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Targeting the EGFR and immune pathways in squamous cell carcinoma of the head and neck (SCCHN): forging a new alliance

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Abstract

Despite the recent approval of immune-modulatory agents, EGFR inhibition continues to be a cornerstone in the management of SCCHN namely in combination with radiotherapy in the treatment of locoregionally advanced disease as well as in platinum sensitive recurrent or metastatic disease in the first line setting.

Importantly, recent evidence has emerged supporting also an immune-modulatory effect of EGFR inhibition, and interest has now focused on utilizing these effects in the current treatment approaches for SCCHN. In this report we review the rationale and evidence supporting the forging of this new alliance in optimizing the treatment of SCCHN.

Keywords

Immunotherapy and EGFR in head and neck cancer; combination cetuximab and immunotherapy; EGFR monoclonal antibodies and immunotherapy; EGFR and immunotherapy in SCCHN; combination of anti-EGFR and immunotherapy in head and neck cancer Immunotherapy

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Rationale for inhibiting EGFR in SCCHN

SCCHN is diagnosed worldwide in more than 500,000 people and is responsible for 380,000 deaths annually [1]. SCCHN is a heterogeneous disease as far as anatomic location and genetic aberrations [2]. Despite advances in multimodal therapy, the survival rates and functional outcomes of patients remain limited with an overall 5 year survival rate that is close to 50% [3]. Novel strategies are urgently needed particularly in patients with recurrent or metastatic disease. Abnormal EGFR signaling in a variety of tumors including SCCHN has been correlated with poor prognosis and lower response to therapy [4-8]. More than 80% of SCCHN tumors show EGFR over-expression [7, 9]. Cetuximab is an anti-EGFR receptor antibody contributing to growth suppression and apoptosis of SCCHN [10] and it has been shown to reduce cancer cell proliferation in xenograft models [11-15]. Even though the immunomodulatory effects of cetuximab have been described and well-established earlier, attention to this particular mechanism has surged more recently given the increased interest of this application in SCCHN [16]. Cetuximab in combination with platinum-based chemotherapy has been shown to result in improved overall survival when given as first-line treatment to patients with recurrent/metastatic SCCHN compared with platinum-based chemotherapy alone [17]. In recently reported two phase III trials comparing cetuximab versus cisplatin in combination with radiation for definitive treatment of HPV related oropharyngeal SCC, cisplatin resulted in a superior overall survival compared to cetuximab [18, 19], therefore confirming that cisplatin radiotherapy is the preferred standard of care for patients with HPV-related oropharyngeal SCC

The limitations of anti-EGFR targeting in SCCHN

The human epidermal growth factor receptor (EGFR) family consists of four types of trans-membrane tyrosine kinase receptors, HER1 (EGFR, ErbB1), HER2 (ErbB2), HER3 (ErbB3), and HER4 (ErbB4). The general structure of ErbB members includes an extracellular ligand-binding region, an α -helical trans-membrane segment, a cytoplasmic tyrosine-kinase-containing domain, and a C-terminal phosphorylation tail [20, 21]. ErbB members are widely expressed in epithelial, mesenchymal, and neuronal tissues and regulate cell division, proliferation, differentiation, and other normal cellular processes [22, 23].

EGFR is expressed in more than 80% of SCCHN [9]. Ligand binding, resulting in homodimerization or heterodimerization with other HER family members, causes phosphorylation of the tyrosine kinase domain and cell proliferation. The EGFR-specific monoclonal antibody drug cetuximab decreases tumor cell line growth, and increases apoptosis of SCCHN [10]. In vitro and xenograft studies have shown that cetuximab effectively decreases tumor cell proliferation [11-14]. Despite its success in combination therapy, the overall response rate to cetuximab as a single-agent treatment approach in recurrent/metastatic SCCHN does not exceed 13% and is associated with a median response duration of less than 70 days [24]. In combination with chemotherapy, the median progression-free survival for patients receiving it as a first-line therapy is around six months [17]. Furthermore, patients frequently demonstrate primary resistance to EGFR monoclonal antibodies and acquired resistance emerges over time [25, 26], which further stresses the need for mechanistic studies to understand resistance to EGFR inhibition. Analyses

