Review

Molecular Cancer Therapeutics

Targeting the EGFR and Immune Pathways in Squamous Cell Carcinoma of the Head and Neck (SCCHN): Forging a New Alliance

Nabil F. Saba[1], Zhuo Gerogia Chen[1], Missak Haigentz[2], Paolo Bossi[3], Alessandra Rinaldo[4], Juan P. Rodrigo[5], Antti A. Mäkitie[6], Robert P. Takes[7], Primoz Strojan[8], Jan B. Vermorken[9], and Alfio Ferlito[10]

Abstract

Despite the recent approval of immune-modulatory agents, EGFR inhibition continues to be a cornerstone in the management of squamous cell carcinoma of the head and neck (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.

Rationale for Inhibiting EGFR in SCCHN

Squamous cell carcinoma of the head and neck (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 overexpression (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 (OS) 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 human papillomavirus (HPV)–related oropharyngeal Squamous Cell Carcinoma (SCC), cisplatin resulted in a superior OS compared with 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 EGFR family consists of four types of transmembrane 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 transmembrane 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

[1]Department of Hematology and Medical oncology, Winship Cancer Institute, Emory University, Atlanta, Georgia. [2]Atlantic Health System, Morristown, New Jersey. [3]Medical Oncology Department, University of Brescia, Brescia, Italy. [4]University of Udine School of Medicine, Udine, Italy. [5]Hospital Universitario Central de Asturias, Oviedo, Spain. [6]Department of Otorhinolaryngology–Head and Neck Surgery, University of Helsinki and HUS Helsinki University Hospital, Helsinki, Finland. [7]Radboud University Medical Center, Nijmegen, the Netherlands. [8]Institute of Oncology, Ljubljana, Slovenia. [9]Antwerp University Hospital, Edegem, Belgium. [10]Coordinator of the International Head and Neck Scientific Group, Padua, Italy.

Corresponding Author: Nabil F. Saba, Winship Cancer Institute, Emory University, 1365 Clifton Road NE, Building C, Atlanta, GA 30322. Phone: 404-778-3126; Fax: 404-778-5676; E-mail: nfsaba@emory.edu

Mol Cancer Ther 2019;18:1909–15

doi: 10.1158/1535-7163.MCT-19-0214

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 6 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 (ref. 37; Fig. 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; ref. 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; refs. 15, 20, 21, 45). Upon binding of HRG1, the physiologic 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 TGFβ–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 TGFβ–IL6 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 antitumor 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 (CTLA-4) 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 Ib 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 1-year OS (36.0% vs. 16.6%, $P = 0.0101$; ref. 65). The median OS was 7.5 versus 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 versus 4.6 months for patients with tumor PD-L1 expression > 1% versus PD-L1 < 1%. The 18-month OS rate was 21.5% versus 8.3%, and overall response was 13.3% versus 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-month 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 with docetaxel, methotrexate, and cetuximab supported the results of checkmate 141, showing a clinically meaningful prolongation of OS favoring pembrolizumab (69). In the first-line setting, Keynote 048 has compared single-agent pembrolizumab as well as pembrolizumab in combination with platinum and 5-Fluorouracil with the cetuximab containing Extreme regimen. The results of this trial were reported at the ESMO 2018 meeting and favored both

