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EGFR-targeted therapies in the post-genomic era

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Abstract Over 90% of head and neck cancers overexpress the epidermal growth factor receptor (EGFR). In diverse tumor types, EGFR overexpression has been associated with poorer prognosis and outcomes. Therapies targeting EGFR include monoclonal antibodies, tyrosine kinase inhibitors, phosphatidylinositol 3-kinase (PI3K) inhibitors, and antisense gene therapy. Few EGFR-targeted therapeutics are approved for clinical use. The monoclonal antibody cetuximab is a Food and Drug Administration (FDA)-approved EGFR-targeted therapy, yet has exhibited modest benefit in clinical trials. The humanized monoclonal antibody nimotuzumab is also approved for head and neck cancers in Cuba, Argentina, Colombia, Peru, India, Ukraine, Ivory Coast, and Gabon in addition to nasopharyngeal cancers in China. Few other EGFR-targeted therapeutics for head and neck cancers have led to as significant responses as seen in lung carcinomas, for instance. Recent genome sequencing of head and neck tumors has helped identify patient subgroups with improved response to EGFR inhibitors, for example, cetuximab in patients with the KRAS-variant and the tyrosine kinase inhibitor erlotinib for tumors harboring $MAPK1^{E322K}$ mutations. Genome sequencing has furthermore broadened our understanding of dysregulated pathways, holding the potential to enhance the benefit derived from therapies targeting EGFR.

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1 Introduction

Stanley Cohen's discovery of epidermal growth factor (EGF) was awarded the 1986 Nobel Prize, heralding the development of epidermal growth factor receptor (EGFR)-targeted therapeutics [1]. In diverse tumor types including head and neck, bladder, ovarian, and cervical cancers, EGFR overexpression has been associated with poorer prognosis and outcomes [2–4]. In 2004, the FDA initially approved the monoclonal antibody cetuximab for metastatic colorectal cancer. Its use was expanded to head and neck squamous cell carcinomas (HNSCC) in 2006. Cetuximab remains the only EGFR-directed treatment FDA-approved for head and neck cancers. Here, we review EGFR-targeted therapies and highlight insights from recent genomic research relevant to head and neck cancers.

2 Receptor pathway and function

2.1 EGFR structure

EGFR, also called HER1 or ErbB1, was the first member of the ErbB family of tyrosine kinase receptors discovered [5]. This family also includes HER2/neu (ErbB2), HER3 (ErbB3), and HER4 (ErbB4). The 170 kDa EGFR receptor spans the membrane once and contains extracellular, transmembrane, and intracellular regions. The extracellular component is comprised of four domains. Domains I and III are leucine rich and structurally similar to domains found in the insulin receptor [6], a cell-surface receptor known to share downstream

signaling pathways with EGFR [7, 8]. Domains II and IV are cysteine rich and similar to laminin [9]. The intracellular region harbors the intrinsic tyrosine kinase activity of EGFR. Existing in both closed monomer and open dimer conformations [10], EGFR is composed of 20% carbohydrates, with N-linked glycosylation affecting receptor structure and stability; increased glycosylation stabilizes and drives the equilibrium toward the extended conformation [4, 11, 12].

2.2 EGFR pathway

EGF, transforming growth factor-alpha (TGF-α), amphiregulin, heparin-binding EGFR, and betacellulin are among the ligands which bind to domains I and III of EGFR. Subsequent exposure of domain II results in receptor dimerization *via* disulfide bonds. After dimerization at the cell surface, autophosphorylation of tyrosine residues in the cytoplasmic region provides docking sites for signal transducers, including proteins such as Ras, to bind and initiate intracellular signaling cascades and gene transcription [4, 13]. Downstream signaling cascades of EGFR can be broadly divided into the following pathways: RAS/RAF/MEK/MAPK/ERK, phosphatidylinositol 3-kinase (PI3K) and Akt, protein kinase C (PKC), Src, and the JAK/STAT pathways (Fig. 1) [14]. These extensively studied signaling cascades influence gene expression, proliferation, angiogenesis, apoptosis inhibition, cell motility, metastasis, adhesion, and angiogenesis [4, 15].

2.3 EGFR function in normal physiology and cancer

Indisputably, EGFR possesses a critical role in development and differentiation, particularly in epithelial and glial cells. Highly expressed in the basal layer of the epidermis and the outer root sheath of hair cells, EGFR influences migration and differentiation of keratinocytes and hair follicle development. Mouse models expressing mutant EGFR develop papillomas and squamous cell carcinomas (SCC) [16]. In neurons, EGFR regulates migration and neurodegeneration, with mutations leading to glioma-like tumors in murine models [16]. Furthermore, in lung tissue, EGFR influences maturation of type II pneumocytes; following lung damage, these cells proliferate into type 1 pneumocytes and replace damaged tissue.

