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Review

Targeting the PI3K/AKT/mTOR pathway in squamous cell carcinoma of the head and neck



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SUMMARY

Despite recent advances in novel therapies, the prognosis for patients with squamous cell carcinoma of the head and neck (SCCHN) remains poor. Progress in understanding the biology of cancer has led to the development of personalized therapy targeted at blocking defective signaling pathways of cancer cells. These drugs aim to act selectively to reduce the adverse effects associated with systemic therapy. Cetuximab (Erbix[®]), an anti-epidermal growth factor receptor gene (*EGFR*)-targeted agent, is the only approved targeted therapy for patients with SCCHN. However, resistance to *EGFR* therapy remains a major obstacle to achieving a positive clinical outcome with cetuximab. Other therapies that offer better clinical outcomes in patients with advanced SCCHN are urgently needed. The phosphoinositide 3-kinase (PI3K)/AKT/mammalian target of rapamycin pathway, which is downstream of *EGFR*, has also been implicated in SCCHN development and progression, and therefore, targeting this pathway offers another rational treatment approach. This review discusses the potential role of PI3K pathway inhibitors in the treatment of patients with advanced SCCHN, both alone and in combination with other therapies.

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Introduction

Head and neck cancer is one of the most common cancer diagnoses in the world with an annual incidence of nearly 600,000 worldwide, with 40,000 of these occurring in the US alone [1–3]. Of those, approximately 90% are squamous cell carcinomas of the head and neck (SCCHN) [1]. SCCHN includes cancers of the oral cavity, oropharynx, nasopharynx, hypopharynx, and larynx. Approximately 75% of head and neck cancers are attributable to tobacco and alcohol consumption [1]. In addition, 40–60% of oropharyngeal cancers are associated with human papilloma virus (HPV) infection, and the incidence of HPV-related SCCHN cancers is increasing [1,4,5].

A combination of surgery, radiotherapy (RT), and chemotherapy (CT) is the standard treatment for SCCHN. Despite aggressive combined modality treatment, outcomes are relatively poor with 5-year cause-specific survival rates of less than 50% [6], although patients with HPV-associated tumors have a better prognosis [7]. Amplification of the epidermal growth factor receptor gene (*EGFR*) contributes to *EGFR* overexpression, which is a common

occurrence in SCCHN [8,9]. Patients with *EGFR* overexpression tend to have a particularly poor prognosis, with higher rates of locoregional recurrence following RT and lower rates of survival [10,11]. *EGFR* overexpression has also been shown to confer radioresistance *in vitro* [12].

Recent progress in understanding the biology of cancer has led to the development of personalized therapy targeted at blocking defective signaling pathways of cancer cells. By targeting specific pathways or pathway components, it is thought that such drugs aim to act selectively to reduce the adverse effects associated with systemic therapy [13]. Systemic chemotherapy for SCCHN is associated with significant toxicities, highlighting the need for more targeted therapeutics [14]. Cetuximab (Erbix[®], ImClone LLC, Somerville, NJ), an anti-*EGFR*-targeted agent, is the only approved targeted therapy for patients with SCCHN. Approval of cetuximab was based on the landmark EXTREME study, which showed improved survival compared with a standard CT regimen [15]. Other clinical studies have shown promising results with the use of cetuximab in combination with platinum-based CT in the recurrent or metastatic setting and as a radiosensitizer as part of definitive RT for medically unfit patients who are not able to receive platinum-based agents [15,16]. The use of cetuximab has become standard in both of these settings [17–19]. Although these early clinical results are promising, resistance to *EGFR* therapy remains

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a major obstacle to achieving a positive clinical outcome with cetuximab [20,21]. Other anti-EGFR agents have not shown much promise in the treatment of SCCHN. Gefitinib (Iressa™, AstraZeneca, London, UK), another EGFR-tyrosine kinase inhibitor (TKI), did not improve survival in a Phase III study of patients with recurrent or metastatic SCCHN with poor prognosis [22].

