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Opportunities and challenges in combining immunotherapy and radiotherapy in head and neck cancers

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ABSTRACT

Locally advanced and recurrent/ metastatic (R/M) head and neck cancers have poor prognosis generally. Radiotherapy (RT) is known to have multiple immunomodulatory effects, and various immune checkpoint inhibitors (ICIs) have been shown to be efficacious in the R/M setting in recent years. Hence, it is logical to combine RT and ICIs to improve the outlook for such patients, especially in view of the promising pre-clinical data on this novel combination. In this review, we highlighted the key mechanisms underlying the immunostimulatory and immunoinhibitory effects of RT, with a view to suggesting strategies to overcome radioresistance. We also discussed how the unique immune landscapes of virus-induced cancers, namely Epstein-Barr virus-induced nasopharyngeal carcinoma and human papillomavirus-mediated oropharyngeal cancer, could be exploited with ICIs. The landmark clinical trials in both the locally advanced and R/M settings were reviewed, and these trials showed that the combination of RT and ICIs is generally well tolerated. The potential reasons behind the largely negative results of these studies were also explored, focusing on various parameters including dose fractionation, sequencing, irradiated volume and the use of predictive biomarkers.

Introduction

Head and neck cancers are common worldwide, with approximately 870,000 new cases and more than 440,000 deaths in the year 2020 according to GLOBOCAN (excluding thyroid and salivary gland cancers) [1]. The vast majority of such cases are squamous cell carcinomas (SCCs), and roughly 60% of patients with SCCs of the head and neck present with locoregionally advanced disease (LA-HNSCC) [2]. Patients with LA-HNSCC frequently undergo multi-modality treatments, consisting of surgery, radiotherapy (RT) and chemotherapy. Despite advances in technology in recent decades, these patients have rather poor prognosis. For instance, patients with stage III-IV laryngeal SCC have a 5-year overall survival (OS) of 44% despite surgical treatments [3]. On the other hand, the two major virally-induced head and neck cancers, namely Epstein-Barr virus (EBV)-related nasopharyngeal carcinoma (NPC) and human papillomavirus (HPV)-positive oropharyngeal carcinoma, have relatively better outlook [4–6].

Clinical research into immune-checkpoint inhibitors (ICIs), such as monoclonal antibodies against programmed cell death receptor 1 and its ligand (PD-1 and PD-L1) or cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), has been a rapidly developing field in oncology over the last decade. Several randomized controlled trials (RCTs) have shown efficacy of ICIs in the palliative treatment of HNSCC [7–9]. However, given the relatively low response rates with ICI monotherapy, many recent trials are evaluating enrichment strategies such as novel predictive biomarkers, and combinatorial strategies with chemotherapy, targeted therapies, other immunotherapies or RT. Combining ICI and RT is a logical approach as radiation has immunomodulatory properties such as the induction of immunogenic cell death (ICD), and may thus have synergistic effects when combined with immunotherapy. In this review, we will explore the rationale behind combining immunotherapy and RT in head and neck cancers, and will highlight the key pre-clinical and clinical data on this novel therapeutic strategy.

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Immunomodulatory effects of radiation (Table 1 and Fig. 1)

Radiation increases antigen release and upregulates MHC class I molecules

Radiation kills cancer cells mainly by inducing clustered DNA damage, including double-strand breaks, which is difficult to repair [10]. Besides this direct cytotoxic process, the effects of radiation are multifold, many of which are immunomodulatory and are essential to the therapeutic efficacy of radiation. Firstly, radiation-induced DNA damage results in cell death, with a resultant increase in neoantigen release from cancer cells which can prime the immune system by acting as a form of 'in situ vaccination' [11]. Tumor-associated antigens (e.g. cancer-testis antigens) are expressed at low levels in some normal tissues, but are overexpressed in malignant cells [12,13]. Neoantigens are immunogenic tumor-associated antigens which are generated by somatic non-synonymous mutations, and are entirely absent from normal human tissues [12,13]. Reits *et al.* showed, using a melanoma cell line, that approximately 1% of major histocompatibility complex (MHC) class I-binding antigenic peptides were unique to irradiated cells after 25 Gy of γ-radiation [14]. Such neoantigens could function as targets for $CD8^+$ T cells and enhance sensitivity to ICIs *in vivo* [15].

MHC-I proteins play a crucial role in the antigen presentation of tumor-associated epitopes, and the downregulation of MHC-I protein expression is one of the key mechanisms of immune evasion in many solid tumors [16]. Radiation has been shown to upregulate MHC-I and MHC-II protein expression in tumor cells [14,17–20], possibly via mechanisms such as the activation of type I interferon (IFN)-mediated signalling [21]. Radiation also enhances intracellular protein degradation [14], thereby diversifying peptide presentation.

Radiation activates dendritic cells and enhances cross-presentation of antigens

Radiation-induced ICD creates an inflammatory microenvironment characterized by the release of tumor antigens and danger-associated molecular patterns (DAMPs), such as calreticulin, high-mobility group protein B1 (HMGB1) and adenosine triphosphate (ATP) [22–24]. When calreticulin is expressed on the surface of malignant cells undergoing ICD, a pro-phagocytic signal is generated that activates low-density lipoprotein receptor-related protein 1 (LRP1) on antigen-presenting cells (APCs) such as dendritic cells [22,25,26]. The subsequent engulfment of dying cells by dendritic cells or their precursors would provide them with an abundant source of antigens, which are essential to the development of an adaptive immune response [22,27]. HMGB1 could bind to pattern recognition receptors on myeloid cells such as Toll-like receptor 4 (TLR4), and this interaction is crucial to tumor antigen processing and presentation [22,26,28,29]. Extracellular ATP is a potent 'find-me' signal and binds to P2X purinoceptor 7 (P2RX7) on dendritic cells with resultant secretion of interleukin (IL)-1β [22,26,29]. Together, activation of these signals results in the recruitment of dendritic cells to the tumor bed, the engulfment of antigens, with dendritic cell activation and migration to the draining lymph nodes, where cross-presentation of tumor-associated epitopes and T-cell priming primarily occur (Fig. 2) [26,30–32].

Cytosolic DNA could be found after exposure to ionizing radiation [33], and this cytosolic DNA is sensed by the cGMP-AMP (cyclic guanosine monophosphate-adenosine monophosphate) synthase/ stimulator of IFN genes (cGAS-STING) pathway which upregulates the production of type-I IFN [34]. An intact STING pathway was shown to be essential to the regression of abscopal tumors when combining RT and an anti-CTLA-4 antibody in a mouse melanoma model [35].