In head and neck cancers, EGFR is overexpressed in over 90% of tumors and correlates with poorer outcomes [17, 18]. In tissue from 91 HNSCC patients, tumor EGFR level was a statistically significant predictor of disease-free survival (DFS) (p = 0.0001) along with tumor site and TGF-α level [17]. In the large phase III RTOG 9003 trial evaluating radiation regimens, retrospective subset analysis of 155 patients reinforced the correlation between EGFR expression and decreased overall survival (OS) along with increased local-regional relapse [19]. In addition to overall increased expression, EGFR copy number was associated with a 91% (20/22 patients) 5-year mortality compared to 29% (30/102 patients) in patients with a

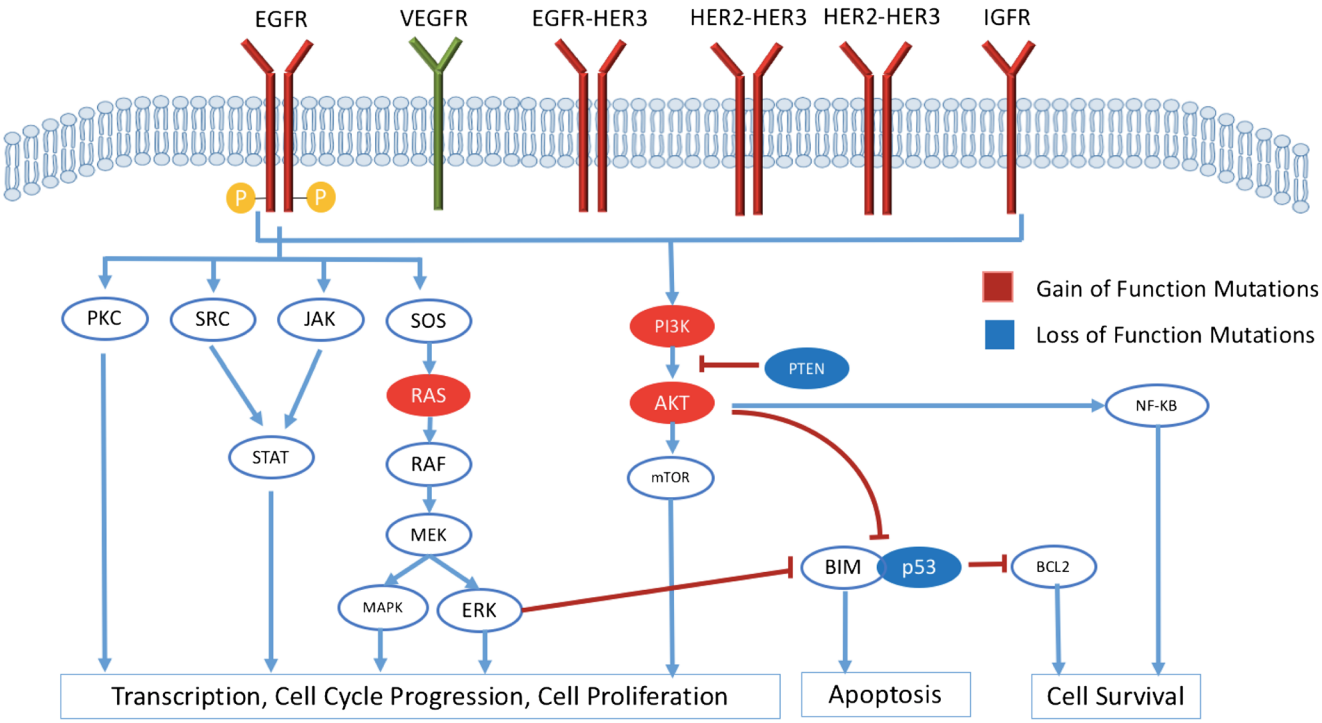

Fig. 1 Epidermal growth factor receptor downstream signaling pathways include RAS/RAF/MEK/MAPK/ERK, phosphatidylinositol 3-kinase (PI3K) and Akt, protein kinase C (PKC), Src, and the JAK/STAT pathways. Subsequent signaling cascades influence gene expression, proliferation, angiogenesis, apoptosis inhibition, cell motility, metastasis, adhesion, and angiogenesis

normal copy number [20]. Similar associations exist for breast, lung, and other tumor types [4, 21, 22].