performed on tissue samples from large randomized trials have disappointingly revealed that EGFR expression does not seem to be a clinically useful predictive biomarker in SCCHN patients [27]. In addition, EGFR copy number was not predictive of cetuximab efficacy in recurrent/metastatic SCCHN[28], despite being useful in other solid tumors[29, 30]. The survival benefit of chemotherapy plus cetuximab over chemotherapy alone was shown to be independent of tumor p16 and HPV status[31] indicating that resistance mechanisms to these regimens may affect both HPV-positive and –negative subtypes of SCCHN. Despite the argument that single agent anti-EGFR therapy may have a lower clinical activity in HPV-related versus -unrelated disease, and data pointing to an inverse relation between HPV status and EGFR expression[32, 33], no definite evidence exists to distinguish the use of cetuximab based on HPV status. These findings suggest that failure of cetuximab therapy is likely linked to alternative pathways, which may include compensatory signaling from other EGFR family receptors, such as HER2 and HER3, or other downstream resistance mechanisms[15] [34-36].

Resistance mechanisms to EGFR inhibition

More than 10 mechanisms of resistance to EGFR targeting have been reported in SCCHN[37] (Figure 1). Possible mechanisms for *de novo* and acquired resistance to EGFR inhibition include mutations in the *KRAS*, *BRAF*, *NRAS*, and *PIK3CA* genes[25, 38], a secondary mutation (S492R) in the extracellular domain of the EGFR receptor[25, 26], overexpression of the MET proto-oncogene (c-Met)[39], and expression of the in-frame deletion mutation of EGFR variant III, in addition to other possible mechanisms [40, 41]. In other tumor types, genetic alterations in the EGFR-RAS-RAF-MEK signaling pathways are mechanisms of acquired resistance to anti-EGFR antibodies through the possible constitutive activation of intracellular downstream signaling pathways, including RAS/RAF/MEK/ERK and PI3K/AKT/mTOR pathways[42-47], some of which are rarely observed in SCCHN. Recent evidence has confirmed a possible role of HRAS in EGFR resistance [48-50] and has led to the resurgence of HRAS inhibitors as possible effective therapeutic targets in HRAS mutant SCCHN[51]. Other mechanisms of EGFR resistance may include the dysregulation of EGFR internalization and subcellular localization, including nuclear localization and degradation of EGFR [52-54].

HER3 (ErbB3) is a member of the human EGFR family, which consists of four types of transmembrane tyrosine kinase receptors: HER1 (EGFR, ErbB1), HER2 (Neu, ErbB2), HER3 (ErbB3), and HER4 (ErbB4)[15, 20, 21, 45]. Upon binding of HRG1, the physiological HER3 receptor ligand, HER3 dimerizes with other ErbB family members, preferentially HER2[55-57]. High HRG1 expression is associated with activation of HER3 and has been correlated with worse clinical outcome in SCCHN[58]. We and others have demonstrated consistently elevated expression levels of HRG1 in SCCHN in comparison with other solid tumors, such as non-small cell lung and breast cancers[34].

It is of interest that some mechanisms of EGFR resistance are immune-mediated; such an example is resistance to EGFR mediated through the TGF-beta IL6 axis arguing for an interaction between EGFR signaling and the immune-microenvironment[59]. This

interaction could potentially be harnessed in future applications focusing on targeting EGFR and the TGF-beta IL-6 axis.

Emergence of immunotherapy as an effective therapeutic modality in SCCHN

Even though the immune-suppressive nature of certain cancers including SCCHN has been long recognized [60], it was not until recently that targeted immunotherapy promoting anti-tumor T-cell activity was demonstrated to induce improved survival and durable objective responses in solid tumors, including advanced melanoma[61]. In addition, preclinical data have suggested a beneficial effect of targeting both the programmed death receptor-1 (PD-1) / PD-ligand 1 (PD-L1) and the cytotoxic T lymphocyte antigen-4 (CTLA4) immune checkpoints in SCCHN [62]. PD-L1 expression has been observed in close to 68% of SCCHN tumors regardless of HPV status [63] and several trials have evaluated the utility of PD-L1:PD-1 blockade for the treatment of recurrent/metastatic SCCHN [64] leading to impressive clinical benefits in heavily pretreated patients.

