases with DTC are re-classified as stage IVB instead of IVC. As a result of these changes a significant number of 45–54 year old patients will be downstaged to stage I, and older patients will be downstaged to either stage I (if ≥55 years old with minor extrathyroidal extension, N0, M0) or stage II (if ≥55 years old and N1 without distant metastases). The changes in staging of thyroid cancer are consistent with the emphasis of the 2015 ATA guidelines that stress de-intensification of treatment for patients with thyroid cancer without adverse features with an expected excellent survival. Even with the improvements in prognostication the AJCC and similar staging systems identify only a small fraction of patients at risk of death. This is likely because of failure to incorporate histologic characteristics, radioiodine and PET avidity, key molecular markers and response to therapy. Additionally, while surgeons should be aware that the new AJCC staging system to inform patient discussions, it does not reflect the risk of recurrence like the ATA staging system. Many patients may require more extensive surgery, RAI therapy and TSH suppression to decrease the risk of recurrence based on the ATA staging system and ATA guidelines.

Initial Surgical Management of Differentiated Thyroid Cancer

Active Surveillance of Select Thyroid Cancer

Prospective studies with prolonged surveillance show that most microPTC do not progress [39]. 2015 ATA guidelines now state that active surveillance can be considered as an alternative to immediate surgery in patients with very low risk tumors DTC <1 cm without other high-risk features [10]. If surgery is planned for patients with microPTC the initial procedure should be thyroid lobectomy unless there is clear indication to remove the contralateral lobe [10]. In Japan there is a 22-year experience with active surveillance for low-risk microPTC in over 2000 patients. Only 8% experienced growth in PTC of >3 mm and 4% experience new lymph node metastasis [40]. Patients with microPTC near the trachea-esophageal groove or that is suspicious for extrathyroidal extension were excluded. Active surveillance was less likely to be successful in younger patients. The Japanese experience has been replicated in the United States with similarly low rates of growth of 4% of patients over 2-years [41]. In the future, molecular testing of microPTC may identify patients with indolent disease that are good candidates for observation and the subset that will likely grow and require surgery.

Initial Management of Low-Risk Disease

A more conservative approach towards surgery for DTC between 1 and 4 cm in size has also been emphasized in the 2015 ATA guidelines. Lobectomy or total thyroidectomy is the recommended initial surgical procedure for DTC from 1 to 4 cm [10]. This shift in management emphasizing lobectomy has been coupled with a more selective approach towards use of RAI in low- or intermediate-risk patients. Using appropriate patient selection, loco-regional recurrence rates of 1–4% and completion thyroidectomy rates of 10% can be achieved with lobectomy alone [42]. In many situations total thyroidectomy can be avoided with an inherent halving in the morbidity of surgery. Total thyroidectomy or near total thyroidectomy is recommend for tumors that display size >4 cm, gross extrathyroidal extension, cN1 or cM1 metastatic disease.

Management of Regional Lymphatic Spread

Indications for elective CND remain controversial. 2015 ATA guidelines for DTC recommend that elective CND may be performed in the clinically N0 central neck in patients with advanced primary tumors (T3-4), clinically involved lateral neck nodes (cN1b) or if the information will guide therapy [10]. However recommendations on prophylactic CND are based on retrospective data and expert opinion. Currently, there is a National Cancer Institute sponsored phase II trial of total thyroidectomy with or without CND in patients with PTC, enrolling patients to provide a higher level of evidence to answer this question (NCT 02408887; https://clinicaltrials.gov/ct2/show/NCT02408887).

Extrathyroidal Extension

As is emphasized in the new AJCC staging guidelines gross extrathyroidal extension is associated with highly aggressive malignancy. Correspondingly these patients require appropriately aggressive surgery. If preoperative nerve function is normal and there is concern for RLN invasion then the RLN should be saved and dissected free of gross disease. Postoperative RAI therapy can be used to treat microscopic disease. If the RLN is not working preoperatively and visually invaded by cancer it is reasonable to sacrifice. As MTC and poorly differentiated cancers are nonresponsive to RAI, RLN sacrifice should be considered for these pathologies in the presence of RLN invasion.

In patients with tracheal or esophageal invasion complete surgical resection should be attempted if feasible. Most patients with tracheal or esophageal invasion display symptoms preoperatively and this possibility should be evaluated with imaging and endoscopy to permit operative planning. If minimal invasion is present then shaving techniques may permit removal of all disease. However if transmural invasion is present then tracheal resection, partial laryngectomy or esophagectomy are required.

Intraoperative Nerve Monitoring

Guidelines consistently agree that the RLN should be identified and preserved and that steps should be taken to preserve the external branch of superior laryngeal nerve (EBSLN) [10, 43]. However guidelines leave the decision about use of nerve monitoring to the surgeon. American Academy of Otolaryngology—Head and Neck Surgery guidelines suggest value in intraoperative nerve monitoring for bilateral thyroid surgery, revision surgery or surgery in a setting of existing RLN paralysis [43]. Many centers routinely use intraoperative nerve monitoring for all thyroid surgery and intraoperative nerve monitoring informs the surgeon before proceeding with bilateral thyroid surgery. Intraoperative nerve monitoring may also improve the accuracy in identification of the RLN or EBSLN during surgery potentially avoiding nerve injury. Reported rates of nerve paralysis with and without nerve monitoring in large meta-analyses suggest a non-significant trend towards decreased rates of nerve paralysis with intraoperative nerve monitoring (4.7% versus 5.7%) [44–46]. When intraoperative nerve monitoring is used guidelines outlining equipment setup, endotracheal tube placement, standardization of signals and troubleshooting algorithms should be followed [47].

