

Immunotherapy in head and neck cancer – scientific rationale, current treatment options and future directions

Rothschild Uta^a, Muller Laurent^b, Lechner Axel^{cde}, Schlößer Hans A.^{ef}, Beutner Dirk^g, Läubli Heinz^{hi}, Zippelius Alfred^{hi}, Rothschild Sacha I.^{hi}

^a Division of Otorhinolaryngology, Head and Neck Surgery, Cantonal Hospital Olten, Switzerland

^b Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital, Basel, Switzerland

^c Department of Otorhinolaryngology, Head and Neck Surgery, University of Cologne, Germany

^d Centre for Molecular Medicine Cologne (CMMC), Cologne, Germany

^e Department of Otorhinolaryngology, Head and Neck Surgery, Grosshadern Medical Centre, Ludwig-Maximilians-University of Munich, Germany

^f Department of General, Visceral and Cancer Surgery, University of Cologne, Germany

^g Department of Otorhinolaryngology, Head and Neck Surgery, University Medical Centre Göttingen, Georg August University, Göttingen, Germany

^h University Hospital Basel, Department of Internal Medicine, Medical Oncology, Basel, Switzerland

ⁱ Cancer Immunology, Department of Biomedicine, University of Basel, Switzerland

Summary

Head and neck squamous cell carcinoma (HNSCC) is a frequent tumour arising from multiple anatomical subsites in the head and neck region. The treatment for early-stage disease is generally single modality, either surgery or radiotherapy. The treatment for locally advanced tumours is multimodal. For recurrent/metastatic HNSCC palliative chemotherapy is standard of care. The prognosis is limited and novel treatment approaches are urgently needed. HNSCC evades immune responses through multiple resistance mechanisms. HNSCC is particularly characterised by an immunosuppressive environment which includes the release of immunosuppressive factors, activation, expansion of immune cells with inhibitory activity and decreased tumour immunogenicity. An in-depth understanding of these mechanisms led to rational design of immunotherapeutic approaches and clinical trials. Currently, only immune checkpoint inhibitors, namely monoclonal antibodies targeting the immune inhibitory receptor programmed cell death 1 (PD-1) and its ligand PD-L1 have proven clinical efficacy in randomised phase III trials. The PD-1 inhibitor nivolumab is the only drug approved for platinum-refractory recurrent/metastatic HNSCC. However, many more immunotherapeutic treatment options are currently under investigation. Ongoing trials are investigating immunotherapeutic approaches also in the curative setting and combination therapies using different immunotherapeutic approaches. This review article summarises current knowledge of the role of the immune system in the development and progression of HNSCC, and provides a comprehensive overview on the development of immunotherapeutic approaches.

Key words: head neck cancer, cancer immunotherapy, checkpoint inhibitor, human papilloma virus

Introduction

Head and neck squamous cell carcinoma (HNSCC) is one of the most common malignancies worldwide, accounting for more than 550,000 new cases and 380,000 deaths per year [1, 2]. These tumours arise from different anatomic subsites in the head and neck region, and include nasopharyngeal carcinoma, oral cavity carcinoma, oropharyngeal carcinoma, laryngeal carcinoma and hypopharyngeal carcinoma. Beside the well-established risk factors such as tobacco smoking and alcohol consumption, human papilloma virus (HPV) infection has recently become an important factor in the epidemiology and prognosis of HNSCC, mainly in the oropharyngeal region [3, 4]. Most patients are diagnosed when they have locally advanced disease and are usually treated with multimodal therapy including surgery, radiotherapy and systemic therapy. Therefore, an up-front interdisciplinary approach is a basic principle for these patients. Despite advances in surgical and radiation techniques, as well as the addition of chemotherapy and epidermal growth factor receptor (EGFR)-targeting monoclonal antibodies in the treatment of locally advanced HNSCC, more than half of patients eventually experience a relapse or distant metastases. Most of these patients are no longer suitable for curative treatment approaches. For locally recurrent disease, local treatment options such as surgery and radiotherapy play an important role. For recurrent disease no longer amenable to local therapy or metastatic HNSCC, cytotoxic chemotherapy remains the standard treatment option. Even with modern multi-agent chemotherapy, the expected median survival for a patient with an incurable or metastatic relapse remains under a year, or marginally longer for patients who develop metastases from an HPV-related HNSCC [5, 6]. The addition of an EGFR-targeting monoclonal antibody has been shown to improve overall survival compared with platinum-based

Correspondence:

Sacha I. Rothschild, MD, PhD, University Hospital Basel, Petersgraben 4, CH-4031 Basel, [sacha.rothschild\[at\]usb.ch](mailto:sacha.rothschild[at]usb.ch)

chemotherapy alone by 2.7 months [7]. Although early clinical immunotherapy trials have yielded mixed results with ambiguous clinical benefit [8], cancer immunotherapy is nowadays regarded as an important pillar of anti-cancer treatment. The increasing knowledge as to how the immune system works, in particular with regard to chronic viral infections and cancer, has paved the way for the rational development of novel treatment strategies [9]. This review focuses on the currently approved immunotherapy, as well as promising immunotherapeutic approaches under clinical investigation.

Head and neck cancer and the immune system

In recent years, a key role for the immune system in control of tumour growth and progression has been well established [10, 11]. The lymphocytic infiltrate of tumours is known to be associated with the prognosis of malignant tumours [12–14]. In HNSCC, a high density of tumour-infiltrating lymphocytes (TILs) is associated with improved outcome of patients [15, 16]. A correlation with outcome has mainly been shown for CD8+ lymphocytes [17]. The rate of CD8+ infiltrating effector lymphocytes is higher in HPV-positive cancers, which might at least partially explain their better clinical outcome [17, 18]. Regulatory T cells (Tregs) control autoreactive lymphocytes, but also downregulate the immune response to tumour-associated antigens. Tregs have been regularly described among TILs and in the peripheral circulation in various solid tumours [19–22]. Our own findings support previous findings of increased rate of Tregs in the microenvironment of HNSCC [23–26]. Furthermore, we found an increased rate of Tregs in the peripheral circulation of HNSCC patients compared with healthy controls, suggesting a systemic immunosuppressive environment in HNSCC patients [23]. Dendritic cells play an important role in the initiation of tumour antigen-specific T cell responses and subgroups of dendritic cells have the unique ability to present captured exogenous tumour antigens on major histocompatibility complex class I molecules (“cross-presentation”). Therefore, both naïve CD4+ and naïve CD8+ T cells are activated and differentiated into tumour-antigen-specific effector T cells by mature dendritic cells in lymph nodes [27]. High dendritic cell infiltration in the tumour is significantly correlated with improved outcome in HNSCC patients [28]. Immunological responses in tumours are carefully regulated through expression of both stimulatory and inhibitory signals. Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and programmed death 1 (PD-1) are two membrane proteins expressed by T cells involved in maturation and suppressive functions of TILs [29, 30]. These immune checkpoint receptors are characteristic of exhausted T cells found after long-term antigen exposure [31, 32]. Exhausted T cells are incapable of robust effector responses upon antigen exposure [33, 34]. PD-1 and CTLA-4 are highly expressed in regulatory Tregs of HNSCC tissue compared with peripheral blood, suggesting a suppressive function of Tregs in the tumour tissue [35, 36]. On the other hand, PD-1+ cells were found to be increased and associated with an improved prognosis in the setting of HPV-positive tumours [18, 26, 37]. The PD-1 receptor has two ligands, PD-L1 and PD-L2 [38]. Both receptors are up-regulated under inflammatory conditions, mediated, in part, by type

I and type II interferons. Furthermore, PD-L1 and, to a lesser extent, PD-L2 are expressed on many human solid and haematological tumours [39]. Several recent studies demonstrated that PD-L1 expression is closely correlated to tumour grade and prognosis in patients with various solid malignancies [40–42]. In HNSCC, high PD-L1 expression was associated with metastasis and poor outcome [43]. In a recently published study we provided the first comprehensive analysis of multiple T cell subsets and the expression of clinically relevant immune checkpoint receptors in treatment-naïve HNSCC in comparison with non-cancerous oral mucosa and peripheral blood of patients and healthy donors [23]. Briefly, our study showed a decrease in naïve T cells and an increase in Tregs in the tumour microenvironment. Inhibitory immune checkpoint receptors (CTLA-4, PD-1 and PD-L1) were increased in TILs. PD-L1 expression in HNSCC was described in 66 to 87% of HNSCC primary tumours [35]. Another recent study showed increased expression of the co-stimulatory checkpoint receptor, OX40 [36]. Additional immune checkpoints (e.g., TIM-3 [T cell immunoglobulin mucin receptor 3], LAG3 [lymphocyte-activation gene 3], TIGIT [T cell immunoreceptor with Ig and ITIM domains]) have been described and their targeting is being tested in early phase clinical trials.

Differences in the immune infiltrate of HPV-positive and HPV-negative tumours have been described. HPV-positive tumours are characterised by an adaptive host immune response directed against viral antigens, as well as specific T cells directed against viral proteins [44–46]. Moreover, the tumour microenvironment of HPV-positive tumours has been shown to contain a higher number of CD8+ T cells and a decreased CD4/CD8 ratio [18, 46]. We and others described higher PD-1 expression in TILs of HPV-positive tumours, probably reflecting a strong immune response induced by HPV [18, 23]. However, another study showed high PD-1 levels in HPV-negative HNSCC [47]. In our study we found a trend towards a higher number of tumour-infiltrating Tregs in HPV-negative tumours, potentially corresponding to a more immunosuppressive tumour microenvironment in HPV-negative tumours [23]. With regard to expression of PD-L1 there are conflicting data concerning its correlation to HPV status [48–50]

Vaccines

The basic principles of therapeutic cancer vaccines are, briefly:

1. Peptide/protein-based vaccines are produced by combining one or more peptides or proteins commonly expressed in the specific tumour with an adjuvant. In response to the adjuvant the immune system will also respond to tumour cells that express the respective antigens.
2. DNA/RNA-based vaccines use exogenously manipulated nucleic acids expressing a tumour-specific antigen when injected. This antigen is processed by antigen-presenting cells, inducing a specific immune response toward tumour cells expressing the same antigen.
3. Attenuated bacteria and viral vectors can serve as vectors to deliver proteins of interest or plasmids encoding genes in vaccines [51, 52].

- For dendritic cell vaccines, dendritic cells are isolated from the blood of cancer patients by leucapheresis and stimulated with a tumour antigen. The cells are reinjected and are supposed to activate tumour-specific T cells.