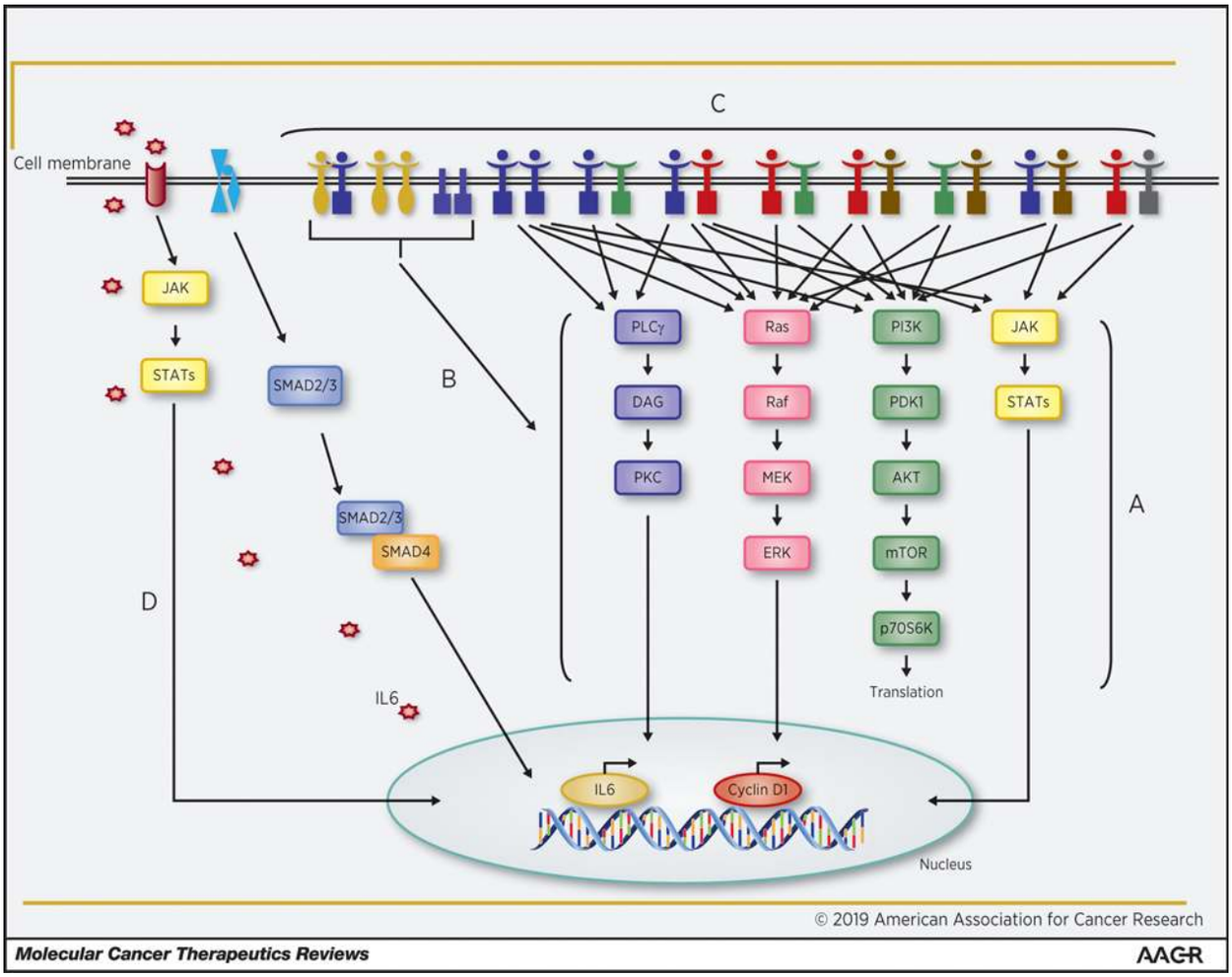

Figure 1.

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**) Heterodimerization between EGFR family members; and (**D**) Activation of TGFβ–IL6 axis.

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 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 IFNγ produced by NK cells via antibody-dependent cellular cytotoxicity, 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 because NK cells themselves can express PD-1 (77). Recently, the intracellular DNA sensor stimulator of interferon genes (STING) has been shown to have a

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	NCT03370276	Recurrent or metastatic
Phase III	Randomized Trial of Avelumab-cetuximab-radiotherapy Versus SOCs in LA SCCHN (REACH)	NCT02999087	Locoregional
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	NCT03349710	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)	NCT 0193592	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.	NCT01836029	Recurrent and metastatic SCCHN (manuscript published; ref. 88).
Phase II	EACH: Evaluating Avelumab in Combination with Cetuximab in Head and Neck Cancer (EACH)	NCT03494322	Locoregional

crucial role in the immune response to viruses and tumors by stimulating cytokine production (78). The evidence also suggests 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 IFNγ-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 chemoattractants (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 biological interactions between EGFR inhibition and the tumor microenvironment remains 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. NCT03370276 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 (Table 1). 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 (NCT02999087; Table 1). Other trials are exploring similar combinations with radiotherapy 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 is also the NK cell and its most common inhibitory receptors, because 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 (NCT02643550) 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; ref. 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 affect OS 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 (ref. 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.