Remote-Access Thyroid Surgery

Traditional thyroid surgery is performed via a cervical Kocher incision. This approach provides the most direct exposure and in experienced hands offers low morbidity. However some patients are left with a prominent scar. With technologic advances novel remote-access approaches to the thyroid have been developed to avoid the midline cervical scar. Young women, many of whom are concerned about a visible cervical scar, compose a significant proportion of thyroid cancer patients. Remote-access thyroid surgery can be categorized based on the instrument as either endoscopic or robotic and based on approach as breast, axillary, facelift or transoral [48]. In the late 1990s endoscopic techniques were first described for thyroid surgery [49]. The introduction of robotic surgery provided another tool that has been utilized for remote access thyroid surgery [50]. Using axillary, breast and facelift

approaches provide limited exposure to the contralateral lobe but in experienced hands can be used for total thyroidectomy. The transoral approach provides a midline approach with equal access to bilateral thyroid lobes. The data on remote-access surgery indicates longer operative times, an arduous learning curve and higher costs compared with conventional thyroid surgery [48]. Despite these limitations the Koreans have particularly documented minimal morbidity and avoidance of cervical incisions using robotic approaches [50, 51]. The ATA evaluated remote-access thyroid surgery and concluded that it has a role in a select group of patients with unilateral, small nodules that are motivated to avoid a neck incision [48]. However adoption of remote-access thyroid surgery in the United States has been slow secondary to technical, financial and patient motivation. When performed, high-volume surgeons with additional expertise in thyroid and robotic surgery should perform remote-access surgery.

Postoperative Management of Differentiated Thyroid Cancer

Pathologic Staging and Risk Stratification

Postoperatively, the presence or absence of persistent disease and the risk for recurrent disease need to be assessed in order to determine the need for RAI therapy. The ATA risk stratification system classifies patients into low-risk (PTC confined to the thyroid), intermediate-risk (regional lymph node metastasis, extrathyroidal extension, aggressive histology or vascular invasion) or high-risk (gross extrathyroidal extension or distant metastases). Risk-stratification can be used to guide postoperative RAI and TSH suppression, however the nuances of RAI and TSH suppression are outside the scope of this chapter.

Persistent/Recurrent Differentiated Thyroid Cancer

Clinically apparent persistent or recurrent disease is identified in 10% of patients [5]. When recurrence is diagnosed it is localized to the cervical lymph nodes, most commonly in level VI, in 60–75% of cases [36]. Recurrence may be detected by clinical examination or rising Tg concentrations. Neck US is the most sensitive technique for anatomically localizing the site of recurrence when first detected biochemically [52]. If unrecognized, recurrence of iodine avid lesions can be detected on radioiodine scan while PET is useful to detect unrecognized metastases in non-iodine avid disease [53]. Signs of recurrence on imaging include central neck lymph nodes that are >8 mm or lateral neck lymph nodes >1.5 to 2 cm, especially if increasing in size or PET avid. Recurrence should be considered a sign of a potentially lethal outcome and warrants intervention [54].

As with initial treatment, surgical resection is typically the primary treatment for recurrent low-volume loco-regional disease. Preoperatively it is essential to review previous operative reports to obtain information on the extent of surgery and parathyroid gland integrity, perform laryngoscopy to examine vocal cord function [43] and evaluate intact PTH and ionized calcium levels. Reoperative surgery in the central neck places the RLN and parathyroid glands at greater risk than during primary surgery. During surgery for recurrent disease consideration of a lateral neck approach may identify less scarring and virgin planes to facilitate dissection. Early identification of the RLN is essential during reoperative surgery and it can be useful to identify the nerve in a previously undissected area. Preservation of parathyroid gland function is especially challenging during reoperative surgery and requires meticulously technique. Examination of the specimen for parathyroid glands and reimplantation after histologic confirmation by frozen section frequently is required.

Poorly Differentiated and Anaplastic Thyroid Cancer

Poorly differentiated thyroid cancer is significant and accounts for 6% of all thyroid cancer [5].Poorly differentiated thyroid carcinomas display multiple histologically aggressive features with a mean survival of 3.2 years [55]. RAI is of limited benefit and aggressive surgical therapy is the primary therapy. Patients frequently require systemic chemotherapy.

Anaplastic thyroid cancers are extremely aggressive undifferentiated tumors from thyroid follicular epithelium with a mean survival of 6 months [56]. Unlike other thyroid tumors that generally carry an excellent prognosis, the mortality with anaplastic thyroid cancer approaches 100%. Many anaplastic thyroid carcinomas arise form pre-existing DTC or poorly differentiated thyroid cancer. Anaplastic thyroid cancer has an extremely high genetic mutational burden with frequent mutations in BRAF, RAS, TP53, TERT, mTOR pathways [57]. When possible, anaplastic thyroid cancers should be resected, however it is uncommon they present with low-volume resectable disease. Patients frequently presents with unresectable disease in which case preservation of the airway is critical. Anaplastic thyroid cancer is typically refractory to RAI, radiotherapy and traditional chemotherapy. Recently the Food and Drug Administration approved combination BRAF inhibitor (dabrafenib) and MEK inhibirtor (trametinib) for BRAF V600E mutant anaplastic thyroid cancer. Anaplastic thyroid cancers should be considered for a clinical trial whenever possible.