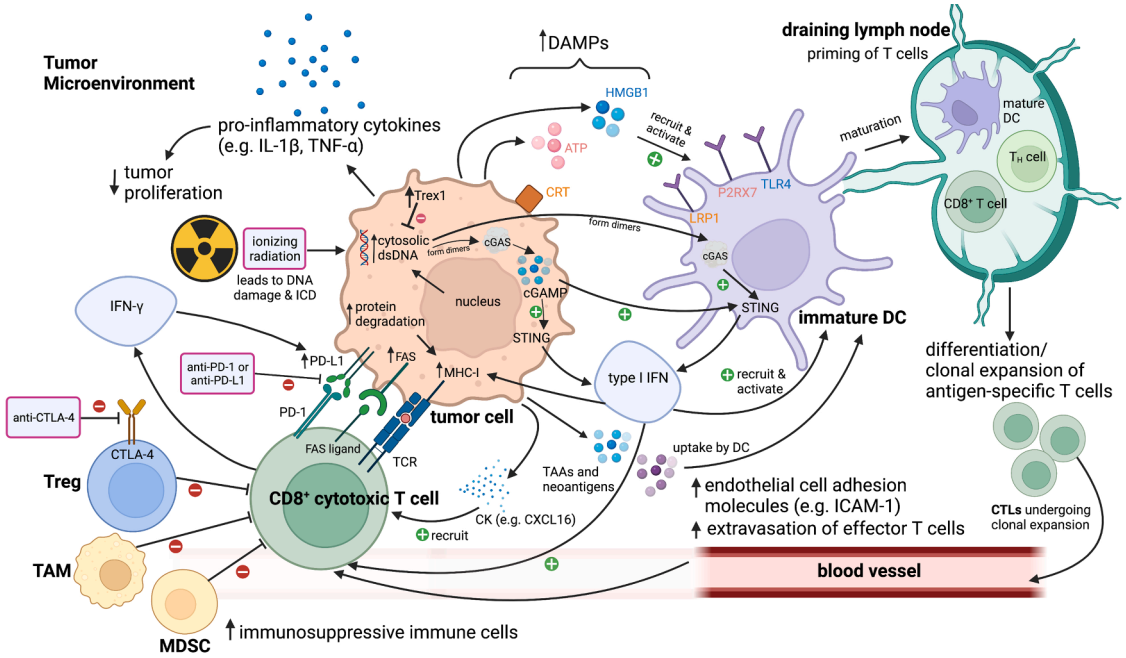

Fig. 1. The immunomodulatory effects of radiation. Radiotherapy leads to clustered DNA damage (e.g. double-strand breaks) and immunogenic cell death in cancer cells, with resultant immunostimulatory and immunoinhibitory effects. The underlying mechanisms are further elaborated in Table 1. This figure was created with BioRender.com. ATP: adenosine triphosphate; cGAMP: cyclic guanosine monophosphate–adenosine monophosphate; cGAS: cyclic GMP-AMP synthase; CK: chemokines; CRT: calreticulin; CTLs: cytotoxic T lymphocytes; CTLA-4: cytotoxic T-lymphocyte-associated antigen 4; CXCL16: C-X-C motif ligand 16; DAMPs: danger-associated molecular patterns; DC: dendritic cell; dsDNA: double-stranded DNA; HMGB1: high-mobility group protein B1; ICAM-1: intercellular adhesion molecule 1; ICD: immunogenic cell death; IFN: interferon; IL-1β: interleukin-1β; LRP1: low-density lipoprotein receptor-related protein 1; MDSC: myeloid-derived suppressor cell; MHC-I: major histocompatibility complex class I; P2RX7: P2X purinoceptor 7; PD-1: programmed cell death receptor 1; PD-L1: programmed cell death ligand 1; STING: stimulator of interferon genes; TAAs: tumor-associated antigens; TAM: tumor-associated macrophage; TCR: T-cell receptor; T_H cell: T helper cell; TLR4: Toll-like receptor 4; TNF-α: tumor necrosis factor alpha; Treg: regulatory T lymphocyte; Trex1: three prime repair exonuclease 1.

Table 1
The immunomodulatory effects of radiation.

Immunostimulatory effects		
1	Radiation increases antigen release and upregulates MHC class I molecules	• Radiation induces DNA damage (e.g. double-strand breaks) and cell death [10] • Results in the release of tumor-associated antigens and neoantigens, which are targets for $CD8^+$ T cells and acting as a form of 'in situ vaccination' [11] • Radiation upregulates MHC-I protein expression [14,17–19,21], and subsequent antigen presentation, via mechanisms such as o activation of type I interferon-mediated signalling [21] o transcriptional upregulation of NLRC5 (a transactivator of MHC-I genes) [18] • Radiation enhances intracellular protein degradation thereby diversifying peptide presentation [14]
2	Radiation activates dendritic cells and enhances cross-presentation of antigens	• Radiation induces ICD, which is characterized by the release of DAMPs (e.g. calreticulin, HMGB1, ATP, type I interferons, IL-1β and heat shock proteins) [22–24] • These signals lead to the recruitment and activation of dendritic cells [26,30] • Dendritic cells will then migrate to the draining lymph nodes where cross-presentation and T-cell priming occur [26,30–32] • Pro-inflammatory cytokines (e.g. IL-1β, tumor necrosis factor alpha) also contribute to the lethality of radiation via a diverse range of mechanisms [23] • Cytosolic DNA is sensed by the cGAS-STING pathway which upregulates type-I interferon [34], which has a crucial role in enhancing cross-presentation by APCs
3	Radiation enhances effector functions by increasing the density of tumor-infiltrating lymphocytes	• Radiation upregulates endothelial cell adhesion molecules (e.g. ICAM-1, E-selectin, VCAM-1, CD31) and facilitates the extravasation of leucocytes with effector functions [37–40] • Radiation induces the secretion of chemokines (e.g. CXCL9, CXCL10, CXCL11, CXCL16) which attract Th1 helper cells and CTLs [41–43] • Intra-tumoral resident T cells also contribute to the antitumor effects of RT by increasing their production of interferon-γ in response to radiation [45] • Radiation increases the surface expression of the death receptor FAS on tumor cells, leading to an increase in apoptosis [31,46]
Immunoinhibitory effects		
		• Radiation leads to PD-L1 upregulation on tumor cells which is mediated by IFN-γ produced by $CD8^+$ T cells [47] • Immunosuppressive immune cells, including Tregs, TAMs and MDSCs, are upregulated with radiation [49,50,52,53] • Radiation-induced Trex1 could degrade cytosolic DNA and thus attenuating its immunogenicity [54] • Radiation could also lead to lymphopenia [57]

NRLC5: NOD-, LRR- and CARD-containing 5; ICD: immunogenic cell death; DAMPs: danger-associated molecular patterns; HMGB1: high-mobility group protein B1; ATP: adenosine triphosphate; cGAS-STING: cyclic GMP-AMP synthase/ stimulator of interferon genes; APCs: antigen-presenting cells; CTLs: cytotoxic T lymphocytes; Tregs: regulatory T lymphocytes; TAMs: tumor-associated macrophages; MDSCs: myeloid-derived suppressor cells.

Radiation enhances effector functions by increasing the density of tumor-infiltrating lymphocytes

High levels of tumor-infiltrating lymphocytes (TILs) have been shown to be independently associated with better OS in HNSCC, especially for patients undergoing chemoradiation [36]. This is in keeping with the notion that effector T cells have to infiltrate the tumor to eradicate target malignant cells bearing specific antigens. One obstacle to this effector phase is the frequently dysfunctional vasculature in the tumor microenvironment (TME), which could be reversed with radiation via the inhibition of ongoing angiogenesis [37]. In addition, endothelial cell adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1), E-selectin, vascular cell adhesion molecule 1 (VCAM-1) and cluster of differentiation 31 (CD31), are required for the extravasation of leucocytes from the circulation, and such molecules are upregulated after irradiation [37–40]. Besides changes in the endothelium, ionizing radiation induces the secretion of chemokines [e.g. C-X-C motif ligand (CXCL)9, CXCL16] which attract Th1 helper cells and cytotoxic T lymphocytes (CTLs) [41–43]. Mice deficient in C-X-C motif chemokine receptor (CXCR)6, which is the only receptor for CXCL16 [44], had reduced infiltration of tumors by $CD8^+$ T cells with subsequent impaired tumor regression with RT combined with anti-CTLA-4 [43]. Besides newly-infiltrating T cells, intra-tumoral resident T cells, whose radioresistance is attributed to TGF-β (transforming growth factor beta)-mediated reprogramming in the TME, also contribute to the antitumor effects of RT by increasing their production of IFN-γ in response to radiation [45]. Moreover, radiation could increase the surface expression of FAS on tumor cells, whereby FAS-L-mediated apoptosis could be triggered in CTLs specific or non-specific for tumor antigens [31,46].

Immunoinhibitory effects of radiation

Despite the many immunostimulatory effects of radiation, it also has various immunoinhibitory effects, and any success in combining RT and immunotherapy would depend on the relative degrees of activation of these opposing forces. Firstly, RT modulates the expression of immune checkpoint molecules, in particular it leads to PD-L1 upregulation which is mediated by IFN-γ produced by $CD8^+$ T cells [47], though whether such PD-L1 upregulation is inhibitory or not remains controversial [24,28] . It was also found that chemoradiation increased the expression of PD-1 on $CD4^+$ T cells by 2.5-fold in patients with LA oropharyngeal cancer [48].

