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Immunomodulatory Therapy in Head and Neck Squamous Cell Carcinoma: Recent Advances and Clinical Prospects

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Abstract

The immune system plays a significant role in the development, invasion, progression, and metastasis of head and neck cancer. Over the last decade, the emergence of immunotherapy has irreversibly altered the paradigm of cancer treatment. The current treatment modalities for head and neck squamous cell carcinoma (HNSCC) include surgery, radiotherapy, and adjuvant or neoadjuvant chemotherapy which has failed to provide satisfactory clinical outcomes. To encounter this, there is a need for a novel or targeted therapy such as immunological targets along with conventional treatment strategy for optimal therapeutic outcomes. The immune system can contribute to promoting metastasis, angiogenesis, and growth by exploiting the tumor's influence on the microenvironment. Immunological targets have been found effective in recent clinical studies and have shown promising results. This review outlines the important immunological targets and the medications acting on them that have already been explored, are currently under clinical trials and are further being targeted.

Keywords

head and neck squamous cell carcinoma, immunomodulatory therapy, human papillomavirus, monoclonal antibodies, cetuximab, pembrolizumab, nivolumab

Introduction

The prevalence of head and neck cancer (HNC) and resultant morbidity and mortality has been rising worldwide. HNC is one of the most widespread malignancies, with an annual incidence of more than 800,000 cases and 400,000 fatalities.[1,2] Head and neck squamous cell carcinoma (HNSCC) is the most prevalent type of HNC, accounting for approximately 90% of all occurrences.[3] Based on anatomical sites, the pyriform fossa, oral cavity, oropharynx, hypopharynx, and larynx are the prominent sites of HNC.[4,5] The prevalence of human papillomavirus (HPV) associated with HNSCC is increasing apart from conventional risk factors such as tobacco chewing, smoking, and alcohol consumption.[6] The treatment of HNSCC is comprised of surgery, radiotherapy, and neoadjuvant or adjuvant chemotherapy.[7] Despite extensive multimodal treatments, the 5-year overall survival (OS) of patients suffering from HNSCC is only 40 to 50%.[8] The conventional first-line treatment comprises of platinum analogs, 5-fluorouracil (5-FU), and cetuximab. This has a median survival of just 10.1 months for recurrent or metastatic HNSCC (R/M HNSCC).[9] The prognosis of locoregional or distant recurrence HNC is very poor and the curative treatment options are limited.[10–12] In addition, these conventional regimen is hazardous, with an 82% rate of grade 3 and 4 adverse events (AEs).[13] Therefore, there is a significant need to prolong the survival of the patients suffering from HNSCC without aggravating toxicity.

To encounter these problems, the development of novel treatment approaches as effective treatment modalities such as immunomodulatory therapy for the management of HNSCC have gained much needed attention.[14] According to cancer immunotherapy, tumors can be detected as foreign rather than internal

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cells/tissue, and an activated immune system can efficiently target them. A better understanding related to the dysregulation and evasion of the immunology might help to optimize current therapies and improve patient outcomes, especially for HNSCC.[15] Anti-tumor immunotherapy is premised on the principle that immune escape is attainable because of changes in immune surveillance and the tumor microenvironment.

The rationale of immunotherapy especially in HNSCC has been strengthened by the fact that the tumor mutation burden (TMB) in HNSCC is relatively high.[16] This is significant because a high TMB has been observed to be predictive of the efficacy of immune checkpoint inhibitors (ICIs), which is mainly because of the synthesis of antigenic altered proteins from mutant DNA that act as tumoral immune targets.[17] Malignant cells release cytokines such as transforming growth factors-β (TGF-β), interleukin(IL)-6, and IL-10, which inhibit the cell-mediated antitumor immune response by activating signal transducer and activator of transcription 1 (STAT1) suppression.[18,19] HNSCC is an immunosuppressive disease characterized by lower absolute lymphocyte counts compared to healthy individuals, diminished natural killer (NK) cell activity, and poor antigen-presenting function.[20–24] In addition, T lymphocytes that infiltrate tumors have been shown in HNSCC and other malignancies, with a significant impact on clinical outcomes.[25,26] Suppressive regulatory T cells (Tregs) produce suppressive cytokines such as TGF-β and IL-10, express cytotoxic T lymphocyte-associated protein 4 (CTLA-4), and are linked with tumor growth.[27]

Thus, an immunomodulatory approach that overcomes immune suppressive signals in HNSCC patients shows potential as a treatment option. Cancer vaccines based on tumor peptide antigens, viral, bacterial, and DNA-based vectors, and tumor antigen-specific monoclonal antibodies (mAbs) are among them. The US Food and Drug Administration has approved mAbs, which has shown promising clinical outcomes that are targeting immunological checkpoint receptors, such as anti-CTLA-4 and anti-programed death-1 (anti-PD-1), suggestive of better clinical outcomes for patients suffering from HNSCC. The most recent breakthroughs in immunotherapy for HNSCC that aimed to integrate immunotherapy into established treatment protocols are discussed in this review. Notably, the integration of immunotherapy as neoadjuvant therapy, definitive concurrent therapy, adjuvant therapy and therapy for R/M HNSCC are covered in this review.

Immunomodulatory Targets for Head and Neck Tumor

Ehrlich was the first to propose the significant role of immunomodulatory therapy in tumor cell growth. The NK cell discovery by Herbermann explained the innate response of the body's immune mechanism to protect from malignancies.[28,29] Moreover, a drastic increase in the incidence of tumor formation was seen in patients suffering from HNSCC with compromised immunity due to organ transplants.[30,31] Thus, the drugs acting on these immune mediators can be theoretically used in patients with HNSCC to achieve better clinical outcomes such as improvement of 1-year disease-free survival (DFS).

HNSCC is an immunosuppressive malignancy. It is mediated by the influence of the developing tumor on the TME, these tumor cells manipulate the immune system that leads to promotion of production of immunosuppressive mediators.[32] Thus, as the tumor grows and subsequently stage advances, further immune suppression is mediated by tumor cells itself. This is responsible for metastasis, angiogenesis and ultimately tumor progression. Thus, it can be inferred that the progression of tumor through the stages of HNSCC goes parallel with the level of immunosuppression.[33] A study (NCT02759575) was conducted to evaluate the efficacy of pembrolizumab in patients with advanced stage HNC. 9 out of 9 patients achieved surgery free survival when assessed for up to 18 months.[34] Based on the above findings it can be stated that immunotherapy might be more effective in advanced stages compared to initial stages. Also, the effectiveness in initial stages is not as strongly established as in advanced stages. Further research is warranted in order to elucidate the role of immunotherapy in early stages of HNC.

The conventional therapies for HNSCC include surgical resection, radiation therapy and chemotherapy. Though it seems unlikely, there might be more to the mechanism of the chemotherapeutic molecules apart from cytotoxicity. There have been distant evidences of the influence of chemotherapeutic agents on immune system and them being immunostimulatory on some levels.[35] Some of these agents have even shown to increase antigen presentation and PD-L2 induced increased T cell activation. These agents are also enhancing the effects of CTLs and thus can induce immunogenic cell death. Thus, every cytotoxic agent also modulates immune responses mediated by different pathways.[36] Some agents act via downregulation of STAT-6 pathway such as docetaxel, TUBB3/TLE protein overexpression by taxanes, TS protein overexpression by fluoropyrimidines.[37] Radiation therapy is known to exert its cytotoxic effects by DNA damage induced by production of reactive oxygen species.[38] But apart from this, radiation is also known to release high mobility group protein B1 (HMGB1) that is a ligand for pattern recognition receptor damage-associated molecular patterns (DAMPs).[39] This further activates innate immune responses and induces immunostimulatory activities in the TME. Radiation also leads to increase expression of MHC-I mediated by interferon (IFN).[40] It also increases the infiltration of TILs leading to CTL mediated tumor cell death.[41] Based on the above findings, combination of chemotherapy and radiation with immune targeted strategies might be proven efficacious.

To understand the utilization of immunomodulatory drugs for the management of patients suffering from HNSCC, an understanding related to the mechanism of these drugs is crucial. Thus, to facilitate the survival of such patients, a proper apprehension of the targets on which these drugs can act and how it affects the growth or progression of the head and neck tumor is required.

These targets could be divided based on their mechanism and clinical importance. The initial targeted therapy includes HRAS, oncogenic endothelial growth factor receptor (EGFR), Vascular endothelial growth factor (VEGF), STAT-1/3, and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3Ca). The novel immune checkpoint inhibitors (ICIs) are also known to play a significant role in HNSCC and can be explored as potential targets. Several oncovaccines that are either peptide or nucleic acid-based targeting HPV have also shown positive impact in the management of HNSCC. Other inflammatory mediators as targets include nuclear factor such as kappa-B (NF-κB), Tregs, human leukocyte antigens (HLA), prostaglandin E2, interferons, Myeloid-derived suppressor cells (MDSCs), and Tumor-associated macrophages.

These targets are further being discussed along with their actions and targeted drug or molecule in regulating the tumor growth in the head and neck region in Table 1.