Summary

Advancements in molecular markers of thyroid cancer are enabling a personalized approach to thyroid cancer. Previously a large percentage of thyroid surgery was performed for benign disease, however molecular diagnostics of FNA specimens

allows patients with benign or malignant disease to be identified preoperatively. This will result in an elimination of the need for diagnostic lobectomy. A further dilemma in thyroid cancer is identifying patients with clinically aggressive disease that warrant more extensive surgery, RAI therapy and TSH suppression. Improvements in the molecular testing of thyroid cancer will enable prognostication selecting patients with indolent versus aggressive DTC to tailor treatments and extent of surgery.

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Part VII
Keynote Address

Tumor Immunology, Immunotherapy and Its Application to Head and Neck Squamous Cell Carcinoma (HNSCC)

Jessica M. Moskovitz and Robert L. Ferris

Adaptive and Innate Immunity

Immunity protects the host by recognizing and eradicating pathogens and other foreign molecules with an immediate and non-specific response, known as innate immunity, and a more precise yet later onset response known as adaptive immunity [1].

Innate immunity includes not only physical and microbiologic barriers, but additionally elements of the immune system. The six immune system elements that comprise the innate response are neutrophils, monocytes, macrophages, complement, cytokines, and acute phase proteins [1]. During initial stages of tissue damage or infection, activated macrophages release cytokines stimulating division of myeloid precursors in the bone marrow that leads to a rapid production and release of neutrophils into circulation [1]. Activation of complement occurs by two pathways-through antigen antibody reactions in the classical pathway and polysaccharides in the alternative pathway. This cascade sequence with amplification stages leads to organism lysis, increased vascular permeability, and targeting of cells for components of the adaptive immune system to recognize and remove. Natural killer (NK) cells provide an important first line of defense and communication capability with cells of the adaptive immune system. NK cells bear immunoglobulin receptors (FcR) that bind antibody coated targets in addition to receptors that bind major histocompatibility class I (MHC I).Adhesion molecules, chemokines, and cytokines,

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also allow for cells of the innate and adaptive immune system to be recruited to sites of inflammation [1].

The hallmark of the adaptive immune response is the ability for T cells to enter a memory pool. The adaptive immune response is initiated upon recognition of a nonself antigen presented by an antigen presenting cell (APC). T cells express surface molecules, T cell receptors (TCR), that bind to antigens on major histocompatibility complex (MHC) on APCs [2]. The second signal between T cells and APCs is an antigen independent co-stimulatory or coinhibitory signal of the B7:CD28 family [3]. In the presence of an inflammatory milieu of activating cytokines, T cells are activated, however, if one of these three signals are missing, T cells will not be activated and will enter a dysfunctional T cell state.

CD8 T cells differentiate into cytotoxic effector cells that secrete cytokines such as tumor necrosis factor alpha (TNF alpha) and cytolytic molecules such as granzymes. After antigen clearance and contraction of the immune response, some CD8 T cells survive as memory cells that can be rapidly reinvigorated with exposure to the same antigen. CD4 T cells also release cytokines that shape the inflammatory milieu as well as playing a role in signaling to B cells which produce antibodies. Regulatory T cells (Treg) play an important role in peripheral tolerance and prevention of autoimmune disease, however, in the setting of tumors, these cells thwart a robust immune response.

Cancer Immunoediting and Its Role in Tumor Biology

The understanding of tumor biology has been eloquently described by the six hallmarks of cancer that enable tumor growth and metastatic dissemination: (1) sustained proliferative signaling (2) evasion of growth suppressors (3) Resistance to cell death (4) induction of angiogenesis (5) enabling of replicative immortality and (6) activation of invasion and metastasis [4, 5]. The acquisition of these six hallmarks have been explained by two enabling characteristics; the development of genomic instability in cancer cells and the inflammatory state of premalignant and malignant disease [4].

This inflammatory state surrounding developing neoplastic cells allows for the immune system to recognize and potentially destroy these tumors, known as immunologic surveillance. This concept of immunologic surveillance proposed by Paul Ehrlich in the late 1950s was confirmed with experimental support in the mid 1960s showing that neonatally thymectomized mice were more susceptible to cancer formation from carcinogens and viral infection [6].