Regulatory T lymphocytes (Tregs) are forkhead box P3 (FOXP3)-positive $CD4^+$ T lymphocytes, that are known to downregulate the intratumoral cytotoxic function of $CD8^+$ T cells via the secretion of TGF-β and IL-10 [23]. Tregs in both immune organs and tumors are upregulated with radiation, which could be related to Tregs being more radioresistant than other lymphocyte subpopulations and their preferential proliferation in the tumor [49,50]. Radiation-induced release of TGF-β is also known to stimulate $CD4^+$ T cells' adopting a Treg phenotype [28]. Interestingly, tumor-infiltrating Tregs have high levels of surface CTLA-4, and anti-CTLA-4 antibodies were shown to lead to intra-tumoral Treg depletion *in vivo*, thereby increasing the $CD8^+$/Treg cell ratio with resultant therapeutic effects [51]. Radiation-induced DNA damage and hypoxia could recruit other immunosuppressive myeloid-derived cells, including tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs) [52,53].

Moreover, radiation at doses above 12–18 Gy could induce the DNA exonuclease three prime repair exonuclease (Trex)1, which could degrade radiation-induced cytosolic DNA and thus attenuating its immunogenicity [54]. Interestingly, in a patient-derived lung adenocarcinoma xenograft, 24 Gy/3 Fr led to significant upregulation of human *Ifnb1* (which encodes IFN-β that plays an important role in antitumor immunity), while 20 Gy/1 Fr upregulated *Trex1*, highlighting the differential effects of different dose fractionation regimens which will be discussed further below. Although an intact DNA-sensing cGAS/

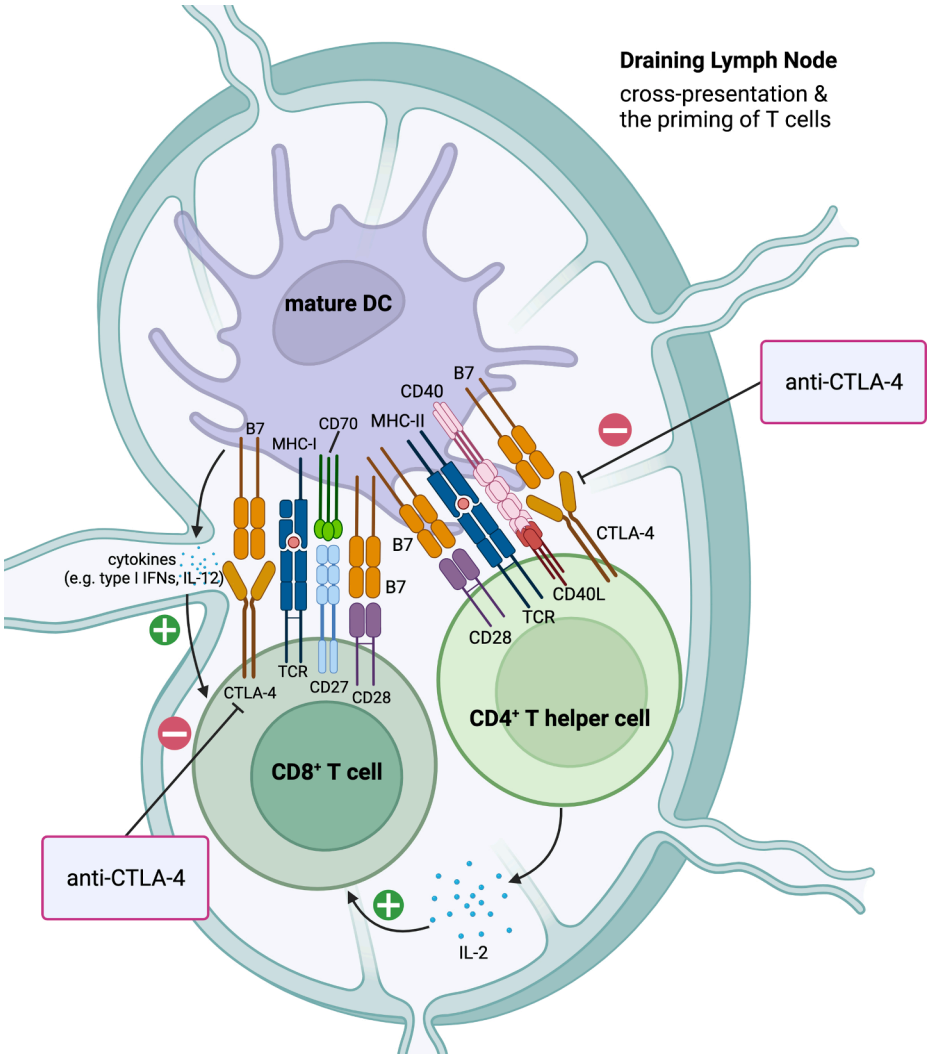

Fig. 2. Cross-presentation and priming of T cells in a draining lymph node. Help signals are relayed from $CD4^+$ T cells to $CD8^+$ T cells, and activation of the latter depends on 3 signals: TCR activation, co-stimulatory signals (e.g. the B7-CD28 and CD70-CD27 co-stimulation) and stimulatory cytokines (e.g. type I IFNs, IL-12). Anti-CTLA-4 monoclonal antibodies could potentiate this process which is regulated by various inhibitory signals including those mediated by CTLA-4. This T-cell priming culminates in the differentiation of activated $CD8^+$ T cells into, and the clonal expansion of, memory and effector CTLs. This figure was created with BioRender.com. CTLA-4: cytotoxic T-lymphocyte-associated antigen 4; DC: dendritic cell; IL-2: interleukin-2; IL-12: interleukin-12; MHC-I: major histocompatibility complex class I; MHC-II: major histocompatibility complex class II; TCR: T-cell receptor; type I IFNs: type I interferons.

STING pathway is essential to antitumor immunity, chronic activation of this pathway could paradoxically lead to an immune-suppressive TME [55]. Interestingly, Bruand et al. showed, in *BRCA1*-deficient tumors, that STING singalling leads to immune resistance via the upregulation of VEGF (vascular endothelial growth factor)-A and neovascularization, and demonstrated the therapeutic efficacy of an anti-VEGF-based regimen [56]. Furthermore, radiation itself could lead to a reduction in lymphocytes. In a retrospective review of patients receiving both palliative RT and either pembrolizumab or nivolumab, palliative RT was shown to reduce the median lymphocyte count by 161 cells/ml, and the presence of severe lymphopenia at the start of ICI therapy was associated with increased mortality (HR 2.1, $p = 0.03$) [57].

Optimization of the radiotherapy-immunotherapy synergy – the impact of dose fractionation, sequencing and irradiated volume

Dose fractionation

Morisada *et al.* showed in a mouse oral cavity carcinoma model that hypofractionated radiation (16 Gy/2 Fr) combined with an anti-PD-1 antibody led to significantly better local and distant (abscopal) tumor control in comparison to conventional fractionation (20 Gy/10 Fr) [58]. Oweida *et al.* revealed, using an orthotopic HNSCC mouse model, that an RT dose of 10 Gy/1 Fr yielded a significant increase in effector $CD8^+$ and $CD4^+$ T cells, but no response was observed with 10 Gy/5 Fr [59]. Taken together, it appears that higher fractional doses generate a larger immune response, though a recent study by Herrera et al. showed that doses as low as 0.5–1 Gy are adequate to reprogramme the TME in immune-cold tumors, with a marked influx of $CD4^+$ T cells and a significant increase in Th1 signatures in those patients who responded to an ICI-based regimen [60].

Sequencing

In the neoadjuvant setting, immunotherapy can prime the TME, enriching the effector and memory T cells within the tumor to exert their antitumor cytotoxic effects, thus potentially reversing the immunosuppressive effects of RT [61]. This may be particularly effective in HPV+ oropharyngeal carcinoma and EBV+ NPC, which have a dense lymphocytic infiltrate. Young *et al.* showed a more pronounced response when anti-CTLA-4 was given 7 days before RT in mice bearing colorectal tumors, when compared with giving the ICI 1 or 5 days post-RT [62]. However, there is limited pre-clinical data in head and neck cancer.