Disclosure of Potential Conflicts of Interest

N.F. Saba has a consultant/advisory board relationship with BMS, Merck, Pfizer, Lilly, Aduro, and Rakuten Medical. M. Haigentz has a consultant/advisory board relationship with AstraZeneca, Takeda Oncology, and Genentech. P. Bossi has a consultant/advisory board relationship with Roche, Merck Serono, MSD, Kyowa Kyrin, Astrazeneca, BMS, Sanofi, and Angelini. J.B. Vermorken is an advisory board member at MSD and received honoraria from the speakers' bureau of BMS, MSD, and Merck AG. No potential conflicts of interest were disclosed by the other authors.

Acknowledgments

This article was written by members and invitees of the International Head and Neck Scientific Group (www.IHNSG.com).

This work was supported by an NIH grant P30 CA 138292 to N.F. Saba and Z.G. Chen.

Received March 7, 2019; revised June 11, 2019; accepted September 4, 2019; published first November 1, 2019.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394–424.
2. Rothenberger NJ, Stabile LP. Hepatocyte growth factor/c-Met signaling in head and neck cancer and implications for treatment. Cancers (Basel) 2017;9. pii: E39.
3. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin 2014;64:9–29.
4. Chen Z, Ke LD, Yuan XH, Adler-Storthz K. Correlation of cisplatin sensitivity with differential alteration of EGFR expression in head and neck cancer cells. Anticancer Res 2000;20:899–902.
5. D'Amico TA, Massey M, Herndon JE 2nd, Moore MB, Harpole DH Jr. A biologic risk model for stage I lung cancer: immunohistochemical analysis of 408 patients with the use of ten molecular markers. J Thorac Cardiovasc Surg 1999;117:736–43.
6. Hansen AR, Siu LL. Epidermal growth factor receptor targeting in head and neck cancer: have we been just skimming the surface? J Clin Oncol 2013;31:1381–3.
7. Ang KK, Andratschke NH, Milas L. Epidermal growth factor receptor and response of head-and-neck carcinoma to therapy. Int J Radiat Oncol Biol Phys 2004;58:959–65.
8. Saba NF, Khuri FR, Shin DM. Targeting the epidermal growth factor receptor. Trials in head and neck and lung cancer. Oncology (Williston Park) 2006;20:153–61.
9. Grandis JR, Falkner D, Melhem MF, Gooding WE, Drenning SD, Morel PA. Human leukocyte antigen class I allelic and haplotype loss in squamous cell carcinoma of the head and neck: clinical and immunogenetic consequences. Clin Cancer Res 2000;6:2794–802.
10. Huang SM, Bock JM, Harari PM. Epidermal growth factor receptor blockade with C225 modulates proliferation, apoptosis, and radiosensitivity in squamous cell carcinomas of the head and neck. Cancer Res 1999;59:1935–40.
11. Kawamoto T, Sato JD, Le A, Polikoff J, Sato GH, Mendelsohn J. Growth stimulation of A431 cells by epidermal growth factor: identification of high-affinity receptors for epidermal growth factor by an anti-receptor monoclonal antibody. Proc Natl Acad Sci U S A 1983;80:1337–41.
12. Sato JD, Kawamoto T, Le AD, Mendelsohn J, Polikoff J, Sato GH. Biological effects in vitro of monoclonal antibodies to human epidermal growth factor receptors. Mol Biol Med 1983;1:511–29.
13. Masui H, Kawamoto T, Sato JD, Wolf B, Sato G, Mendelsohn J, et al. Growth inhibition of human tumor cells in athymic mice by anti-epidermal growth factor receptor monoclonal antibodies. Cancer Res 1984;44:1002–7.
14. Gill GN, Kawamoto T, Cochet C, Le A, Sato JD, Masui H, et al. Monoclonal anti-epidermal growth factor receptor antibodies which are inhibitors of epidermal growth factor binding and antagonists of epidermal growth factor binding and antagonists of epidermal growth factor-stimulated tyrosine protein kinase activity. J Biol Chem 1984;259:7755–60.
15. Jiang N, Wang D, Hu Z, Shin HJ, Qian G, Rahman MA, et al. Combination of anti-HER3 antibody MM-121/SAR256212 and cetuximab inhibits tumor growth in preclinical models of head and neck squamous cell carcinoma. Mol Cancer Ther 2014;13:1826–36.