Immunologic surveillance involves a dynamic process allowing for immunocompetent lymphocytes to guard against the emergence of neoplastic clones. The existence of tumor antigens (TA) that could be recognized by the immune system was demonstrated when mice immunized with chemically induced tumors were protected against subsequent re-challenge with the same tumor [7]. Further support of this immunosurveillance concept developed in the 1970s with the realization that

post-transplant immunosuppressed humans had an increased incidence of tumors such as skin cancer and lymphoma [8], although it was unclear if this increased incidence was due to decreased immune surveillance or propensity for oncogenic viral infection. Studies in athymic mice lacked evidence for the development of spontaneous tumors [9], yet RAG deficient mice (lacking T cells, B cells, and NK cells) did show a propensity for spontaneous development of gastrointestinal epithelial malignancies [10]. Tumors from mice without an immune system, and therefore not edited or altered by immune cells, showed qualitative differences compared to tumors in immunocompetent mice [10]. Collectively, these studies led to important information about the role of the immune system in cancer development and the emergence of a new cancer hallmark: immune system evasion [4]. We now know that there is a dual role of host immunity both as a tumor suppressor and as a facilitator of tumor growth—a process coined cancer immunoediting. Immunoediting is comprised of three sequential phases-elimination, equilibrium, and escape [11].

Cancer Immunoediting: Elimination, Equilibrium, and Escape

In the elimination phase of cancer immunoediting, the innate and adaptive immune response work simultaneously to destroy tumors. Inflammatory cells of the innate response, such as neutrophils and macrophages, are involved in wound healing and tissue remodeling. These cells are often subverted towards promoting tumor development with the use of angiogenic and stromal growth factors that normally are required for wound healing [12]. Tumor cell death by necroptosis, in contrast to apoptosis, leads to release of tumor neoantigens and proinflammatory signals into the surrounding tissue [13–15]. Necrotic cells also release factors such as interleukin 1 (IL-1) that signals neighboring living cells to proliferate [16]. Inflammatory cells release reactive oxygen species that can lead to genetic alterations that can accelerate tumorigenesis [16].

Processing of mutated proteins, presentation of the mutant peptide by MHC, and recognizing of the mutant peptide-MHC by a T cell in the vicinity are required steps in developing the adaptive immune response. Mutated peptides released by tumor cells undergoing necroptosis, termed neoantigens, are presented to T cells by MHC class I and MHC class II [17, 18]. Tumors with large mutational burden, such as melanoma, frequently have a large pool of neoantigens [17, 19]. Propagation of antigen specific effector T cells tips the balance towards anti-tumorigenesis, however, this balance can be disrupted if a cancer cell is not eliminated and enters into the equilibrium phase. During this phase, the adaptive immune system continues to work to prevent tumor outgrowth (Fig. 1) [4, 5, 11]. Dense immune cell infiltrates have long been noted on standard histochemical stains of neoplastic disease [16]. The development of more accurate immune cell markers has clarified that almost all tumors contain immune cells at various densities [20, 21].

Cell signaling and interactions within the developing tumor microenvironment (TME) allow a tumor to escape immune system recognition in the final phase of