Though the potential of vaccines for therapeutic efficacy has been extensively demonstrated in tumour models, clinical trials with vaccines have failed in melanoma and non-small-cell lung cancer [9]. Therefore, vaccination approaches are currently tested in combination with other immunotherapeutic approaches.

Peptide/protein-based vaccines

Although the incidence of alcohol- and smoking-induced HNSCC is steadily declining, the incidence of HPV-associated oropharyngeal carcinoma has rapidly increased during the last decades in developed countries worldwide [53]. Approximately 90% of these cases are caused by HPV16 and the remainder by other oncogenic HPV types [53, 54]. It is estimated that the number of HPV-positive HNSCCs will surpass the annual number of cervical cancers diagnosed within the US by the year 2020 [53]. Randomised trials of prophylactic HPV vaccines have demonstrated effective prevention of high-grade cervical lesions associated with HPV16 and HPV18 [55]. Therefore, prophylactic HPV vaccination with the bivalent (HPV16/18) or quadrivalent (HPV16/18/6/11) vaccine is currently recommended for young females to prevent genital warts, and cervical, anal, vulvar and vaginal precancerous lesions worldwide, including in Switzerland [56]. Further randomised trials also showed significantly reduced HPV-associated anogenital lesions in male [57]. This led to the approval of prophylactic vaccination also for boys and young males [56]. Very recently, a cross-sectional study showed that HPV vaccination significantly reduced the prevalence of oral HPV16/18/6/11 infections (0.11 vs 1.61%, $p = 0.008$) [58]. This corresponds to an estimated 88.2% reduction in prevalence after model adjustment for age, gender and race. The effect was more pronounced in males (prevalence 0.0 vs 2.13%, $p = 0.007$). Despite the effectiveness of prophylactic HPV vaccination, widespread general adoption and administration of HPV vaccination is poor [59].

The cyclin-dependent kinase inhibitor p16(INK4a) is strongly and consistently overexpressed in HPV-associated cancers. A recently published study investigated a p16(INK4a)-based peptide vaccine in patients with advanced HPV-positive HNSCC [60]. A total of 20 patients received at least four injections and were evaluated for immune responses. CD4+ T cells were detected in 14 of 20 patients, CD8+ T cells were detected in 5 of 20 patients, and antibodies were detected in 14 of 20 patients. Vaccination was safe with low toxicity rates. Tumour response could be assessed in 14 patients. Of these, nine patients (64%) had stable disease as their best overall response. In the VICORYX-2 trial this vaccination is being investigated in combination with cisplatin-based chemotherapy in patients with advanced HPV-positive cervical, vulvar, vaginal, penile, anal or head and neck cancer (NCT02526316). A study with an immunomodulatory peptide vaccine for HPV-positive and melanoma antigen A3-positive tumours elicited antigen-specific T cells and antibody responses to the respective vaccines, but lacked clinical efficacy [61].

In another phase I/II study, the safety profile of AlloVax, a vaccine composed of a patient-specific tumour antigen derived from chaperone-rich tumour cell lysate is being investigated in patients with recurrent/metastatic HNSCC (NCT01998542). Recently, personalised neoantigen vaccines have been developed and investigated [62]. This approach is based on massively parallel sequencing for detection of all coding mutations within tumours, and of machine learning approaches to reliably predict which mutated peptides have high-affinity binding of autologous human leukocyte antigen (HLA) molecules.

DNA/RNA-based vaccines

Several attempts to use therapeutic HPV vaccines have been made. MEDI0457 (previously called INO-3112) is a DNA-based cancer vaccine with two main components: VGX-3100, a DNA plasmid containing modified sequences for E6 and E7, and INO-9012, a DNA plasmid containing the immune activator interleukin-12. Preliminary results from a phase I/II trial demonstrated that this DNA-based immunotherapy can safely generate HPV-specific CD8 T cell immunity in patients with locally advanced HPV-related HNSCC [63]. All tested patients had positive cellular immune responses in at least one assay. In an ongoing phase I/II study MEDI0457 is being investigated in combination with the anti-PD-L1 antibody durvalumab in patients with HPV-positive recurrent/metastatic HNSCC (NCT03162224).

Recently, a phase II trial with a multi-peptide vaccine for three cancer-testis-antigens (LY6K, CDCA1 and IMP3) showed a significant survival benefit (median overall survival 4.9 vs 3.5 months, $p < 0.05$) compared with an untreated cohort in recurrent/metastatic HNSCC [64].

Attenuated bacteria and viral vectors

ADXS11-001 is a live attenuated *Listeria monocytogenes* (Lm)-listeriolysin O (LLO) immunotherapy bioengineered to secrete an HPV-E7 tumour antigen as a truncated LLO-E7 fusion protein in cells capable of presenting antigen. An ongoing study includes HPV-positive oropharyngeal cancer patients prior to surgery (NCT02002182). A currently ongoing phase I/II trial is investigating the combination of ADXS11-001 with durvalumab and comparing efficacy with durvalumab alone (NCT02291055). DPX-E7 is a HPV16-E711-19 nanomer vaccine currently investigated in HPV-positive cancers (NCT02865135)

TG4001 is based on the modified vaccinia virus Ankara that carries and expresses mutation-inactivated HPV16 E6 and E7 oncoproteins. A phase Ib/II study is investigating this vaccine in combination with the anti-PD-L1 antibody avelumab (NCT03260023).

Dendritic cell vaccines

HPV-targeting dendritic cell vaccines have been investigated in cervical cancer. Although HPV-specific cytotoxic T lymphocytes could be measured, no clinical responses were observed [65, 66]. In preclinical models, the activity of dendritic cell vaccines has been improved by using a recombinant adenovirus expressing codon-optimised HPV E6/E7 fusion proteins [67]. A dendritic cell-based vaccination against p53 was tested in a phase I trial in patients with resected HNSCC [68]. Monocyte-derived dendritic cells from 16 patients were loaded with two modified HLA-

class I p53 peptides and were injected into inguinal lymph nodes. The frequencies of p53-specific T cells were increased in 11 of 16 patients (69%), with interferon- γ secretion detected in 4 of 16 patients. Moreover, the rate of Tregs was decreased after vaccination.

In conclusion, the currently most successful vaccination strategy is preventive vaccination for HPV. Therapeutic vaccines for HPV-associated HNSCC has been investigated using different approaches. Although immune responses have been seen in most studies, these vaccines are currently not effective enough for clinical use. However, their combination with other immunotherapeutic strategies, namely immune checkpoint inhibitors might be more promising.

Cytokines

Interleukin-2 is one of the major proinflammatory cytokines produced by T cells. It enhances the proliferation and cytotoxic response of activated T cells [69]. Early trials using interleukin-2 in HNSCC patients demonstrated increased levels of cytokines, intratumoural levels of natural killer cells and activity of TILs [70, 71]. Perilymphatic injection of interleukin-2 significantly prolonged disease-free survival and overall survival of HNSCC patients and was well tolerated [72]. However, an earlier trial investigating combination therapy with interferon- α 2a and interleukin-2 showed substantial toxicity [73]. Although the response rate of 18% was encouraging, this concept is no longer pursued.

Furthermore, the use of interferon- γ demonstrated immunological and some level of clinical response [74]. Intratumoural injection of interleukin-12 led to increased infiltration of B cells into the tumour and higher levels of interferon- γ . A shift in the plasma antibody profile towards a Th1 phenotype and a redistribution of lymphocytes, monocytes and natural killer cells from the peripheral blood to lymph nodes was detected [75, 76]. IRX-2 is a cell-derived mixture of cytokines including interleukins-1 β , -2, -6 and -8, interferon- γ , tumour necrosis factor- α , and granulocyte and granulocyte-macrophage colony stimulating factors (G-CSF and GM-CSF). In preclinical models IRX-2 treatment of human monocyte-derived dendritic cells resulted in morphologic, phenotypic and functional changes consistent with the development of mature activated dendritic cells leading to increased ability to stimulate T cells and increased interleukin-12 production [77, 78]. Furthermore, IRX-2 reduced intratumoural levels of immunosuppressive cytokines (interleukin-10, transforming growth factor- β) while increasing levels of interferon- γ [78]. In an early clinical trial, IRX-2 was injected peritumourally in 27 patients prior to surgery. During a 21-day neoadjuvant regimen, subcutaneous immunotherapy injections were administered over 10 days (Monday to Friday over a 2-week period) as two bilateral injections of 115 U each in the mastoid region of the left and right neck in close proximity to the regional nodal basins. The regimen also included a single infusion of a non-cytotoxic dose of cyclophosphamide (300 mg/m²) on day 1, and daily oral indomethacin (25 mg three times daily) and zinc gluconate (24 mg once daily). The neoadjuvant immunotherapy regimen was well tolerated with minimal toxicity [79]. Overall, 45.8% of patients showed a decrease in tumour size. Overall survival was

92, 73 and 69% at 12, 24 and 36 months, respectively. Compared with the initial biopsy, tumour resection specimens showed an increased level of TILs. CD3+ CD4+ T cells and CD20+ B cells were primarily found in the peritumoural stroma and CD3+ CD8+ T cells and CD68+ macrophages intratumourally. High frequencies of TILs were associated with good clinical outcome. Therapy also led to changes in the peripheral blood lymphocyte subsets, with a decrease in B cells, natural killer cells and naïve T cells [80]. The INSPIRE trial (NCT02609386) is a randomised phase II trial investigating the neoadjuvant IRX-2 regimen in patients with stage II to IVA resectable squamous cell carcinoma of the oral cavity and is currently recruiting patients also at two sites in Switzerland.

In conclusion, cytokine immunotherapeutic strategies have shown some encouraging results in HNSCC patients. Systemic use is associated with substantial toxicity. However, local injection into the tumour or the regional nodal basin might be a promising alternative and is currently being investigated in ongoing studies. Targeted cytokine therapy with chimeric constructs that show tumour specificity are potentially less toxic, since lower doses of the cytokines are needed. This approach is currently being tested in early phase clinical trials.

Toll-like receptors

Toll-like receptors (TLRs) are a subclass of pattern-recognition receptors that recognise pathogen-associated molecules and have emerged as key mediators of immune functions. TLRs are usually expressed on immune cells and trigger local inflammation in response to binding particular molecules from pathogens (bacteria, viruses) such as lipopolysaccharide and double-strand RNA. This mediates protective function, but under certain circumstances this may promote tumour progression by induction of cell proliferation, activation of the NF- κ B signalling pathway and resistance to natural killer cells [81] (NF- κ B = nuclear factor “kappa-light-chain-enhancer” of activated B-cells).