As discussed previously, RT-induced cell death culminates in antigen presentation and the priming of T cells. It may take hours or days to reach a maximum level of interaction between APCs and T cells [63]. This event may also increase the infiltration of CTLs, and increase PD-L1 expression in tumors in response to T-cell-derived IFNγ [64]. This provides a rationale for concurrent treatment. Dovedi and colleagues showed in mice models of melanoma and colon/ breast cancer that the

synergistic effects of RT and anti-PD-L1 antibodies were only seen when the treatments were given concurrently [45].

In the adjuvant setting, there is evidence from pre-clinical studies that chemoradiotherapy-induced inflammation may promote the priming of dendritic cells in lymph nodes, CTL entry and re-activation of T cells. These factors may potentially bolster the activity of ICIs in the adjuvant setting [65].

Irradiated volume

In head and neck cancers, the adjacent nodal regions are often treated prophylactically with RT to a dose of around 50 Gy in conventional fractionation (i.e. 2 Gy per fraction) even if not clinically involved. However, induction of an antigen-specific, antitumor response relies on the migration of APCs to the draining lymph nodes for the priming of T cells after radiation is given to the primary lesion. In addition, using a murine model of HPV+ HNSCC, Kim *et al.* demonstrated that RT and an anti-PD-L1 antibody could synergistically enhance the development of a B-cell-mediated adaptive immune response in the tumor-draining lymph nodes [66]. Theoretically, nodal irradiation could attenuate this lymphocyte priming. In the study by Morisada *et al.* mentioned above, irradiation of the primary and draining lymph nodes with a dose of 20 Gy/10 Fr suppressed tumor-specific IFN-γ production within the nodes [58]. Marciscano *et al.* demonstrated in murine models that the addition of nodal irradiation attenuated adaptive immune responses through reduced chemokine expression and $CD8^+$-T-cell trafficking [67]. Further studies are certainly required to ascertain the optimal target volume when combining RT with ICIs.

Unique immune landscapes of virus-induced cancers and their clinical significance

EBV-related nasopharyngeal carcinoma

EBV-related NPC is a typical example of an "immune-hot" tumor with a stroma that is densely infiltrated by immune cells. EBV exists in a state of type II latency in NPC cells, thereby expressing a limited repertoire of non-coding RNAs and oncogenic EBV-related proteins that are poorly immunogenic and can evade immune surveillance by the host [68]. The TME is infiltrated by dysfunctional and exhausted $CD8^+$ T cells and effector cells that overexpress inhibitory immune-checkpoint proteins such as PD-L1 and CTLA-4 [69,70]. Other immunosuppressive cells such as Tregs, M2 macrophages and MDSCs, and various chemokines and cytokines all contribute to the immunosuppressive environment. In addition, in a whole-genome sequencing study, NPC has a mutational burden that is comparable to that of HNSCC [71].

The ubiquitous nature of EBV in non-keratinizing NPC has made EBV antigens an ideal target for EBV-directed immunotherapy such as therapeutic vaccines, for which several approaches have been evaluated clinically. These include autologous dendritic cells primed with EBV antigens, modified or recombinant viruses encoding latent EBV antigens such as EBNA1 and/or LMP2 or LMP1, peptide vaccines and plasmid DNA vaccines [4]. Clinical reports have shown that these vaccines are fairly well tolerated albeit with limited clinical efficacy in patients with high tumor burden. Therefore, vaccines have been investigated as an adjuvant following definitive chemoradiotherapy in patients with locally advanced NPC. Elevation in the percentage of natural killer (NK) and $CD4^+$ T cells was observed in patients treated with an LMP2-primed autologous dendritic cell vaccine [72], while LMP2 and EBNA1-specific T-cell responses were seen in patients treated with a Modified Vaccinia Ankara virus-based EBNA1/LMP2 vaccine [73]. Therefore, adjuvant EBV-specific vaccines have the potential of augmenting cellular immunity following definitive radiotherapy especially in patients with high level of plasma EBV DNA post-radiotherapy, thus further studies are warranted.

HPV-related (p16+) oropharyngeal carcinoma

HPV+ oropharyngeal cancer has different biological and clinical behavior to its HPV- counterparts, and it is possible that the immune TME may play a part. The oropharynx is highly enriched with lymphoid tissue, and transcriptional data from tumors profiled by the Cancer Genome Project has shown that oropharyngeal tumors display generally higher levels of T-cell infiltration and immune activation compared to other subsites [74]. Viral oncoproteins activate the adaptive immune response, and HPV+ HNSCC is enriched in $CD8^+$ and $CD4^+$ T cells compared to HPV- tumors. PD-L1 and PD-1 expression were found to be similar between HPV- and HPV+ tumors, though there was higher expression of CTLA-4 and Treg in the HPV+ subset [74]. Further work has demonstrated that HPV+ tumors are associated with increased T-cell receptor diversity, higher levels of immune cytolytic activity, and an overall enriched inflammatory response [75,76]. Taken together, HPV+ HNSCC is associated with high levels of immune infiltration and immune activation, making it more vulnerable to immunotherapy.

Translation from bench to bedside – data from clinical trials

Immunotherapy has proven efficacy in recurrent/ metastatic (R/M) head and neck cancer. The KEYNOTE-040 and CheckMate 141 studies established pembrolizumab and nivolumab as the standard-of-care options in the second-line setting after failure of platinum-based chemotherapy [8,9]. In the KEYNOTE-048 study, pembrolizumab, either alone or in combination with chemotherapy, was compared with the EXTREME regimen in patients with previously untreated R/M HNSCC [7]. Pembrolizumab monotherapy was associated with better OS and fewer serious toxicities than the EXTREME regimen in patients with tumors expressing PD-L1 combined positive scores (CPS) of ≥ 20 (HR 0.61, $p = 0.0007$) and ≥ 1 (HR 0.78, $p = 0.0086$).

For R/M NPC, there are at least 6 PD-1/PD-L1 inhibitors with single-agent activity in heavily pre-treated patients [4]. In the first line R/M setting, 3 PD-1 inhibitors (toripalimab [77], camrelizumab [78], tislelizumab [79]) have recently been shown to improve progression-free survival (PFS) and objective response rate (ORR) when combined with platinum-gemcitabine.

Combining radiation and immunotherapy in the metastatic setting

Most retrospective and prospective analyses have shown that the concurrent/ sequential administration of RT and ICIs is generally well tolerated [80]. For example, in a phase I trial investigating stereotactic body radiation therapy (SBRT) in combination with pembrolizumab in patients with different metastatic cancers (including 4 patients with head and neck cancer), dose-limiting immune-related toxicities occurred in 9.7% of patients including colitis, hepatitis and pneumonitis [81]. There have been reports of patients developing radiation recall, which is a rare and unpredictable inflammatory reaction in previously irradiated tissues, after administration of immunotherapy, as well as an increased risk of radionecrosis when combining immunotherapy and stereotactic RT for brain metastases [82–84].

Radiation may help to stimulate a systemic antitumor immune response in synergy with immunotherapy, resulting in an abscopal effect which is characterised by the regression of non-irradiated distant lesions [85]. However, this is still a very rare phenomenon. In a randomized phase II study of 62 patients with metastatic HNSCC who were given nivolumab with or without SBRT to a single metastatic site (27 Gy/3 Fr, between the first and second doses of nivolumab), there was no improvement in ORR (34.5% for nivolumab alone versus 29.0% for nivolumab with SBRT, $p = 0.86$) [86]. More studies are ongoing (Table 2), though few are randomized.

Patients with oligometastases from HNSCC and NPC may benefit from local ablative treatments in addition to systemic treatments. There is increasing interest in using SBRT as a non-invasive treatment for

Table 2
Ongoing trials in metastatic head and neck squamous cell carcinoma.