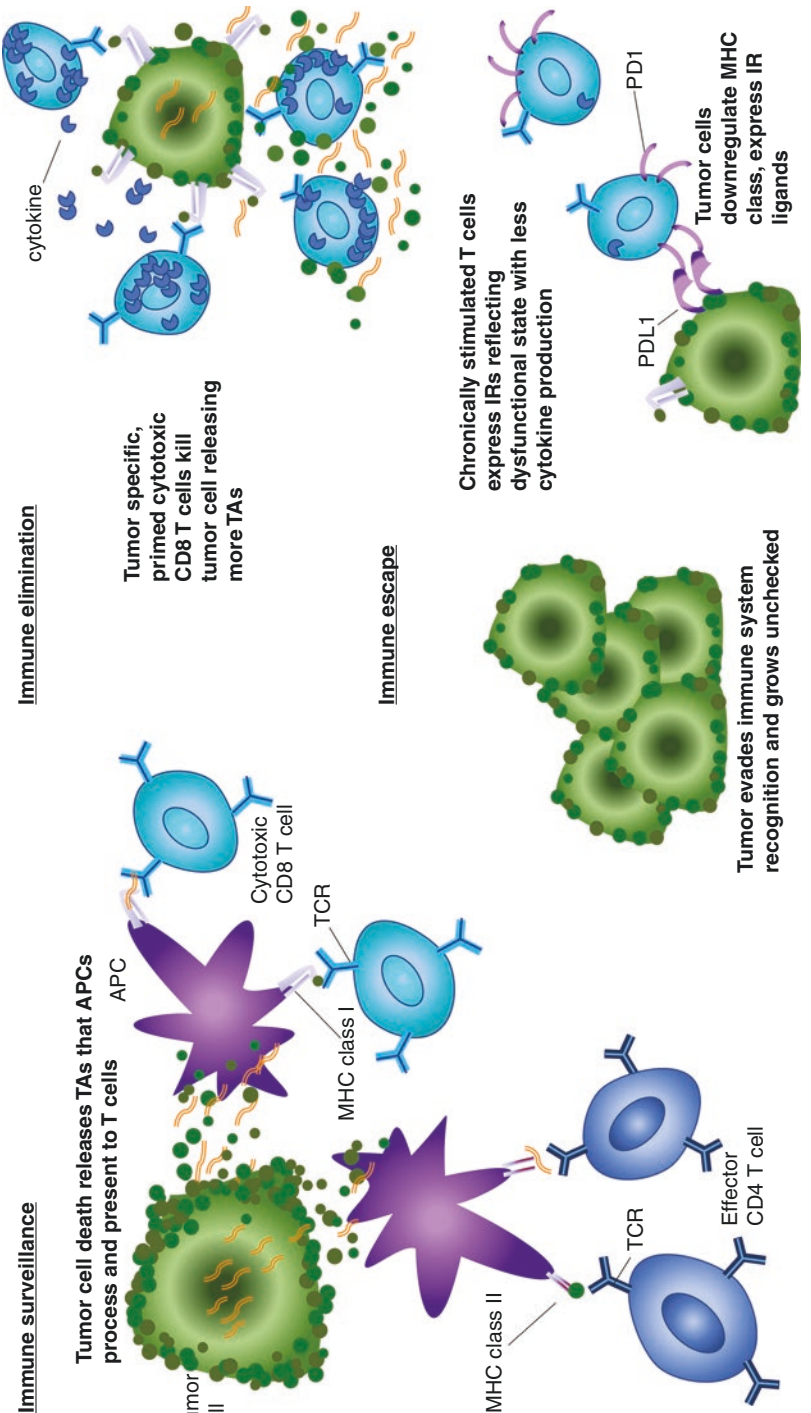

Fig. 1 Phases of cancer immunoediting : tumor cell death leads to antigen presentation and cell specific response to combat growing tumor cells. The balance of tumor cell growth and immune cell suppression of tumor growth can tip this balance to favor tumor escape leading to uncontrolled growth of the tumor

cancer immunoediting. In the presence of chronic antigen exposure, as in the setting of viral infection and cancer, a constant low level of stimulus can lead to a T cell state termed exhaustion [22, 23]. Exhaustion is characterized by an increase in expression and number of inhibitory receptors (IRs), a decrease in production of effector molecules such as interferon gamma, and a decrease in transcriptional factors such as T-bet and Eomesoderin (Eomes) [24–26]. This dysfunctional T cell state is one of many events that allow for developing tumors to escape recognition by the immune system (Fig. 1). Several IRs have been identified for targeting with therapeutic agents to reverse this dysfunctional, exhausted state. IRs such as program death 1 (PD1) and cytotoxic T lymphocyte antigen 4 (CTLA4) have been extensively studied in the preclinical and clinical setting. Other IRs, such as lymphocyte activating gene 3 (LAG3) and T cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif (ITIM) domains (TIGIT), have been studied in the preclinical setting and are beginning to enter the clinical setting.

Monoclonal Antibodies for Treatment of HNSCC

Tumors develop immune resistance using different mechanisms; the goal of immunotherapy is to counteract these resistance mechanisms, allowing the endogenous immune system to reject tumors. Identification of molecularly defined TAs has provided well defined moieties to be used as immunogens and as markers to monitor the immune response. Molecular studies of human TAs demonstrated these antigens to be products of mutated cellular genes, aberrantly expressed normal genes, or genes encoding viral proteins [11]. Immunotherapy encompasses a group of therapeutic agents called monoclonal antibodies (mAb). These mAbs target cell surface molecules and can be grouped into molecular targeted mAb, such as the epidermal growth factor receptor (EGFR) blocking mAb cetuximab, or those that target immune checkpoint receptors referred to as immune checkpoint blockade (ICB) therapy.

Prior to 2006, novel agents for the treatment of HNSCC were sparse. The approval of cetuximab, a targeted mAb to EGFR, for treatment in combination with radiation therapy for locally advanced (LA) HNSCC resulted from a phase III clinical trial showing improved median overall survival (OS) and progression free survival (PFS) with combination therapy compared to radiation alone [27]. Further studies in the platinum resistant recurrent and/or metastatic (R/M) setting showed a 13% response rate [28], and the prolonged median OS with the addition of cetuximab to platinum and 5-fluorouracil (5-FU), led to this regimen being approved in 2008 in Europe and in the United States (US) in 2011 as first line treatment for patients with R/M HNSCC [29]. Although the overall survival increased compared to cytotoxic chemotherapy alone, both arms of the study still showed low overall survival. Additionally, rapid development of therapeutic resistance to cetuximab in this setting highlighted the need for additional therapeutic options for patients with R/M HNSCC [30–32].

Preclinical Models and Early Clinical Trials Evaluating Blockade of Inhibitory Receptors

In the early 1980s, the interest in CTLA4 as a therapeutic target developed from earlier work improving understanding of how antigen recognition leads to T cell activation. Studies identified CTLA4 to be a competitive antagonist for CD28 through transduction of negative signaling that prevents immune system overactivation [33–36]. Knockout of CTLA4 in mice led to lethal lymphoproliferation within the first weeks of life suggestive of a key role of CTLA4 in restraining the T cell immune response. However, studies using CTLA4 blocking antibodies revealed tumor response without fatality in immune competent animal models [37].

Initial phase I studies in melanoma showed durable response with ipilimumab, a mAb targeting CTLA4, alone and when used in conjunction with a melanoma antigen vaccine [38]. A phase III trial comparing ipilimumab to a peptide vaccine for metastatic melanoma evaluated overall survival rather than response rate as the primary endpoint. This was the first phase 3 randomized trial that showed improved overall survival in patients with metastatic melanoma [39]. These results opened the door for applicability of ICB to other solid tumors as well as generated interest in other immunomodulatory pathways.