16. Szturz P, Vermorken JB. Immunotherapy in head and neck cancer: aiming at EXTREME precision. BMC Med 2017;15:110.
17. Vermorken JB, Mesia R, Rivera F, Remenar E, Kawecki A, Rottey S, et al. Platinum-based chemotherapy plus cetuximab in head and neck cancer. N Engl J Med 2008;359:1116–27.
18. Gillison ML, Trotti AM, Harris J, Eisbruch A, Harari PM, Adelstein DJ, et al. Radiotherapy plus cetuximab or cisplatin in human papillomavirus-positive oropharyngeal cancer (NRG Oncology RTOG 1016): a randomised, multicentre, non-inferiority trial. Lancet 2019;393:40–50.
19. Mehanna H, Robinson M, Hartley A, Kong A, Foran B, Fulton-Lieuw T, et al. Radiotherapy plus cisplatin or cetuximab in low-risk human papillomavirus-positive oropharyngeal cancer (De-ESCALaTE HPV): an open-label randomised controlled phase 3 trial. Lancet 2019;393:51–60.
20. Olayioye MA, Neve RM, Lane HA, Hynes NE. The ErbB signaling network: receptor heterodimerization in development and cancer. EMBO J 2000;19:3159–67.
21. Kim HH, Vijapurkar U, Hellyer NJ, Bravo D, Koland JG. Signal transduction by epidermal growth factor and heregulin via the kinase-deficient ErbB3 protein. Biochem J 1998;334(Pt 1):189–95.
22. Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer 2005;5:341–54.
23. Mendelsohn J, Baselga J. Status of epidermal growth factor receptor antagonists in the biology and treatment of cancer. J Clin Oncol 2003;21:2787–99.
24. Vermorken JB, Trigo J, Hitt R, Koralewski P, Diaz-Rubio E, Rolland F, et al. Open-label, uncontrolled, multicenter phase II study to evaluate the efficacy and toxicity of cetuximab as a single agent in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck who failed to respond to platinum-based therapy. J Clin Oncol 2007;25:2171–7.
25. Bardelli A, Janne PA. The road to resistance: EGFR mutation and cetuximab. Nat Med 2012;18:199–200.
26. Montagut C, Dalmases A, Bellosillo B, Crespo M, Pairet S, Iglesias M, et al. Identification of a mutation in the extracellular domain of the epidermal growth factor receptor conferring cetuximab resistance in colorectal cancer. Nat Med 2012;18:221–3.
27. Licitra L, Störkel S, Kerr KM, Van Cutsem E, Pirker R, Hirsch FR, et al. Predictive value of epidermal growth factor receptor expression for first-line chemotherapy plus cetuximab in patients with head and neck and colorectal cancer: analysis of data from the EXTREME and CRYSTAL studies. Eur J Cancer 2013;49:1161–8.
28. Licitra L, Mesia R, Rivera F, Remenár E, Hitt R, Erfán J, et al. Evaluation of EGFR gene copy number as a predictive biomarker for the efficacy of cetuximab in combination with chemotherapy in the first-line treatment of recurrent and/or metastatic squamous cell carcinoma of the head and neck: EXTREME study. Ann Oncol 2011;22:1078–87.
29. Wheeler DL, Dunn EF, Harari PM. Understanding resistance to EGFR inhibitors-impact on future treatment strategies. Nat Rev Clin Oncol 2010;7:493–507.
30. Moroni M, Veronese S, Benvenuti S, Marrapese G, Sartore-Bianchi A, Di Nicolantonio F, et al. Gene copy number for epidermal growth factor receptor (EGFR) and clinical response to antiEGFR treatment in colorectal cancer: a cohort study. Lancet Oncol 2005;6:279–86.
31. Vermorken JB, Psyrri A, Mesía R, Peyrade F, Beier F, de Blas B, et al. Impact of tumor HPV status on outcome in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck receiving chemotherapy with or without cetuximab: retrospective analysis of the phase III EXTREME trial. Ann Oncol 2014;25:801–7.
32. Szturz P, Seiwert TY, Vermorken JB. How standard is second-line cetuximab in recurrent or metastatic head and neck cancer in 2017? J Clin Oncol 2017;35:2229–31.
33. Keck MK, Zuo Z, Khattri A, Stricker TP, Brown CD, Imanguli M, et al. Integrative analysis of head and neck cancer identifies two biologically