Trial identifier	Study title	Phase	Patient selection	Timing with respect to RT	Estimated sample size	Primary endpoint
NCT03539198	Study of Proton SBRT and Immunotherapy (Nivolumab) for Recurrent/Progressive Locoregional or Metastatic Head and Neck Cancer	N/A	Recurrent/metastatic HNSCC, ≥ 2 metastatic sites	Induction/ maintenance	91	ORR
NCT03844763	CONFRONT: Targeting the Tumor Microenvironment in R/M SCCHN (avelumab, cyclophosphamide)	1/2	Recurrent/metastatic HNSCC	Concurrent/ maintenance	71	Adverse events, ORR
NCT03522584	Durvalumab, Tremelimumab and Hypofractionated Radiation Therapy in Treating Patients With Recurrent or Metastatic Head and Neck Squamous Cell Carcinoma	1/2	Recurrent/metastatic HNSCC, progression through prior PD-1/ PD- L1 inhibitor	Concurrent	6	Adverse events
NCT03283605	Immunotherapy (durvalumab) and SBRT for Metastatic Head and Neck Carcinomas	1/2	Metastatic HNSCC, ≥ 2 metastatic sites	Concurrent/ maintenance	45	Acute toxicities of treatment, PFS
NCT03317327	REPORT: REirradiation and Programmed Cell Death Protein 1 (PD-1) Blockade (nivolumab) on Recurrent Squamous Cell Head and Neck Tumors	1/2	Recurrent HNSCC after prior radiation or second primary HNSCC	Concurrent/ maintenance	20	Adverse events
NCT04340258	Trial Combining Pembrolizumab and Cesium 131 Brachytherapy With Salvage Surgery in HNSCC	1/2	Recurrent HNSCC after prior radiation or second primary HNSCC	Concurrent/ maintenance	50	Overall safety measured by dose-limiting toxicities, DFS
NCT03474497	UCDCC#272: IL-2, Radiotherapy, and Pembrolizumab in Patients Refractory to Checkpoint Blockade	1/2	Recurrent/metastatic HNSCC, progression through prior PD-1/ PD- L1 inhibitor	Concurrent	45	Abscopal response rate
NCT03313804	Priming Immunotherapy in Advanced Disease With Radiation	2	Recurrent/metastatic HNSCC	Induction	57	PFS
NCT04454489	Quad Shot Radiotherapy in Combination With Immune Checkpoint Inhibition (pembrolizumab)	2	Recurrent/metastatic HNSCC	Concurrent/ maintenance	15	Overall response
NCT03546582	KEYSTROKE: SBRT +/− Pembrolizumab in Patients With Local-Regionally Recurrent or Second Primary Head and Neck Carcinoma	2	Recurrent HNSCC after prior radiation or second primary HNSCC	Adjuvant	102	PFS
NCT03386357	Radiotherapy With Pembrolizumab in Metastatic HNSCC	2	Recurrent/metastatic HNSCC, ≥ 2 metastatic sites, progression through platinum-based therapy	Concurrent/ maintenance	130	Best response according to iRECIST criteria
NCT03085719	Targeting PD-1 Therapy Resistance (pembrolizumab) With Focused High or High and Low Dose Radiation in SCCHN	2	Metastatic HNSCC, progression through prior PD-1 inhibition, ≥ 3 metastatic sites	Concurrent	26	ORR
NCT03511391	CHEERS: CHEckpoint Inhibition (Nivolumab) in Combination With an Immunoboost of External Body Radiotherapy in Solid Tumors	2	Recurrent/metastatic HNSCC, progression through platinum-based therapy	Concurrent	99	PFS
NCT02289209	Reirradiation With Pembrolizumab in Locoregional Inoperable Recurrence or Second Primary Squamous Cell CA of the Head and Neck	2	Unresectable recurrent HNSCC after prior radiation or second primary HNSCC	Concurrent/ adjuvant	48	PFS
NCT03521570	Intensity-Modulated Radiation Therapy & Nivolumab for Recurrent or Second Primary Head & Neck Squamous Cell Cancer	2	Recurrent HNSCC after prior radiation or second primary HNSCC	Concurrent/ adjuvant	51	PFS

RT: radiotherapy; SBRT: stereotactic body radiation therapy; N/A; not available; HNSCC: head and neck squamous cell carcinoma; ORR: objective response rate; R/M: recurrent/ metastatic; SCCHN: squamous cell carcinoma of the head and neck; PD-1: programmed cell death protein 1; PD-L1: programmed cell death ligand 1; PFS: progression-free survival; DFS: disease-free survival; IL-2: interleukin-2; iRECIST: Response Evaluation Criteria in Solid Tumours (for immunotherapy); CA: carcinoma.

patients with distant metastases from HNSCC [87,88], though prospective data is limited. In a single-centre prospective observational study of 10 patients (including 1 patient with NPC), Chua et al. showed that RT given to an oligoprogressive/ symptomatic site after prior response to immune-checkpoint inhibition would elicit an immunostimulatory effect by increasing circulating Ki67+ $CD8^+$ T cells, and these cells were further upregulated by the re-introduction of immunotherapy [89]. There is an ongoing phase I/II trial which examines the use of durvalumab, tremelimumab [a CTLA-4 inhibitor] and SBRT in patients with HNSCC with ≤ 10 metastases (NCT03283605). Randomized phase III trials are required to definitively establish a survival benefit of SBRT in metastatic HNSCC, and in particular to determine the optimal sequencing and timing with immunotherapy.

Combining radiation and immune-checkpoint inhibitors in the locally advanced setting

The combination of ICIs and RT is being actively investigated in intensifying the definitive treatment of HNSCC and NPC, as induction, concurrent and/or adjuvant treatments (Tables 3-5). ICIs are also being used to de-escalate the definitive treatment of HPV+ HNSCC in order to minimize treatment-related toxicities, and are given in cisplatin-ineligible patients with LA-HNSCC.

Use of immunotherapy in the intensification of treatment of HNSCC

The literature reported to date has shown that the addition of PD-1/ L1 inhibitors does not seem to significantly increase the risk of acute toxicity or reduce compliance with RT [90–92]. For example, in a single-arm phase Ib trial investigating the addition of concurrent and adjuvant pembrolizumab to RT with concurrent weekly cisplatin in LA-HNSCC, 98.3% of 56 patients were able to complete the planned course of RT, and only 8.8% of patients had to discontinue pembrolizumab due to immune-related adverse events (irAEs) [90].

The largest reported trial to date investigating the addition of immunotherapy to definitive chemoradiotherapy in HNSCC is the JAVELIN Head and Neck 100 study [93]. It is a double-blind, placebo-controlled phase III study that randomized 697 patients with LA-HNSCC who were unselected for PD-L1/ HPV status to cisplatin-based chemoradiotherapy with concurrent avelumab (a PD-L1 inhibitor) or placebo, followed by one year of adjuvant avelumab or placebo. The primary endpoint of the study was PFS. Unfortunately, the trial was discontinued early for likely futility, after interim results showing a stratified hazard ratio of 1.21 [95% confidence interval (CI) 0.93–1.57, p = 0.92] favouring the placebo group, though there was no substantial difference

Table 3
Completed randomized controlled trials for the combination of radiotherapy and immunotherapy in head and neck squamous cell carcinoma.