Completed Clinical Trials Using ICB in HNSCC

Prior to the approval of ICB therapy, the majority of patients with R/M HNSCC who qualified for palliative therapy had an expected survival of 6–10 months [29, 40]. Immunotherapy for treatment of R/M HNSCC has been approved by the US Federal Drug Administration (FDA) for platinum refractory disease. The two approved agents, pembrolizumab and nivolumab, disrupt the interaction between PD1 and its ligands.

Pembrolizumab for R/M HNSCC

The KEYNOTE 055 trial, a phase II single arm study, evaluated patients with both platinum and cetuximab resistance. Patients received pembrolizumab at a dose of 200 milligrams (mg) intravenously (IV) every 3 weeks for up to 24 months unless intolerable toxicities, progressive disease or patient/physician decision for withdrawal of therapy. Radiographic disease progression required confirmation with repeat imaging at least four weeks later. Imaging was performed at baseline, 9 weeks after the first dose, and subsequently every 6 weeks for the first year and every 9 weeks for the second year [41].

The primary study endpoint was overall response rate (ORR) with secondary endpoints included PFS, OS, and duration of response. ORR was defined as a primary endpoint by the proportion of patients with complete or partial response by Response Evaluation in Solid Tumors (RECIST) 1.1 criteria, and by PD-L1 and HPV status as a prespecified secondary endpoint. A combined positive score (CPS) for PD-L1 immunohistochemistry was determined by expression of PD-L1 on tumor cells and mononuclear inflammatory cells. PD-L1 positivity was defined as a CPS of greater than or equal to 1%.

171 patients were enrolled in the trial and received at least one dose of pembrolizumab. ORR was 16% [95% confidence interval (CI), 11–23%, $p < 0.001$); 19% and 51% of patients experienced stable or progressive disease, respectively. The majority of patients had HPV negative disease (77%), but response rates were similar regardless of HPV status. The majority of patients had a positive CPS with 28% of patients having a CPS over 50% [41].

Adverse events (AE) were followed for 30–90 days after cessation of pembrolizumab depending on the severity of the AE. The majority of AEs were grade 1 or 2 with only 4% of patients discontinuing because of treatment related AEs. Hypothyroidism and pneumonitis were the only immune mediated AEs seen in 16% and 4%, respectively. One patient died of treatment related pneumonitis [41].

Accelerated approval of pembrolizumab by the US FDA resulted from the KEYNOTE 055 study. Preliminary results from a randomized phase III trial, KEYNOTE-040, showed that although the study did not meet the prespecified difference for statistical significance, there was still an OS improvement of 19% in the pembrolizumab group [42]. It is possible that subsequent immunotherapy in the standard of care arm may have confounded the OS analysis. This phase III trial randomized patients to receive pembrolizumab or investigator's choice (methotrexate, docetaxel, or cetuximab). Median OS was only marginally improved with the pembrolizumab treated group compared to investigator's choice [8.4 versus 7.1 months; hazard ratio (HR) 95% CI 0.66–0.99, $p = 0.0204$], however in patients with a PD-L1 CPS of $\geq$50%, median OS improved to 11.6 months in pembrolizumab treated patients. Treatment with pembrolizumab was also tolerated better than standard therapy, however, the incidence of hypothyroidism was 13% in the pembrolizumab group compared to 1% in the standard therapy group [42].

Nivolumab for R/M HNSCC

Nivolumab received approval for platinum refractory HNSCC as a result of the Checkmate 141 trial. This randomized (2:1), open label phase 3 trial assessed 361 patients assigned to receive nivolumab 3 mg/kg every 2 weeks or standard single agent systemic therapy. Standard therapy consisted of weekly IV administration of one of the following: methotrexate, docetaxel, or cetuximab. Patients that progressed within 6 months after platinum-based chemotherapy were eligible, and the

primary end point of the study was OS. Additional end points included safety and patient reported quality of life. The European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire 30 module (QLQ-30) and the head and neck specific module (QLQ-H&N35) were used for patient reported outcomes including symptoms and quality of life. Biomarker analysis included tumor PD-L1 and for oropharyngeal tumors, HPV status (as defined by p16 immunohistochemistry) [43].

OS was 7.5 months (95% CI: 5.5–9.1) in the nivolumab group compared to 5.1 months (95% CI, 4.0–6.0) in the standard therapy group [43]. Nivolumab treated patients had a 30% lower risk of death compared to patients treated with standard therapy. PD-L1 expression was evaluated in 72% of patients, and 57.3% of patients had PD-L1 expression of ≥1%. The HR for death in patients treated with nivolumab compared to standard therapy was 0.55 (95% CI: 0.36–0.83) and 0.89 (95% CI: 0.54–1.45) for patients with PD-L1 expression of ≥1% and patients with PD-L1 expression of <1%, respectively. Patients with PD-L1 expression levels of ≥5% and of ≥10% had similar HR estimates to patients with PD-L1 expression of ≥1%.

The median OS performed in a post hoc exploratory analysis of 178 patients with reported p16 status appeared slightly longer with nivolumab regardless of p16 status. However, there was a non-statistically significant trend demonstrating a greater magnitude of nivolumab effect in patients with tumor PD-L1 expression of 1% or more or p16 positive tumors (or both) compared to those with PD-L1 level less than 1% or who had p16 negative tumors.