Other therapies that offer better clinical outcomes in patients with advanced SCCHN are therefore urgently needed. One potential approach to improve the response to cetuximab treatment, and consequently patient outcomes, is through combination treatment with other targeted therapies, including downstream of EGFR in the phosphoinositide 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway. Indeed, emerging evidence indicates a fundamental role for the PI3K pathway in SCCHN tumors, paving the way for a wide range of novel therapies or combination therapies for the treatment of this type of cancer. This review focuses on the PI3K pathway and its involvement in SCCHN tumors, revealing the potential role for PI3K pathway inhibitors in targeted molecular therapy for patients with SCCHN.

The PI3K/AKT/mTOR pathway

The PI3K/AKT/mTOR intracellular signaling pathway plays a diverse role in normal physiologic processes, including cellular survival, migration, proliferation, and differentiation, as well as angiogenesis, protein synthesis, and glucose metabolism. In addition, the pathway is associated with a number of oncogenic processes (Fig. 1). PI3K/AKT/mTOR is one of the most frequently dysregulated signaling pathways in cancer, including SCCHN [23]. The PI3K family of enzymes can be divided into three classes (I–III) based on their structure and substrate specificity (Table 1) [24]. Class I PI3Ks are heterodimers comprising regulatory and catalytic subunits, of which there are four isoforms: p110 α (encoded by *PIK3CA* gene), p110 β , p110 δ (Class IA), and p110 γ (Class IB). Class II PI3Ks are involved in the regulation of membrane trafficking and class III PI3K is involved in autophagy, the process by which cytoplasmic material is delivered to the lysosomes for degradation [25]. Class IA PI3Ks are commonly implicated in cancer, and thus represent potential therapeutic targets in SCCHN that will be reviewed in detail here.

PI3K is a downstream mediator of cell membrane receptor tyrosine kinases (RTKs). RTKs are phosphorylated following growth factor binding. Class IA PI3K isoforms are activated by various RTKs, including EGFR, ErbB3, Met, platelet-derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR), and insulin-like growth factor 1 receptor (IGF-1R), while p110 γ is activated by G-protein-coupled receptors. In SCCHN, overexpression of EGFR and its heterodimerization with other erbB family members suggests that PI3K may be readily activated. The activated form of PI3K phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP₂), generating phosphatidylinositol [3–5]-trisphosphate (PIP₃) – these are the key lipids that are responsible for mediating cellular responses to growth factor or cytokine stimulation, integrin ligation, and signaling via integrin-linked kinase or focal adhesion kinase [24]. PIP₃ initiates AKT activation by its translocation to the plasma membrane, leading to a conformational change in, and phosphorylation of, AKT. AKT, a serine/threonine kinase, is a central mediator of the PI3K pathway and activation of AKT further phosphorylates multiple proteins that regulate numerous cellular responses, including apoptosis, metabolism, cell proliferation and cell growth. Activated AKT promotes protein synthesis and cell growth by activation of the mTOR complex (mTORC) 1. Phosphorylated mTORC1 activates p70 S6 kinase, which enhances messenger RNA translation and drives cell growth by activating the ribosomal protein S6 and elongation factor 2. A