Targeted Therapy

Monoclonal antibodies (mAbs) have emerged as a widely accepted immunotherapy for HNSCC.[89] These mAbs are target specific, which includes tumor antigen (TA)-targeted, tumor necrosis factor receptors (TNFR) targeted, cytokine-targeted, and immune checkpoint-targeted. Among all these mAbs, the TA-targeted mAbs namely cetuximab (an anti-EGFR mAb) have been approved and utilized mostly for the management of HNSCC.[90] The mAbs can be targeted to various overexpressed receptors or cytokines in HNSCC including but not limited to EGFR, VEGF, various ILs, PD-1, CTLA-4.[91] Numerous such antibodies are being assessed in various stages of preclinical and clinical trials. For the use in HNSCC, three mAbs have been approved namely cetuximab, pembrolizumab and nivolumab. The former targets EGFR whereas the latter two, targets PD-1.[92] The maximum researched target is EGFR for which many mAbs are still being developed such as futaximab, modotuximab, zalutumumab, nimotuzumab, panitumumab. Other important targets such as PD-1 has many mAbs being currently studied in the pipeline such as spartalizumab, cemiplimab, budigalimab, cosibelimab, teslelizumab. Apart from these other targets are also explores such as human hepatocyte growth factor (HGF), cluster of differentiation (CD)-70/ 137/ 276, activin receptor like kinase 1 (ALK 1), VEGF, HER3, IL-6, CTLA4. Despite being promising strategies theoretically these mAbs have various gaps in their safety and efficacy which is yet to be bridged by extensive research and trials. Evidences have shown that some of the immunotherapy drugs impairs the functions of DCs thus, leading to decreased immune response.[93] The mAb cetuximab has been showed to have effectivity in only 10–20% of patients whereas the overexpression of EGFR is seen in 80–90% of HNSCC tumors. The major cause for these limitations might be that the mechanism of anti-tumor activity of these agents might not be solely dependent on modifying immune response such as mechanism of ADCC related responses in cetuximab.[94]

Anti-EGFR Monoclonal Anti-Bodies (MAbs)

The anti-EGFR mAbs are responsible for complement fixation, or opsonization of tumors. This induces specific cytotoxic T lymphocyte response via phagocytosis, and subsequent antigen processing. Cetuximab, a chimeric monoclonal antibody of class immunoglobulin (Ig)-G1, binds to the extracellular domain of human EGFR with high affinity. Cetuximab was approved by the FDA in March 2006 for its use in treating HNSCC or as a single agent in patients who have had prior platinum-based therapy. A lot of trials were conducted to establish the safety and efficacy of cetuximab alone as well as in different combinations before its use in patients with HNSCC. Cetuximab provided better clinical outcomes when used in combination with radiation therapy or as a single agent in patients treated priory with platinum-based therapy. Lastly, the addition of cetuximab to platinum-based chemotherapy prolongs survival in patients with Recurrent/Metastatic HNSCC (R/M HNSCC).

The EGFR is a type of Tyrosine kinase receptor and belongs to the ErbB family.[95] These receptors have an important role in cell physiology and are discovered to be overexpressed or mutated in the early carcinogenesis of HNSCC.[96] Biochemical, genetic, and structural studies have concluded that receptor trans-phosphorylation occurring in response to ligand (EGF, TGFα) binding and stimulation, leads to further consequent activation of the intracellular signalling cascade.[95] Ligand binding facilitates the dimerization of the receptor[97] which leads to activation of the EGFR kinase and trans-autophosphorylation of a critical tyrosine residue in the cytoplasmic receptor tail.[98] This, in turn, activates the multiple signalling cascades including the Ras/mitogen activated protein kinase (MAPK) pathway, the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signalling pathway, and Phospholipase C(PLC)/ protein kinase C (PKC) signalling cascade.[99] These signals are important for various functions of cells like proliferation, differentiation, survival, and motility. Thus, when in malignant conditions EGFR is overexpressed, it leads to tumor progression.

Drugs belonging to the mAb IgG1 class binds with high affinity with the extracellular domain of EGFR. Moreover, numerous clinical trials have shown the highest affinity of cetuximab to stimulate antibody-dependent cell-mediated cytotoxicity (ADCC).[100] It acts by inhibiting intracellular signalling pathway of EGFR along with stimulation of ADCC by binding to the Fc receptors on NK cells which leads to activation of NK cells.[101] These activated NK cells have the capacity to carry out serial lyses of tumor cells.[102] Along with tumor lyses these NK cells leads to the release of tumor antigens which are than presented to the cytotoxic T-cells by the antigen presenting DCs.[103] Thus, multiple cytotoxic immunological cells are activated by cetuximab in order to carry out immune mediated tumor cell death.[104] The graphical representation of the mechanism of action of cetuximab is shown is Figure 1.

The binding affinity of cetuximab to the extracellular domain of EGFR is 5 to 10 times higher than the endogenous

Table 1. Novel Immune Targets and Targeted-Therapy in Head and Neck Cancer.

Name of Target	Brief Mechanism of Action	Drugs Action on Targets (mAbs/Immunotherapy)
Targeted therapy		
HRAS (HRas Proto-Oncogene, GTPase)	• HRAS is a proto-oncogene when mutated it promotes tumor growth.[42] • This type of mutations mostly seen in 4 to 8% of R/M HNSCC patients.[43]	Tipifarnib
Oncogenic EGFR	• Downregulation of HLA, APM components, and STAT1 activation. • Suppressive STAT3 signalling which leads to tumor cell proliferation and invasion causing angiogenesis and tumor survival.[15]	Cetuximab Panitumumab Zalutumumab Afatinib Gefitinib
VEGF	• It is a promoter of angiogenesis and leads to an increase in the ratio of immature to mature DCs in the tumor microenvironment [44] • Thus, VEGF contributes to immune suppression by binding of VEGFR1 onto myeloid derived stem cells. This prevents their differentiation into mature cells by inducing PD-L1 expression on DCs which leads to T-cell activation, adhesion and extravasation along with Tregs differentiation[45,46]	Lenvatinib Bevacizumab Ramucirumab
STAT-1/3	• STAT-3 is activated by EGFR as well as IL-6 expression induced by NF-κB.[47] • It is overexpressed in malignant cells of head and neck region subsequently causing alteration in cell cycle. This prevents apoptosis and mediates proliferation leading to survival of tumor cells[48,49] • STAT-1 on the other hand, is involved immune stimulation by Th1 as well as shows proapoptotic functions thus showing tumor suppressor activities by counteracting effects of STAT-3.[50]	Danvatirsen
PIK3Ca	• PIK3Ca gene alteration has been observed in many HNSCC patients with the prevalence of 56% in HPV positive and 39% in HPV negative HNSCC patients.[51]	Buparlisib
Immune checkpoint inhibitors		
TLRs • TLR-9	• They stimulate the production of proinflammatory cytokines such as TNF-α, IFN-γ with a T-cell-stimulating effect. • In addition it induces the maturation and cross-priming of DCs and have been shown to induce NK cell-dependent lysis of tumor cells [52]	Intratumoral SDS-101
LAG-3 or the KIRs	• KIRs are basically important and key receptors for NK cells.[53] • They interact with MHCI molecules and regulate immune response. • It enhances Treg function.[54]	Eftilagimod alpha Relatlimab
IDO	• IDO is responsible for the depletion of tryptophan and accumulation of its metabolite kynurenines. • This is the main mechanism behind its immunosuppressive mechanism that diminish the immune function and promotes T-cell death. • Thus, the tumor escapes immunosurveillance because of upregulation of IDO.[55,56]	Epacadostat
Costimulatory agents • ICOS	• HNSCC patients have shown lower expression of costimulatory receptors, which are essential for T cell activation.	GSK609 (ICOS agonist)

(continued)

Table 1. (continued)

Name of Target	Brief Mechanism of Action	Drugs Action on Targets (mAbs/Immunotherapy)
	• Therefore, in absence of this stimulation immune response will diminish because of abnormal T cell apoptosis. • Thus, it contributes in tumor progression and metastasis.[57–59]	
B7-H3 (CD276)	• This is a member of B7 ligand family which is overexpressed in several malignant cells and has a normal presence in nonmalignant cells.[60,61] • B7-H3 has both immunogenic and nonimmunogenic effects. • It diminishes immune response against tumor-specific antigen. • Its nonimmunogenic functions include tumor growth, invasion, angiogenesis and chemoresistance.	Enoblituzumab
NK cell receptor NKG2A	• NK cell receptor NKG2A is a inhibitory receptor as it contains a tyrosine-based inhibitory motif.[62] • Both T and NK-cells express this receptor in the peripheral blood. • However, cytokines such as interleukin-15 and antigenic stimulation can upregulate its expression on NK-cells.[63,64] • This upregulated receptor can promote tumor growth and its inhibition has shown beneficiary anti-tumor activity.[65]	Monalizumab
TGF-β	• It suppresses cell-mediated antitumor immunity while inducing suppression of STAT1 and NK cells and T-cell activation. • It is a key cytokine in the differentiation of Tregs.[66]	Bintrafusp alfa
TILs	• A few solid malignancies have shown clinical activity in response to TILs derived from primary tumors and genetically engineered T-cell receptor/ T-cell based treatments.[67]	LN-145
CTLA-4	• It competes with CD28 to bind to stimulatory ligands CD80 and CD86.[68] • CTLA4 has an important role in downregulation of T-cell activation in order to maintain immune homeostasis thus, its absence leads to lethal immune hyperactivity.[69]	Ipilimumab Tremelimumab
PD-1, PD-L1	• It induces a loss of function of cytotoxic T lymphocytes. • It has a greater overall antitumor immune response, because proinflammatory conditions can stimulate PD-L1 expression. • Also, causes downregulation of T-cell activation on binding to PD-1 in both murine and human systems by suppressing type 1–based antitumor immunity [70–72]	Pembrolizumab Nivolumab Durvalumab Atezolizumab Avelumab
Onco-vaccines. The overexpression of the HPV oncogenes E6-7 in majority of the malignant cells has provided the opportunity for vaccine development.[73]		
Live-vector vaccines	• Live-vector vaccine is attenuated vaccine which carries specific antigen that mimic the immunogenic response.[74]	Axalimogene filolisbac (Terminated due to systemic listeria infection)
Peptide/protein-based vaccines	• A synthetic tumor-related epitope found in peptide vaccines is identified by immune cells when they are complexed with MHC I or II on APCs. • This results in immune activation, memory formation, and targeting/ clearance of tumor cells.	ISA101b

(continued)

Table 1. (continued)

Name of Target	Brief Mechanism of Action	Drugs Action on Targets (mAbs/Immunotherapy)
Nucleic acid-based vaccines	• The transfer of plasmid DNA or messenger RNA and subsequent transcription of a target protein are made possible by nucleic acid vaccines.[75]	MEDI0457
Oncolytic virus	• By deleting important virulence genes required for replication in the normal host, oncolytic viruses can specifically multiply in tumor cells.	Intratumoral talimogene laherparepvec
Miscellaneous cytokines and inflammatory mediators involved in tumor growth and progression		
IFNs	• IFN-α induces immunosuppression in HNSCC by expression of PD-1 and PD-L1[76]. Its synergistic antitumor actions are also revealed along with EGFR- targeting HNSCC therapies[77]. • On the other hand, INF-γ which is one of the important Th1 pathway cytokine has a proapoptotic effect on tumor growth which activates leukocyte migration, antigen presentation, and inflammation thus showing a protective and positive prognostic effects in patients.[78–80]	None
NF-κB	• It is activated in malignant cells of head and neck region proving its involvement in invasion as well as metastasis.[81,82] • It is of critical importance in carcinogenesis via chemoresistance as well as protection from apoptosis.[83]	None
TIM-3	• It is a negative immune checkpoint initially expressed on CD4 Th1 and CD8 cells. • It plays an important role in immune suppression mediated by tumor in HNSCC.[84] • Thus, it is a marker or a mediator for immunosuppression.[85]	None
MDSCs	• It has a diverse cellular population of myeloid origin with T-cell suppressive functions.[86] • It produces nitric oxide and reactive oxygen species, which interacts to catalyse the nitration of the T-cell receptor. • This inhibits T-cell receptor and HLA interaction, signalling, and subsequent activation [87]	None
TAMs	• It has a strong antitumor activity and possess a so-called M1 phenotype, which is characterized by the production of IFN-γ and other type 1 cytokines. • It produces EGF, IL-6, and IL-10 and have been associated with angiogenesis, local tumor progression, and metastasis [88]	None