Title	Phase	Number of patients	Eligible patients	Experimental arm	Control arm	RT fractionation	Sequencing with respect to RT	Primary endpoint	PFS experimental vs. control arm	OS experimental vs. control arm	Trial result
Debio 1143 study [102,103]	2	110	Stage III-IVB HNSCC	Debio 1143 every 3 weeks for 3 cycles + 3-weekly cisplatin + RT	Placebo + 3-weekly cisplatin + RT	Conventional (70 Gy in 35 fractions)	Concurrent	Rate of LRC at 18 months	3-year PFS: 72% vs. 36% (HR 0.34, p = 0.0023)	NR vs. 36.1 months (HR 0.49, p = 0.0261)	Improved LRC: 54% vs. 33% at 18 months (odds ratio 2.69, p = 0.026)
PembroRad study [108]	2	131	Stage III-IVB HNSCC, cisplatin-ineligible	Pembrolizumab every 3 weeks for 3 cycles + RT	Weekly cetuximab + RT	Conventional (69.96 Gy in 33 fractions)	Concurrent	LRC	2-year PFS: 42% vs. 40% (HR 0.83, p = 0.41)	2-year OS: 62% vs. 55% (HR 0.83, p = 0.49)	No difference in 2-year LRC/ OS/ PFS between arms
Nivolumab With SBRT Versus Nivolumab Alone in Patients With Metastatic HNSCC [86]	2	62	Metastatic HNSCC with at least 2 metastatic sites	Nivolumab every 2 weeks up to 96 weeks + SBRT	Nivolumab alone	SBRT (27 Gy in 3 fractions every other day)	Concurrent	ORR	Median PFS: 2.6 vs. 1.9 months (p = 0.79)	Median OS: 13.9 vs. 14.2 months (p = 0.75)	No difference in ORR, PFS, OS
JAVELIN Head and Neck 100 study [93]	3	697	Stage III-IVB HNSCC	Avelumab every 2 weeks with up to 12 months of maintenance therapy + 3-weekly cisplatin + RT	Placebo + 3-weekly cisplatin + RT	Conventional (70 Gy in 35 fractions)	Concurrent + adjuvant	PFS	Median NR in either arm, stratified HR 1.21 (one-sided p = 0.92)	Median NR in either arm, stratified HR 1.31 (one-sided p = 0.94)	Negative, no difference in PFS
REACH: Randomized Trial of Avelumab-cetuximab-radiotherapy Versus SOCs in LA-SCCHN [109]	3	707	Stage III-IVB HNSCC (cisplatin-eligible and cisplatin-ineligible)	2-weekly avelumab + weekly cetuximab + RT, followed by 2-weekly maintenance avelumab for up to 12 months	3-weekly cisplatin + RT (cisplatin-eligible) Weekly cetuximab + RT (cisplatin-ineligible)	Conventional (69.96 Gy in 33 fractions)	Concurrent + adjuvant	PFS	1-year PFS in cisplatin-eligible patients: 64% vs. 73%, HR 1.27 (95 % CI 0.83–1.93); 2-year PFS in cisplatin-ineligible patients: 44% vs. 31% (HR 0.85, p = 0.15)	2-year OS for cisplatin-ineligible patients: 58% vs. 54% (HR 1.08, p = 0.69)	Negative, no difference in PFS

RT: radiotherapy; PFS: progression-free survival; OS: overall survival; HNSCC: head and neck squamous cell carcinoma; LRC: locoregional control; HR: hazard ratio; SBRT: stereotactic body radiation therapy; ORR: objective response rate; NR: not reached, SOCs: standards of care; LA-SCCHN: locally advanced squamous cell carcinoma of the head and neck.

Table 4
Ongoing randomized clinical trials for the definitive management of locally advanced head and neck squamous cell carcinoma.

Trial identifier	Study title	Phase	Patient selection	Timing with respect to RT	Expected sample size	Primary endpoint
NCT02841748	PATHWay study: A Randomized, Double-Blind Phase II Study of Adjuvant Pembrolizumab Versus Placebo in Head and Neck Cancers at High Risk for Recurrence	2	Stage III-IVB HNSCC	Adjuvant	100	PFS
NCT03410615	Cisplatin + Radiotherapy vs Durvalumab + Radiotherapy Followed by Durvalumab vs Durvalumab + Radiotherapy Followed by Tremelimumab + Durvalumab in Intermediate-Risk HPV-Positive Oropharyngeal SCC	2	T1-2 N1 (smoking ≥ 10 pack-years), T3N0-1 (smoking ≥ 10 pack-years), T1-3 N2 (any smoking history)	Concurrent + adjuvant	180	EFS
NCT03811015	Testing Immunotherapy (nivolumab) Versus Observation in Patients With HPV Throat Cancer	2/3	Locally advanced p16+ OPSCC	Adjuvant	636	OS, negative 12-week post-therapy PET-CT
NCT03952585	De-intensified Radiation Therapy With Chemotherapy (Cisplatin) or Immunotherapy (Nivolumab) in Treating Patients With Early-Stage, HPV-Positive, Non-Smoking Associated Oropharyngeal Cancer	2/3	T1-2, N1, M0 or T3, N0-1, M0 p16+ OPSCC, with ≤ 10 pack-years of smoking	Concurrent	711	PFS, QOL
NCT03576417	NIVOPOSTOP: A Trial Evaluating the Addition of Nivolumab to Cisplatin-RT for Treatment of Cancers of the Head and Neck	3	Resected LA-HNSCC, with ENE, positive margins, or multiple positive nodes	Concurrent + adjuvant	680	DFS
NCT03452137	Study of Atezolizumab as Adjuvant Therapy After Definitive Local Therapy in Patients With High-Risk LA-HNSCC	3	LA-HNSCC	Adjuvant	406	EFS
NCT03765918	KEYNOTE 689: Study of pembrolizumab given prior to surgery and in combination with radiotherapy given post-surgery for LA-HNSCC	3	Resectable LA-HNSCC	Concurrent + adjuvant	704	mPR, EFS
NCT03040999	KEYNOTE-412: Study of Pembrolizumab or Placebo With Chemoradiation in Participants With Locally Advanced Head and Neck Squamous Cell Carcinoma	3	LA-HNSCC	Concurrent + adjuvant	780	EFS

RT: radiotherapy; PFS: progression-free survival; HPV: human papilloma virus; SCC: squamous cell carcinoma; EFS: event-free survival; OPSCC: oropharyngeal squamous cell carcinoma; OS: overall survival; PET-CT: positron emission tomography-computed tomography; QOL: quality of life; LA-HNSCC: locally advanced head and neck squamous cell carcinoma; ENE: extra-nodal extension; DFS: disease-free survival; mPR: major pathological response.

Table 5
Ongoing clinical trials in locally advanced nasopharyngeal carcinoma.

Trial identifier	Study title	Phase	Patient selection	Timing with respect to RT	Expected sample size	Primary endpoint
NCT03734809	NEOSPACE: Pembrolizumab and induction cisplatin-gemcitabine and CRT, followed by maintenance	2	Stage IVA (T4 or N3)	Induction + concurrent + adjuvant	46	PFS
NCT03984357	CANIRA: Concurrent and adjuvant Nivolumab combined with induction chemotherapy and radiotherapy	2	T4N1 or N2-3	Induction + concurrent + adjuvant	146	FFS
NCT03925090	Phase II trial of neoadjuvant and adjuvant anti-PD-1 antibody Toripalimab combined with CRT in NPC patients	2	Stage III-IVA, plasma EBV DNA ≥ 1500 copies/ml	Induction + adjuvant	138	PFS
NCT03383094	Chemoradiation vs Immunotherapy (pembrolizumab) and Radiation for Head and Neck Cancer	2	Stage III-IVB p16+ squamous cell NPC	Concurrent + adjuvant	114	PFS
NCT03267498	Nivolumab + Chemoradiation in Stage II-IVB	2	Stage II-IVB, WHO type II/III	Concurrent + adjuvant	40	Feasibility of treatment completion
NCT04143984	Carbon-Ion Radiotherapy Plus Camrelizumab for Locally Recurrent NPC	2/3	Recurrent non-metastatic NPC, completed definitive course of IMRT to a total dose of ≥ 66 Gy	Concurrent + adjuvant	180	PFS
NCT03427827	PACIFIC: Camrelizumab after CRT in locoregionally advanced NPC	3	Stage III-IVA (except T3-4 N0 or T3N1)	Adjuvant	417	DFS
NCT03700476	Sintilimab (PD-1 antibody) and CRT in Locoregionally-advanced NPC	3	Stage III/IVA (except T3N0-1 or T4N0)	Induction + concurrent + adjuvant	417	FFS

RT: radiotherapy; CRT: chemoradiation; PFS: progression-free survival; FFS: failure-free survival; PD-1: programmed cell death protein 1; NPC: nasopharyngeal carcinoma; EBV: Epstein-Barr virus; WHO: World Health Organization; IMRT: intensity-modulated radiotherapy; DFS: disease-free survival.

in toxicity.