Agonists of TLRs have profound immune stimulatory effects in preclinical models. Motolimod (VTX-2337) is a small molecule TLR-8 agonist that activates monocytes, dendritic cells and natural killer cells [82]. In a phase Ib study in patients with HNSCC, motolimod was combined with cetuximab and demonstrated an overall response rate of 17% and a disease control rate of 50% [83]. These data and preclinical work showing synergistic activity of motolimod with platinum and fluorouracil led to the design of a randomised phase II trial investigating the additional benefit of motolimod together with the EXTREME regimen (NCT01836029).

The TLR-9 agonist EMD1201081 has been compared with cetuximab in a randomised phase II trial involving recurrent/metastatic HNSCC patients after failure of one cytotoxic regimen [84]. There was no incremental clinical efficacy of the TLR-9 agonist. Another trial with the same TLR-9 agonist in combination with cisplatin was stopped early because of safety concerns (NCT01360827).

Cell-based therapies

Cell-based therapies include adoptive cell transfer and chimeric antigen receptor (CAR) T cells. Adoptive cell transfer involves the expansion of autologous antigen-spe-

cific cytotoxic T lymphocytes *ex vivo* and the option to genetically modify these cells prior to reinfusion. CAR T cells are used to induce a stronger antitumour T cell effect. This novel technique involves the use of naïve T cells isolated from patients' peripheral blood. CARs are engineered membrane proteins composed of three parts: an extracellular antigen-recognition region, a hinge and transmembrane region, and an intracellular T cell activation domain [85]. The antigen-recognition part consists of a single-chain variable fragment derived from hypervariable regions of an antibody's immunoglobulin heavy and light chains. This fragment has a higher affinity for tumour-associated antigens than the classical T cell receptor. This fragment is linked to the intracellular signalling region that is usually derived from the cytoplasmic domain of the CD3 ζ chain. Furthermore, co-stimulatory molecules are linked between the single-chain variable fragment and the CD3 ζ chain to improve T cell activation. In CAR therapy, CARs are overexpressed in patient-derived T cells using a retroviral or lentiviral vector.

Adoptive cell transfer

In an initial study, E6 and E7 reactive cytotoxic T lymphocytes were used in nine patients with HPV-associated cervical cancer, leading to complete remission in two of them [86]. More recently, genetically modified cytotoxic T lymphocytes expressing receptors against an HPV E6 and E7 epitope were shown to kill HPV-positive cells from cervical and head and neck cancer cell lines [87]. This model was tested in a phase I/II trial in 12 patients with HPV-associated epithelial cancer (1 patient with oropharyngeal carcinoma). E6-receptor T cell therapy was safe and led to tumour regression in two patients with anal cancer [88]. In another trial, patients with recurrent HNSCC received adoptive therapy with autologous peripheral blood mononuclear leukocytes that had been opsonised during culture with catumaxomab, an antibody that with one arm binds epithelial cell adhesion molecule on tumours and with the other arm binds CD3 $^{+}$ T cells [89]. This approach showed significant toxicity with a high cell dose, but was well tolerated and led to clinical response with lower numbers of CD3 $^{+}$ cells. In a combination of active and adoptive immunisation, HNSCC patients were vaccinated with irradiated autologous tumour plus GM-CSF and then received adoptive transfer of their *in vitro* expanded lymph node cells, including CD4 $^{+}$ and CD8 $^{+}$ cells [90]. This led to tumour response in 5 out of 17 patients with limited toxicity. In a trial in patients with surgically resected HNSCC, autologous tumour cells antigenically modified by infection with Newcastle disease virus were used in combination with interleukin-2 and compared with interleukin-2 treatment alone [91]. Vaccination plus interleukin-2 increased levels of reactive T cells and prolonged overall survival.

CAR T cells

In HNSCC, CARs targeting latent membrane protein (LMP)1-HELA/CAR in nasopharyngeal carcinoma [92] or chondroitin sulphate proteoglycan-4 [93] induced an antitumour effect *in vitro* and *in vivo*. In a preclinical model, HER-2-specific CAR T cells were investigated after pretreatment with an oncolytic adenovirus that delivered a construct encoding the PD-L1 blocking antibody and inter-

leukin-12p70 with a high response rate in a xenograft model [94]. There is currently an ongoing phase I trial investigating an ErbB-specific CAR [95] (NCT01818323).

Immune checkpoint inhibitors

A broad range of immune checkpoint receptors have recently been characterised and can be targeted by monoclonal antibodies [96]. So far, mainly antibodies targeting inhibitory checkpoint receptors have been investigated in clinical trials. These drugs re-activate the antitumoural immune response and have recently shown impressive results in different solid tumours, such as melanoma, non-small cell lung cancer, and renal cell carcinoma [97–99]. Unlike conventional tumour cell-directed antibodies or chemotherapeutic agents, immune checkpoint inhibitors do not target cancer cells directly, but bind receptors or their ligands on immune cells and therefore modulate their activity. Interestingly, these drugs might induce long-lasting remissions in advanced and metastatic solid tumours even after discontinuation of therapy [100].

The role of CTLA-4 inhibitors is not yet established in HNSCC. However, in a recent study it was demonstrated that cetuximab increased CTLA-4 expression in the majority of intratumoural Tregs and it was also shown that the CTLA-4 inhibitor ipilimumab decreased suppression of natural killer cells from Tregs [101]. Ipilimumab and another CTLA-4-targeting monoclonal antibody, tremelimumab, are currently being investigated in combination with cetuximab and radiotherapy in patients with advanced HNSCC.

Metastatic disease

Nivolumab and pembrolizumab are anti-PD-1 antibodies. So far, the only fully published randomised phase III trial (CheckMate 141) compared nivolumab with standard of care therapy in patients with recurrent/metastatic HNSCC refractory to platinum therapy [102]. A total of 361 patients with disease progression or recurrence within 6 months after the last dose of platinum-based chemotherapy – either in the curative setting combined with radiotherapy or the palliative setting as part of multiagent chemotherapy – were randomised in a 2:1 manner between nivolumab 3 mg/kg every 2 weeks and single agent chemotherapy at the investigator's discretion (methotrexate, docetaxel or cetuximab). After an interim analysis, the trial was stopped early and patients in the chemotherapy arm were allowed to cross over to nivolumab, on the recommendation of the independent data monitoring committee. More than half of the patients had two or more previous therapy lines. At the final analysis, nivolumab significantly prolonged overall survival (7.5 vs 5.1 months; hazard ratio 0.70, $p = 0.0101$), whereas progression-free survival was not different. Survival rates after one year were more than doubled with nivolumab (16.6 vs 36%). Survival benefit was independent of HPV status but seemed to be stronger in p16 positive patients. The effect of nivolumab on overall survival was more pronounced in PD-L1 positive patients. The study included extensive quality of life analysis and patient-reported outcomes [103]. Nivolumab stabilised symptoms, whereas investigator's choice therapy led to clinically meaningful deterioration. Furthermore, nivolum-

ab delayed time to deterioration of patient-reported outcomes compared with chemotherapy.

Pembrolizumab was approved for HNSCC in the US on the basis of the Keynote-012 study [104]. In this phase Ib study, 60 patients with recurrent/metastatic HNSCC, irrespective of previous therapy, with a PD-L1 expression of at least 1% of tumour cells were treated with pembrolizumab 10 mg/kg every two weeks. The majority of patients were heavily pretreated with two or more previous treatment lines. Half of the patients experienced reduction of tumour burden with an overall response rate based on Response Evaluation Criteria In Solid Tumours (RECIST) criteria of 18%. Median duration of response was 53 weeks. progression-free and overall survival were 2 and 13 months, respectively. PD-L1 positivity was assessed using two different methods: (1) the combined positive score, defined as $\geq 1\%$ of expression in both tumour and mononuclear inflammatory cells and (2) the tumour proportion score defined as $\geq 1\%$ of expression only in tumour cells. The study showed a relation between PD-L1 positivity and efficacy only with the combined positive score. HPV status determined by p16 immunohistochemistry was not predictive for response to pembrolizumab. The authors reported on six interferon- γ -related genes associated with tumour response. In an expansion cohort of Keynote-012, the results were confirmed in a total of 132 patients with an overall response rate of 18%, and progression-free and overall survival of 2 and 8 months, respectively [105]. In a recently presented analysis of the Keynote-012 study, patients with a T cell inflamed gene expression profiling showed a higher response rate irrespective of their HPV status [106]. A recent study analysed the gene expression profile in the tumour microenvironment, using RNA from different solid tumours treated with pembrolizumab [107]. An 18-gene T cell inflamed gene expression profile was predictive of response to pembrolizumab independently of PD-L1 expression. In a follow-up phase II trial (Keynote-055), 171 patients refractory to platinum and cetuximab (disease progression within 6 months of therapy) were treated with pembrolizumab 200 mg every 3 weeks [108]. Seventy-five percent of patients received two or more prior lines of therapy for metastatic disease. Overall response rate was 16%, with a median duration of response of 8 months; 75% of responses were ongoing at the time of analysis. Response rates were similar in all HPV and PD-L1 subgroups. Median progression-free survival was 2.1 months, and median overall survival was 8 months. Keynote-040 was a randomised phase 3 trial investigating pembrolizumab in patients with recurrent/metastatic HNSCC after platinum-based first-line chemotherapy [109]. A total of 495 patients were randomised in a 1:1 manner to either pembrolizumab 200 mg every 3 weeks or treatment of the

investigator's choice (methotrexate, docetaxel, or cetuximab). Pembrolizumab prolonged overall survival, but the difference did not achieve significance (8.4 vs 7.1 months; hazard ratio 0.81; 95% confidence interval 0.66–0.99; $p = 0.0204$). There was no difference in progression-free survival. Among patients with a PD-L1 combined positive score of at least 1%, median overall survival was 8.7 months with pembrolizumab versus 7.1 months with standard therapy (hazard ratio 0.75; $p = 0.0078$), and among patients with combined positive score expression in more than 50% of their cancer cells, median overall survival was 11.6 versus 7.9 months, respectively (hazard ratio 0.54; $p = 0.0017$).

Durvalumab is a monoclonal antibody against PD-L1. In the 1108 study, 62 patients with recurrent/metastatic HNSCC and with at least one prior therapy received durvalumab monotherapy [110]. Overall response rate was 11.3%, with six of seven patients showing a response duration of more than 1 year. The one-year survival rate was reported to be 42%.