Abbreviations: HLA, human leucocyte antigen; APM, antigen-presenting machinery; STAT, signal transducer and activator of transcription; HPV, human papilloma virus; moAbs, monoclonal antibodies; TGF, transforming growth factor; NK, natural killer cells; IL, interleukins; INF, interferons; CTLA, cytotoxic T-lymphocyte associated protein; DC, dendritic cells; Treg, T regulatory cells; CD, cluster of differentiation; PD, programmed cell death protein; LAG, lymphocyte-activation gene 3; TIM, transmembrane immunoglobulin and mucin domain; MDSC, myeloid-derived suppressor cells; TAM, tumor-associated macrophages; EGF, endothelial growth factor; MHC, major histocompatibility complex; APCs, antigen-presenting cells; HNSCC, head and neck squamous cell carcinoma; EGFR, endothelial growth factor receptor; VEGF, vascular endothelial growth factor; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; TLR, toll-like receptors; KIR, killer-cell immunoglobulin-like receptor; IDO, indoleamine 2, 3-dioxygenase 1; ICOS, inducible T-cell co-stimulator; IFN, interferon; NF-κB, nuclear factor κ light chain-enhancer of activated B cells.

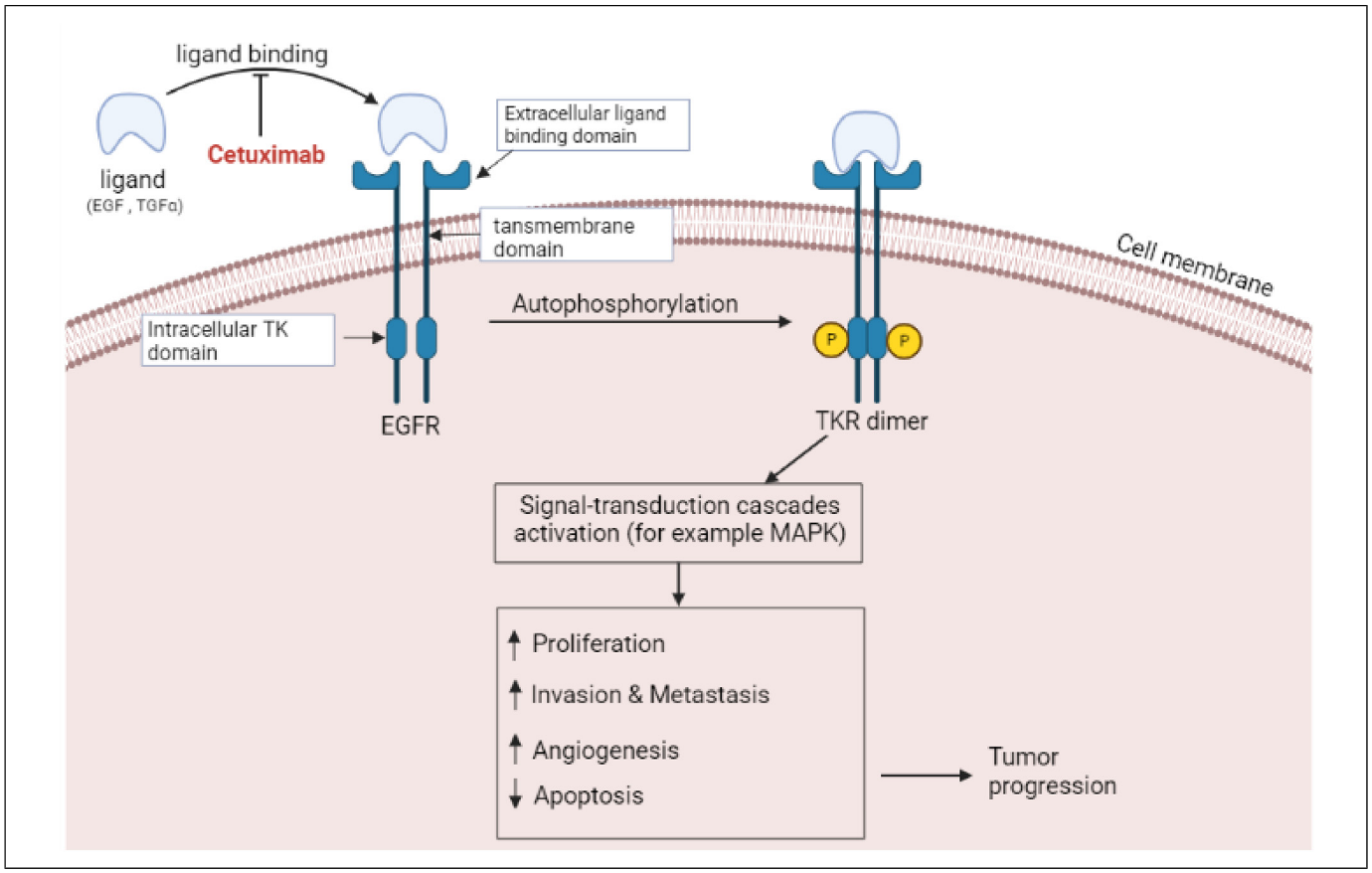

Figure 1. Mechanism of action of cetuximab, blocks and interferes with the ligand binding to EGFR (tyrosine kinase receptor) leading to decreased cellular functions like proliferation, invasion, and survival. Thus, reducing tumor progression. Abbreviations: TK, tyrosine kinase; EGF, epidermal growth factor; TGF, transforming growth factor; EGFR, epidermal growth factor receptor).

ligands. Thus, it blocks or interferes with the binding of these ligands leading to competitive inhibition of the receptors. Cetuximab also internalizes the EGFR leading to its downregulation, resulting into an overall control in tumor progression.[105,106]

Erin MB *et al* conducted a phase 1 trial in order to evaluate the ADCC of cetuximab and lenalidomide in colorectal cancer as well as HNSCC. A total of 22 patients were enrolled out of which 3 patients were of HNSCC and were evaluated on the basis of measurement of ADCC, FcγRIIIA polymorphism genotyping, serum cytokine levels and flow cytometric analysis of immune cells. The trial concluded that the cetuximab based combination have shown clinical benefits with good efficacy and well tolerated.[107]

Similarly, panitumumab which was an IgG2 isotype of EGFR, however, it doesn't lead to ADCC and activation of NK cells which is the main difference between these two mAbs. Moreover, the greater level of immune mediated cell death was reported by IgG1 mAb compared to IgG2 mAb.[108]

The mutation in the EGFR is the most common and maximum targeted therapy research has been focused on developing molecules targeting this mutation. Apart from this various other immune target have an impact in HNSCC.

HRAS gene mutations are prevalent up to 4–8% in R/M HNSCC. It is a type of mutation in Ras proto-oncogene. They are of three types namely, KRAS, NRAS and HRAS. Out of these only HRAS mutation is solely dependent upon farnesylation for its pathway activation whereas the other two mutations can bypass farneylation by other prenylation pathways. Thus, HRAS mutations are uniquely susceptible to blocking of farnesyl pathway by farnesyl transferase inhibitors (FTI). Thus FTIs such as tipifarnib had significant effects on HRAS mutated HNSCC but not on wild type Ras mutated HNSCC.[109]

Another interesting approach to target the HNSCC tumors is targeting the angiogenesis via anti-angiogenic agents. The proximal angiogenic factor VEGF can be targeted to manipulate angiogenesis. As mentioned above the HPV-negative HNSCC have higher expression of EGFR which is associated angiogenesis via STAT activation. The association of VEGF is also associated with STAT activation. The tumor is responsible for secretion of VEGF which stimulates tumor associated angiogenesis. VEGF expression is also associated with HPV status but there is no significant evidence pertaining to it. The overexpression of VEGF is also associated with COX-2 expression suggesting the possible role of COX inhibitors as an adjuvant in VEGF mutated HNSCC.[110]

STAT family of proteins are majorly responsible for various host responses. The upregulation of STAT-3 is seen in all cases of drug resistant HNSCC. STAT inhibition has been known to reverse the immunosuppression in HNSCC. STAT3 is activated in HNSCC tumors by its upstream factors including VEGF and EGFR. Activation subsequent to phosphorylation leads to its dimerization with STAT3 or hetero dimerization with STAT1. This leads to translation of proteins responsible for formation of immunosuppressive cytokines as well as proto-oncogenes. Thus, blocking of the activation of STAT activation directly by targeting its transcription or direct inhibition as well as indirectly by targeting upstream regulators responsible for STAT activation such as inhibitors of Jak pathway or mAbs against IL-6 can prove to of significant importance.[111]

Mutation in PIK3CA is also quite commonly seen not only in HNSCC but also in a lot of human malignancies. These mutations are associated more frequently in HPV positive oropharyngeal cancer and also responsible for poorer disease prognosis and renders the tumor resistant to chemotherapy. Evidence have shown the that PI3K/mTOR inhibitors have a positive effect on outcomes of HNSCC. Thus, dual targeting at this pathway can also be explored for HNSCC.[112]

Labetuzumab, a mAb targeting specific surface marker, carcinoembryonic antigen that is present on the surface of many solid tumors including HNSCC. This was explored via clinical trials in thyroid tumors along with conventional chemotherapy. Similarly, numerous mAbs specific to different targets have been extensively studied.[113]

Immune Checkpoint Inhibitors (ICIs)