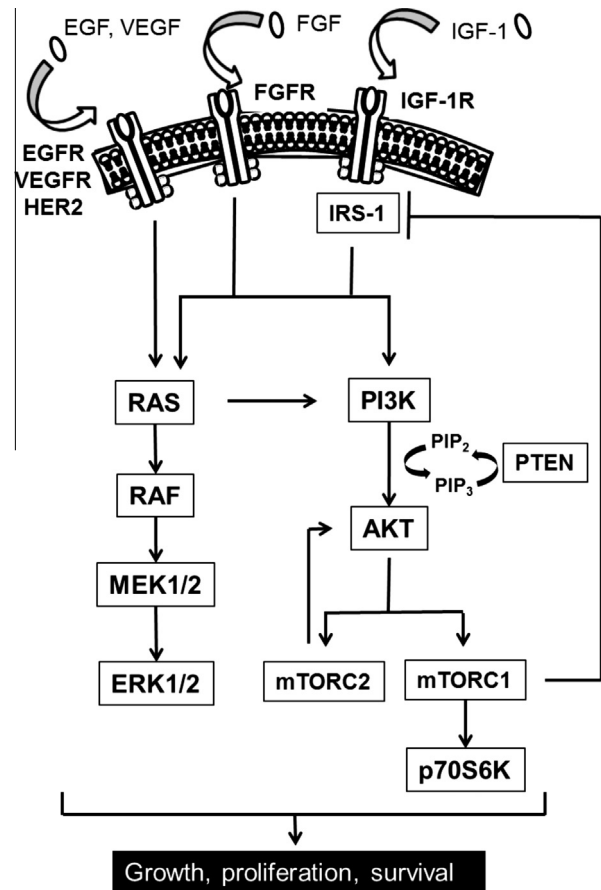


Fig. 1. The PI3K/AKT/mTOR pathway and associated signaling pathways. AKT, protein kinase B; EGFR, epidermal growth factor receptor; ERK 1/2, extracellular signal-regulated kinase 1/2; FGFR, fibroblast growth factor receptor; HER2, human epidermal growth factor 2; IGF, insulin-like growth factor; IRS-1, insulin receptor substrate 1; MEK 1/2, mitogen-activated protein kinase 1/2; mTORC, mammalian target of rapamycin complex; PI3K, phosphatidylinositol 3-kinase; PIP₂, phosphatidylinositol 4,5-bisphosphate; PIP₃, phosphatidylinositol (3,4,5)-trisphosphate; PTEN, phosphatase and tensin homolog; p70S6K, p70S6 kinase; VEGFR, vascular endothelial growth factor.

Table 1
Phosphatidylinositol 3-kinase enzyme family [24].

Class	Isoform	Subunits		Substrate
		Catalytic	Regulatory	
IA	p110 α	+	+	PIP ₂ , PIP, PI
	p110 β	+	+	PIP ₂ , PIP, PI
	p110 δ	+	+	PIP ₂ , PIP, PI
IB	p110 γ	+	+	PIP ₂ , PIP, PI
II	–	+	–	PIP ₂ , PIP, PI
III	–	+	+	PI

PI, phosphatidylinositol; PIP, phosphatidylinositol phosphate; PIP₂, phosphatidylinositol 4,5-bisphosphate.

second mTOR complex, mTORC2, also directly phosphorylates AKT on serine 473 [24]. AKT also stimulates cell proliferation by inactivating cell cycle inhibitors, such as p27^{Kip1}, RBL2 and p21^{cip1/WAF1}, and stabilizing cell cycle proteins, such as c-Myc and cyclin D1 [24,25]. Finally, activated AKT promotes cell survival by inhibiting proapoptosis proteins and degradation of the tumor suppressor TP53 [25]. The PI3K pathway has also been implicated in insulin signaling and adipose metabolism [26]. In mouse models, defective PI3K pathway signaling in muscle cells leads to insulin resistance, glucose intolerance, hyperlipidemia and increased

adiposity [26]. PI3K activity is kept in check by negative feedback loops. Although several negative feedback mechanisms act at different nodes of the PI3K pathway, a key regulator of PI3K is the tumor suppressor, phosphatase and tensin homolog (PTEN), which antagonizes PI3K function by dephosphorylating PIP₃ to PIP₂ [24,25].

Alterations in the PI3K/AKT/mTOR pathway in SCCHN

The PI3K pathway is frequently mutated in human cancers, including SCCHN. Gain of function mutations in the *PIK3CA* gene have been described and are associated with increased AKT activity and oncogenic transformation [27]. Amplification of *PIK3CA* and *AKT* as well as overexpression of AKT have also been implicated in human cancers [25]. Inactivating mutations or loss of *PTEN* impairs its lipid phosphatase activity and relieves its inhibitory effect on the PI3K pathway [25,28]. Such aberrations lead to an upregulation of AKT and an increase in cell proliferation rates.