Based on these results, PD-1 inhibitors can be considered a new standard of care in the second-line treatment of patients with recurrent/metastatic HNSCC. Published results for this setting are summarised in [table 1](#). Randomised trials are currently investigating the role of these drugs in the first-line setting. KEYNOTE-048 (NCT02358031) is a randomised study with three arms. In the standard treatment arm patients receive chemotherapy (platinum and 5-fluorouracil) in combination with cetuximab based on the EXTREME protocol [7]. In the two experimental arms patients either receive pembrolizumab in combination with platinum plus 5-fluorouracil or pembrolizumab alone. Pembrolizumab is given at a dose of 200 mg every 3 weeks for up to 24 months. The primary endpoint of the study is progression-free and overall survival. The study enrolled 825 patients and recruitment is currently closed. So far, no results have been presented. CheckMate 651 (NCT02741570) is another randomised trial investigating the combination of nivolumab and ipilimumab compared to standard chemotherapy (platinum + 5-fluorouracil) combined with cetuximab. The study is currently recruiting, with a target enrollment of 700 patients. Primary endpoint of the trial is overall and progression-free survival. In the Kestrel study (NCT02551159), with a similar design, more than 800 patients will be randomised to either the EXTREME regimen, durvalumab monotherapy, or durvalumab in combination with the anti-CTLA-4 antibody tremelimumab. [Table 2](#) provides an overview on ongoing randomised phase III trials.

Combination therapies including different immune checkpoint inhibitors have recently been shown to improve response rates and prognosis compared with PD-1/PD-L1

Table 1: Immune checkpoint inhibitors for recurrent/metastatic head and neck squamous cell carcinoma.

Study	Phase	Drug	No. patients	ORR (%)	Median PFS (months)	Median OS (months)
CheckMate 141 [102]	III	Nivolumab SOC	240	13.3%	2.0	7.5
			121	5.8%	2.3	5.1
Keynote-012 [104]	Ib	Pembrolizumab	60	18%	2	13
Keynote-012 [105]	Ib expansion	Pembrolizumab	132	18%	2	8
Keynote-055 [108]	II	Pembrolizumab	171	16%	2.1	8
Keynote-040 [109]	III	Pembrolizumab SOC	247	14.6%	2.1	8.4
			248	10.1%	2.3	7.1
1108 [110]	Ib	Durvalumab	62	11.3%	NR	42% after 1 year

ORR = overall response rate; OS = overall survival; PFS = progression-free survival; SOC = standard of care (methotrexate, docetaxel or cetuximab)

monotherapy in patients with various solid tumours. Recently, first clinical study data for immune checkpoint combinations have been presented for patients with HNSCC. Epcadostat is an oral inhibitor of indoleamine-2,3-dioxygenase 1, an intracellular enzyme that initiates the first and rate-limiting step of tryptophan degradation in the kynurenine pathway [111]. Indoleamine-dioxygenase supports inflammation in the tumour microenvironment, development of immune tolerance in stromal and immune cells, suppression of T and natural killer cells, generation and activation of Tregs and myeloid-derived suppressor cells, and promotion of tumour angiogenesis [112]. In a recently presented phase II trial (ECHO-202/Keynote-037), 38 patients with recurrent/metastatic HNSCC after failure of at least one prior chemotherapy regimen including a platinum agent were treated with epcadostat 100 mg daily and pembrolizumab 200 mg every 3 weeks [113]. The combination therapy led to a disease response in 34% of patients and a disease control rate of 39% with most of the responses ongoing at the time point of analysis. Responses were seen independently from HPV and PD-L1 status. Toxicities of grade 3 or 4 were seen in 18% of patients, including one patient with pneumonitis. One patient died as a result of aspiration pneumonia. However, pneumonitis could not be ruled out. PD-1/PD-L1 and CTLA-4 inhibition showed synergistic activity in various tumours [114, 115].

Monalizumab (previously IPH2201) is a IgG4 antibody targeting NKG2A, an inhibitory receptor expressed on tumour infiltrating cytotoxic natural killer and CD8 T lymphocytes. In a phase Ib/II study, monalizumab was tested in combination with cetuximab in recurrent/metastatic HNSCC. In a preliminary report of the phase Ib part of the trial, combination therapy showed a good safety profile in 17 patients [116].

Localised and locally advanced disease

The current standard of care for patients with locally advanced HNSCC is tumour resection followed by radiotherapy or platinum-based chemoradiotherapy (CRT). Organ preservation by means of definitive radiotherapy in combination with platinum chemotherapy or cetuximab is a well-established alternative. Recently, a study investigating the combination of pembrolizumab with definitive CRT was presented [117]. In this trial, 27 patients with stage III-IVB HNSCC of the oro- or hypopharynx, or larynx, irrespective of HPV status, were treated with pembrolizumab at a fixed dose of 200 mg 4 to 7 days prior to initiation of CRT and then every 3 weeks during CRT (two concomitant doses) and then after CRT for five additional doses.

CRT consisted of six doses weekly cisplatin 40 mg/m² given concurrently with radiation at a dose of 2 Gy once daily for 35 fractions (cumulative dose 70 Gy). At the time of the presented interim analysis, 21 patients (78%) had received all planned doses of pembrolizumab. Three patients discontinued therapy because of immune-related adverse events and three patients for protocol reasons. All patients completed the full dose of radiotherapy without significant delays (defined as >5 days). Twenty-three patients (85%) received the goal target dose of cisplatin (≥200 mg/m²). At day 150 after the initiation of CRT, 21 patients (78%) had a complete response, 4 patients had a partial response and 1 patient developed disease progression. The study (NCT02586207) is currently recruiting patients and further efficacy analyses are expected. Another trial included patients with stage III or IV HPV-negative HNSCC. Patients were treated with one dose of pembrolizumab 200 mg prior to tumour resection [118]. Patients with high-risk pathological features (positive resection margin and/or extracapsular extension in lymph node metastasis) were given postoperative cisplatin and radiotherapy followed by pembrolizumab. At the time of the first report of this study, 21 patients were enrolled. Importantly, this study did not show any serious study-drug related adverse event, unexpected surgical delay, or postoperative complications. None of the 14 patients with a follow-up of at least 1 year experienced loco-regional recurrence or disease specific death. Forty-two percent of patients experienced a pathological treatment response to neoadjuvant pembrolizumab, defined as tumour necrosis and/or a giant cell or histiocytic reaction to keratinous debris in >10% of the tumour area. The authors reported a significant correlation between baseline PD-L1 expression on tumour cells and pathological treatment effect in the tumour. Currently, several ongoing clinical studies are investigating immune checkpoint inhibitors in the setting of locally advanced HNSCC. In Switzerland, JAVELIN Head and Neck 100 (NCT02952586) is currently recruiting patients. This phase III randomised, placebo-controlled study evaluates the safety and antitumour activity of the anti-PD-L1 antibody avelumab in combination with standard of care CRT versus CRT alone.

Other immune checkpoint proteins that can be expressed on T cells include BTLA (B- and T-lymphocyte attenuator), LAG3 and TIM3. Antibodies that block the binding of these proteins to their ligands are being developed and tested in clinical trials.

Table 2: Ongoing phase III trials with immune checkpoint inhibitors.

Study	NCT number	Setting	Treatment	No. patients
CheckMate 651	NCT02741570	1st-line	Nivolumab + ipilimumab vs EXTREME regimen	700
KEYNOTE-048	NCT02358031	1st-line	Pembrolizumab vs EXTREME regimen + pembrolizumab vs EXTREME regimen	825
Kestrel	NCT02551159	1st-line	Durvalumab vs durvalumab + tremelimumab vs EXTREME regimen	823
Eagle	NCT02369874	2nd-line	Durvalumab vs durvalumab + tremelimumab vs SOC	736

EXTREME regimen = cisplatin + 5-fluorouracil + cetuximab; SOC = standard of care (methotrexate, docetaxel or cetuximab)

Immune checkpoint activators

Compared with the inhibitory checkpoint receptor antibodies, an immunostimulatory effect is induced through the activation of member of the tumour necrosis factor-receptor superfamily on T cells [119]. The stimulatory checkpoint receptors and their ligands most investigated so far are OX40 (OX40-ligand), CD137 (4-1BB ligand), and CD27 (CD70). OX40 is expressed on activated T cell. Stimulation of OX40 by activating antibodies suppresses T cell apoptosis and induces production of immunostimulatory cytokines. An ongoing clinical trial is investigating the OX40 agonist MEDI6469 in a neoadjuvant setting in HNSCC patients (NCT02274155). Other OX40 agonists (MEDI0562, PF-04518600) are currently in phase I trials in different solid tumours. Urelumab is an agonistic antibody towards CD137 that is expressed on the surface of different immune cells including natural killer cells. In a phase I study urelumab is being investigated in combination with cetuximab in patients with HNSCC and colorectal carcinoma (NCT02110082). The study has terminated accrual but no results have been reported so far. In a pre-clinical study, it was shown that urelumab enhanced cetuximab-activated natural killer cell survival, dendritic cell maturation and tumour antigen cross-presentation [120]. Varlilumab is a CD27 agonist and is currently being investigated in combination with nivolumab (NCT02335918), as well as with atezolizumab (NCT02543645). The JAVELIN Medley trial (NCT02554812) is a phase Ib/II trial investigating the inhibitory antibody against PD-L1 avelumab in combination with different other cancer immunotherapies including PF-04518600 and the anti-4-1BB antibody utomilumab.

Conclusions

Introduction of novel technologies to assess the tumour microenvironment has led to a profound understanding of immunosuppressive mechanisms in HNSCC tissue. Immune checkpoint inhibitors targeting the PD-1/PD-L1 axis represent a new standard of care for platinum-refractory recurrent/metastatic HNSCC patients. Ongoing clinical studies are investigating their role in the first-line palliative as well as in the curative setting. For future development it will be crucial to identify markers predictive of immunotherapy response to better characterise patients who will eventually benefit from these drugs.

Prophylactic HPV vaccination showed significant reduction of oral HPV infections and seems to be an effective strategy in the prevention of HPV-related HNSCC, which has recently shown rising incidence. Therapeutic vaccination and cell-based therapies have shown interesting response rates in early clinical trials and are currently being further investigated in different settings in HNSCC patients. Combination of different immunotherapeutic approaches could affect different phases of immune response, eventually leading to an improved response and outcome compared with monotherapy. Rational treatment combination strategies are currently under investigation and might further improve the outcome of HNSCC patients.

Financial disclosure

HL: research support: BMS, Palleon Pharmaceuticals; honoraria (to the institution): BMS, MSD. AZ: research support: Roche, BMS, Seccarna, Beyondsprings; honoraria: BMS, MSD. SIR: research support:

AstraZeneca, BMS, Merck; honoraria for advisory boards (to the institution): AstraZeneca, BMS, Merck, MSD, Novartis, Pfizer, Roche.