Immune checkpoints are important and can be altered to support tumor immune escape.[114] The novel immune checkpoint inhibitors (ICIs) namely toll-like receptors (TLRs), lymphocyte-activation gene (LAG-3) or the killer-cell immunoglobulin-like receptors (KIRs), indoleamine 2, 3-dioxygenase 1, Costimulatory agents, B7-H3, NK cell receptor NKG2A, transforming growth factor-β (TGF-β), Tumor-infiltrating lymphocytes (TILs), CTLA-4, and PD-1, PD-L1 are responsible for the tumor growth and progression. A T-cell can be activated only when all the signals are present, namely T-cell receptor engagement as well as the costimulatory signals. Immune checkpoints modulate the immune response. These checkpoints are controlled by receptor-ligand binding for example CTLA-4 with its ligands CD80 and CD84, and PD-1 with its ligands PD-L1 and PD-L2. Studies have demonstrated that anti-CTLA4 treatment leads to cancer rejection.[115] Patients with tumors expressing these specific receptors shows significant efficacy when administered these targeted antibodies, it is possible to give immunotherapy in a personalized way based on the tumor expression of different antigens. Anti-CTLA-4 drugs include Ipilimumab[116] and Tremelimumab, whereas anti-PD-1 drugs include Pembrolizumab and Nivolumab.[117,118] PD-L1 and PD-L2 downregulate T-cell activation on binding to PD-1, a receptor present on activated T-Cells, B-Cells, and myeloid cells.[119] PD-1 is responsible for tumor growth inhibition thus when blocked by either of its ligands may lead to tumor immune evasion. Based on the outcomes of the clinical trials, a combination of two ICIs seems to provide better clinical outcomes in HNSCC compared to using a single agent. A combination of PD-1 blocker and CTLA-4 blocker has been approved for melanoma and can also be considered for use in HNSCC after being evaluated.[120] Cost benefit analysis of ICIs being used as second line agents have been shown to have significant efficacy around 15% objective response rate .[121] Apart from PD-1 and CTLA-4 various other receptors are targeted such as TILs, TGF-β and TLRs. The inhibitors of these receptors are also a part of ICIs. The ICIs can be important targets in HNSCC as they directly target the abnormal tumor cells without affecting the normal cells thus, reducing the major toxicities of conventional therapies.

Preventive/Prophylactic HPV-Vaccines

Apart from the molecular differences between HPV positive and negative HNSCC tumors, there is a difference in the immune responses exerted by them. The major difference is in the tumor antigen expression. In case of HPV positive tumors, the major antigen will be the viral particles and proteins whereas for HPV negative tumors, there is major overexpression of various genes due to random mutations.[122,123] Another point of difference is based on the prognostic factor TILs. There is higher level of expression of TILs with HPV positive tumors that is associated with better clinical response compared to HPV negative tumors.[124,125] Also, the overexpression of immune checkpoint PD-1 that inhibits the antitumor functions of T cells is more significant in HPV positive tumors. In contrast, higher expression of PD-L1 is seen in HPV negative tumors and is associated with better prognostic outcomes.[126] A higher level of expression of various T cell exhaustion markers is present on HPV positive tumors. Thus, recognition of HPV positive markers and reactivation of the exhausted T cells can be used as a strategy against HPV positive tumors.[127] The expression of MHC-I is still controversial in HPV positive tumors.[128,129] However, it is generally downregulated in HPV negative tumors.[130] Another positive prognostic marker is the B cell infiltration in TME which is distinctively higher in HPV positive tumors than HPV negative tumors.[131,132] On the contrary, a negative prognostic marker, C reactive protein is highly expressed on HPV positive tumors.[133] Thus, the understanding of these differences between the HPV positive and negative tumors is necessary as it guides the prognosis and the treatment of the patient.

HPV may increase a person's risk of HNC, especially oropharyngeal cancer. Approximately 63% of oropharyngeal cancer is caused due to HPV infections.[134] HPV is also a major cause of tonsil cancer. The most successful HNSCC-targeted immunotherapy will likely be HPV-targeted immune-preventive vaccines. Inhibition of viral infection is the main function of preventive vaccines which subsequently hinders cancer formation. Additionally, vaccination is unlikely to result in

Table 2. Summary of Clinical Studies Investigating Effectiveness of Therapeutic Vaccines in HNSCC.

Study (trial No. or name)	No. of Patients	Trial Phase	Clinical Protocol	Treatment Arm 1	Treatment Arm 2	Primary Endpoint	Secondary Endpoint
Live-attenuated vaccine							
NCT01598792	2	I	Oropharyngeal cancer	Axalimogene filolisbac	-	The study has been terminated early because of systemic listeria infection	
Peptide/protein-based vaccines							
NCT02426892	24	II	Incurable HPV-related HNSCC	ISA101 100 μg/peptide + nivolumab 3 mg/kg	-	ORR: 33%	DoR: 10.3 months PFS: 2.7 months OS: 17.5 months
NCT03258008 (On-going study)	3	II	Incurable HPV-related oropharyngeal cancer	ISA101b 100 mcg/peptide + Utomilumab	-	ORR	AEs, immune related PFS
NCT02865135 (On-going study)	11	I/II	Incurable HPV-related oropharyngeal cancer	DPX-E7 vaccine	-	Treatment related AEs	ORR, OSR, PFS
NCT03978689 (On-going study)	85	I	R/M HNSCC	CUE-101	CUE-101 + pembrolizumab	Dose limiting toxicities, pharmacokinetic parameters	ORR
Nucleic acid-based vaccines							
NCT03162224	35	Ib/IIa	HPV-associated R/M HNSCC	MEDI0457 7 mg + durvalumab 1500 mg	-	Safety (treatment related AEs): 77.1% ORR: 22.2%	Induction of antibodies, HPV-specific T cells
Aggarwal C *et al* (2019) (On-going study)	21	Ib/II	Locally advanced HPV-associated HNSCC	MEDI0457	-	Safety, tolerability, immunogenicity	-
Oncolytic immunotherapy							
NCT02626000	36	Ib	R/M HNSCC	Talimogene laherparepvec + pembrolizumab	-	Dose limiting toxicity: 55.6% patients	PFS: 3 months OS: 5.8 months ORR: 5 patients Safety

Abbreviations: ORR, overall response rate; AE, adverse event; PCR, polymerase chain reaction; DFS, disease-free survival; DRR, durable response rate; MPR, major pathologic response; EFS, event-free survival; SOC, standard of care; PFS, progression-free survival; OS, overall survival; OSR, overall survival rate; DoR, duration of response: HNSCC, head and neck squamous cell carcinoma.

immunological tolerance because the proteins present in the vaccine are exogenous.[135]

Currently, a recombinant vaccine approved by the FDA named Gardasil 9, for the prevention of throat cancer is based on a study recruiting 6000 patients suggesting that the subjects receiving vaccines were at a lesser risk of developing throat infection by HPV.[136]

There are approximately 200 genotypes of the HPV based on the genome sequence.[137] The open reading frames (ORFs) in the HPV genome are based on; the time when the genes are expressed in accordance with the viral life cycle: early (E1-E7) and late (L1 and L2) genes.[138] E6 and E7 play a major role in the initial steps of HPV infection contributing to carcinogenesis in head and neck squamous cells by causing cell cycle dysregulation. E6 and E7 are oncoproteins that are overexpressed before significant viral replication.[139] The viral deoxyribonucleic acid (DNA) gets incorporated into the host DNA with consequent production of E6 and E7 oncoproteins. E6 oncoprotein targets a tumor protective gene, p53 (a protein responsible for cell division and cell death) and causes its degradation.[140] E7 oncoprotein targets retinoblastoma protein (pRb) leading to its proteolytic destruction and inhibits its activity leading to excessive cell growth.[141] Various other proteins transcribed also leads to cell membrane signal transduction dysfunction as well as the acceleration of the G1-S phase, all leading to malignant proliferation and carcinogenesis.[142]

Moreover, HPV is a factor that can induce carcinogenesis in the head and neck, HPV-targeted immunoprevention vaccines will prevent viral infection by activation of immune B-cells and formation of antibodies that will act and fight the infection, thus hindering the cancer formation.[143] Role of HPV infection in development of HNSCC and the protective action of prophylactic HPV vaccines is depicted in Figure 2.

Therapeutic Vaccines

The overexpression of the HPV oncogenes E6–7 in majority of the malignant cells has provided the opportunity for therapeutic vaccine development.[73] These vaccines are investigated for their therapeutic applications along with other ICIs for the better clinical outcomes in HNSCC. Apart from the above