3 EGFR-targeted therapies

Until the development of targeted therapeutics, chemotherapy for head and neck cancers was predominated by non-specific inhibitors of cellular division and proliferation. FDA-approved therapies included cisplatin, methotrexate, 5-fluorouracil (5-FU), bleomycin, and docetaxel, all of which produced clinical response rates ranging from 20 to 40% [22]. Common side effects included dysphagia, odynophagia, nausea, vomiting, and hematologic suppression [22, 23]. EGFR-targeted therapies approved and under-development includes monoclonal antibodies (Table 1), tyrosine kinase inhibitors (Table 2), PI3K inhibitors, and antisense gene therapy.

3.1 Monoclonal antibodies

In 2006, cetuximab was the first targeted treatment for head and neck cancers approved by the FDA (Table 1). A chimeric murine antibody linked to human IgG, cetuximab was approved in combination with radiation (XRT) in locally advanced (LA) disease, as a single agent for recurrent or metastatic HNSCC after failure of platinum therapies, and in combination with 5-FU and platinum-based therapies for first-line recurrent or metastatic HNSCC [14]. In addition to inhibiting ligand binding, alternative mechanisms of action involve initiating receptor endocytosis, activating antibody-dependent cell-mediated cytotoxicity (ADCC), and inhibiting repair of radiation-induced damage [23, 24].

In clinical care, cetuximab improved patient outcomes when combined with radiotherapy (Table 3). Randomized, phase III, multicenter trials assessing the addition of cetuximab to radiotherapy noted increased local-regional control and increased median OS from 29.3 months (95% CI 20.6–41.4) to 49.0 months (95% CI 32.8–69.5) [25, 26]. Importantly, patients experienced unchanged rates of treatment-related toxicities. However, higher grade of acneiform rash, a common side effect, was associated with improved OS and thought indicative of an inflammatory response [26].

Cetuximab also conferred additional benefit in combination with chemotherapy (Table 3). In a phase II multicenter study, patients with recurrent or metastatic HNSCC were started on cetuximab therapy; cisplatin was subsequently added following disease progression. Of the 103 patients, 46% benefited from cetuximab with either disease control or stabilization with a mean time to progression of 70 days [27]. Similarly, in a phase III trial, addition of cetuximab to platinum-based and 5-FU therapies increased median OS from

Table 1 EGFR-targeted monoclonal antibodies

Compound	Company	Description	Approval and clinical indications
Cetuximab Erbitux (IMC-C225)	ImClone Systems Incorporated Bristol-Myers Squibb Eli Lily Merck KGaA	Chimeric, murine antibody, and human IgG1	2004: FDA approval for metastatic CRC 2006: FDA approval for use in combination with XRT for locally or regionally advanced HNSCC or as monotherapy for platinum refractory, recurrent, or metastatic HNSCC 2009: FDA approval for KRAS wild-type CRC 2011: FDA approval for use as first-line treatment in combination with platinum-based chemotherapeutics and 5-FU for recurrent local-regional or metastatic HNSCC
Panitumumab Vectibix (ABX-EGF)	Amgen Takeda	Humanized mAb	2006: FDA approval for metastatic CRC 2007: European Medicines Agency approval for use in combination with FOLFIRI chemotherapy for metastatic colon cancer 2008: Health Canada approval for refractory EGFR-expressive metastatic CRC with wild-type KRAS 2014: FDA approval in combination with FOLFOX for first-line treatment of wild-type KRAS CRC
Nimotuzumab	YM Biosciences	Humanized mAb	2006: Approval for HNSCC in India 2008: Approval in combination with XRT for NPC in China Phase II and III studies for cancers including HNSCC, esophageal, gastric, CRC, and gliomas
Zalutumumab Genmab	Genmab MATOS Pharma	Human IgG1	Phase I, II, and III for HNSCC, NSCLC, and CRC
Duligotuzumab	Roche	Humanized dual EGFR/HER3 mAb	Phase I and II studies in HNSCC

CRC colorectal cancer, *FDA* Food and Drug Administration, *FOLFOX* a chemotherapy combination of leucovorin, fluorouracil, and oxaliplatin, *HNSCC* head and neck squamous cell carcinoma, *NPC* nasopharyngeal carcinoma, *NSCLC* non-small cell lung cancer, *XRT* radiotherapy