distinct HPV and three non-HPV subtypes. Clin Cancer Res 2015;21: 870–81.
34. Qian G, Jiang N, Wang D, Newman S, Kim S, Chen Z, et al. Heregulin and HER3 are prognostic biomarkers in oropharyngeal squamous cell carcinoma. Cancer 2015;121:3600–11.
35. De Pauw I, Lardon F, Van den Bossche J, Baysal H, Fransen E, Deschoolmeester V, et al. Simultaneous targeting of EGFR, HER2, and HER4 by afatinib overcomes intrinsic and acquired cetuximab resistance in head and neck squamous cell carcinoma cell lines. Mol Oncol 2018;12: 830–54.
36. Pollock NI, Wang L, Wallweber G, Gooding WE, Huang W, Chenna A, et al. Increased expression of HER2, HER3, and HER2:HER3 heterodimers in HPV-positive HNSCC using a novel proximity-based assay: implications for targeted therapies. Clin Cancer Res 2015;21:4597–606.
37. Boeckx C, Baay M, Wouters A, Specenier P, Vermorken JB, Peeters M, et al. Anti-epidermal growth factor receptor therapy in head and neck squamous cell carcinoma: focus on potential molecular mechanisms of drug resistance. Oncologist 2013;18:850–64.
38. Lievre A, Bachet JB, Le Corre D, Boige V, Landi B, Emile JF, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res 2006;66:3992–5.
39. Krumbach R, Schüler J, Hofmann M, Giesemann T, Fiebig HH, Beckers T. Primary resistance to cetuximab in a panel of patient-derived tumour xenograft models: activation of MET as one mechanism for drug resistance. Eur J Cancer 2011;47:1231–43.
40. Wheeler SE, Suzuki S, Thomas SM, Sen M, Leeman-Neill RJ, Chiosea SI, et al. Epidermal growth factor receptor variant III mediates head and neck cancer cell invasion via STAT3 activation. Oncogene 2010; 29:5135–45.
41. Lee JW, Soung YH, Kim SY, Nam HK, Park WS, Nam SW, et al. Somatic mutations of EGFR gene in squamous cell carcinoma of the head and neck. Clin Cancer Res 2005;11:2879–82.
42. Iglesias-Bartolome R, Martin D, Gutkind JS. Exploiting the head and neck cancer oncogenome: widespread PI3K-mTOR pathway alterations and novel molecular targets. Cancer Discov 2013;3:722–5.
43. Leeman RJ, Lui VW, Grandis JR. STAT3 as a therapeutic target in head and neck cancer. Expert Opin Biol Ther 2006;6:231–41.
44. Sen M, Thomas SM, Kim S, Yeh JI, Ferris RL, Johnson JT, et al. First-in-human trial of a STAT3 decoy oligonucleotide in head and neck tumors: implications for cancer therapy. Cancer Discov 2012;2:694–705.
45. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol 2001;2:127–37.
46. Van Emburgh BO, Sartore-Bianchi A, Di Nicolantonio F, Siena S, Bardelli A. Acquired resistance to EGFR-targeted therapies in colorectal cancer. Mol Oncol 2014;8:1084–94.
47. Rampias T, Giagini A, Siolos S, Matsuzaki H, Sasaki C, Scorilas A, et al. RAS/PI3K crosstalk and cetuximab resistance in head and neck squamous cell carcinoma. Clin Cancer Res 2014;20:2933–46.