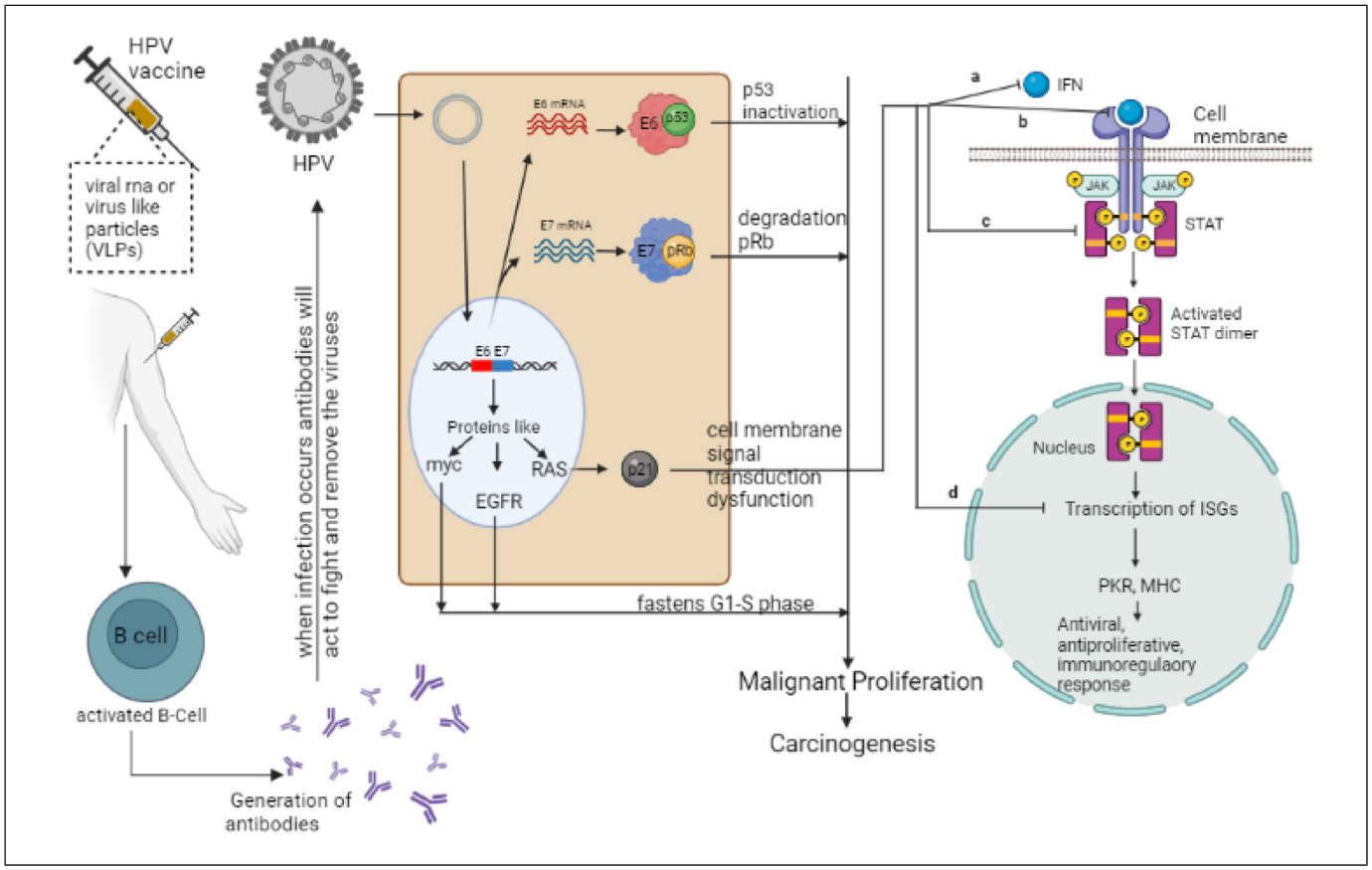

Figure 2. Pathology of HPV infection in the development of HNSCC and effect of vaccination in the prevention of HPV-associated HNSCC. After infection, the viral DNA is integrated with the host genome resulting in altered expression of Tumor suppressor genes, and overexpression of E6 and E7 mRNA that is translated to oncoprotein E6 and E7 respectively leading to dysregulation of the cell cycle and malignant proliferation. Abbreviations: HPV, human papillomavirus; VLP, virus-like proteins; pRb, retinoblastoma protein; IFN, interferon; STAT, signal transducer and activator of transcription; ISGs, interferon stimulated genes; EGFR, epidermal growth factor receptor; MHC, major histocompatibility complex; PKR, protein kinase R; HNSCC, head and neck squamous cell carcinoma.

mentioned viral based vaccine, other strategies of therapeutic vaccines are also being researched such as live vector based, DNA/RNA based, DC based or peptide-based vaccines. Live vector-based vaccine uses bacteria such as lactobacilli or viruses such as adenovirus as vectors to trigger innate or adaptive response in hosts. Gene based vaccines combines the DNA/RNA strands with a lipid carrier which acts as immunogenic agents activating the innate responses via immune receptors. DNA based vaccines are preferred over RNA based vaccines as they are easier to prepare and are much more stable. A DC based vaccine initiates adaptive immune response and cross-presents the antigens to immune cells. Peptide based vaccines contains epitopes that are capable to induce an immune response which will be recognized by the APCs.[144] The outcome of these therapeutic vaccines clinical studies has been mentioned in the table 2.[145–148]

Miscellaneous Cytokines and Inflammatory Mediators Involved in Tumor Growth and Progression

Cytokines

The tumors of HNC possess high inflammatory properties and express a lot of cytokines and other factors involved in inflammation.[149] Recently, a lot of research has shown that imbalance in the expression of pro-inflammatory and anti-inflammatory cytokines occurs in patients having HNSCC. Thus, theoretically, it may have a role in tumor formation, immunomodulatory targets for therapy or aid in diagnostics. IL-8, a pro-inflammatory cytokine is raised in the serum of patients having HNSCC proving its role in the invasion, metastasis, and angiogenesis of cancer.[150] The mAbs against IL-6, tocilizumab is also being studied for a treatment option in HNSCC.[151]

HNSCC cells produce cytokines that lead to immune suppression.[152] Major cytokines involved in immune suppression, that can be targeted are TGF-β, IL-6, and IL-10. The TGF-β produced by HNSCC cells suppresses the activation of T-Cells and NK cells. It also plays an important role in the differentiation of Tregs which are involved in tumor development and progression by inhibition of antitumor immunity.[66] IL-6 leads to activation of STAT3 signalling which further inhibits activation of NK cells, T-Cells, neutrophils, macrophages, and maturation of dendritic cells (DCs).[48] This inhibition of activation and maturation has been correlated with survival and recurrence.[153] STAT3 signalling is also responsible for further activation of immunosuppressive pathways like IL-10,[154] downregulation of IL-12,[155] generation of Tregs,[156] and suppression of DCs.[157] IL-10 further leads to the activation of the STAT-1 signalling pathway. HNSCC cells produce proangiogenic molecules like Prostaglandin E (PGE)[158–160] and promoters of angiogenesis like VGEF.[161,162] This leads to an increase in immature cells resulting into dysfunction and inactivation of T cells.[44] Mechanism of the cytokine mediated development and progression of HNSCC is shown in Figure 3.

Inflammatory Mediators

Inflammation that occurs in the tumor microenvironment is due to the production of inflammatory mediators produced by the tumor itself and its infiltrating and stromal cells.[149] NF-κB is an injury signal transcription factor that contributes to cell survival, proliferation, invasion, inflammation, and angiogenesis which is activated in many tumors. Inhibition of NF-κB function in HNSCC results in reduced tumor growth by decreasing the expression of IL-6 and IL-8 along with many other pro-inflammatory cytokines and chemokines.

Inhibition of apoptosis by upregulation of B-cell lymphoma 2 (Bcl-2) is carried out by one of the members of the prostaglandins class, namely PGE2 which is increased in HNSCC. It also induces the production of angiogenic factors and causes invasive and metastatic growth of tumors, thus promoting tumor growth.[159] A specific cyclooxygenase (COX-2) inhibitor, celecoxib is currently being studied in various trials to prevent the recurrence of HNC in high-risk patients.[163]

A preclinical model in phase 1 has demonstrated that STAT3 decoy oligonucleotides possess the angiogenic activity and that it inhibits STAT3. Since EGFR and IL-6 activate STAT3,

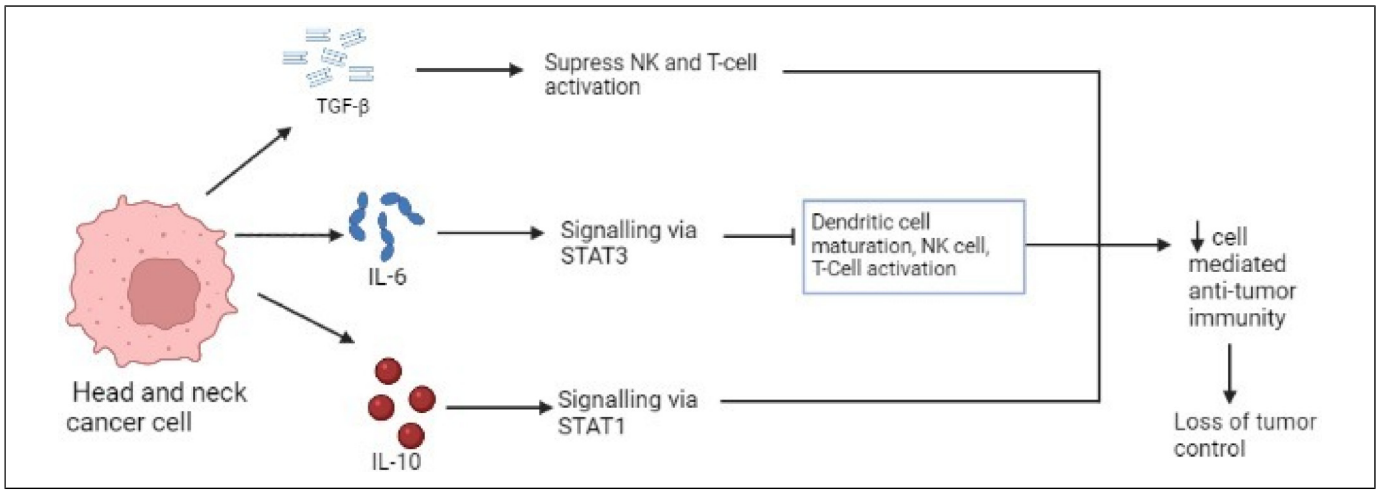

Figure 3. Process of cytokine-mediated loss of anti-tumor immunity by activation of signaling pathways by cytokines produced by the cancer cells.

STAT decoy oligonucleotides can be used in synergism with EGFR or IL-6 antagonists.[164]

Clinical Trials: Neoadjuvant, Definitive, Adjuvant and R/M HNSCC Including Immunotherapy for the Management of HNSCC

The numerous clinical trials have been conducted in HNSCC to evaluate safety and efficacy of neoadjuvant, adjuvant as well as definitive immunotherapy. The significant landmark clinical trials have been mentioned in the table 3 which are completed and table 4 which are currently on-going to explain about the efficacy and safety of immunotherapy agents.

Neoadjuvant Immunotherapy

The immunomodulatory agent against PD-1 receptor, nivolumab has been approved along with other chemotherapy or immunotherapy. Its use as neoadjuvant ie, without surgical resection of the tumor has been explored through various clinical trials.

In a phase-II clinical trial (eligible for ≥ T2 or node-positive) NCT02919683, Schoenfeld and his colleagues investigated neoadjuvant Nivolumab (N) or Nivolumab plus Ipilimumab (N+I) in 29 untreated oral cavity cancer patients. Patients received Nivolumab 3 mg/kg on weeks 1 and 3 and Ipilimumab 1 mg/kg was given only on week 1. There were no surgical delays even though 21 patients had AEs, including grade 3 or 4 AEs in two (N) and five (N+I) patients. The volumetric response rate was seen in three patients more for N+I group compared to N group. Four patients (N, n=1; N+I, n=3) had shown a complete response which suggests a positive response in both the arms. Objective response was seen in 38 patients receiving N+I and 13 patients receiving N only. These indicate the clinical tolerability and efficacy of immunotherapy. We can also conclude that combining two immunotherapy agents would have an improved response when compared to monotherapy of immunotherapy. Also, addition of another agent did not show significant increase in toxicity.