Nivolumab therapy was tolerated well in the Checkmate 141 trial; the most frequently reported AEs were fatigue, nausea, decreased appetite and pruritus. Fewer grade 3 or 4 events were reported in the nivolumab group compared to standard therapy (13.1% vs 35.1%, respectively). AEs of the skin (rash and pruritus) and endocrine system (hypothyroidism) were more common with nivolumab. Patient reported outcomes from the QLQ-C30 and QLQ-H&N35 demonstrated increased pain and sensory problems with worsening of physical and social functioning in the standard therapy group, reaching statistical significance at weeks 9 and 15 [43].

Upcoming Clinical Trials with ICB: Combinatorial Regimens for R/M HNSCC and ICB in LA HNSCC

Clinical trials using combinatorial ICB will assess synergistic mechanisms of IRs thus potentially improving patient response without too much additional toxicity (Table 1). Anti-LAG3 combined with anti-PD1 agents to overcome PD1 resistance in non-responder patients is being assessed in multiple solid tumor types including HNSCC, melanoma, and NSCLC. Completed trials evaluating the combination of anti-PD1 with anti-CTLA4 agents in advanced melanoma demonstrated that this combination was superior to either monotherapy alone [44, 45].

Table 1 Selected combination immunotherapy trials for HNSCC

Target	Clinical trial number (NCT)/acronym	Therapeutic agent	Phase	Patient eligibility	Status
PD1 and CTLA4					
	NCT02551159/ KESTREL	Durva (MEDI4736) ± treme vs SOC EXTREME regimen (cetux + cis/ carbo + 5FU	III	R/M HNSCC	Active, not recruiting
	NCT02369874/ EAGLE	Durva (MEDI4736) ± treme vs SOC	III	R/M HNSCC	Active, not recruiting
	NCT02741570/ CHECKMATE-651	Nivo + ipi vs SOC [EXTREME regimen]	III	R/M HNSCC	Recruiting
	NCT02823574/ CHECKMATE-714	Nivo + ipi vs nivo + ipi placebo	II	R/M HNSCC	Active, not recruiting
PD1 and LAG3					
	NCT01968109	Nivo + relat vs relat	I/II	Advanced solid tumors (including HNSCC)	Recruiting
	NCT02966548	Nivo + relat vs relat	I	Advanced solid tumors (including HNSCC)	Recruiting
PD1, LAG3, and CTLA4					
	NCT03459222	Relat + nivo + ipi vs relat + nivo + BMS-986205 (IDO1 inhibitor)	I/II	Multiple solid tumors, including HNSCC	Recruiting

Key: *R/M* recurrent and/or metastatic, *HNSCC* head and neck squamous cell carcinoma, *PD1* program death 1, *CTLA4* cytotoxic T lymphocyte antigen 4, *Vs* versus, *Durva* durvalumab, *Treme* tremelimumab, *SOC* standard of care, *Cetux* cetuximab, *Cis* cisplatin, *Carbo* carboplatin, *5FU* 5 fluoruracil, *Nivo* nivolumab, *Mtx* methotrexate, *Ipi* ipilimumab, *LAG3* lymphocyte activating gene 3, *Relat* relatilimab, *IDO1* indoleamine 2,3 dioxygenase 1

Current clinical trials are evaluating ICB for R/M and LA HNSCC in addition to traditional standard of care (SOC) therapies (Table 2). For example, the addition of nivolumab to 2 different dose regimens of cisplatin prior to definitive chemotherapy and radiation (CRT) evaluates optimal timing and dose for combining ICB to traditional modalities. Lower doses of cytotoxic therapy, such as cyclophosphamide, have been shown to increase the immune cell infiltrate in the TME, potentially allowing for improved response to ICB [46].

One concern for treatment of solid tumors in the LA setting relates to when to time ICB therapy and at what dose regimen such that it does not interfere or delay SOC treatments. In trials using neoadjuvant ICB prior to surgery, evaluation of intraoperative and postoperative complications, such as bleeding and infection, as well as evidence for delay of surgery due to complications related to immunotherapy are

Table 2 Selected cytotoxic therapy with immunotherapy trials for LA and R/M HNSCC