The anti-PD-1 agents pembrolizumab and nivolumab were approved recently for treatment of recurrent metastatic SCCHN based on the results from phase I, II and III studies[65, 66]. The phase 1b Keynote-012 trial using pembrolizumab showed an unprecedented 1-year survival benefit of 18%, which resulted in FDA approval of the drug for the treatment of platinum-resistant recurrent metastatic SSCHN in August 2016 [67]. The results from Checkmate-141, a phase III trial randomizing patients with recurrent or metastatic, platinum-refractory SCCHN to nivolumab versus investigator's choice of chemotherapy (weekly cetuximab, docetaxel or methotrexate), demonstrated a doubling of one-year overall survival (OS) (36.0% versus 16.6%, $p = 0.0101$)[65]. The median OS was 7.5 vs 5.1 months for patients treated with nivolumab versus chemotherapy (HR 0.70, $p = 0.01$). The median OS by PD-L1 status was 8.7 vs. 4.6 months for patients with tumor PD-L1 expression $> 1\%$ vs. PD-L1 $< 1\%$. The 18-month OS rate was 21.5% vs. 8.3% and overall response was 13.3% vs. 5.8%. Nivolumab also doubled the median duration of response versus chemotherapy (9.7 vs 4.0 months). Furthermore, immunotherapy was better tolerated with lower grade 3–4 treatment-related adverse event rates for nivolumab versus chemotherapy (15.3% vs 36.0%). Longer-term follow-up data continued to favor nivolumab with a significant survival benefit (estimated 24-months OS rate 16.7% vs. 6.0%) and better tolerability versus chemotherapy in patients with platinum-refractory disease [68]. The Keynote 040 phase III trial comparing pembrolizumab to docetaxel, methotrexate and cetuximab, supported the results of checkmate 141, showing a clinically meaningful prolongation of overall survival favoring pembrolizumab [69]. In the first line setting Keynote 048 has compared single agent pembrolizumab as well as pembrolizumab in combination with platinum and 5FU to the cetuximab containing Extreme regimen. The results of this trial were reported at the ESMO 2018 meeting and favored both immunotherapy containing versus the cetuximab arm[70]. Though these benefits are groundbreaking in the treatment of recurrent or metastatic SSCHN, there is still a large proportion of the patients who do not benefit from this therapy and might require alternative immune-(re)activation or combination therapies. As immunotherapy continues to be a

rapidly evolving field in the treatment of various malignancies including SCCHN, a growing interest in the examination of immune mechanisms to various targeted therapies including EGFR targeting is currently evident. With the move of immunotherapy to the first line setting in SCCHN as well as its application in the definitive treatment setting, exploring methods to combine these agents with EGFR inhibitors and understanding their interactions is strongly warranted.

Immune implications of EGFR targeting

Although EGFR targeting with cetuximab has been approved for more than a decade as an effective treatment modality for SCCHN, recent evidence has emerged showing that immune modulation represents an alternative mechanism by which EGFR inhibition, specifically with cetuximab, elicits clinical activity in SCCHN [71, 72]. Cetuximab and panitumumab, both monoclonal antibodies to EGFR, were shown to activate natural killer (NK) cells, with cetuximab being a more potent activator[73]. Of further interest however, is that myeloid cell-mediated antibody-dependent cellular phagocytosis (ADCP) may differ based on whether the clinical agent is an IgG1 (cetuximab) versus IgG2 (panitumumab) monoclonal antibody, which may have implications on the use of these agents as part of future combinatorial approaches [74].

In addition to blocking EGFR signaling, cetuximab has been shown to increase IFN- γ produced by NK cells via antibody-dependent cellular cytotoxicity (ADCC), which in tumor cells as well as on immune cells within the tumor microenvironment can induce PD-L1 expression [75, 76] arguing for a possible synergistic effect of EGFR and PD-1 inhibition, also since NK cells themselves can express PD-1.[77]. Recently, the intracellular DNA sensor stimulator of interferon genes (STING) has been shown to have a crucial role in the immune response to viruses and tumors by stimulating cytokine production.[78] The evidence also suggesting that cetuximab coupled with the activation of STING enhances the antitumor activity in SCCHN [79]. All these recent findings strongly suggest that EGFR inhibition interacts closely with the tumor microenvironment affecting the cytokine milieu and leading to an immune modulatory effect.