The IMCISION study was conducted in stage II-Iva HNSCC patients to examine neo-adjuvant nivolumab and ipilimumab (NCT03003637) which were presented at the European Society of Medical Oncology (ESMO) 2020. Patients undergoing either definitive or salvage therapy were included in this study and found that the therapy was safe, with 16/26 patients (61.5%) having pathologic responses and 8/26 patients (31%) having a complete response, respectively.

Another similar clinical trial NCT03238365 that is currently ongoing, to compare the efficacy and safety of nivolumab along with ipilimumab.

These indicate the clinical tolerability and efficacy of immunotherapy. We can also conclude that combining nivolumab with ipilimumab would have an improved response when compared to monotherapy of immunotherapy. Also, addition of another agent did not show significant increase in toxicity.

Similar other trials have shown significant response in HNSCC when nivolumab was combined with conventional chemotherapy. Nivolumab alone was used in presurgical oral cavity cancer in a clinical trial NCT03021993.

As per the results of the above trials it can be inferred that nivolumab in combination with other immunotherapy drugs should be evaluated as it has the potential to show promising results in terms of safety and efficacy.

Another PD-1 targeted immunomodulatory agent pembrolizumab is also assessed alone as well as in combination. Pembrolizumab (neoadjuvant/adjuvant) was investigated in patients with locally advanced, resectable HPV-negative HNSCC in a phase-II clinical trial NCT02296684. A total of 36 patients were included (T3/T4; 80%, stage IV; 92%) and were given a single dose of neoadjuvant Pembrolizumab 200 mg followed by surgery two or three weeks. The safety, pathologic tumor response rate (pTRR), and relapse rate with Pembrolizumab were assessed. Patients with positive surgical margins or extra-nodal extension were treated with adjuvant pembrolizumab and postoperative RT or concurrent chemoradiation therapy (CCRT) were performed as standard of care. There were no grade 3 or 4 major AEs or surgery delays, demonstrating the safety of neoadjuvant immunotherapy. Moreover, in high-risk patients, the one-year relapse rate was 16.7%. Two independent pathologists have examined the pTR scores and graded them on a scale of pTR-0 (<10%), pTR-1 (10-49%), and pTR-2 (≥50%). The pTR-0 and pTR-1 were observed in 44% of patients, while pTR-2 was found in 22%. Primarily, pTR following neo-adjuvant Pembrolizumab was associated with baseline tumor, PD-L1, immune infiltration, and IFN-activity, but not with TMB.[165] These findings demonstrate that the reactivity of neoadjuvant immunotherapy is associated with the immunogenic phenotype before treatment, implying that patients could be selected for neoadjuvant immunotherapy before surgery in the future. The efficacy and safety of neoadjuvant pembrolizumab can thus be established. Another clinical trial NCT03765918 which is currently ongoing also tests pembrolizumab along with radiation therapy. As shown in the above trial it is also expected to get results proving the safety and efficacy of pembrolizumab as neoadjuvant therapy.

Apart from this various other PD-1 inhibitors such as durvalumab studied with or without tremelimumab and atezolizumab are under clinical trials

Based on the above results clinical trials it can be inferred that these PD-1 inhibitors alone or in combination with other PD-1 inhibitors have acceptable efficacy and safety profiles. Still a lot of combinations can be explored to find the safest and most effective combination.

Adjuvant Immunotherapy

Nivolumab as an adjuvant therapy has not been studied much until lately. Two clinical trials are currently ongoing using nivolumab in combination with conventional chemotherapy or ipilimumab.

Table 3. Summary of Completed Clinical Studies Investigating Effectiveness of Targeted/Immunotherapy in HNSCC.

Study (Trial No. or Name)	No. of Patients	Trial Phase	Clinical Protocol	Treatment Arm 1	Treatment Arm 2	Primary Endpoint	Secondary Endpoint
Neoadjuvant targeted/immunotherapy							
NCT03238365	25	Early phase I	Neoadjuvant	Nivolumab 240 mg + conventional surgery	Nivolumab + Tadalafil 10 mg + conventional surgery	≥20% Pathological treatment response: 54% Complete pathological response: 7%	Prevalence of intra tumoral immune cell population: augmented immune microenvironment
NCT03003637	26	Ib/II	Neoadjuvant	Nivolumab 240 mg	Nivolumab 240 mg + Ipilimumab 1 mg/kg	Pathological response: 61.5% Complete response: 31%	-
NCT03341936	28	II	Neoadjuvant	Nivolumab 240 mg + Lirilumab 240 mg	-	DFS: 55.2% at 1 year	Response at time of salvage surgery: 27 patients, Median DFS: 12.9 months, OS: 85.7% at 1 year
NCT03342911	27	II	Neoadjuvant	Nivolumab 240 mg + paclitaxel 100 mg/m^2 + carboplatin (AUC: 2)	-	Pathologic complete response rate: 42% patients	Major pathologic response: 69% patients
NCT02296684 (Uppaluri et. al)	46	2	Neoadjuvant	Pembrolizumab 10 mg/kg	-	LRR/DM rate: 15%	AEs
NCT03021993 (Xiong et. al)	17	2	Neoadjuvant	Nivolumab	-	Pathological ORR: 33%	Level of Treg cells, activated T-cells
NCT03144778 (Ferrarotto et. al)	28	1	Neoadjuvant	Durvalumab 1500 mg	Durvalumab 1500 mg + Tremelimumab 75 mg	Pre and post treatment Difference in CD8 +TILS: no significant difference	Safety, toxicity: No grade >3 AEs ORR: 43%
Adjuvant targeted/immunotherapy							
NCT03700905	276	III	Adjuvant/ neoadjuvant	Surgical resection + cisplatin 100 mg/m^2 + radiation 55-65 Gy + Nivolumab 3 mg/kg + Ipilimumab 1 mg/kg	Surgical resection + cisplatin 100 mg/m^2 + radiation 55-65 Gy	DFS (approximately 71 months)	LRC, DMFS, OS, acute toxicity and late morbidity, quality of life, survival depending on PD-L1
Definitive targeted/immunotherapy							
NCT03349710	74	3	Definitive	Cohort 1: Nivolumab + Radiotherapy (Arm A) Cetuximab + Radiotherapy	Cohort 2: Cisplatin + Nivolumab + Radiotherapy (Arm C) Cisplatin + Radiotherapy (Arm D)	SAE: Arm A: 50%; Arm B: 50%; Arm C: 37.04%; Arm D: 29.17%	-

(continued)

Table 3. (continued)

Study (Trial No. or Name)	No. of Patients	Trial Phase	Clinical Protocol	Treatment Arm 1	Treatment Arm 2	Primary Endpoint	Secondary Endpoint
				(Arm B)			
R/M HNC targeted/immunotherapy							
KEYNOTE-048 comparison 1: pembrolizumab plus chemo versus EXTREME	882	III	First-line R/M	Pembrolizumab 200 mg + cisplatin 100 mg/m^2 + 5-FU 1000 mg/ m^2 + carboplatin (AUC=5)	Cetuximab 400 mg/m^2 + cisplatin 100 mg/m^2 + 5-FU 1000 mg/m^2 + carboplatin (AUC=5)	Arm 1 PFS: 4.9 months OS: 13 months Arm 2 PFS: 5.2 months OS: 10.7 months	Arm 1 ORR: 36.4 patients QoL score: 1.17 Arm 2 ORR: 35.7 patients QoL score: 0.77
NCT02517398	32	I	Advanced HNSCC	Bintrafusp alfa 1200 mg	-	ORR: 13%	Total clinical response: 22% Treatment related AEs: 34%
NCT02521870	26	Ib/II	R/M HNSCC	SD-101 8 mg + pembrolizumab 200 mg	-	This trial was terminated.	
NCT02643550	40	II	R/M HNSCC	Monalizumab 10 mg/kg + cetuximab	-	ORR: 27.5% Dose limiting toxicities	OS: 44% PFS, DoR
ECHO-202/ KEYNOTE-037	62	I/II	Advanced HNSCC	Epacadostat (25, 50, 100, or 300 mg) + pembrolizumab 2 mg/ kg	-	Treatment related AEs:84% ORR:55%	-
NCT02501096	137	Ib/II	Advanced HNSCC	Lenvatinib + pembrolizumab 200 mg	-	ORR: 46% DLT, maximum tolerated dose	Treatment emergent AEs, PFS: 4.7 months OS, DoR, DCR, clinical benefit rate
NCT03695510 (ALPHA study)	29	II	R/M HNSCC	Afatinib and pembrolizumab	-	ORR: 41.4%	PFS: 4.1 months OS: 8.9 months
Ferris et.al checkmate 141 NCT02105636	361	III	Second-line R/M	Nivolumab 3 mg/kg	Methotrexate 40 mg/m^2 + docetaxel 30 mg/m^2 + cetuximab 400 mg/m^2	Arm 1 OS: 7.49 months Arm 2 OS: 5.06 months	Arm 1 PFS: 2 months ORR:13.3% drug related serious AEs: 127 patients Arm 2 PFS: 2.3 months ORR: 5.8% drug related serious AEs: 66 patients
Cohen et.al KEYNOTE-040 NCT02252042	495	III	Second-line R/M	Pembrolizumab 200 mg	Methotrexate 40 mg/m^2 + docetaxel 75 mg/m^2 + cetuximab 400 mg/m^2	Arm 1 OS: 8.4 months Arm 2 OS: 6.9 months	PFS, ORR, duration of response, time to progression, AEs

(continued)

Table 3. (continued)