48. Hah JH, Zhao M, Pickering CR, Frederick MJ, Andrews GA, Jasser SA, et al. HRAS mutations and resistance to the epidermal growth factor receptor tyrosine kinase inhibitor erlotinib in head and neck squamous cell carcinoma cells. Head Neck 2014;36:1547–54.
49. Braig F, Voigtlaender M, Schieferdecker A, Busch CJ, Laban S, Grob T, et al. Liquid biopsy monitoring uncovers acquired RAS-mediated resistance to cetuximab in a substantial proportion of patients with head and neck squamous cell carcinoma. Oncotarget 2016;7:42988–95.
50. Bossi P, Bergamini C, Siano M, Cossu Rocca M, Sponghini AP, Favales F, et al. Functional genomics uncover the biology behind the responsiveness of head and neck squamous cell cancer patients to cetuximab. Clin Cancer Res 2016;22:3961–70.
51. Ho AL, Chau N, Bauman J, Bible K, Chintakuntlawar A, Cabanillas ME, et al. Preliminary results from a phase 2 trial of tipifarnib in squamous cell carcinomas (SCCs) with HRAS mutations. Ann Oncol 2018; 29 (suppl_8): viii372–viii399.
52. Wheeler DL, Huang S, Kruser TJ, Nechrebecki MM, Armstrong EA, Benavente S, et al. Mechanisms of acquired resistance to cetuximab: role of HER (ErbB) family members. Oncogene 2008;27:3944–56.
53. Lu Y, Li X, Liang K, Luwor R, Siddik ZH, Mills GB, et al. Epidermal growth factor receptor (EGFR) ubiquitination as a mechanism of acquired resistance escaping treatment by the anti-EGFR monoclonal antibody cetuximab. Cancer Res 2007;67:8240–7.
54. Li C, Iida M, Dunn EF, Ghia AJ, Wheeler DL. Nuclear EGFR contributes to acquired resistance to cetuximab. Oncogene 2009;28:3801–13.
55. Atlas E, Bojanowski K, Mehmi I, Lupu R. A deletion mutant of heregulin increases the sensitivity of breast cancer cells to chemotherapy without promoting tumorigenicity. Oncogene 2003;22:3441–51.
56. Tzahar E, Waterman H, Chen X, Levkowitz G, Karunagaran D, Lavi S, et al. A hierarchical network of interreceptor interactions determines signal transduction by Neu differentiation factor/neuregulin and epidermal growth factor. Mol Cell Biol 1996;16:5276–87.
57. Kol A, Terwisscha van Scheltinga AG, Timmer-Bosscha H, Lamberts LE, Bensch F, de Vries EG, et al. HER3, serious partner in crime: therapeutic approaches and potential biomarkers for effect of HER3-targeting. Pharmacol Ther 2014;143:1–11.
58. Shames DS, Carbon J, Walter K, Jubb AM, Kozlowski C, Januario T, et al. High heregulin expression is associated with activated HER3 and may define an actionable biomarker in patients with squamous cell carcinomas of the head and neck. PLoS One 2013;8:e56765.
59. Yao Z, Fenoglio S, Gao DC, Camiolo M, Stiles B, Lindsted T, et al. TGF-beta IL-6 axis mediates selective and adaptive mechanisms of resistance to molecular targeted therapy in lung cancer. Proc Natl Acad Sci U S A 2010; 107:15535–40.
60. Young MR, Wright MA, Lozano Y, Matthews JP, Benefield J, Prechel MM. Mechanisms of immune suppression in patients with head and neck cancer: influence on the immune infiltrate of the cancer. Int J Cancer 1996;67:333–8.