In addition, blocking EGFR might affect mechanisms of resistance to immunotherapy. In fact, EGF was shown to induce overexpression of PD-L1 by increasing the protein levels of STAT1 to enforce the IFN γ -JAK1/2-mediated signaling axis[80]; this could ultimately reduce the response to PD-L1 inhibitors.

Moreover, activation of the EGFR pathway is involved in suppressing the immune response through activation of Tregs or reducing the level of T cell chemo-attractants [81].

Forging the alliance

Given that both EGFR-targeted therapy and immunotherapy are, or are becoming, important cornerstones for treatment of advanced SCCHN and the scientific rationale supporting an immune modulatory effect of EGFR blockade, research focusing on combining these two modalities is clearly warranted and is already underway. Of importance is the observation that an immune-mediated component seems to be a characteristic of monoclonal antibodies

to EGFR such as cetuximab, panitumumab, zalutumumab, matuzumab or nimotuzumab rather than small molecule EGFR inhibitors. This increases the interest of forging such an alliance in SCCHN given the noted importance of both classes of agents in this disease. Furthermore, exploration of the biologic interactions between EGFR inhibition and the tumor microenvironment remain highly justified. Of note is that such efforts may have significant implications on the therapeutic approaches in different malignancies given the benefit derived from each of these modalities in different tumor types. is an example of a multicenter clinical trial focusing on the combination of cetuximab and nivolumab, both approved for the treatment of recurrent/metastatic SCCHN (Table1). Patients in this trial will be allowed to have prior exposure of either immune checkpoint inhibitor or cetuximab provided these agents are not administered simultaneously, hence exploring a possible beneficial combinatorial effect of these agents. Another example is the REACH study in locoregionally advanced disease, comparing cisplatin with radiation versus cetuximab, avelumab and radiation, versus cetuximab and radiation () (Table 1). Other trials are exploring similar combinations with radiation therapy in patients who are not eligible to receive cisplatin. Even though these trials will take time to mature, we expect to learn of the clinical benefit of these combinations in a relatively short period of time. An important cell to focus on in this alliance are also the NK cells and their most common inhibitory receptors, since they are key players in the immune-related mechanisms of cetuximab[82]. It has been shown that in SSCHN, NK cells can be highly suppressed in their function due to high presence of inhibitory ligands preventing NK cell infiltration within the tumor [83]. An alternative could be combining cetuximab with NK cell adoptive cell therapy[84]. or targeting other NK cell inhibitory receptors besides PD-1. Recent preclinical work and interim analyses of a phase II clinical study [] showed benefit of blocking the inhibitory receptor NKG2A, on NK cells and T cells, using Monalizumab combined with anti-EGFR targeting in patients with recurrent or metastatic SSNHN, showing 31% partial responses (8/26 patients)[85]. On the other side, combining anti-EGFR-tyrosine kinase inhibitors with immunotherapy did not show a favorable safety profile, with an elevated incidence of interstitial lung disease and an increase of alanine aminotransferase/aspartate aminotransferase levels [86]. Therefore, the anti-EGFR drug choice is crucial to ensure the optimal therapeutic ratio when added to immunotherapy. Recently, also the TLR8 agonist motolimod was shown to recruit circulating EGFR-specific T cells as well as CD8⁺ T-cells into SCCHN tumors[87]. Despite these promising observations in early phase trials, when motolimod was added to the EXTREME regimen in a large phase II randomized study, it did not seem to impact overall or progression-free survival in patients with recurrent/metastatic SCCHN. Despite these disappointing results, a significant benefit was observed in HPV-positive patients and patients with injection site reactions, suggesting that a subset of patients might benefit from EGFR inhibition combined with TLR8 stimulation[88] (Table 1). More profound biomarker research will have to explore on which criteria this selection should be based. Numerous other therapeutic trials in SCCHN are actively exploring novel combinations of immunotherapy and EGFR inhibition.