Study (Trial No. or Name)	No. of Patients	Trial Phase	Clinical Protocol	Treatment Arm 1	Treatment Arm 2	Primary Endpoint	Secondary Endpoint
EAGLE# (NCT02369874)	487	III	Second-line R/M	Durvalumab 10 mg/kg	Durvalumab 20 mg/kg + tremelimumab 1 mg/kg or standard of care	OS: No statistically significant improvement	PFS, objective response rate, duration of response.
KEYNOTE-012 (expansion cohort) NCT01848834	132	I	Advanced solid tumors	Pembrolizumab 200 mg IV every 3 weeks	-	ORR: 18.2% AE: 47% participants withdrawing from the study due to AEs	No. of participants with log fold change of cytokines from baseline
NCT01462838	20	I/IIa	Advanced HPV associated cancers	P16_37-63 vaccine 100 μg + Montanide ISA-51 0.3 mL	-	Immune response (CD4: 11, CD8: 4, antibodies:14 patients)	Tumor response: stable disease in 10 patients, Safety: No unacceptable toxicities
ECOG	96	II	Stage III/IV HNSCC	Paclitaxel + carboplatin followed by standard radiation	-	OS: 95%	Risk of progression: 0.27
CheckMate 651 (NCT02741570)	947	III	R/M HNSCC	Nivolumab 3 mg/kg + Ipilimumab 1 mg/kg	EXTREME (cetuximab + cisplatin/carboplatin + fluorouracil ≤6 cycles, then cetuximab maintenance)	Arm 1 OS:15.7 months Arm 2 OS:14.6 months	Arm 1 PFS: 34 months ORR: 98% DoR: 18.3 months Arm 2 PFS: 33 months ORR: 68% DoR: 7.05 months
NCT02111460	126	III	Metastatic nasopharyngeal carcinoma	fluorouracil 5 g/m^2 + cisplatin 100 mg/m^2	fluorouracil 5 g/m^2 + cisplatin 100 mg/m^2 + intensity-modulated radiotherapy	Arm 1 OS: 54.5 Arm 2 OS: 76.4	PFS
NCT02684253	62	II	Metastatic HNSCC	Nivolumab 3 mg/kg	Nivolumab 3 mg/kg + SBRT	Arm 1 ORR: 34.5% Arm 2 ORR: 29%	PFS, AEs, OS, response duration
NCT02383927	22	II	R/M HNSCC	Tipifarnib 600 or 900 mg	-	ORR: 55%	PFS: 5.6 months OS: 15.4 months Safety, tolerability
NCT03740100	8	II	R/M HNSCC	Bimiralisib		The trial was closed because the sponsor became insolvent.	
NCT01852292 BERIL-1	158	II	R/M HNSCC	Buparlisib 100 mg + paclitaxel 80 mg/m^2	Placebo + paclitaxel 80 mg/m^2	Arm 1 PFS: 4.63 months Arm 2 PFS: 3.45 months	Arm 1 OS: 10.41 months ORR: 39.2 patients Time to response: 1.02 months

(continued)

Table 3. (continued)

Study (Trial No. or Name)	No. of Patients	Trial Phase	Clinical Protocol	Treatment Arm 1	Treatment Arm 2	Primary Endpoint	Secondary Endpoint
							Arm 2 OS: 6.45 months ORR: 13.9 patients Time to response: 0.99 months
NCT03522584	6	I/II	R/M HNSCC	Tremelimumab + durvalumab + Hypo fractionated Image-Guided Radiation Therapy + SBRT	-	Terminated by the sponsor due to lack of efficacy in study treatment.	

Abbreviations: ORR, overall response rate; AE, adverse event; PCR, polymerase chain reaction; DFS, disease-free survival; DRR, durable response rate; MPR, major pathologic response; EFS, event-free survival; SOC, standard of care; PFS, progression-free survival; OS, overall survival; OSR, overall survival rate; DoR, duration of response; HNSCC, head and neck squamous cell carcinoma; CD, cluster of differentiation; ECOG, Eastern Cooperative Oncology Group; SBRT, stereotactic body radiotherapy.

Table 4. Summary of on-Going Clinical Studies Investigating Effectiveness of Targeted/Immunotherapy in HNSCC.

Study (Trial No. or Name)	No. of Patients	Trial Phase	Clinical Protocol	Treatment Arm 1	Treatment Arm 2	Primary Endpoint	Secondary Endpoint
Neoadjuvant targeted/immunotherapy							
KEYNOTE-689 (NCT03765918)	704 (Recruitment ongoing)	III	Neo-adjuvant	Neoadjuvant pembrolizumab and adjuvant pembrolizumab added to surgery and standard risk-based adjuvant therapy	Surgery and standard risk-based adjuvant therapy	MPR, EFS	OS, PCR, AEs
NCT02488759	578 (Not recruiting)	I/II	Neo-adjuvant	Nivolumab	-	AEs, SAEs, rate of surgical delay, ORR	DoR, OS, PFS
NCT02919683 (Schoenfeld et. al)	30 (not recruiting)	II	Neoadjuvant	Nivolumab	Nivolumab Ipilimumab	Safety, volume response	PFS, OS, PR
NCT04080804	60 (recruiting)	II	Neoadjuvant	Nivolumab + Relatlimab (Arm 1) Nivolumab + Ipilimumab (Arm 2)	Nivolumab (Arm 3)	AEs	Radiographic response, levels of TILS, levels of PBL, CD4+, CD8+
NCT03708224	55 (Recruiting)	II	Neoadjuvant	Atezolizumab Monotherapy (Arm 1) Atezolizumab (Adjuvant therapy) (Arm 2)	Atezolizumab + Tiragolumab (Arm 3) Atezolizumab + Tocilizumab (Arm 4)	CD3 counts, R0 resection rate	AEs
NCT03247712 (Leidner et. al)	22	I/II	Neoadjuvant/ adjuvant	Nivolumab	-	Surgical delay, pCR, MPR, pathological downstaging	-
Adjuvant targeted/Immunotherapy							
WO420242 (NCT03452137)	406 (Not recruiting)	III	Adjuvant Immunotherapy after definitive CRT	Adjuvant atezolizumab (1200 mg)	Placebo after definitive local therapy (surgery or RT)	EFS	OS, IRF-assessed EFS
ECOG ACRIN EA3161 (NCT03811015)	636 (recruiting)	II/III	Adjuvant Immunotherapy after definitive CRT	Nivolumab plus cisplatin/RT	Cisplatin/RT	OS	PFS
NCT03700905	276	III	Adjuvant/ neoadjuvant	Surgical resection + cisplatin 100 mg/m^2 + radiation 55-65 Gy + Nivolumab	Surgical resection + cisplatin 100 mg/m^2 + radiation 55-65 Gy	DFS	LRC, DMFS, OS, acute toxicity and late morbidity, quality of life, survival depending on PD-L1

(continued)

Table 4. (continued)

Study (Trial No. or Name)	No. of Patients	Trial Phase	Clinical Protocol	Treatment Arm 1	Treatment Arm 2	Primary Endpoint	Secondary Endpoint
				3 mg/kg + Ipilimumab 1 mg/kg			
NCT03174275	39	II	Adjuvant/ Neoadjuvant	Durvalumab 750mg + carboplatin + nab-paclitaxel + surgical resection		pCRR	Clinical CRR, patients with change in estimated risk, PFS, OS, toxicity profile.
Definitive targeted/Immunotherapy							
GORTEC 2017-01 (REACH) (NCT02999087)	707 (not recruiting)	III	Definitive	Cisplatin eligible patients: Avelumab Plus cetuximab/RT	Cisplatin/RT	PFS	OS, Safety
NRG HN-004 (NCT03258554)	493 (recruitment suspended)	II/III	Definitive	Durvalumab/RT	Cetuximab/RT	DLT, PFS, OS	LRF, DM, CM
NRG HN-005 (NCT03952585)	711 (Recruitment ongoing)	II/III	Definitive	Nivolumab/reduced dose RT (60 Gy) (Arm 1) Cisplatin/reduced dose RT (60 Gy) (Arm 2)	Cisplatin/standard dose RT (70 Gy) (Arm 3)	PFS, QOL	OS, LRF
KEYNOTE-412 (NCT03040999)	804 (not recruiting)	III	Definitive	Pembrolizumab + cisplatin/RT	Cisplatin/RT	EFS	OS, AEs
NCT03944915	36 (recruiting)	II	Definitive	Carboplatin + Paclitaxel (100 mg) + Nivolumab 360 mg	Paclitaxel (100 mg) + radiation + hydroxyurea + 5-fluorouracil +filgrastim + cisplatin	DRR	PFS, OS, LRC, DC
R/M HNC targeted/immunotherapy							
NCT04862455	60	II	R/M HNSCC	NBTXR3 + radiation Therapy + pembrolizumab	-	PFS, local/ regional/ distant failure, ORR	DoR, OS, treatment related AEs
NCT03283605 OZM-088	45	I/II	Metastatic HNSCC	Durvalumab 1500 mg + tremelimumab 75 mg + SBRT	-	Acute toxicities, PFS	Local control, OS, abscopal events
LM-HNSCC NCT05136768	50	II	Limited metastatic HNSCC	Sintilimab 200 mg + platinum-based chemotherapy + SBRT	-	PFS	OS, ORR, DCR, DoR, time to progression, AEs
NCT04338399 BURAN	483	III	R/M HNSCC	Buparlisib 100 mg + Paclitaxel 80 mg/m^2	Paclitaxel 80 mg/m^2	OS	PFS, ORR, QoL, safety and tolerability, plasma concentration-time profile
NCT03494322 922P EACH	130	II	R/M HNSCC	Avelumab 10 mg/kg + cetuximab 500 mg/m^2	-	Dose limiting toxicity	ORR: 50% DCR: 6 months
NCT03524326	24	I	Advanced HNSCC	Lenvatinib (dose de-escalation) 24-4 mg + cetuximab 400 mg/m^2	-	Maximum tolerated dose	-
	500	III	R/M HNSCC			ORR, PFS, OS	DOR, AEs

(continued)

Table 4. (continued)

Study (Trial No. or Name)	No. of Patients	Trial Phase	Clinical Protocol	Treatment Arm 1	Treatment Arm 2	Primary Endpoint	Secondary Endpoint
LEAP-010 NCT04199104				Pembrolizumab 200 mg + Lenvatinib 20 mg	Pembrolizumab 200 mg + placebo		
LEAP-009 NCT04428151	400	II	R/M HNSCC	Pembrolizumab 200 mg + Lenvatinib 20 mg	Standard of care (docetaxel, paclitaxel, cetuximab, or capecitabine)	ORR	PFS, OS, DoR, AEs
NCT05063552 ECOG-ACRIN	216	III	Advanced HNSCC	Cetuximab, Docetaxel, Cisplatin/Carboplatin	Chemotherapy, Bevacizumab, Atezolizumab	PFS, OS	AEs, treatment response
NCT04634825	80	II	R/M HNSCC	Enoblituzumab 15 mg/kg + Retifanlimab 375 mg	Enoblituzumab 15 mg/kg + Tebotelimab 600 mg	ORR, AEs	PFS, DCR, DoR, OS, Antidrug antibody development
NCT04590963	624	III	R/M HNSCC	Monalizumab + cetuximab	placebo + cetuximab	OS	PFS, immunogenicity, AEs, ORR, DoR, pharmacokinetics
NCT03625323	18	II	Metastatic HNSCC	Eftilagimod alpha 30 mg + pembrolizumab 200 mg	-	ORR: 39%	Tolerability, DCR, PFS, OS, pharmacokinetics, immunogenicity

Abbreviations: OS, overall survival; PCR, pathological complete response; MPR, major pathological response; EFS, event-free survival; IRF-assessed EFS, independent review facility-assessed event free survival; DLT, dose-limiting toxicity; LRF, locoregional failure; DM, distant metastasis; CM, competing mortality; QOL, quality of life; AE, adverse events; ORR, objective response rate; SAE, serious adverse events; DoR, duration of response; PR, pathological response; LRC, locoregional control; DRR, deep response rate; DC, distant control; HNSCC, head and neck squamous cell carcinoma; TILs, tumor-infiltrating lymphocytes; CD, cluster of differentiation; SBRT, stereotactic body radiotherapy.