61. Weber JS, O'Day S, Urba W, Powderly J, Nichol G, Yellin M, et al. Phase I/II study of ipilimumab for patients with metastatic J Clin Oncol2008;26: 5950–6.
62. Swanson MS, Sinha UK. Rationale for combined blockade of PD-1 and CTLA-4 in advanced head and neck squamous cell cancer—review of current data. Oral Oncol 2015;51:12–5.
63. Kim HS, Lee JY, Lim SH, Park K, Sun JM, Ko YH, et al. Association between PD-L1 and HPV status and the prognostic value of PD-L1 in oropharyngeal squamous cell carcinoma. Cancer Res Treat 2016;48:527–36.
64. Zandberg DP, Strome SE. The role of the PD-L1:PD-1 pathway in squamous cell carcinoma of the head and neck. Oral Oncol 2014;50:627–32.
65. Ferris RL, Blumenschein G Jr, Fayette J, Guigay J, Colevas AD, Licitra L, et al. Nivolumab for recurrent squamous-cell carcinoma of the head and neck. N Engl J Med 2016;375:1856–67.
66. Bauml J, Seiwert TY, Pfister DG, Worden F, Liu SV, Gilbert J, et al. Pembrolizumab for platinum- and cetuximab-refractory head and neck cancer: results from a single-arm, phase II study. J Clin Oncol 2017;35: 1542–9.
67. Seiwert TY, Burtness B, Mehra R, Weiss J, Berger R, Eder JP, et al. Safety and clinical activity of pembrolizumab for treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-012): an open-label, multicentre, phase 1b trial. Lancet Oncol 2016;17:956–65.
68. Ferris RL, Blumenschein G Jr, Fayette J, Guigay J, Colevas AD, Licitra L, et al. Nivolumab vs. investigator's choice in recurrent or metastatic squamous cell carcinoma of the head and neck: 2-year long-term survival update of CheckMate 141 with analyses by tumor PD-L1 expression. Oral Oncol 2018;81:45–51.
69. Cohen EEW, Soulières D, Le Tourneau C, Dinis J, Licitra L, Ahn MJ, et al. Pembrolizumab versus methotrexate, docetaxel, or cetuximab for recurrent or metastatic head-and-neck squamous cell carcinoma (KEYNOTE-040): a randomised, open-label, phase 3 study. Lancet 2019;393:156–67.
70. Burtness B, Harrington KJ, Greil R, Soulières D, Tahara M, De Castro Jr. G, et al. Phase 3 study of first-line pembrolizumab (P) for recurrent/metastatic head and neck squamous cell carcinoma (R/M HNSCC);. NCT02358031, ESMO 2019 Abstract and oral presentation 2018.
71. Concha-Benavente F, Ferris RL. Oncogenic growth factor signaling mediating tumor escape from cellular immunity. Curr Opin Immunol 2017;45: 52–9.
72. Srivastava RM, Trivedi S, Concha-Benavente F, Gibson SP, Reeder C, Ferrone S, et al. CD137 stimulation enhances cetuximab-induced natural killer: dendritic cell priming of antitumor T-cell immunity in patients with head and neck cancer. Clin Cancer Res 2017;23:707–16.
73. Trivedi S, Srivastava RM, Concha-Benavente F, Ferrone S, Garcia-Bates TM, Li J, et al. Anti-EGFR targeted monoclonal antibody isotype influences antitumor cellular immunity in head and neck cancer patients. Clin Cancer Res 2016;22:5229–37.