The failure of this trial shows that we still have much to learn about the interactions between immunotherapy and RT. A possible explanation for the failure of this trial is there may be dysregulation or depletion of T cells or changes in the TME after RT which negatively affect the ability of the immune system to eradicate microscopic disease [94]. It is interesting to compare the positive PACIFIC trial in non-small cell lung cancer (NSCLC) [95] and the CheckMate 577 trial in esophageal cancer [96], both of which had a proportion of patients with squamous cell cancers. In clinical practice, only the gross tumor in NSCLC and esophageal cancer is irradiated, whereas in LA-HNSCC the cervical nodal basins are covered prophylactically to encompass possible microscopic disease. It is possible that irradiating these drainage lymph nodes may hinder T-cell priming by APCs. Furthermore, this trial consisted of both concurrent and adjuvant treatments, and there is no way to tease out which sequence of checkpoint inhibition would have more benefit;

hopefully future trials may give us more insight into this. Of note, the ongoing KEYNOTE-412 trial is investigating concurrent and adjuvant pembrolizumab combined with chemoradiation in LA-HNSCC (NCT03040999).

In the neoadjuvant setting, a phase II trial investigating neoadjuvant pembrolizumab prior to surgery followed by adjuvant concurrent pembrolizumab and RT +/- cisplatin for high-risk, resectable LA-HNSCC (T3/T4, ≥ N2 or with extracapsular spread in nodal disease) has been recently reported [97]. The trial showed that the 1-year disease-free survival (DFS) was significantly superior in patients who experienced partial or major pathological response to neoadjuvant pembrolizumab, compared to patients without pathological response (1-year DFS 100% versus 68%, p = 0.01). Several neoadjuvant trials are ongoing, of note the phase III KEYNOTE-689 is comparing neoadjuvant pembrolizumab followed by surgery and adjuvant treatment plus pembrolizumab, versus surgery and standard-of-care adjuvant treatment (NCT03765918). The combination of immunotherapy and RT prior to surgery may also play a role. The phase Ib Neoadjuvant Immuno-Radiotherapy Trial (NIRT) investigated the feasibility of neoadjuvant SBRT with nivolumab prior to surgery in LA-HNSCC patients, followed by adjuvant nivolumab [98]. There were no treatment delays and there was an 86% major pathological response rate, a 67% pathological complete response rate, with 90% achieving clinical to pathological down-staging. Further studies are needed to see if this approach could translate into an improvement in clinical outcome, furthermore it is questionable whether this approach is needed in all patients especially for HPV+ disease.

Another approach that may hold promise is targeting the negative regulators of the apoptosis pathway that allow tumor cells to evade programmed cell death. The inhibitors of apoptosis proteins (IAPs) have been shown to negatively regulate apoptosis, and they also modulate NF-κB signalling, which in turn plays an important role in T-cell activation and proliferation. Antagonising IAPs can promote apoptosis, and reactivation of the innate and adaptive immunity [99]. Debio 1143, an oral small-molecule antagonist of IAPs, was shown to be an effective radiosensitizer in pre-clinical models of HNSCC, and following a phase I trial demonstrating a good safety profile, a phase II trial was conducted and recently reported [100–102]. In this double-blind controlled trial of stage III/IV HNSCC, patients were randomized to standard radical chemoradiotherapy with Debio 1143 versus standard chemoradiotherapy with placebo. All 96 patients had a history of smoking and were predominantly p16- oropharyngeal carcinoma patients (58% in both arms). Results showed significantly improved locoregional control in the experimental arm (54% versus 33%, odds ratio 2.69, p = 0.026), and recently updated results also showed superior OS favouring the experimental arm (HR 0.49, p = 0.0261) [103]. Patients in the experimental arm experienced higher rates of grade 3–4 dysphagia, mucositis and anaemia, however no treatment-related deaths were reported as opposed to 2 deaths in the placebo group. A confirmatory phase III trial is ongoing, and the results could potentially be practice-changing (NCT04459715).

Alternatives to cisplatin

Cetuximab is an epidermal growth factor receptor (EGFR) antibody that inhibits EGFR signal transduction in cancer cells and activates both innate and NK cell-mediated immune responses [104]. Weekly cetuximab combined with radiotherapy is a standard treatment option for stage III/IV HNSCC patients who are ineligible for cisplatin [105]. Cetuximab is also known to have immunosuppressive effects by the expansion of regulatory T cells and increasing PD-L1 expression on tumor cells in the TME [106]. Combining immunotherapy with cetuximab may counteract the potential resistant mechanisms of cetuximab [107]. There are 2 randomized studies that have investigated, respectively, replacing cetuximab with immunotherapy and adding immunotherapy to cetuximab (Table 3).

The PembroRad randomized phase II trial compared concurrent cetuximab-RT versus pembrolizumab-RT in 131 cisplatin-ineligible patients with stage III-IVA/B SCC of the oral cavity, oropharynx, hypopharynx and larynx [108]. Pembrolizumab failed to show superior locoregional control (OR 1.05, p = 0.91), and there were also no statistically significant differences between the two arms in terms of PFS (HR 0.83, p = 0.41) and OS (HR 0.83, p = 0.49). There was a reduction in acute toxicity in the pembrolizumab-RT arm versus the cetuximab-RT arm (74% versus 92% of patients with at least one grade ≥ 3 acute adverse event, p = 0.006).

The Phase III GORTEC-REACH trial investigated avelumab and cetuximab combined with RT with interim results presented at the European Society of Medical Oncology (ESMO) Congress 2021 [109]. The trial enrolled patients with stage III/IV HNSCC who were divided into cohorts according to the eligibility of receiving cisplatin. Cisplatin-ineligible patients (n = 277) received concurrent cetuximab-IMRT (intensity-modulated radiotherapy), or concurrent avelumab and cetuximab-IMRT followed by adjuvant avelumab for 12 months. Cisplatin-eligible patients (n = 430) received cisplatin-IMRT with or without avelumab during the concurrent and adjuvant phases. After a median follow-up of 21.3 months in the cisplatin-ineligible cohort, both the 2-year PFS (primary endpoint) and locoregional progression rates favoured the avelumab arm, but the differences did not reach statistical significance (2-year PFS rate: 44% versus 31%, HR 0.84, 95% CI 0.62–1.15, p = 0.15; 2-year locoregional progression rate: 34% versus 44%, HR 0.83, 95% CI 0.56–1.22, p = 0.34). There was a statistically significant decrease in the distant metastasis rate favouring the experimental arm (5.4% versus 14.3%, HR 0.31, 95% CI 0.13–0.72, p = 0.007). However, there was an increased cumulative incidence of death in the experimental versus the control arm of 16% versus 11%, respectively (HR, 1.81; 95% CI, 0.90–3.6; p = 0.1). In the cisplatin-eligible group, a planned interim analysis showed 1-year PFS rates of 73% versus 64% in the control arm and the avelumab arm respectively, which translated into a hazard ratio of 1.27, crossing the boundary for futility and favouring the control arm. Overall, cetuximab combined with RT remains the standard of care in cisplatin-ineligible HNSCC patients, although it would be intriguing to see which subsets of patients may have derived benefit in the final publication.

In general, HPV+ disease has better prognosis than HPV- HNSCC, and there are ongoing trials investigating treatment de-escalation with immunotherapy, given concurrently with RT, in early-stage HPV+ HNSCC (NCT03952585, NCT03410615). Augmentation of chemoradiotherapy with immunotherapy is also being actively investigated in NPC. In a single-arm phase II trial, toripalimab in combination with IMRT in locally recurrent, inoperable NPC showed a promising 1-year PFS of 91.8% (95% CI 91.7% − 91.9%) [110]. In addition, there are several ongoing studies investigating pembrolizumab, nivolumab, toripalimab, camrelizumab and sintilimab in the induction, concurrent and/or adjuvant settings (Table 5). Given the different TMEs between HNSCC and NPC, hopefully these approaches may prove to be more successful.