Table 2 EGFR-targeted tyrosine kinase inhibitors

Compound	Company	Description	Approval and clinical indications
Gefitinib Iressa (ZD1839)	AstraZeneca Pharmaceuticals	Reversible binding EGFR specific Oral medicine	2003: advanced or metastatic NSCLC
Erlotinib Tarceva (OSI-774)	Genentech Astellas	Reversible binding EGFR specific	2004: locally advanced or metastatic NSCLC; approved in combination with gemcitabine for locally advanced or metastatic pancreatic cancer
Lapatinib Tykerb	GlaxoSmithKline	Reversible binding Inhibition of HER2/neu and EGFR	2007: in combination for breast cancer patient on capecitabine 2010: in combination with an aromatase inhibitor for HER2 and hormone receptor-positive metastatic breast cancer
Afatinib	Boehringer Ingelheim Pharmaceuticals	Irreversible Pan-ErbB binding	2013: first-line treatment of metastatic NSCLC with EGFR exon 19 deletions or exon 21 (L858R) substitutions
Dasatinib (Sprycel)	Bristol-Myers Squibb	c-Scr kinases; thought to interfere with nuclear localization and of EGFR (Raju 2012)	2006: adult chromosome-positive chronic myelogenous eukemia (CP-CML) for which imatinib was ineffective 2010: newly diagnosed CP-CML
Dacomitinib	Pfizer	Irreversible Pan-ErbB binding	Phase I, II, and III trials for cancers including HNSCC, NSCLC, and glioblastoma multiforme
ASP8273	Astellas Pharma	Irreversible binding Affinity higher for EGFR activating and T790 M mutations compared to wild type	Phase I, II, and III trials in NSCLC and solid malignancies

7.4 to 10.1 months and progression-free survival (PFS) from 3.3 to 5.6 months [28]. Though the improvements observed were modest, these trials prompted FDA approval for cetuximab in combination with XRT for locally or regionally advanced HNSCC or as monotherapy for platinum refractory, recurrent, or metastatic HNSCC in 2006. The latter trial, of note, led to expansion of cetuximab from treatment of only platinum-refractory to any untreated recurrent or metastatic tumors. While addition of cetuximab to radiotherapy or chemotherapy increased survival, the addition of cetuximab to both radiotherapy and cisplatin in combination did not amplify clinical benefit [29].

Ongoing research and development are focused more on fully humanized EGFR-targeted antibodies (Table 1). Panitumumab, FDA approved for colorectal cancers, has led to modest outcomes for HNSCC. In the phase III randomized SPECTRUM trial, OS was not significantly improved for patients with late stage disease randomized to cisplatin and 5-FU with or without panitumumab; PFS was modestly increased from 4.6 to 5.8 months [30]. Similarly, in the CONCERT-1 phase II trial, the addition of panitumumab to cisplatin-based therapy for late stage HNSCC did not improve 2-year local-regional control though led to increased rates of grades 3 and 4 side effects [31]. Ongoing trials are assessing the role of panitumumab in adjuvant treatment (NCT00798655). Zalutumumab has a decreased immunogenic profile with a lower risk of hypersensitivity; however, OS was not significantly improved following treatment for patients with incurable HNSCC [32]. Ongoing trials will assess the role of zalutumumab in curative chemoradiation (C-XRT) (NCT00496652). Finally, nimotuzumab is an antibody which requires bivalent binding to EGFR and thus selectively binds to cells with higher EGFR expression. Clinical trials showed improved clinical response rates when nimotuzumab was added to XRT (59.5 *versus* 34.2%) [33]. Rash was rarely detected and increased EGFR expression correlated with improved survival [33, 34]. Nimotuzumab is approved for HNSCC in countries including Cuba, Argentina, Colombia, Peru, India, Ukraine, Ivory Coast, and Gabon. In China, nimotuzumab is administered in combination with radiation for nasopharyngeal carcinomas. It is still being assessed in clinical trials in the USA.

To amplify the therapeutic response of targeting EGFR, duligotuzumab was developed to target both EGFR and HER3. However, a phase II trial showed no significant improvement in PFS nor OS when compared to cetuximab (Table 3) [35].

3.2 Tyrosine kinase inhibitors

Tyrosine kinase inhibitors (TKI) target the intracellular catalytic domain of receptor tyrosine kinases (Table 2). Reversible-binding TKIs, including gefitinib and erlotinib, were initially approved for non-small cell lung cancer (NSCLC) but have yet to enhance outcomes for HNSCC. Irreversible-binding TKIs, which were subsequently developed and include afatinib, appear clinically promising.