An analysis of whole-exome sequencing data from 151 primary SCCHN tumors revealed that the PI3K pathway is the most frequently mutated oncogenic pathway in SCCHN, found in 31% of tumors [29]. Numerous mutations or alterations in the PI3K pathway have now been identified in human SCCHN, at the level of both gene expression and function (Table 2) [29]. These genes include, but are not limited to: *PIK3CA*, *PIK3CD*, *PTEN*, *PDK1*, *AKT*, and *mTOR* [8,30–35]. Mutations and copy number alterations of PI3K pathway components, including *PIK3CA* amplifications and *PTEN* inactivation by loss or inactivation of gene copy, are particularly prevalent in HPV-positive tumors [36]. Indeed, in a study of patients with HPV-positive oropharyngeal cancer, *PIK3CA* mutations were observed in 31% of cases, with 20% of cases harboring *PIK3CA* amplifications and 33% of cases showing *PTEN* loss [37]. A separate study investigating genetic aberrations of SCCHN tumors also showed differences depending on HPV status. HPV-positive tumors were associated with aberrations in *DDX3X*, *FGFR2/3*, *PIK3CA*, *KRAS*, *MLL2/3* and *NOTCH1*, whereas HPV-negative tumors were associated with mutations in *TP53*, *CDKN2A*, *MLL2*, *CUL3*, *NSD1*, *PIK3CA* and *NOTCH* genes and amplification of *EGFR*, *FGFR1* and *CCND1* genes [38]. HPV infection is an important factor in SCCHN development and has been associated with increased levels of PI3K, EGFR and MAPK, even in precancerous papillomas [24], which may explain some of these differences.

A recent genomic analysis of tumor tissue and healthy tissue from 279 patients with previously untreated SCCHN highlighted 15 genes that were significantly mutated across all tumor samples, including *CDKN2A*, *TP53*, *PIK3CA*, *NOTCH1*, *HRAS*, and *NFE2L2* [39].

Interestingly, *PIK3CA* was activated in approximately 21% of all samples, the highest frequency among mutations against which inhibitors are now available. Among HPV-positive samples, there was a 40–50% rate of *PIK3CA* alterations linked with very low rates of *EGFR* alterations, suggesting *EGFR* mutations are not universally expressed in SCCHN. Taken together, these findings point to mechanisms outside the EGFR pathway, e.g. PI3K pathway aberrations, which also contribute to the development of SCCHN, particularly in the HPV-positive setting.

Alterations in *TP53* and *CDKN2A* are found in the earliest precancerous lesions, whereas *CCND1* amplification and *PTEN* inactivation are associated with higher grades of dysplasia and SCC [40]. *PIK3CA* amplification has also been observed in dysplastic as well as malignant lesions and may contribute to progression towards SCCHN carcinogenesis [24]. Tumors of patients with advanced SCCHN have been shown to carry multiple aberrations in PI3K pathway components, such as *PIK3CA* and *mTOR* or *PIK3CA* and *PTEN* [41]. This raises the possibility that single mutations in the PI3K pathway may be responsible for SCCHN formation whereas multiple mutations in the pathway components cause disease progression. Indeed, a recent Phase II study determined whether genetic variations in the genes for *PIK3CA*, *PTEN*, *AKT1*, *AKT2*, and *FRAP1* (mTOR) were associated with differences in disease progression, survival, and response to therapy in a cohort of patients with recurrent and/or metastatic SCCHN treated with docetaxel plus cetuximab [42]. They demonstrated a correlation between the single-nucleotide polymorphisms *PTEN*:rs12569998 and *AKT2*:rs8100018 and risk of progression and PFS. Patients with the variants of *PTEN*:rs12569998 and *AKT2*:rs8100018 were at higher risk of progression after docetaxel plus cetuximab and experienced a shorter PFS. Further validation of these results is required in larger clinical trials.

Targeting the PI3K/AKT/mTOR pathway in SCCHN

SCCHN is highly heterogeneous, and its large number of genetic alterations renders it resistant to specific targeted treatments. Activation of the PI3K pathway may mediate resistance to RT, CT, and targeted agents such as EGFR inhibitors. SCCHN is relatively sensitive to RT; however, activation of the PI3K pathway has been implicated in radioresistance [43]. Blocking this pathway therefore has the potential to enhance the effectiveness of RT for patients with SCCHN. Indeed, PI3K inhibition has been seen to sensitize the FaDu model of SCCHN to RT [44]. Activation of the PI3K pathway is also a potential mechanism of resistance to anti-EGFR treatment [21]. Cetuximab-resistant tumors have higher PI3K/AKT pathway gene expression and protein activation compared with cetuximab-sensitive tumors [45]. Overcoming resistance to cetuximab may lead to increased response rates and potentially decrease the need for traditional cytotoxic CT in SCCHN.