Potential competing interests

No other potential conflict of interest relevant to this article was reported.

References

- Vos T, Abajobir AA, Abate KH, Abbafati C, Abbas KM, Abd-Allah F, et al., GBD 2016 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet*. 2017;390(10100):1211–59. doi: [http://dx.doi.org/10.1016/S0140-6736\(17\)32154-2](http://dx.doi.org/10.1016/S0140-6736(17)32154-2). PubMed.
- Naghavi M, Abajobir AA, Abbafati C, Abbas KM, Abd-Allah F, Abera SF, et al., GBD 2016 Causes of Death Collaborators. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet*. 2017;390(10100):1151–210. doi: [http://dx.doi.org/10.1016/S0140-6736\(17\)32152-9](http://dx.doi.org/10.1016/S0140-6736(17)32152-9). PubMed.
- Gillison ML, Chaturvedi AK, Anderson WF, Fakhry C. Epidemiology of Human Papillomavirus-Positive Head and Neck Squamous Cell Carcinoma. *J Clin Oncol*. 2015;33(29):3235–42. doi: <http://dx.doi.org/10.1200/JCO.2015.61.6995>. PubMed.
- Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tân PF, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med*. 2010;363(1):24–35. doi: <http://dx.doi.org/10.1056/NEJMoa0912217>. PubMed.
- Argiris A, Li S, Ghebremichael M, Egloff AM, Wang L, Forastiere AA, et al. Prognostic significance of human papillomavirus in recurrent or metastatic head and neck cancer: an analysis of Eastern Cooperative Oncology Group trials. *Ann Oncol*. 2014;25(7):1410–6. doi: <http://dx.doi.org/10.1093/annonc/mdu167>. PubMed.
- Fakhry C, Zhang Q, Nguyen-Tan PF, Rosenthal D, El-Naggar A, Garden AS, et al. Human papillomavirus and overall survival after progression of oropharyngeal squamous cell carcinoma. *J Clin Oncol*. 2014;32(30):3365–73. doi: <http://dx.doi.org/10.1200/JCO.2014.55.1937>. PubMed.
- Vermorken JB, Mesia R, Rivera F, Remenar E, Kawecki A, Rottey S, et al. Platinum-based chemotherapy plus cetuximab in head and neck cancer. *N Engl J Med*. 2008;359(11):1116–27. doi: <http://dx.doi.org/10.1056/NEJMoa0802656>. PubMed.
- Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. *Nat Med*. 2004;10(9):909–15. doi: <http://dx.doi.org/10.1038/nm1100>. PubMed.
- Rothschild SI, Thommen DS, Moersig W, Müller P, Zippelius A. Cancer immunology - development of novel anticancer therapies. *Swiss Med Wkly*. 2015;145:w14066. doi: <https://doi.org/10.4414/smw.2015.14066>.
- Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646–74. doi: <http://dx.doi.org/10.1016/j.cell.2011.02.013>. PubMed.
- Vesely MD, Kershaw MH, Schreiber RD, Smyth MJ. Natural innate and adaptive immunity to cancer. *Annu Rev Immunol*. 2011;29(1):235–71. doi: <http://dx.doi.org/10.1146/annurev-immunol-031210-101324>. PubMed.
- Gooden MJM, de Bock GH, Leffers N, Daemen T, Nijman HW. The prognostic influence of tumour-infiltrating lymphocytes in cancer: a systematic review with meta-analysis. *Br J Cancer*. 2011;105(1):93–103. doi: <http://dx.doi.org/10.1038/bjc.2011.189>. PubMed.
- Ogino S, Nosho K, Irahara N, Meyerhardt JA, Baba Y, Shima K, et al. Lymphocytic reaction to colorectal cancer is associated with longer survival, independent of lymph node count, microsatellite instability, and CpG island methylator phenotype. *Clin Cancer Res*. 2009;15(20):6412–20. doi: <http://dx.doi.org/10.1158/1078-0432.CCR-09-1438>. PubMed.
- de Miranda NFCC, Goudkade D, Jordanova ES, Tops CMJ, Hes FJ, Vasen HFA, et al. Infiltration of Lynch colorectal cancers by activated immune cells associates with early staging of the primary tumor and absence of lymph node metastases. *Clin Cancer Res*. 2012;18(5):1237–45. doi: <http://dx.doi.org/10.1158/1078-0432.CCR-11-1997>. PubMed.
- Balermas P, Michel Y, Wagenblast J, Seitz O, Weiss C, Rödel F, et al. Tumour-infiltrating lymphocytes predict response to definitive chemoradiotherapy in head and neck cancer. *Br J Cancer*. 2014;110(2):501–9. doi: <http://dx.doi.org/10.1038/bjc.2013.640>. PubMed.
- Ward MJ, Thirdborough SM, Mellows T, Riley C, Harris S, Suchak K, et al. Tumour-infiltrating lymphocytes predict for outcome in HPV-posi-