Table 3 Select clinical trials of EGFR therapeutics

Lead author (year published)	Phase	Study	Patient and disease demographics				Outcomes
			Number	Stage	Tumor characteristics	EGFR expression (% of total patients)	
Bonner et al. [25]	III	High-dose XRT with and without cetuximab	424	III or IV	Nonmetastatic, measurable SCC Oropharynx (56%), hypopharynx (17%), larynx (27%)	79	- Addition of cetuximab-improved median duration of local-regional control (24.4 months cetuximab with XRT *vs.* 14.9 months XRT alone)
Bonner et al. [26]	III	High-dose XRT with and without cetuximab Update on Bonner et al. (2006) study	424	III or IV	Nonmetastatic, measurable SCC Oropharynx (56%), hypopharynx (17%), larynx (27%)	79	- 5-year OS 5-year overall survival 45.6% on cetuximab and XRT *vs.* 36.4% with radiotherapy alone - Median OS 49.0 on cetuximab and XRT 49.0 months (95% CI 32.8–69.5) *vs.* 29. 3 months (20.6–41.4) on XRT alone - Presence of cetuximab-induced rash was associated with improved survival
Vermorken et al. [27]	II	Treatment with cetuximab and subsequent combination of cetuximab and platinum therapy in the setting of disease progression Open-label, no control arm	103	III or IV	Recurrent or metastatic SCC Pharynx (38%), larynx (20%), paranasal sinuses (3%); other (39%) Progression after a 2–6 cycles of platinum-based therapy	97	- 103 patients started on single agent cetuximab; 53 progressed and began combination therapy - Median OS 178 days in the single-agent phase - During the single-agent phase, disease was controlled (any response or stabilized disease) in 46% of patients and time to progression 70 days - During the dual-agent phase, disease was controlled in 26% of patients and time to progression 50 days
Vermorken et al. [28]	III	Platinum-based therapy and fluorouracil with and without cetuximab	442	NR	Metastatic or local-regionally recurrent SCC Oral cavity (20%), oropharynx (34%), hypopharynx (14%), larynx (25%), other (7%)	92	- Addition of cetuximab lead to an increased median OS from 7.4 to 10.1 months - Addition of cetuximab increased the progression-free survival time from 3.3 to 5.6 months
Ang et al. [29]	III	Cisplatin-based C-XRT with and without cetuximab	891	III or IV	Oropharynx (70%), hypopharynx (7%), larynx (23%)	NR	- Cetuximab did not improve 3-year OS (72.9% without cetuximab *vs.* 75.8% with cetuximab; $P = .32$) - Addition of cetuximab led to increased radiation treatment interruptions and increased severe grade mucositis, rash, fatigue, anorexia, and hypokalemia - Patients with HPV+ tumor had improved survival with 3-year OS 85.6% *vs.* with HPV-tumors 60.1% ($p < 0.001$)
Weidhass et al. [57]	III	Cisplatin-based C-XRT with and without cetuximab Patients subcategorized by KRAS mutation	413	III or IV	Oropharynx (72%), hypopharynx/larynx (28%) 17% KRAS-variant (70/413)	NR	- Cetuximab improved 1- and 2-year OS with in patients with KRAS-variant (HR, 0.19; 95% CI, 0.04–0.86; $p = 0.03$)
	II		150	III or IV	Locally advanced SCC	NR	

Table 3 (continued)

Lead author (year published)	Phase	Study	Patient and disease demographics				Outcomes
			Number	Stage	Tumor characteristics	EGFR expression (% of total patients)	
Mesia et al. [31] CONCERT-1		Cisplatin-based C-XRT compared to a dose-reduced cisplatin-based C-XRT with panitumumab			Oral cavity (9%), oropharynx (53%), hypopharynx (19%), larynx (18%)		- Panitumumab did not improve 2-year local-regional control (68% without *vs.* 61% with panitumumab) - Addition of panitumumab was associated with increased rates of grades 3–4 mucosal inflammation, dysphagia, and radiation-related skin toxicity
Fayette et al. [35] MEHGAN study	II	Duligotuzumab compared to cetuximab following progressing on/after cisplatin-based chemotherapy	121	III or IV	Recurrent or metastatic SCC Oral cavity (29%), oropharynx (30%), hypopharynx (10%), larynx (16%), unspecified (10%), unknown (6%)	NR	- OS was statistically similar between duligotuzumab (7.2 months) compared to cetuximab (8.7 months; HR 1.15, 90% CI 0.81–1.63) - Expression level of neuregulin 1 (NRG1, ligand to HER3) nor ERBB3 expression (encodes HER3) did not influence response lai
Martins et al. [36]	II	Cisplatin and XRT with and without erlotinib Randomized	204	III or IV	Locally advanced SCC Oral cavity (7%), oropharynx (67%), hypopharynx (6%), larynx (18%), nasopharynx (1%), other (1%)	4/90 samples assessed had EGFR amplification	- Addition of erlotinib did not increase toxicity - The TKI erlotinib did not confer additional tumor response or survival
Argiris et al. [37]	III	Docetaxel with or without gefitinib Randomized	270	NR	Recurrent or metastatic SCC Oral cavity (22%), oropharynx (33%), larynx (26%), multiple (5%), other (14%)	NR	- The TKI gefitinib did not lead to improved survival or outcomes
Kim et al. [58]	II	Dacomitinib monotherapy	48	NR	Local-regionally recurrent or metastatic SCC Progression on or intolerance to platinum therapy Oral cavity (37%), oropharynx (23%), hypopharynx(17%), larynx (19%), maxillary sinus (4%)	NR	- 20.8% (10) of patients with partial response and 65% (31) of patients with stable disease - OS 6.6 months and PFS 3.9 months - in the cohort, the patients with PI3K pathway mutations
Machiels et al. [41]	III	Afatinib or methotrexate as a second-line therapy following prior platinum-based therapy and disease progression	483	NR	Recurrent or metastatic SCC Progression after or on platinum-based therapy Oral cavity (28%), oropharynx (32%), hypopharynx (19%), larynx (21%)	NR	- PFS improved with afatinib (median 2.6 months) compared to methotrexate (median 1.7 months), hazard ratio 0.80 (95% CI 0.65–0.98, $p = 0.03$) - Of note, 59% of patients were previously treated with EGFR-targeted therapy
Harrington et al. [43]	III	Adjuvant C-XRT with lapatinib or placebo followed by 1 year of lapatinib or placebo	688	II, III, IVA	Surgical margin <5 mm or ECE Oral cavity (41%), oropharynx (19%), hypopharynx (13%), larynx (23%), multiple sites (4%)	70 (IHC 3+)	- Addition of lapatinib did not improve overall survival (HR 0.96, 95% CI 0.73 to 1.25) nor disease-free survival (HR 1.10, 0.85 to 1.43) - Lapatinib was associated with increased grades 3–4 adverse events (75%) compared to placebo (67%, $p = 0.019$)
Soulières et al. [49] BERIL-1	II	Buparlisib, oral pan-PI3K inhibitor, or placebo with paclitaxel as	158	NR	Recurrent or metastatic SCC	NR	- Median PFS was improved with second-line buparlisib and paclitaxel (4.6 months)