Targeting the PI3K/AKT/mTOR pathway has become a major focus of clinical research in SCCHN and there are several agents that target the PI3K/AKT/mTOR pathway in SCCHN in various ways. These agents are currently being tested in clinical trials, both as single agents and in combination with other treatments [30,35] (Table 3).

Pan-PI3K inhibition

Agents that target all four class I PI3K isoforms, including buparlisib (BKM120; Novartis Pharmaceuticals Corporation, East Hanover, NJ) and PX-866 (Oncothyreon, Seattle, WA), are currently under investigation in SCCHN. Buparlisib has demonstrated antiproliferative, proapoptotic, and antiangiogenic activity in multiple preclinical cancer models [46]. Buparlisib demonstrates

Table 2
Mutations or alterations in the PI3K pathway associated with SCCHN [29].

Gene	Approximate frequency (%)
PTEN	4
PIK3CA	13
PIK3AP1	2.7
PIK3C2A	1
PIK3C2B	1
PIK3C2G	2
PIK3CD	1
PIK3CG	4
PIK3R1/2/3/4/5/6	1–3
AKT 1/2/3	1.3
mTOR	1.3
TSC1/2	1
RICTOR	2
RPTOR	1

SCCHN, squamous cell carcinoma of the head and neck.

Table 3
Ongoing clinical trials of agents targeting the PI3K/AKT/mTOR pathway in SCCHN (clinicaltrials.gov).

Drug	Trial number	Phase	Regimen	Patient group
<i>PI3K inhibitors</i>				
Buparlisib	NCT02113878	Ib	Combination therapy with cisplatin and radiotherapy	High risk locally advanced SCCHN
	NCT01816984	I/II	Combination therapy with cetuximab	Recurrent or metastatic SCCHN
	NCT01852292	II	Combination therapy with paclitaxel	Platinum-refractory recurrent or metastatic SCCHN
	NCT01527877	II	Single agent	Recurrent or metastatic SCCHN
	NCT01737450	II	Single agent	Recurrent or progressive metastatic SCCHN
PX-866	NCT01252628	I/II	Combination therapy with cetuximab	Recurrent or metastatic SCCHN
	NCT01204099	II	Combination therapy with docetaxel	Recurrent or metastatic SCCHN
BYL719	NCT02051751	I	Combination with paclitaxel	Recurrent or metastatic SCCHN and breast cancer
	NCT01822613	I/II	Combination with LJM716	Recurrent or metastatic esophageal squamous cell carcinoma
	NCT01602315	I/II	Combination therapy with cetuximab	Platinum-refractory recurrent or metastatic SCCHN
	NCT02145312	II	Single agent	Recurrent or metastatic SCCHN
<i>mTOR inhibitors</i>				
Temsirolimus	NCT01016769	I/II	Combination therapy with paclitaxel and carboplatin	Recurrent or metastatic SCCHN
	NCT01256385	II	With or without cetuximab	Recurrent or metastatic SCCHN
Everolimus	NCT01333085	I/II	Combination induction therapy with carboplatin and paclitaxel	Unresectable or inoperable locally advanced SCCHN
	NCT01283334	II	Combination therapy with carboplatin and cetuximab	Recurrent or metastatic SCCHN
	NCT00942734	II	Combination with erlotinib	Recurrent SCCHN
	NCT01111058	II	Single agent	Recurrent or metastatic SCCHN
	NCT01051791	II	Single agent	Recurrent or metastatic SCCHN
	NCT01133678	II	Single agent	Locoregional advanced SCCHN
Sirolimus	NCT01195922	I/II	Single agent	Advanced stage SCCHN
<i>AKT inhibitors</i>				
MK2206	NCT01370070	II	Single agent	Recurrent or metastatic nasopharyngeal carcinoma
Panitumumab	NCT00513383	I	Combination with CT plus induction CT	Locally advanced SCCHN
	NCT00454779	II	Combination with docetaxel and cisplatin	Metastatic and/or recurrent SCCHN
	NCT00798655	II	Postoperative RT, cisplatin, and panitumumab	Locally advanced SCCHN
	NCT01264328	II	Combination with paclitaxel	First-line metastatic or recurrent SCCHN
	NCT00820248	III	Combination with RT versus RT and cisplatin	Advanced stage III or IV SCCHN
	NCT01305772	II	Single agent	Untreated, locally advanced SCCHN
	NCT00446446	II	Single agent	Second-line SCCHN