IMVoke010 (NCT03452137) is a phase 3 study currently ongoing in patients who have received a definitive local/regional therapy for stage III HPV-positive oropharyngeal cancer or stage IVA/IVB HPV-negative HNSCC to assess the safety and efficacy of atezolizumab as adjuvant therapy. The primary endpoint of this study includes IRF-assessed EFS and OS whereas the secondary outcomes are investigator-assessed EFS, safety and patient reported outcomes.

Apart from this durvalumab is also assessed for its efficacy via measuring pathologic and clinical CRR, PFS and OS along with its safety for adjuvant use in HNSCC.

The clinical trials for adjuvant immunotherapy are currently ongoing and thus it cannot be said that these PD-L1 inhibitors can be effective or not. The results of these clinical trials might help in giving an idea about the practical applicability of PD-L1 inhibitors as adjuvants in HNSCC.

Definitive Immunotherapy

A comparison of nivolumab and cetuximab alone as well as in combination with conventional chemotherapy and radiotherapy was carried out via a clinical trial (NCT03349710). The main objective was to measure the safety of these drugs alone and in combination. There was a higher chance of adverse events and immune mediated toxicities with combining cetuximab and nivolumab. However no serious toxicities were seen with the combination so as to cause dose reduction or death. Thus, it might be helpful to target two immune targets at a same time. Trials measuring the efficacy of nivolumab along with conventional chemotherapy are currently ongoing. Durvalumab and cetuximab as monotherapy is also assessed (NCT03258554) in combination with radiation therapy. This trail measures the efficacy and safety of these agents. Another phase-III randomized trial, KEYNOTE-412/MK-3475–412, recently completed enrolment of neo-adjuvant and adjuvant pembrolizumab versus placebo in definitive CRT (NCT03040999). But efficacy studies regarding combinations are yet to be done to see the magnitude of outcome improvement. Efficacy study combining cetuximab and avelumab is currently ongoing measuring PFS and OS as safety measures. These results might help to extrapolate the efficacy parameters to the nivolumab combination.

Recurrent/ Metastatic HNSCC Immunotherapy

Comparison of pembrolizumab and cetuximab along with definitive chemotherapy was carried out through a clinical trial (KEYNOTE-048). The subjects received either pembrolizumb or cetuximab along with cisplatin, 5-FU and carboplatin. The primary outcome of this study was progression free survival (PFS) and overall survival (OS) wherein the observation showed a better PFS and OS in the arm receiving cetuximab. Thus, both the treatments can be effective in patients with R/M HNSCC. Another study (KEYNOTE-037) evaluating the safety of combination epacadostat and pembrolizumab in patients with advance HNSCC exhibited a good tolerability and anti-tumor activity thus encouraging the use of this combination. Apart from this pembrolizumab alone against the combination of methotrexate, docetaxel and cetuximab was assessed for OS in a phase-III trial (NCT02252042).

A phase III clinical trial comparing Nivolumab and the standard combination of methotrexate, docetaxel and cetuximab showed better overall survival with nivolumab than the standard therapy. Thus, patients with PD-L1 expression may respond better with nivolumab. Other phase III trial comparing combination of nivolumab and ipilimumab with standard therapy showed no significant difference in OS. However, the study supports the indication of the combination due to favourable safety profile than the standard therapy.

Durvalumab as a monotherapy was compared with durvalumab and tremelimumab combination for OS in another phase III study (NCT02369874). However, no significant difference was observed between both the groups indicating that durvalumab alone may suffice the outcome as compared to the combination.

Various other trials evaluated Overall response rate for Bintrafusp (NCT02517398), monalizumab and cetuximab combination (NCT02643550) and Tipifarnib (NCT02383927).

Efficacy studies of pembrolizumab in combination with radiation therapy (NCT04862455) or Lenvatinib (NCT04199104) or eftilagimod alpha (NCT03625323) are still ongoing whose result may give a better understanding for the drug to be best combined with pembrolizumab for the management of R/M HNSCC.

Shortcomings of Immunotherapy

Progression and response to immunotherapy may vary in patients such as progression, pseudoprogression or hyperprogression.[166] A response following progression is termed as pseudoprogression. It can also be stated as increase in primary tumor size followed by a response to immunotherapy. Thus, it is not a genuine tumor progression.[167,168] This may be due to delayed immunologic response along with inflammatory response.[168] On the other hand, hyperprogression is when the disease appears to grow at a faster rate after starting the immunotherapy.[168]

In a randomized phase 3 study CheckMate141 (NCT0210 5636), 240 patients with R/M HNSCC received nivolumab. Among them 146 (61%) noticed progression. Of the 146 patients with progression nivolumab was continued in 62 (41%) patients and discontinued in the remaining 84(58%) patients. The study concludes that treatment beyond progression in patients with R/M HNSCC was tolerable and also correlated to reduction in tumor size.[169]

There are four basic immunotherapeutic strategies in treatment of HNSCC including oncolytic viral therapy, monoclonal antibodies, CAR-T cell therapy and therapeutic vaccines. Majorly immunotherapy works in patients with tumor showing some expression of checkpoints or that is positive for some specific mutation. For instance, patients with PD-1 positive status are sensitive to ICIs targeting PD-1 receptor or patients with positive EGFR status are sensitive to TKIs.[170] This seems to be a major challenge of monoclonal antibodies-based immunotherapy. Patients that lack particular immune islands or predictive biomarkers cannot be reliably given

these antibodies. Though there are case reports showing effectiveness of PD-1 targeted ICI pembrolizumab in patients with PD-1 negative tumors .[171] In order to overcome the unpredictability of response in these patients, an approach is being developed that corelates microbiota as a biomarker for response to immunotherapy. This has been evaluated in two preclinical studies on murine models that revealed an impact of commensal intestinal microbiota on the effectiveness of ICIs.[172,173] A single trial CHECKMATE-141 corelated the influence of oral microbiota with the response to nivolumab therapy in R/M HNSCC. This study showed a no significant correlation with efficacy or survival.[174] Thus, studies with better designs and wider perspectives are yet to be carried out in order to establish a reliable correlation and thus help in predicting response of immunotherapy in patients with negative expression of immune islands.

Immunotherapy is comparatively safer compared to chemotherapy. However, it is also associated with numerous toxicities. A meta-analysis was carried out in order to elucidate the toxicity profile of these immunotherapeutic agents. The results showed 16.5% patients with more than grade 3 adverse events.[175] ICIs are commonly associated with gastrointestinal (GI) side effects and hepatic complications such as hepatitis.[176] The GI side effects vary from diarrhoea to enterocolitis, intestinal perforation, and even death.[177] GI complications are most significant. Colitis caused due to ICIs is reversible and could be treated as per immune mediated adverse reaction management guidelines. The frequency of ICIs induced hepatic adverse events (AEs) are comparatively less common.[178] Reports about acute pancreatitis have been received but its clinical occurrence is rare.[176] Other Immune-related adverse events (irAEs) associated with ICIs are cutaneous irAEs, pulmonary irAEs, endocrinological SEs like hypophysitis and thyroid irAEs, cardiac irAEs, neurological irAEs, hematological irAEs and renal irAEs.[179,180]

Conclusion

Immunotherapy in cancer is a fast-expanding arena where researchers have recently begun to shed some light on the complex interaction between cancer and the host immune system. The immunomodulatory drugs have the benefit of specific targeting that is a major drawback of the conventional chemotherapy. These agents can clearly discriminate between healthy and cancerous cells and thus show a lot of promising use in the future. The drugs acting on immunologic targets are emerging into routine practice. Immunotherapy is currently being explored through clinical trials. It is thus advancing from an alternative therapeutic option to first-line drugs of choice in the treatment of HNSCC as an adjuvant to existing chemotherapeutic regimens. Despite current advances in immunotherapy, there are many immune targets which are yet to be studied closely.

Future Approaches to Develop MAbs/Immunotherapy for HNSCC

Monoclonal antibodies are a type of targeted therapy in cancer which marks various tumor cells thus leading to their destruction. Currently, three mAbs are approved for use in HNSCC by the FDA that are cetuximab, pembrolizumab and nivolumab. The numerous other targets and their corresponding antibodies are under development. Apart from cetuximab, various other mAbs acts on EGFR which includes tomuzotuximab, Sym004, Zalutumumab, nimotuzumab, GA201 (RG7160), GBR 1372, and panitumumab. Moreover, the different biosimilar versions of cetuximab are also currently being developed by various pharmaceutical industries. Asciminib is another mAb acting on VEGF receptors apart from bevacizumab.[181] Apart from extensive research regarding development of newer drugs acting on the immunologic targets, a nano-technological approach is also drawing attention. Active or passive targeting through nanoparticles can be carried out by coating them with antibodies. Moreover, HNSCC is majorly a heterogenous tumor and the immunotherapy targets very specific mutations which might not be applicable in a generalized pattern to all the tumors. Thus, very specific biomarkers showing the impact of these agents on the tumor are required to prevent unwanted toxicity and achieve proper disease related outcomes to the patients. Looking at the results of various studies and preclinical as well as clinical trials it can be said that immunotherapy might be an answer to the limitations and shortcomings of the current strategies in treatment of HNSCC. Thus, further work in development of mAbs based strategies will provide paradigm shifting treatment options for HNSCC.[182]

Authors' Note

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