Table 3 (continued)

Lead author (year published)	Phase	Study	Patient and disease demographics				Outcomes
			Number	Stage	Tumor characteristics	EGFR expression (% of total patients)	
		second-line therapy after progression with platinum-based treatment			Progression after or on platinum-based therapy Oral cavity (29%), oropharynx (28%), hypopharynx (18%), larynx (16%), nasopharynx (3%), other/unknown (6%)		compared to placebo and paclitaxel (3.5 months; HR 0.65 [95% CI 0.45–0.95) - Of note, 46% of patients were previously treated with EGFR-targeted therapy

Gefitinib and erlotinib were approved for NSCLC in 2003 and 2004, respectively. In a randomized phase II trial of 204 late stage HNSCC patients, the addition of erlotinib to cisplatin and XRT did not confer additional tumor response or patient survival [36]. Gefitinib also did not improve survival or outcomes in a phase III randomized trial of 270 metastatic or recurrent HNSCC patients [37]. For comparison, in NSCLC, these reversible-binding TKIs exhibit RECIST (Response Evaluation Criteria in Solid Tumors) response rates of 55 to 75% for patients harboring an EGFR tyrosine kinase domain mutation [38, 39].

A new generation of TKIs with multiple targets and irreversible binding has shown clinical potential in HNSCC. Afatinib, an irreversible inhibitor of EGFR, HER2, and HER4 kinases, exhibited comparable outcomes to cetuximab. In a randomized, phase II study assessing afatinib *versus* cetuximab for treatment of recurrent or metastatic HNSCC in 124 patients, median OS was 35.9 weeks with afatinib and 47.1 weeks for cetuximab ($p = 0.78$) [40]. Following treatment failure in each arm, patients were transferred to the other treatment arm, during which disease control was 38.9% with afatinib and 18.8% with cetuximab. In light of these promising results, a phase III trial involving 483 patients following treatment failure on platinum-based therapy noted improved PFS with use of afatinib (median 2.6 months) compared to methotrexate (median 1.7 months) for second-line treatment (hazard ratio (HR) 0.80, 95% CI 0.65–0.98, $p = 0.03$) [41].

Dacomitinib, another irreversible multi-targeted TKI, and lapatinib, an oral reversible inhibitor of EGFR and HER2, have exhibited limited effects in early studies [42–46].

3.3 Phosphatidylinositol 3-kinase inhibitors

PI3K mutations are prevalent in head and neck cancers, noted in 34% of HPV-negative HNSCC and 56% of HPV-positive samples [46, 47]. Buparlisib is an oral, pan-PI3K inhibitor noted to modestly improve PFS in recurrent and metastatic head and neck cancer patients (Table 3). In a phase II trial of 158 patients assessing buparlisib as a second-line therapy following progression on platinum-based chemotherapy, buparlisib improved median PFS to 4.6 months with buparlisib and paclitaxel compared to 3.5 months with placebo and paclitaxel (HR 0.65, 95% CI 0.45–0.95) [49]. Of note, 46% of patients were previously treated with EGFR-targeted therapy. Future studies in varying patient populations may elicit more marked improvements in survival.