5-FU, 5-fluorouracil; CT, chemotherapy; EGFR, epidermal growth factor; HPV, human papilloma virus; IL, interleukin; IMRT, intensity-modulated radiotherapy; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; RT, radiotherapy; SCCHN, squamous cell carcinoma of the head and neck.

synergistic activity with cytotoxic and targeted agents [46,47], and enhances sensitivity to these drugs in resistant cancer models [48]. Buparlisib has also been shown to enhance the *in vitro* cytotoxicity of histone deacetylase inhibitors in SCCHN cancer cells and to inhibit tumor growth in xenograft models of SCCHN [49]. PX-866 is another agent that has demonstrated antitumor efficacy in SCCHN models with *PIK3CA* alterations [45]. Current clinical trials of buparlisib and PX-866 in SCCHN are listed in Table 3. Other pan-PI3K inhibitors currently in clinical development include XL147 (pilaralisib; Sanofi-Aventis, Paris, France), GDC0941 (pictilisib; Genentech, San Francisco, CA), and BAY-80-6946 (Bayer Healthcare, Leverkusen, Germany); however, these are not yet being tested in patients with SCCHN. The involvement of the PI3K pathway in insulin signaling means that hyperglycemia and hyperlipidemia are common side effects of PI3K inhibition. Indeed, the use of buparlisib in advanced solid tumors has been associated with hyperglycemia in addition to rash, nausea, fatigue and mood alterations [50].

Isoform-specific PI3K inhibition

As most alterations in PI3K occur in *PIK3CA*, tumors with PI3K pathway activation should ideally be studied in clinical trials of α -isoform-specific inhibitors. Furthermore, inhibition of the α isoform of PI3K may enhance sensitivity to RT. Isoform-specific inhibition may also allow drug administration at therapeutic doses without being limited by off-target effects such as immunotoxicity,

which is typically associated with inhibition of the PI3K δ isoform. As this PI3K isoform is predominantly expressed in the immune system, using an inhibitor that lacks specificity for PI3K δ may help avoid immune defects associated with comprehensive PI3K inhibition [51,52]. However, as with the pan-PI3K inhibitors, the central role of p110 in insulin regulation and signaling renders hyperglycemia a frequent 'on-target' toxicity of isoform-specific PI3K inhibitors [50,53].

BYL719 (Novartis Pharmaceuticals Corporation, East Hanover, NJ) is an oral inhibitor that selectively targets the α isoform of class I PI3K. BYL719 inhibits wild-type and the most common somatic mutants of p110 α [54]. In the first-in-man study of BYL719 in patients with *PIK3CA*-mutated tumors, BYL719 was well tolerated, and preliminary signs of clinical activity were observed [53,55]. Nine patients achieved partial responses, including patients with SCCHN. Patients with SCCHN were also shown to have the greatest reductions in tumor volume [53]. In addition, BYL719 also appears to be synergistic with cetuximab *in vitro* [56].

In another preclinical evaluation, both buparlisib and BYL719 were found to sensitize SCCHN cells to erlotinib, and the beneficial effect was similar between these inhibitors [57]. This supports the notion that PI3K blockade might be an effective strategy to overcome resistance of SCCHN cells toward EGFR TKIs.

There are multiple open clinical studies examining the use of PI3K inhibitors in various malignancies. A Phase I/II study of BYL719 and cetuximab in patients with recurrent or metastatic

SCCHN who are platinum-resistant is ongoing (NCT01602315). A Phase I/II study of BYL719 in combination with LJM716 versus taxanes or irinotecan in patients with previously treated esophageal squamous cell carcinoma is open for patient recruitment (NCT01822613). GS-1101 (Idelalisib; Gilead, Stockley Park, UK), GDC0032 (Taselisib; Genentech, San Francisco, CA), and AMG319 (Amgen, Thousand Oaks, CA) are other isoform-specific PI3K inhibitors that are in clinical development, although trials in SCCHN have not yet been initiated.