- tive oropharyngeal cancer. *Br J Cancer*. 2014;110(2):489–500. doi: <http://dx.doi.org/10.1038/bjc.2013.639>. PubMed.
- 17 Näsman A, Romanitan M, Nordfors C, Grün N, Johansson H, Hammarstedt L, et al. Tumor infiltrating CD8+ and Foxp3+ lymphocytes correlate to clinical outcome and human papillomavirus (HPV) status in tonsillar cancer. *PLoS One*. 2012;7(6):e38711. doi: <http://dx.doi.org/10.1371/journal.pone.0038711>. PubMed.
 - 18 Partlová S, Bouček J, Kloudová K, Lukešová E, Zábrodský M, Grega M, et al. Distinct patterns of intratumoral immune cell infiltrates in patients with HPV-associated compared to non-virally induced head and neck squamous cell carcinoma. *OncoImmunology*. 2015;4(1):e965570. doi: <http://dx.doi.org/10.4161/21624011.2014.965570>. PubMed.
 - 19 Woo EY, Yeh H, Chu CS, Schlienger K, Carroll RG, Riley JL, et al. Cutting edge: Regulatory T cells from lung cancer patients directly inhibit autologous T cell proliferation. *J Immunol*. 2002;168(9):4272–6. doi: <http://dx.doi.org/10.4049/jimmunol.168.9.4272>. PubMed.
 - 20 Woo EY, Chu CS, Goletz TJ, Schlienger K, Yeh H, Coukos G, et al. Regulatory CD4(+)/CD25(+) T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. *Cancer Res*. 2001;61(12):4766–72. PubMed.
 - 21 Liyanage UK, Moore TT, Joo H-G, Tanaka Y, Herrmann V, Doherty G, et al. Prevalence of regulatory T cells is increased in peripheral blood and tumor microenvironment of patients with pancreas or breast adenocarcinoma. *J Immunol*. 2002;169(5):2756–61. doi: <http://dx.doi.org/10.4049/jimmunol.169.5.2756>. PubMed.
 - 22 Wolf AM, Wolf D, Steurer M, Gastl G, Gunsilius E, Grubeck-Loebenstein B. Increase of regulatory T cells in the peripheral blood of cancer patients. *Clin Cancer Res*. 2003;9(2):606–12. PubMed.
 - 23 Lechner A, Schlöber H, Rothschild SI, Thelen M, Reuter S, Zentis P, et al. Characterization of tumor-associated T-lymphocyte subsets and immune checkpoint molecules in head and neck squamous cell carcinoma. *Oncotarget*. 2017;8(27):44418–33. doi: <http://dx.doi.org/10.18632/oncotarget.17901>. PubMed.
 - 24 Badoual C, Hans S, Rodriguez J, Peyrard S, Klein C, Agueznay NH, et al. Prognostic value of tumor-infiltrating CD4+ T-cell subpopulations in head and neck cancers. *Clin Cancer Res*. 2006;12(2):465–72. doi: <http://dx.doi.org/10.1158/1078-0432.CCR-05-1886>. PubMed.
 - 25 Schaefer C, Kim GG, Albers A, Hoermann K, Myers EN, Whiteside TL. Characteristics of CD4+CD25+ regulatory T cells in the peripheral circulation of patients with head and neck cancer. *Br J Cancer*. 2005;92(5):913–20. doi: <http://dx.doi.org/10.1038/sj.bjc.6602407>. PubMed.
 - 26 Jie H-B, Gildener-Leapman N, Li J, Srivastava RM, Gibson SP, Whiteside TL, et al. Intratumoral regulatory T cells upregulate immunosuppressive molecules in head and neck cancer patients. *Br J Cancer*. 2013;109(10):2629–35. doi: <http://dx.doi.org/10.1038/bjc.2013.645>. PubMed.
 - 27 Palucka K, Banchereau J. Cancer immunotherapy via dendritic cells. *Nat Rev Cancer*. 2012;12(4):265–77. doi: <http://dx.doi.org/10.1038/nrc3258>. PubMed.
 - 28 Ma Y, Shurin GV, Peiyuan Z, Shurin MR. Dendritic cells in the cancer microenvironment. *J Cancer*. 2013;4(1):36–44. doi: <http://dx.doi.org/10.7150/jca.5046>. PubMed.
 - 29 Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer*. 2012;12(4):252–64. doi: <http://dx.doi.org/10.1038/nrc3239>. PubMed.
 - 30 Fife BT, Bluestone JA. Control of peripheral T-cell tolerance and autoimmunity via the CTLA-4 and PD-1 pathways. *Immunol Rev*. 2008;224(1):166–82. doi: <http://dx.doi.org/10.1111/j.1600-065X.2008.00662.x>. PubMed.
 - 31 Melero I, Hervas-Stubbs S, Glennie M, Pardoll DM, Chen L. Immunostimulatory monoclonal antibodies for cancer therapy. *Nat Rev Cancer*. 2007;7(2):95–106. doi: <http://dx.doi.org/10.1038/nrc2051>. PubMed.
 - 32 Mueller SN, Ahmed R. High antigen levels are the cause of T cell exhaustion during chronic viral infection. *Proc Natl Acad Sci USA*. 2009;106(21):8623–8. doi: <http://dx.doi.org/10.1073/pnas.0809818106>. PubMed.
 - 33 Wherry EJ. T cell exhaustion. *Nat Immunol*. 2011;12(6):492–9. doi: <http://dx.doi.org/10.1038/ni.2035>. PubMed.
 - 34 Yi JS, Cox MA, Zajac AJ. T-cell exhaustion: characteristics, causes and conversion. *Immunology*. 2010;129(4):474–81. doi: <http://dx.doi.org/10.1111/j.1365-2567.2010.03255.x>. PubMed.
 - 35 Zandberg DP, Strome SE. The role of the PD-L1:PD-1 pathway in squamous cell carcinoma of the head and neck. *Oral Oncol*. 2014;50(7):627–32. doi: <http://dx.doi.org/10.1016/j.oraloncology.2014.04.003>. PubMed.
 - 36 Montler R, Bell RB, Thalhoffer C, Leidner R, Feng Z, Fox BA, et al. OX40, PD-1 and CTLA-4 are selectively expressed on tumor-infiltrating T cells in head and neck cancer. *Clin Transl Immunology*. 2016;5(4):e70. doi: <http://dx.doi.org/10.1038/cti.2016.16>. PubMed.
 - 37 Badoual C, Hans S, Merillon N, Van Ryswyck C, Ravel P, Benhamouda N, et al. PD-1-expressing tumor-infiltrating T cells are a favorable prognostic biomarker in HPV-associated head and neck cancer. *Cancer Res*. 2013;73(1):128–38. doi: <http://dx.doi.org/10.1158/0008-5472.CAN-12-2606>. PubMed.
 - 38 He Y-F, Zhang G-M, Wang X-H, Zhang H, Yuan Y, Li D, et al. Blocking programmed death-1 ligand-PD-1 interactions by local gene therapy results in enhancement of antitumor effect of secondary lymphoid tissue chemokine. *J Immunol*. 2004;173(8):4919–28. doi: <http://dx.doi.org/10.4049/jimmunol.173.8.4919>. PubMed.
 - 39 Callahan MK, Wolchok JD. At the bedside: CTLA-4- and PD-1-blocked antibodies in cancer immunotherapy. *J Leukoc Biol*. 2013;94(1):41–53. doi: <http://dx.doi.org/10.1189/jlb.1212631>. PubMed.
 - 40 Chang Y-L, Yang C-Y, Huang Y-L, Wu C-T, Yang P-C. High PD-L1 expression is associated with stage IV disease and poorer overall survival in 186 cases of small cell lung cancers. *Oncotarget*. 2017;8(11):18021–30. doi: <http://dx.doi.org/10.18632/oncotarget.14935>. PubMed.
 - 41 Wang A, Wang HY, Liu Y, Zhao MC, Zhang HJ, Lu ZY, et al. The prognostic value of PD-L1 expression for non-small cell lung cancer patients: a meta-analysis. *Eur J Surg Oncol*. 2015;41(4):450–6. doi: <http://dx.doi.org/10.1016/j.ejso.2015.01.020>. PubMed.
 - 42 Schlöber HA, Dreber U, Kloth M, Thelen M, Rothschild SI, Haase S, et al. Immune checkpoints programmed death 1 ligand 1 and cytotoxic T lymphocyte associated molecule 4 in gastric adenocarcinoma. *OncoImmunology*. 2015;5(5):e1100789. doi: <http://dx.doi.org/10.1080/2162402X.2015.1100789>. PubMed.
 - 43 Lin Y-M, Sung W-W, Hsieh M-J, Tsai S-C, Lai H-W, Yang S-M, et al. High PD-L1 Expression Correlates with Metastasis and Poor Prognosis in Oral Squamous Cell Carcinoma. *PLoS One*. 2015;10(11):e0142656. doi: <http://dx.doi.org/10.1371/journal.pone.0142656>. PubMed.
 - 44 Albers A, Abe K, Hunt J, Wang J, Lopez-Albaitero A, Schaefer C, et al. Antitumor activity of human papillomavirus type 16 E7-specific T cells against virally infected squamous cell carcinoma of the head and neck. *Cancer Res*. 2005;65(23):11146–55. doi: <http://dx.doi.org/10.1158/0008-5472.CAN-05-0772>. PubMed.
 - 45 Wansom D, Light E, Thomas D, Worden F, Prince M, Urba S, et al.; UM Head Neck SPORE Program. Infiltrating lymphocytes and human papillomavirus-16-associated oropharyngeal cancer. *Laryngoscope*. 2012;122(1):121–7. doi: <http://dx.doi.org/10.1002/lary.22133>. PubMed.
 - 46 Wansom D, Light E, Worden F, Prince M, Urba S, Chepeha DB, et al. Correlation of cellular immunity with human papillomavirus 16 status and outcome in patients with advanced oropharyngeal cancer. *Arch Otolaryngol Head Neck Surg*. 2010;136(12):1267–73. doi: <http://dx.doi.org/10.1001/archoto.2010.211>. PubMed.
 - 47 Malm I-J, Bruno TC, Fu J, Zeng Q, Taube JM, Westra W, et al. Expression profile and in vitro blockade of programmed death-1 in human papillomavirus-negative head and neck squamous cell carcinoma. *Head Neck*. 2015;37(8):1088–95. doi: <http://dx.doi.org/10.1002/hed.23706>. PubMed.
 - 48 Kim HS, Lee JY, Lim SH, Park K, Sun J-M, Ko YH, et al. Association Between PD-L1 and HPV Status and the Prognostic Value of PD-L1 in Oropharyngeal Squamous Cell Carcinoma. *Cancer Res Treat*. 2016;48(2):527–36. PubMed.
 - 49 Lyford-Pike S, Peng S, Young GD, Taube JM, Westra WH, Akpeng B, et al. Evidence for a role of the PD-1:PD-L1 pathway in immune resistance of HPV-associated head and neck squamous cell carcinoma. *Cancer Res*. 2013;73(6):1733–41. doi: <http://dx.doi.org/10.1158/0008-5472.CAN-12-2384>. PubMed.
 - 50 Ukpo OC, Thorstad WL, Lewis JS, Jr. B7-H1 expression model for immune evasion in human papillomavirus-related oropharyngeal squamous cell carcinoma. *Head Neck Pathol*. 2013;7(2):113–21. doi: <http://dx.doi.org/10.1007/s12105-012-0406-z>. PubMed.
 - 51 Paterson Y, Maciag PC. Listeria-based vaccines for cancer treatment. *Curr Opin Mol Ther*. 2005;7(5):454–60. PubMed.
 - 52 Carleton HA. Pathogenic bacteria as vaccine vectors: teaching old bugs new tricks. *Yale J Biol Med*. 2010;83(4):217–22. PubMed.
 - 53 Chaturvedi AK, Engels EA, Pfeiffer RM, Hernandez BY, Xiao W, Kim E, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *J Clin Oncol*. 2011;29(32):4294–301. doi: <http://dx.doi.org/10.1200/JCO.2011.36.4596>. PubMed.
 - 54 Saraiya M, Unger ER, Thompson TD, Lynch CF, Hernandez BY, Lyu CW, et al.; HPV Typing of Cancers Workgroup. US assessment of HPV types in cancers: implications for current and 9-valent HPV vaccines. *J Natl Cancer Inst*. 2015;107(6):djv086. doi: <http://dx.doi.org/10.1093/jnci/djv086>. PubMed.