3.4 Antisense gene therapy

Antisense therapy centers on inhibiting messenger RNA (mRNA) by binding complementary, engineered nucleic acids. This is thought to lead to inhibition of transcription,

Table 4 Frequency of mutations in commonly deregulated head and neck cancer pathways

Cell Cycle			RTK/RAS/PI(3)K			Cell Death			Differentiation		
Gene	HPV -	HPV +	Gene	HPV -	HPV +	Gene	HPV -	HPV +	Gene	HPV -	HPV +
Pathway	96	100	Pathway	62	61	Pathway	44	31	Pathway	64	44
CCND1	31	3	*EFGR*	15	6	*CASP8*	11	3	*NOTCH*	26	17
CDK6	8	0	*ERBB2*	4	3	*TP53*	84	3	*TP63*	19	28
CDKN2A	57	0	*FGFR1*	10	0				*FAT1*	32	3
RB1	4	6	*FGFR3*	2	11						
E2F1	2	19	*IGF1R*	4	0						
MYC	14	3	*HRAS*	5	0						
TP53	84	3	*PIK3CA*	34	56				Activated Gene		
			PTEN	12	6				Inactivation Gene		

splicing, and mRNA modification. An additional mechanism described is RNase H-mediated cleavage [50].

EGFR-targeted antisense therapy has completed early phase clinical testing. In a phase I trial of 17 HNSCC patients, antisense DNA targeting EGFR was directly injected into patients' tumors. Seven patients demonstrated either stable or clinically responsive disease noted by decreased tumor volume [51]. A phase I/II trial combining EGFR antisense with radiation and cetuximab was recently completed (NCT01592721). Future research will also need to address systemic activity of EGFR-targeted antisense activity.

4 Insights from genomic research

Despite the widespread overexpression of EGFR in cancers, cetuximab treatment leads to only a modest response in HNSCC [52]. As a novel tool, genome sequencing has restructured our understanding of dysregulated pathways and provided deeper insight into EGFR-targeted therapies.

Given the broad landscape of mutations in HNSCC, mutations in four major classes of proteins/pathways have been identified: (1) mitogenic pathways (PI3K/mTOR); (2) differentiation and NOTCH pathways; (3) regulators of cell cycle proliferation through p16 and cyclin D1; and (4) regulators of apoptosis, including p53, whose loss of function is found almost universally in smoking-related HNSCC (Table 4) [47]. Whole-exome sequencing of 151 head and neck tumor samples revealed that, aside from p53, the PI3K pathway, which promotes mitogenic signaling, was the most commonly mutated pathway, with mutations occurring in 30.5% of samples [47, 48]. Additional sequencing efforts discovered novel mutations in NOTCH1, functioning as a tumor suppressor gene [53, 54].

Sequencing of HNSCC tumors has not identified recurrent EGFR driving mutations. In contrast to NSCLC in which EGFR mutations are clustered in exons 18–21, the region encoding the tyrosine kinase domain, EGFR mutations in head and neck cancers appear more dispersed across the gene (Fig. 2) [54]. Chang et al. [56] assessed 11,119 human tumor samples and 41 types of cancers to create an algorithm

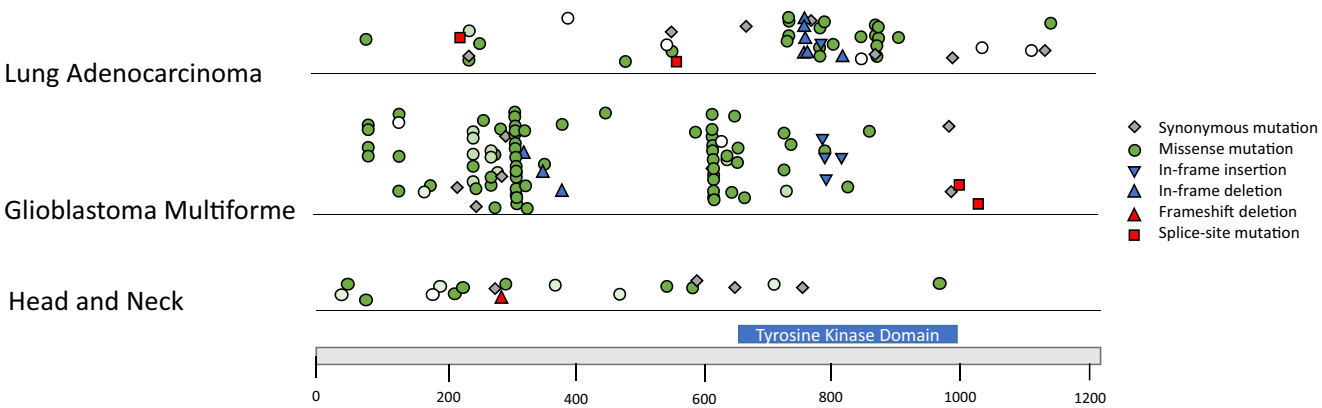

Fig. 2 EGFR mutation patterns. Mutation patterns in EGFR across tumor types. EGFR mutations appear recurrent and localized in lung cancer and glioblastoma multiforme, in contrast to the pattern seen in head and neck carcinomas. Missense mutations, represented by circles, are colored by a degree of conservation of base pair, dark green is conserved, and white is not conserved [55]