Clinical trial number (NCT)/ acronym	Therapeutic agent	Phase	Patient eligibility	Status
AntiPD1				
NCT02952586/ JAVELIN 100	Avelumab + cis/RT vs cis/ RT alone	III	LA HNSCC	Recruiting
NCT03040999/ KEYNOTE-412	Pembro + chemo/RT vs chemo/RT alone	III	LA HNSCC	Recruiting
NCT02764593/ RTOG 3504	Cis/RT or cetux/RT + nivo vs nivo + RT	III (phase I lead in)	Intermediate to high risk HNSCC	Recruiting
NCT02358031/ KEYNOTE 048	Pembro vs pembro + cetux vs pembro + platinum/5FU	III	R/M HNSCC	Active, not recruiting
NCT02641093	Adjuvant cis/ pembrolizumab/RT	II	Surgically resected, high risk (positive margin and/or ECS)	Recruiting
NCT02777385	Concurrent vs sequential pembro with cis/IMRT	II	Previously untreated, intermediate to high risk HNSCC	Recruiting
NCT03085719	Pembro with high vs high and low dose RT	II	R/M HNSCC (progressive or stable disease on prior anti-PD1)	Recruiting
NCT03082534	Pembro + cetux	II	R/M HNSCC	Recruiting
NCT03355560	Adjuvant nivo	II	Recurrent HNSCC after salvage surgery [failed prior definitive therapy]	Recruiting
NCT03193931	Pembro + mtx	II	R/M HNSCC (cis ineligible or elderly)	Recruiting
NCT02759575	Pembro + cis/IMRT	I/II	LA HNSCC (laryngeal only)	Recruiting
NCT03247712	Neoadjuvant nivo + RT followed by surgery	I/II	LA HNSCC	Recruiting
NCT03162731	Nivo + ipi + IMRT	I	LA HNSCC	Recruiting
NCT02764593	Nivo + IMRT with either cis, high dose cis or cetux	I	High risk LA HNSCC	Recruiting

Key: *R/M* recurrent and/or metastatic, *LA* locally advanced, *HNSCC* head and neck squamous cell carcinoma, *PD1* program death 1, *Cis* cisplatin, *Pembro* pembrolizumab, *Cetux* cetuximab, *RT* radiation therapy, *5FU* 5 fluoruracil, *ECS* extracapsular spread, *IMRT* intensity modulated radiation therapy, *Nivo* nivolumab, *Mtx* methotrexate, *Ipi* ipilimumab

assessed (Table 2). Preliminary evidence from pilot studies in lung cancer suggests that these agents are safe to use in the perioperative setting [47].

Evaluation of Response and Disease Monitoring with Immunotherapy

The number of clinical trials containing an immunotherapeutic regimen has increased substantially over the past decade. Accurate interpretation of clinical trial results and correlative studies will therefore be important for clinicians treating oncologic disease. For example, knowledge of variation in reporting techniques for positivity of program death ligand 1 (PD-L1) is important as some assays report positivity from tumor cells alone yet others report tumor cells and immune cells as positive. Additionally, different trials have used various percentages (i.e. 1% or 5%) to denote PD-L1 positivity.

There is a need for prospectively validated correlative studies that will assist in predicting response to ICB. Correlative prognostic biomarkers of response have been categorized into five groups: tumor related, peripheral blood mononuclear cell related (circulating Tregs), serum related (cytokines and antibodies), imaging related (PET/CT), and microbiome related (stool and saliva) [48–50]. Tumor related biomarkers include PD1/PDL1 expression on tumor and immune cells as well as an interferon gamma (IFNγ) gene signature [51, 52].

Disease Monitoring During Checkpoint Blockade Treatment

Unlike cytotoxic therapy, response to ICB may not be appreciated soon after treatment initiation. Initial tumor progression prior to response, a phenomenon called "pseudo-progression", is thought to result from increased tumor immune cell infiltrate. Because of this phenomenon, investigators have begun to evaluate response to ICB with alternative clinical endpoints and radiologic criteria such as immune response criteria (iRC) [53–57] that differs from the traditional assessment of tumor response using RECIST criteria. Pseudo-progression has been observed in about 10% of melanoma patients soon after ICB initiation, however, this is a rare phenomenon in HNSCC [43]. Increased tumor size therefore should not always prompt immediate change in therapeutic management, and a knowledge of response patterns in a given malignancy is important.

Immunotherapy AEs differ from those seen with traditional cytotoxic therapy such as renal failure and anemia. Immune system activation with ICB is not organ specific and therefore can affect any system in the body. The majority of autoimmune reactions that occur with ICB typically resolve with cessation of ICB with or without the addition of corticosteroid therapy. However, the length of time to resolution can vary. Treatment with anti-tumor necrosis factor (anti-TNF) agents may be needed if AEs persist in spite of steroids and ICB cessation [58, 59].

Resistance Mechanisms to Checkpoint Blockade

Since the approval of ipilimumab for metastatic melanoma in 2011, there has been considerable development in the understanding of acquired and innate resistance mechanisms to checkpoint blockade. For example, early on treatment tumor biopsies in metastatic melanoma patients treated with ipilimumab showed a higher density of CD8+ T cells in patients that responded compared to nonresponders [60].

Patients with tumors that do not initially respond to ICB have an innate mechanism of resistance termed primary resistance. For example, primary resistance to ICB is seen in tumor types that have a low infiltrate of immune cells and thus may not have tumor specific immune responses that renders ICB beneficial. Although patients that initially respond to ICB often have a prolonged response, a subset of patients develop recurrent disease through acquired resistance [49, 50, 61].

Conclusions

Response to ICB has garnered excitement due to its applicability to multiple malignancy types, durability of response, and response in patients that had failed regimens with cetuximab and/or platinum regimens. Unfortunately, the majority of patients are nonresponders to ICB monotherapy, but the mechanistic insight into both primary and acquired resistance to ICB has assisted with development of intelligent clinical trial designs. Improvement in biomarker analysis will assist with understanding of the appropriate use of ICB alone or in combination with SOC regimens or other immunomodulatory regimens.

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