- 55 FUTURE II Study Group. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. *N Engl J Med*. 2007;356(19):1915–27. doi: <http://dx.doi.org/10.1056/NEJMoa061741>. PubMed.
- 56 Bundesamt für Gesundheit, Eidgenössische Kommission für Impffragen (EKIF). Schweizerischer Impfplan 2017 Richtlinien und Empfehlungen. Bern: Bundesamt für Gesundheit; 2017.
- 57 Giuliano AR, Palefsky JM, Goldstone S, Moreira ED, Jr, Penny ME, Aranda C, et al. Efficacy of quadrivalent HPV vaccine against HPV Infection and disease in males. *N Engl J Med*. 2011;364(5):401–11. doi: <http://dx.doi.org/10.1056/NEJMoa0909537>. PubMed.
- 58 Chaturvedi AK, Graubard BI, Broutian T, Pickard RKL, Tong Z-Y, Xiao W, et al. Effect of Prophylactic Human Papillomavirus (HPV) Vaccination on Oral HPV Infections Among Young Adults in the United States. *J Clin Oncol*. 2018;36(3):262–7. doi: <http://dx.doi.org/10.1200/JCO.2017.75.0141>. PubMed.
- 59 Stokley S, Jeyarajah J, Yankey D, Cano M, Gee J, Roark J, et al.; Immunization Services Division, National Center for Immunization and Respiratory Diseases, CDC; Centers for Disease Control and Prevention (CDC). Human papillomavirus vaccination coverage among adolescents, 2007–2013, and postlicensure vaccine safety monitoring, 2006–2014—United States. *MMWR Morb Mortal Wkly Rep*. 2014;63(29):620–4. PubMed.
- 60 Reuschenbach M, Pauligk C, Karbach J, Rafiyan M-R, Kloor M, Prigge E-S, et al. A phase I/2a study to test the safety and immunogenicity of a p16^(INK4a) peptide vaccine in patients with advanced human papillomavirus-associated cancers. *Cancer*. 2016;122(9):1425–33. doi: <http://dx.doi.org/10.1002/ncr.29925>. PubMed.
- 61 Zandberg DP, Rollins S, Goloubeva O, Morales RE, Tan M, Taylor R, et al. A phase I dose escalation trial of MAGE-A3- and HPV16-specific peptide immunomodulatory vaccines in patients with recurrent/metastatic (RM) squamous cell carcinoma of the head and neck (SCCHN). *Cancer Immunol Immunother*. 2015;64(3):367–79. doi: <http://dx.doi.org/10.1007/s00262-014-1640-x>. PubMed.
- 62 Ott PA, Hu Z, Keskin DB, Shukla SA, Sun J, Bozym DJ, et al. An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature*. 2017;547(7662):217–21. doi: <http://dx.doi.org/10.1038/nature22991>. PubMed.
- 63 Aggarwal C, Cohen R, Morrow MP, Bauml J, Weinstein G, Boyer J, et al. Immunotherapy with VGX-3100 (HPV16 and HPV18 plasmids) + INO-9012 (DNA encoding IL-12) in human papillomavirus (HPV) associated head and neck squamous cell carcinoma (HNSCCa): interim safety and immunogenicity results. *J Immunother Cancer*. 2015;3(Suppl 2):P426. doi: <http://dx.doi.org/10.1186/2051-1426-3-S2-P426>.
- 64 Yoshitake Y, Fukuma D, Yuno A, Hirayama M, Nakayama H, Tanaka T, et al. Phase II clinical trial of multiple peptide vaccination for advanced head and neck cancer patients revealed induction of immune responses and improved OS. *Clin Cancer Res*. 2015;21(2):312–21. doi: <http://dx.doi.org/10.1158/1078-0432.CCR-14-0202>. PubMed.
- 65 Ferrara A, Nonn M, Sehr P, Schreckenberger C, Pawlita M, Dürst M, et al. Dendritic cell-based tumor vaccine for cervical cancer II: results of a clinical pilot study in 15 individual patients. *J Cancer Res Clin Oncol*. 2003;129(9):521–30. doi: <http://dx.doi.org/10.1007/s00432-003-0463-5>. PubMed.
- 66 Santin AD, Bellone S, Palmieri M, Ravaggi A, Romani C, Tassi R, et al. HPV16/18 E7-pulsed dendritic cell vaccination in cervical cancer patients with recurrent disease refractory to standard treatment modalities. *Gynecol Oncol*. 2006;100(3):469–78. doi: <http://dx.doi.org/10.1016/j.ygyno.2005.09.040>. PubMed.
- 67 Zhou Z-X, Li D, Guan S-S, Zhao C, Li Z-L, Zeng Y. Immunotherapeutic Effects of Dendritic Cells Pulsed with a Coden-optimized HPV 16 E6 and E7 Fusion Gene in Vivo and in Vitro. *Asian Pac J Cancer Prev*. 2015;16(9):3843–7. doi: <http://dx.doi.org/10.7314/APJCP.2015.16.9.3843>. PubMed.
- 68 Schuler PJ, Harasymczuk M, Visus C, Deleo A, Trivedi S, Lei Y, et al. Phase I dendritic cell p53 peptide vaccine for head and neck cancer. *Clin Cancer Res*. 2014;20(9):2433–44. doi: <http://dx.doi.org/10.1158/1078-0432.CCR-13-2617>. PubMed.
- 69 Grande C, Firvida JL, Navas V, Casal J. Interleukin-2 for the treatment of solid tumors other than melanoma and renal cell carcinoma. *Anti-cancer Drugs*. 2006;17(1):1–12. doi: <http://dx.doi.org/10.1097/01.cad.0000182748.47353.51>. PubMed.
- 70 Whiteside TL, Letessier E, Hirabayashi H, Vitolo D, Bryant J, Barnes L, et al. Evidence for local and systemic activation of immune cells by peritumoral injections of interleukin 2 in patients with advanced squamous cell carcinoma of the head and neck. *Cancer Res*. 1993;53(23):5654–62. PubMed.
- 71 Dadian G, Riches PG, Henderson DC, MacLennan K, Lorentzos A, Moore J, et al. Immune changes in peripheral blood resulting from locally directed interleukin-2 therapy in squamous cell carcinoma of the head and neck. *Eur J Cancer B Oral Oncol*. 1993;29(1):29–34. doi: [http://dx.doi.org/10.1016/0964-1955\(93\)90007-2](http://dx.doi.org/10.1016/0964-1955(93)90007-2). PubMed.
- 72 De Stefani A, Forni G, Ragona R, Cavallo G, Bussi M, Usai A, et al. Improved survival with perilymphatic interleukin 2 in patients with resectable squamous cell carcinoma of the oral cavity and oropharynx. *Cancer*. 2002;95(1):90–7. doi: <http://dx.doi.org/10.1002/ncr.10654>. PubMed.
- 73 Urba SG, Forastiere AA, Wolf GT, Amrein PC. Intensive recombinant interleukin-2 and alpha-interferon therapy in patients with advanced head and neck squamous carcinoma. *Cancer*. 1993;71(7):2326–31. doi: [http://dx.doi.org/10.1002/1097-0142\(19930401\)71:7<2326::AID-CNCR2820710725>3.0.CO;2-H](http://dx.doi.org/10.1002/1097-0142(19930401)71:7<2326::AID-CNCR2820710725>3.0.CO;2-H). PubMed.
- 74 Richtsmeier WJ, Koch WM, McGuire WP, Poole ME, Chang EH. Phase I-II study of advanced head and neck squamous cell carcinoma patients treated with recombinant human interferon gamma. *Arch Otolaryngol Head Neck Surg*. 1990;116(11):1271–7. doi: <http://dx.doi.org/10.1001/archotol.1990.01870110043004>. PubMed.
- 75 van Herpen CM, Looman M, Zonneveld M, Scharenborg N, de Wilde PC, van de Locht L, et al. Intratumoral administration of recombinant human interleukin 12 in head and neck squamous cell carcinoma patients elicits a T-helper 1 profile in the locoregional lymph nodes. *Clin Cancer Res*. 2004;10(8):2626–35. doi: <http://dx.doi.org/10.1158/1078-0432.CCR-03-0304>. PubMed.
- 76 van Herpen CML, van der Voort R, van der Laak JAWM, Klases IS, de Graaf AO, van Kempen LCL, et al. Intratumoral rhIL-12 administration in head and neck squamous cell carcinoma patients induces B cell activation. *Int J Cancer*. 2008;123(10):2354–61. doi: <http://dx.doi.org/10.1002/ijc.23756>. PubMed.
- 77 Egan JE, Quadri KJ, Santiago-Schwarz F, Hadden JW, Brandwein HJ, Signorelli KL. IRX-2, a novel in vivo immunotherapeutic, induces maturation and activation of human dendritic cells in vitro. *J Immunother*. 2007;30(6):624–33. doi: <http://dx.doi.org/10.1097/CJI.0b013e3180691593>. PubMed.
- 78 Schilling B, Harasymczuk M, Schuler P, Egan J, Ferrone S, Whiteside TL. IRX-2, a novel immunotherapeutic, enhances functions of human dendritic cells. *PLoS One*. 2013;8(2):e47234. doi: <http://dx.doi.org/10.1371/journal.pone.0047234>. PubMed.
- 79 Wolf GT, Fee WE, Jr, Dolan RW, Moyer JS, Kaplan MJ, Spring PM, et al. Novel neoadjuvant immunotherapy regimen safety and survival in head and neck squamous cell cancer. *Head Neck*. 2011;33(12):1666–74. doi: <http://dx.doi.org/10.1002/hed.21660>. PubMed.
- 80 Whiteside TL, Butterfield LH, Naylor PH, Egan JE, Hadden JW, Baltzer L, et al. A short course of neoadjuvant IRX-2 induces changes in peripheral blood lymphocyte subsets of patients with head and neck squamous cell carcinoma. *Cancer Immunol Immunother*. 2012;61(6):783–8. doi: <http://dx.doi.org/10.1007/s00262-011-1136-x>. PubMed.
- 81 Szczepanski MJ, Czystowska M, Szajnik M, Harasymczuk M, Boyiadzis M, Kruk-Zagajewska A, et al. Triggering of Toll-like receptor 4 expressed on human head and neck squamous cell carcinoma promotes tumor development and protects the tumor from immune attack. *Cancer Res*. 2009;69(7):3105–13. doi: <http://dx.doi.org/10.1158/0008-5472.CAN-08-3838>. PubMed.
- 82 Dietsch GN, Lu H, Yang Y, Morishima C, Chow LQ, Disis ML, et al. Coordinated Activation of Toll-Like Receptor8 (TLR8) and NLRP3 by the TLR8 Agonist, VTX-2337, Ignites Tumoricidal Natural Killer Cell Activity. *PLoS One*. 2016;11(2):e0148764. doi: <http://dx.doi.org/10.1371/journal.pone.0148764>. PubMed.
- 83 Chow LQM, Morishima C, Eaton KD, Baik CS, Goulart BH, Anderson LN, et al. Phase Ib Trial of the Toll-like Receptor 8 Agonist, Motolimod (VTX-2337), Combined with Cetuximab in Patients with Recurrent or Metastatic SCCHN. *Clin Cancer Res*. 2017;23(10):2442–50. doi: <http://dx.doi.org/10.1158/1078-0432.CCR-16-1934>. PubMed.
- 84 Ruzsa A, Sen M, Evans M, Lee LW, Hideghety K, Rottey S, et al.; NA EMD 1201081 Study Group. Phase 2, open-label, 1:1 randomized controlled trial exploring the efficacy of EMD 1201081 in combination with cetuximab in second-line cetuximab-naïve patients with recurrent or metastatic squamous cell carcinoma of the head and neck (R/M SCCHN). *Invest New Drugs*. 2014;32(6):1278–84. doi: <http://dx.doi.org/10.1007/s10637-014-0117-2>. PubMed.
- 85 Li H, Zhao Y. Increasing the safety and efficacy of chimeric antigen receptor T cell therapy. *Protein Cell*. 2017;8(8):573–89. doi: <http://dx.doi.org/10.1007/s13238-017-0411-9>. PubMed.
- 86 Stevanović S, Draper LM, Langhan MM, Campbell TE, Kwong ML, Wunderlich JR, et al. Complete regression of metastatic cervical cancer after treatment with human papillomavirus-targeted tumor-infiltrating T cells. *J Clin Oncol*. 2015;33(14):1543–50. doi: <http://dx.doi.org/10.1200/JCO.2014.58.9093>. PubMed.