identifying frequently mutated residues; hot spots were noted in HRAS and PIK3CA in head and neck cancers but not in EGFR [55]. Perhaps, lack of recurrent EGFR mutations contributes to the limited effects of TKIs and cetuximab in HNSCC. In contrast, TKIs for the treatment of NSCLC which harbor tyrosine kinase domain mutations exhibit RECIST response rates of 55 to 75% [39].

With the lack of driving mutations and the global upregulation of EGFR, the vast landscape of mutations implicates co-activation of additional pathways. Notably the *KRAS*-variant germline and MAPK1^{E322K} mutation were highlighted in recent literature. Patients harboring a germline mutation in the micro-RNA binding site of *KRAS* have poorer overall survival [57]. Surprisingly, in a phase III trial in which cetuximab did not confer benefit when added to chemoradiation in unselected HNSCC patients [29], patients with the *KRAS*-variant (70 of 413 patients tested) had increased OS in the first 2 years following treatment with cetuximab (HR 0.19; 95% CI, 0.04–0.86; $P = 0.03$) [57]. This improvement in survival from cetuximab was not seen for wild-type *KRAS* patients. In *KRAS*-variant patients, TFG-β1 was found to be upregulated; this cytokine has been implicated in suppressing antitumor immunity through regulatory T-cell induction [59]. Authors of this study proposed that through ADCC and improved dendritic cell priming of cytotoxic T lymphocytes, cetuximab bolstered the antitumor immunity otherwise inhibited in *KRAS*-variant patients [57].

In addition to the *KRAS*-variant, genomic sequencing revealed that tumor samples from a patient with a MAPK1^{E322K} mutation were exquisitely sensitive to EGFR TKIs. In a window-of-opportunity clinical trial, a patient with a stage IVA tongue carcinoma who received a 13-day course of erlotinib experienced remarkable disease reduction from initial clinical T1N2c disease with bulky lymphadenopathy to pathological T1 N0 disease. Following surgery, the patient has remained disease-free for more than 4 years without additional treatment [60]. No EGFR mutation was identified in this patient. However, the patient's MAPK1^{E322K} mutation was studied in *in vitro* and *in vivo* models and found to be associated with upregulation of amphiregulin and stimulation of an autocrine feedback loop involving EGFR, ERK, and amphiregulin. Remarkably, upregulated amphiregulin increased tumor sensitivity to erlotinib, an effect emphasized by the loss of erlotinib sensitivity following amphiregulin knockdown in MAPK1^{E322K} models [61].

Improved response to EGFR inhibitors (cetuximab in HNSCC tumors with the *KRAS*-variant and erlotinib in HNSCCs harboring MAPK1^{E322K} mutations) emphasizes the importance of patient selection for EGFR-targeted therapies. These studies suggest that genomic sequencing will further elicit predictive biomarkers of EGFR therapeutic response and deepen our understanding of EGFR-related cellular dysfunction that can be exploited in the clinic.

In summary, the clinical benefit of EGFR-targeted therapies in head and neck tumors has been more modest than expected given the near universal upregulation of EGFR. No dominant EGFR driver mutation has been discovered in HNSCC as in NSCLC, and KRAS mutations do not clearly indicate endogenous cetuximab resistance as they have in colon cancer. Most HNSCC cohorts sequenced to date have been performed on primary tumors without accompanying information on cetuximab treatment and clinical outcome. The coexistence of multiple deregulated pathways, in the absence of driver EGFR mutations, strongly supports the co-activation of alternative signaling pathways as a mechanism of *de novo* or acquired cetuximab resistance. As with *KRAS*-variant tumors and MAPK1^{E322K} mutations, opportunities to exploit these pathways may lead to improved patient selection and therapeutic strategies.

5 Conclusion

Cetuximab remains the only FDA-approved EGFR-targeted therapy for HNSCC and provides improved survival in a subset of patients when used in combination with chemotherapy or radiation. However, long-term survival rates for head and neck cancers have remained unchanged despite increased use of EGFR-targeted therapies. Continued genomic research understanding, the dysregulated and co-activated pathways will improve patient selection and future EGFR-targeted strategies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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