Based on the aforementioned it is clear that the new alliance between EGFR and immune targeting approaches has been forged and this development may provide novel additions to the treatment guidelines of advanced SCCHN.

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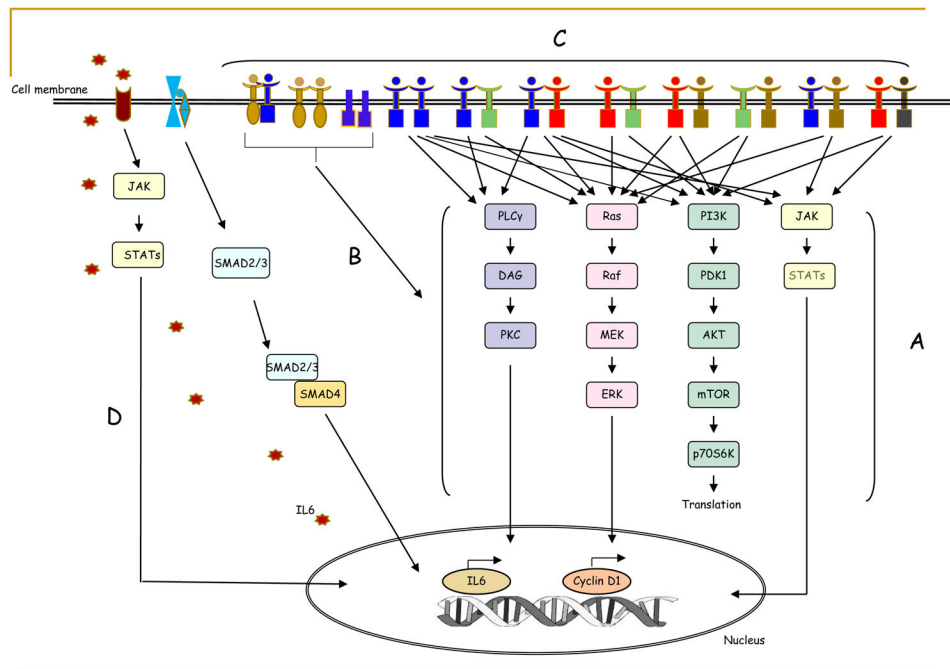


Figure 1. Resistant mechanisms of EGFR targeted therapy. The following molecular activities will result in bypassing EGFR blockade: (A) Activation mutations or amplification of EGFR downstream signaling effectors; (B) Overexpression of MET proto-oncogene and expression of EGFR variant III; (C) Hetero-dimerization between EGFR family members; and (D) Activation of TGF-beta IL-6 axis

Table 1

Ongoing and published clinical trials combining EGFR monoclonal antibodies with immunotherapeutic agents in SCCHN.

Design	Title	Trial number	Status
Phase II	A Phase I/II Study of Concurrent Cetuximab and Nivolumab in Patients with Recurrent and/or Metastatic Head and Neck Squamous Cell Carcinoma		Recurrent or metastatic
Phase III	Randomized Trial of Avelumab-cetuximab-radiotherapy Versus SOC in LA SCCHN (REACH)		Loco-regional
Phase III (terminated)	Nivolumab or Nivolumab Plus Cisplatin or Cetuximab, in Combination with Radiotherapy in Patients with Cisplatin-ineligible or Eligible Locally Advanced Squamous Cell Head and Neck Cancer		Locoregional
Phase I	Phase I trial of cetuximab, intensity modulated radiotherapy (IMRT), and the anti-CTLA-4 monoclonal antibody (mAb) ipilimumab in previously untreated, locally advanced head and neck squamous cell carcinoma (PULA HNSCC)		Locoregional
Phase II	Motolimod and Standard Combination Chemotherapy with Cetuximab in Treatment of Patients with Squamous Cell Carcinoma of the Head and Neck: The Active8 Randomized Clinical Trial.		Recurrent and metastatic SCCHN (manuscript published [88]).
Phase II	EACH: Evaluating Avelumab in Combination with Cetuximab in Head and Neck Cancer (EACH)		Locoregional