- 87 Draper LM, Kwong MLM, Gros A, Stevanović S, Tran E, Kerkar S, et al. Targeting of HPV-16+ Epithelial Cancer Cells by TCR Gene Engineered T Cells Directed against E6. *Clin Cancer Res.* 2015;21(19):4431–9. doi: <http://dx.doi.org/10.1158/1078-0432.CCR-14-3341>. PubMed.
- 88 Hinrichs CS, Doran SL, Stevanovic S, Adhikary S, Mojaidi M, Kwong ML. A phase I/II clinical trial of E6 T-cell receptor gene therapy for human papillomavirus (HPV)-associated epithelial cancers. *J Clin Oncol.* 2017;35(15):3009.
- 89 Riechelmann H, Wiesneth M, Schauwecker P, Reinhardt P, Gronau S, Schmitt A, et al. Adoptive therapy of head and neck squamous cell carcinoma with antibody coated immune cells: a pilot clinical trial. *Cancer Immunol Immunother.* 2007;56(9):1397–406. doi: <http://dx.doi.org/10.1007/s00262-007-0283-6>. PubMed.
- 90 To WC, Wood BG, Krauss JC, Strome M, Escamado RM, Lavertu P, et al. Systemic adoptive T-cell immunotherapy in recurrent and metastatic carcinoma of the head and neck: a phase I study. *Arch Otolaryngol Head Neck Surg.* 2000;126(10):1225–31. doi: <http://dx.doi.org/10.1001/archotol.126.10.1225>. PubMed.
- 91 Herold-Mende C, Karcher J, Dyckhoff G, Schirmacher V. Antitumor Immunization of Head and Neck Squamous Cell Carcinoma Patients with a Virus-Modified Autologous Tumor Cell Vaccine. In: *Current Research in Head and Neck Cancer*. Basel: KARGER; 2004. p. 173–83.
- 92 Tang X, Zhou Y, Li W, Tang Q, Chen R, Zhu J, et al. T cells expressing a LMP1-specific chimeric antigen receptor mediate antitumor effects against LMP1-positive nasopharyngeal carcinoma cells in vitro and in vivo. *J Biomed Res.* 2014;28(6):468–75. PubMed.
- 93 Geldres C, Savoldo B, Hoyos V, Caruana I, Zhang M, Yvon E, et al. T lymphocytes redirected against the chondroitin sulfate proteoglycan-4 control the growth of multiple solid tumors both in vitro and in vivo. *Clin Cancer Res.* 2014;20(4):962–71. doi: <http://dx.doi.org/10.1158/1078-0432.CCR-13-2218>. PubMed.
- 94 Rosewell Shaw A, Porter CE, Watanabe N, Tanoue K, Sikora A, Gottschalk S, et al. Adenovirotherapy Delivering Cytokine and Checkpoint Inhibitor Augments CAR T Cells against Metastatic Head and Neck Cancer. *Mol Ther.* 2017;25(11):2440–51. doi: <http://dx.doi.org/10.1016/j.yjth.2017.09.010>. PubMed.
- 95 Papa S, van Schalkwyk M, Maher J. Clinical Evaluation of ErbB-Targeted CAR T-Cells, Following Intracavity Delivery in Patients with ErbB-Expressing Solid Tumors. In 2015. p. 365–82.
- 96 Farkona S, Diamandis EP, Blasutig IM. Cancer immunotherapy: the beginning of the end of cancer? *BMC Med.* 2016;14(1):73. doi: <http://dx.doi.org/10.1186/s12916-016-0623-5>. PubMed.
- 97 Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob J-J, Cowey CL, et al. Overall Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma. *N Engl J Med.* 2017;377(14):1345–56. doi: <http://dx.doi.org/10.1056/NEJMoa1709684>. PubMed.
- 98 Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al.; KEYNOTE-024 Investigators. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. *N Engl J Med.* 2016;375(19):1823–33. doi: <http://dx.doi.org/10.1056/NEJMoa1606774>. PubMed.
- 99 Motzer RJ, Escudier B, McDermott DF, George S, Hammers HJ, Srinivas S, et al.; CheckMate 025 Investigators. Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma. *N Engl J Med.* 2015;373(19):1803–13. doi: <http://dx.doi.org/10.1056/NEJMoa1510665>. PubMed.
- 100 Robert C, Ribas A, Hamid O, Daud A, Wolchok JD, Joshua AM, et al. Durable Complete Response After Discontinuation of Pembrolizumab in Patients With Metastatic Melanoma. *J Clin Oncol.* 2017;75:JCO2017756270. [Epub ahead of print] PubMed.
- 101 Jie H-B, Schuler PJ, Lee SC, Srivastava RM, Argiris A, Ferrone S, et al. CTLA-4⁺ Regulatory T Cells Increased in Cetuximab-Treated Head and Neck Cancer Patients Suppress NK Cell Cytotoxicity and Correlate with Poor Prognosis. *Cancer Res.* 2015;75(11):2200–10. doi: <http://dx.doi.org/10.1158/0008-5472.CAN-14-2788>. PubMed.
- 102 Ferris RL, Blumenschein G, Jr, Fayette J, Guigay J, Colevas AD, Licitra L, et al. Nivolumab for Recurrent Squamous-Cell Carcinoma of the Head and Neck. *N Engl J Med.* 2016;375(19):1856–67. doi: <http://dx.doi.org/10.1056/NEJMoa1602252>. PubMed.
- 103 Harrington KJ, Ferris RL, Blumenschein G, Jr, Colevas AD, Fayette J, Licitra L, et al. Nivolumab versus standard, single-agent therapy of investigator's choice in recurrent or metastatic squamous cell carcinoma of the head and neck (CheckMate 141): health-related quality-of-life results from a randomised, phase 3 trial. *Lancet Oncol.* 2017;18(8):1104–15. doi: [http://dx.doi.org/10.1016/S1470-2045\(17\)30421-7](http://dx.doi.org/10.1016/S1470-2045(17)30421-7). PubMed.
- 104 Seiwert TY, Burtneis B, Mehra R, Weiss J, Berger R, Eder JP, et al. Safety and clinical activity of pembrolizumab for treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-012): an open-label, multicentre, phase 1b trial. *Lancet Oncol.* 2016;17(7):956–65. doi: [http://dx.doi.org/10.1016/S1470-2045\(16\)30066-3](http://dx.doi.org/10.1016/S1470-2045(16)30066-3). PubMed.
- 105 Chow LQM, Haddad R, Gupta S, Mahipal A, Mehra R, Tahara M, et al. Antitumor Activity of Pembrolizumab in Biomarker-Unselected Patients With Recurrent and/or Metastatic Head and Neck Squamous Cell Carcinoma: Results From the Phase Ib KEYNOTE-012 Expansion Cohort. *J Clin Oncol.* 2016;34(32):3838–45. doi: <http://dx.doi.org/10.1200/JCO.2016.68.1478>. PubMed.
- 106 Haddad RI, Seiwert TY, Chow LQM, Gupta S, Weiss J, Gluck I, et al. Genomic Determinants of Response to Pembrolizumab in Head and Neck Squamous Cell Carcinoma. *J Clin Oncol.* 2017;(ASCO annual meeting):abstract 6009.
- 107 Ayers M, Luceford J, Nebozhyn M, Murphy E, Loboda A, Kaufman DR, et al. IFN- γ -related mRNA profile predicts clinical response to PD-1 blockade. *J Clin Invest.* 2017;127(8):2930–40. doi: <http://dx.doi.org/10.1172/JCI91190>. PubMed.
- 108 Bauml J, Seiwert TY, Pfister DG, Worden F, Liu SV, Gilbert J, et al. Pembrolizumab for Platinum- and Cetuximab-Refractory Head and Neck Cancer: Results From a Single-Arm, Phase II Study. *J Clin Oncol.* 2017;35(14):1542–9. doi: <http://dx.doi.org/10.1200/JCO.2016.70.1524>. PubMed.
- 109 Cohen EE, Harrington KJ, Le Tourneau C, Dinis J, Licitra L, Ahn M-J, et al. Pembrolizumab (pembro) vs standard of care (SOC) for recurrent or metastatic head and neck squamous cell carcinoma (R/M HNSCC): Phase 3 KEYNOTE-040 trial. *Ann Oncol.* 2017;28(suppl_5):abstract LBA45_PR.
- 110 Segal NH, Ou SI, Balmanoukian AS, Massarelli E, Brahmer JR, Weiss J, et al. Updated safety and efficacy of durvalumab (MEDI4736), an anti-PD-L1 antibody, in patients from a squamous cell carcinoma of the head and neck (SCCHN) expansion cohort. *Ann Oncol.* 2016;27(suppl. 6):9490. doi: <http://dx.doi.org/10.1093/annonc/mdw376.01>.
- 111 Moffett JR, Nambodiri MA. Tryptophan and the immune response. *Immunol Cell Biol.* 2003;81(4):247–65. doi: <http://dx.doi.org/10.1046/j.1440-1711.2003.t01-i-01177.x>. PubMed.
- 112 Prendergast GC, Smith C, Thomas S, Mandik-Nayak L, Laury-Kleintop L, Metz R, et al. Indoleamine 2,3-dioxygenase pathways of pathogenic inflammation and immune escape in cancer. *Cancer Immunol Immunother.* 2014;63(7):721–35. doi: <http://dx.doi.org/10.1007/s00262-014-1549-4>. PubMed.
- 113 Hamid O, Bauer TM, Spira AI, Olszanski AJ, Patel SP, Wasser JS, et al. Epcadostat plus pembrolizumab in patients with SCCHN: Preliminary phase 1/2 results from ECHO-202/KEYNOTE-037. *J Clin Oncol.* 2017;(ASCO annual meeting):abstract 6010.
- 114 Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. *N Engl J Med.* 2015;373(1):23–34. doi: <http://dx.doi.org/10.1056/NEJMoa1504030>. PubMed.
- 115 Hellmann MD, Rizvi NA, Goldman JW, Gettinger SN, Borghaei H, Brahmer JR, et al. Nivolumab plus ipilimumab as first-line treatment for advanced non-small-cell lung cancer (CheckMate 012): results of an open-label, phase 1, multicohort study. *Lancet Oncol.* 2017;18(1):31–41. doi: [http://dx.doi.org/10.1016/S1470-2045\(16\)30624-6](http://dx.doi.org/10.1016/S1470-2045(16)30624-6). PubMed.
- 116 Cohen RB, Salas S, Even C, Kotecki N, Jimeno A, Soulié A-M, et al. Safety of the first-in-class anti-NKG2A monoclonal antibody monalizumab in combination with cetuximab: a phase Ib/II study in recurrent or metastatic squamous cell carcinoma of the head and neck (R/M SCCHN). *AACR Annu Meet.* 2017;abstract 5666.
- 117 Powell SF, Gitau MM, Sumey CJ, Reynolds JT, Lohr M, McGraw S, et al. Safety of pembrolizumab with chemoradiation (CRT) in locally advanced squamous cell carcinoma of the head and neck (LA-SCCHN). *J Clin Oncol.* 2017;(ASCO annual meeting):abstract 6011.
- 118 Uppaluri R, Zolkind P, Lin T, Nussenbaum B, Jackson RS, Rich J, et al. Neoadjuvant pembrolizumab in surgically resectable, HPV negative, locally advanced head and neck squamous cell carcinoma (HNSCC). *J Clin Oncol.* 2017;(ASCO annual meeting):abstract 6012.
- 119 Croft M, Benedict CA, Ware CF. Clinical targeting of the TNF and TNFR superfamily. *Nat Rev Drug Discov.* 2013;12(2):147–68. doi: <http://dx.doi.org/10.1038/nrd3930>. PubMed.
- 120 Srivastava RM, Trivedi S, Concha-Benavente F, Gibson SP, Reeder C, Ferrone S, et al. CD137 Stimulation Enhances Cetuximab-Induced Natural Killer: Dendritic Cell Priming of Antitumor T-Cell Immunity in Patients with Head and Neck Cancer. *Clin Cancer Res.* 2017;23(3):707–16. doi: <http://dx.doi.org/10.1158/1078-0432.CCR-16-0879>. PubMed.