74. Schneider-Merck T, Lammerts van Bueren JJ, Berger S, Rossen K, van Berkel PH, Derer S, et al. Human IgG2 antibodies against epidermal growth factor receptor effectively trigger antibody-dependent cellular cytotoxicity but, in contrast to IgG1, only by cells of myeloid lineage. J Immunol 2010;184: 512–20.
75. Bauman JE, Ferris RL. Integrating novel therapeutic monoclonal antibodies into the management of head and neck cancer. Cancer 2014;120:624–32.
76. Fu J, Malm IJ, Kadayakkara DK, Levitsky H, Pardoll D, Kim YJ. Preclinical evidence that PD1 blockade cooperates with cancer vaccine TEGVAX to elicit regression of established tumors. Cancer Res 2014;74:4042–52.
77. Beldi-Ferchiou A, Caillat-Zucman S. Control of NK cell activation by immune checkpoint molecules. Int J Mol Sci 2017;18. pii: E2129.
78. Zhang CX, Ye SB, Ni JJ, Cai TT, Liu YN, Huang DJ, et al. STING signaling remodels the tumor microenvironment by antagonizing myeloid-derived suppressor cell expansion. Cell Death Differ 2019 Feb 28. [Epub ahead of print].
79. Lu S, Concha-Benavente F, Shayan G, Srivastava RM, Gibson SP, Wang L, et al. STING activation enhances cetuximab-mediated NK cell activation and DC maturation and correlates with HPV+ status in head and neck cancer. Oral Oncol 2018;78:186–93.
80. Cheng CC, Lin HC, Tsai KJ, Chiang YW, Lim KH, Chen CG, et al. Epidermal growth factor induces STAT1 expression to exacerbate the IFNr-mediated PD-L1 axis in epidermal growth factor receptor-positive cancers. Mol Carcinog 2018;57:1588–98.
81. MacDonald F, Zaiss DMW. The immune system's contribution to the clinical efficacy of EGFR antagonist treatment. Front Pharmacol 2017;8: 575.
82. Moy JD, Moskovitz JM, Ferris RL. Biological mechanisms of immune escape and implications for immunotherapy in head and neck squamous cell carcinoma. Eur J Cancer 2017;76:152–66.
83. Weil S, Memmer S, Lechner A, Huppert V, Giannattasio A, Becker T, et al. Natural killer group 2D ligand depletion reconstitutes natural killer cell immunosurveillance of head and neck squamous cell carcinoma. Front Immunol 2017;8:387.
84. Veluchamy JP, Spanholtz J, Tordoir M, Thijssen VL, Heideman DA, Verheul HM, et al. Combination of NK cells and cetuximab to enhance anti-tumor responses in RAS mutant metastatic colorectal cancer. PLoS One 2016;13: e0203402.
85. André P, Denis C, Soulas C, Bourbon-Caillet C, Lopez J, Arnoux T, et al. Anti-NKG2A mAb is a checkpoint inhibitor that promotes anti-tumor immunity by unleashing both T and NK cells. Cell 2018;175: 1731–43.e13.
86. Liang H, Liu X, Wang M. Immunotherapy combined with epidermal growth factor receptor-tyrosine kinase inhibitors in non-small-cell lung cancer treatment. Onco Targets Ther 2018;11:6189–96.
87. Shayan G, Kansy BA, Gibson SP, Srivastava RM, Bryan JK, Bauman JE, et al. Phase Ib study of immune biomarker modulation with neoadjuvant cetuximab and TLR8 stimulation in head and neck cancer to overcome suppressive myeloid signals. Clin Cancer Res 2018;24:62–72.
88. Ferris RL, Saba NF, Gitlitz BJ, Haddad R, Sukari A, Neupane P, et al. Effect of adding motolimod to standard combination chemotherapy and cetuximab treatment of patients with squamous cell carcinoma of the head and neck: the Active8 Randomized Clinical Trial. JAMA Oncol 2018;4:1583–8.