Opportunities and challenges in combining radiation and immunotherapy

As RT leads to both immunostimulatory and immunoinhibitory effects, the major challenge is how to tilt this balance in patients' favor. Tregs are a major inhibitory regulator of the RT-induced antitumor immune response, and combining anti-CTLA-4 with RT is a promising approach as CTLA-4 is highly expressed on Tregs. This leads to Treg depletion and an increase in the ratio of $CD8^+$ T cells to Tregs in the TME. Moreover, the actions of anti-CTLA-4 and anti-PD-1/ anti-PD-L1 ICIs are complementary. It was shown that the resistance to RT and anti-CTLA-4 treatment in a murine melanoma model was due to upregulation of PD-L1 on melanoma cells with associated T-cell exhaustion [111]. PD-L1 blockade could reverse the latter, and RT is able to enhance the diversity of the T-cell-receptor repertoire of intra-tumoral T cells. The combination of RT and dual immune checkpoint blockade is currently

being studied in multiple clinical trials (NCT03283605, NCT03700905, NCT03426657, NCT03522584).

In addition to PD-1/PD-L1 and CTLA-4 blockade, other molecular pathways may also be harnessed to improve the synergism between RT and ICIs. Besides antagonists of IAPs as previously discussed, an inhibitor of ATR (ataxia telangiectasia and rad3 related), a key component in the DNA damage response pathway, has been shown to sensitize HPV-driven tumors to RT via diverse mechanisms, including an increase in innate immune cell infiltration and an upregulation of type I/II IFN responses [112]. Using a murine model of HPV-associated head and neck cancer, cyclophosphamide and an inhibitor of inducible nitric oxide synthase (iNOS) were found to be associated with remarkable antitumor activity when given in combination with dual immune checkpoint blockade (against PD-1 and CTLA-4) and radiation, with $CD8^+$ T cell activity essential to the efficacy of this 'CPR' regimen [113]. Such novel multi-drug combinations are, however, challenging to be applied clinically due to potential toxicities and the tolerance to these treatments has to be meticulously studied in clinical trials.

With regard to toxicities, the combination of RT and ICIs is generally well tolerated. In LA-HNSCC patients who could not tolerate cisplatin, pembrolizumab-RT is a less toxic alternative to standard-of-care cetuximab-RT, though it is similar in efficacy to the latter as per the PembroRad study [108]. Nevertheless, concurrent chemoradiotherapy alone is associated with significant toxicities, with 74% of patients developing grade 3 or worse treatment-related toxicities in the control arm of the JAVELIN Head and Neck 100 study [93]. Combining immunotherapy with chemoradiotherapy may lead to additional toxicities (the proportion became 80% according to the same study) and could potentially impair the delivery of chemoradiotherapy. Longer-term follow-up is required to see if ICIs combined with RT with or without chemotherapy in head and neck cancers are associated with more late toxicities such as hypophysitis.

Several syngeneic HNSCC mouse models (e.g. MOC1, MOC2, 4MOSC) are being utilized to mimic the complex interactions between the mutational landscape, TME and a functioning immune system [114]. Such models are invaluable for studies investigating the mechansims underlying the efficacy of, and resistance to, immunotherapy and how the latter should be combined with other treatment modalities such as RT. It is rather disappointing that most clinical studies have so far failed to demonstrate the additional benefits of ICIs when added to radiation or chemoradiotherapy, despite the promising data obtained in pre-clinical models. This is partly attributed to the inaccuracies of such models. For example, Wisdom *et al.* demonstrated the considerable differences in the immune landscapes of transplant and autochthonous sarcomas, with the latter not being enriched for activated $CD8^+$ T cells which led to the resistance to immunotherapy-RT combination treatments [115]. The tumors in animal models also have greater doubling times with respect to their human counterparts [28]. Other factors leading to this discrepancy between pre-clinical and clinical studies include the uncertainties regarding the appropriate dose fractionation, optimal sequencing, and the target volume to be irradiated. When radiation oncologists give RT at radical doses to the head and neck region, the fractional dose is generally around 2 Gy, with total doses up to 60–66 Gy and 70 Gy in the adjuvant and definitive settings respectively. Such doses are strikingly different from the hypofractionated dose schedules commonly employed in pre-clinical studies, though in the palliative setting SBRT is frequently used to treat metastatic foci at high fractional doses. Recent de-escalation studies also showed that doses as low as 30 Gy/15 Fr could be adequate for elective or even definitive nodal irradiation in HPV+ oropharyngeal cancer [116,117]; this could potentially reduce the inhibitory effects of RT in the draining lymph nodes. With regard to the optimal sequencing, ICIs given at different time points relative to RT exploit different, independent mechanisms for their synergistic effects, and may thus require different immunotherapeutic agents at different junctures in time [61].

Another major obstacle to successfully combining RT and ICIs is the lack of a reliable predictive biomarker. PD-L1 expression remains the most widely used biomarker in cancer immunotherapy. Although high PD-L1 expression is independently associated with poorer OS in HNSCC [118], its correlation with the clinical response to ICIs has not been consistently demonstrated in head and neck cancers. For instance, in the KEYNOTE-040 study where pembrolizumab was compared with standard of care in advanced HNSCC in the post-platinum setting, OS was significantly improved with pembrolizumab in those with PD-L1 tumor proportion score of $\geq$ 50% (HR 0.53, $p = 0.0014$) and such survival benefit was not seen if the score was $<$ 50% (HR 0.93, $p = 0.2675$) [9]. In the JAVELIN Head and Neck 100 trial discussed above, subgroup analysis showed that patients with tumors of high PD-L1 expression ($\geq$ 25%) may derive greater PFS benefits from the addition of avelumab (HR 0.59, versus 1.37 for tumors with low PD-L1 expression), though the 95% CI for the HR (0.28 – 1.22) crossed 1 due to the small number of patients [93]. However, in a phase 2 study investigating the role of neoadjuvant nivolumab with or without ipilimumab in untreated oral cavity SCC, PD-L1 expression did not correlate with either volumetric or pathologic response, though the number of $CD4^+$ T cells in pre-treatment samples correlated with pathologic response in the total population ($p = 0.02$) and in those receiving both ICIs ($p = 0.008$) [119]. Similarly, PD-L1 expression has as yet a limited role in predicting the response to ICIs in NPC [4], though the NCI-9742 study showed a trend towards higher ORRs in those with tumors having greater than 1% expression of PD-L1 [120]. Other commonly employed predictive biomarkers include microsatellite instability (MSI)/ deficiency in mismatch repair (dMMR) and tumor mutational burden (TMB), with pembrolizumab being approved by the US Food and Drug Administration in MSI-high (MSI-H)/ dMMR or TMB-high (TMB-H) [$\geq$ 10 mutations/ megabase (mut/Mb)] solid tumors that are unresectable or metastatic. However, several studies showed that the prevalence of MSI-H is $\simeq$1% in HNSCC and NPC [121–123]. Samstein *et al.* reported that $\geq$ 20% of HNSCC patients had a TMB of $\geq$ 10 mut/Mb [124], and although a pre-specified exploratory analysis of the KEYNOTE-158 study showed an ORR of 29% in those with TMB-H tumors ($\geq$ 10 mut/Mb), none of the 102 TMB-H patients in the efficacy population had HNSCC [125]. The optimal TMB cut-off to use in HNSCC or NPC is largely undefined [124], and it is possible that a combination of predictive biomarkers would have to be used to accurately guide treatment decisions [126].

In conclusion, although RT has multiple immunostimulatory effects which could be enhanced with ICIs, there are multiple immunoinhibitory pathways that are upregulated at the same time which limit the success of this promising strategy. Through decades of translational research, we are gaining in-depth understanding of the mechanisms underlying this complex network of intra- and inter-cellular signalling, and of how tumor cells evade the immune response. Researchers are now better equipped than ever to design the next generation of clinical trials, with optimized selection of patients with biomarkers, drug combinations and their sequencing with respect to RT.

CRediT authorship contribution statement

Kenneth C.W. Wong: Conceptualization, Writing – original draft, Writing – review & editing. **David Johnson:** Writing – original draft, Writing – review & editing. **Edwin P. Hui:** Writing – review & editing, Supervision. **Rachel C.T. Lam:** Writing – original draft. **Brigette B.Y. Ma:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition. **Anthony T.C. Chan:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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