AKT inhibition

AKT inhibitors, including MK-2206 (Merck, Whitehouse Station, NJ) and perifosine (Aeterna Zentaris, Quebec, Canada), constitute another class of drugs that has gained recent interest. Inhibitors of AKT fall into two classes: catalytic inhibitors, which compete for the ATP-binding site, and allosteric inhibitors, which act away from this catalytic site. Subtherapeutic doses of the allosteric AKT inhibitor MK-2206 have been shown to significantly reduce cell migration in the FaDu model, with 100% survival of treated mice after 2 weeks compared with 70% survival in a control group ($p < 0.05$) [58]. Another study demonstrated a synergistic effect of MK-2206 and paclitaxel in combination in SCCHN cell lines through an interaction with autophagy trafficking, leading to increased apoptosis [59]. Perifosine, a lipid-based allosteric inhibitor of AKT, has been shown to induce apoptosis and/or cell cycle arrest in several head and neck cancer cell lines [60]. A Phase II study with perifosine lacked antitumor activity as a single agent in patients with recurrent or metastatic SCCHN [61]. Multiple clinical trials investigating the use of other AKT inhibitors have recently opened (Table 3). In a Phase I study of MK-2206 in combination with carboplatin/paclitaxel (NCT00848718), there was a partial response in one patient with SCCHN [62]. MK-2206 is currently being tested in a Phase II study in patients with recurrent or metastatic SCCHN (NCT01349933). The catalytic AKT inhibitor, GDC0068 (Roche Genentech), is also in clinical development. Common toxicities associated with AKT inhibitors include fatigue, gastrointestinal problems, neutropenia, leukopenia and rash [61,62].

mTOR inhibition

Everolimus (Afinitor[®], Novartis Pharmaceuticals Corporation, East Hanover, NJ), an mTORC1 inhibitor, sensitizes cancer cell lines to platinum and taxane CT [63]. Despite much interest in the use of induction CT, well-designed studies have found no improvement in overall survival with induction CT followed by chemoradiation versus chemoradiation alone (DeCIDE study [64], PARADIGM study [65]). Everolimus is being tested as part of a Phase I/II study (CAPRA) investigating induction CT with weekly everolimus plus carboplatin and paclitaxel in unresectable or inoperable locally advanced SCCHN (NCT01333085). Preliminary data suggest that treatment is well tolerated with an overall response rate of 79% [66]. Mild to moderate hypercholesterolemia was a common toxicity associated with everolimus use [66]. A range of other studies of everolimus in head and neck cancer are also underway (Table 3).

Temsirolimus (TORISEL[®], Wyeth Pharmaceuticals Inc., Madison, NJ), another mTORC1 inhibitor, has been shown to inhibit cell proliferation of head and neck cancer cell lines PCI-1 and PCI-13 *in vitro* [67]. In a Phase I study assessing a combination of temsirolimus, carboplatin, and paclitaxel in patients with SCCHN (NCT01016769), the confirmed objective partial response rate was 22% [68]. In a Phase II study in patients with recurrent or metastatic, platinum-refractory SCCHN, the combination of temsirolimus plus erlotinib was poorly tolerated [69], suggesting that investigation of more tolerable combinations of EGFR and PI3K/AKT/mTOR pathway inhibitors in selected patients with SCCHN is

warranted. Common toxicities associated with temsirolimus included fatigue, hyperglycemia, thrombocytopenia, diarrhea, peritonitis and pneumonia [68,69]. Temsirolimus is currently being tested in a Phase II study with or without cetuximab in patients with recurrent or metastatic head and neck cancer (NCT01256385) and in combination therapy with paclitaxel and carboplatin (NCT01016769). One drawback of selectively inhibiting mTOR is the consequent activation of PI3K, which can ultimately enhance tumor growth due to feedback through mTOR [70]; thus, more effective inhibition might be expected by targeting both PI3K and mTOR concurrently [44,56].

Dual PI3K/mTOR inhibition

In order to overcome resistance to single-target inhibition and potentially enhance response rates, dual target inhibitors have been developed. BEZ235 (dual PI3K/mTOR inhibitor; Novartis Pharmaceuticals Corporation, East Hanover) is one such inhibitor that has been shown to radiosensitize head and neck carcinoma cells [44,71,72] by interfering with both homologous and non-homologous recombination DNA repair pathways [73]. Thus, BEZ235 may add potential benefit for patients undergoing RT. SCCHN cell lines and tumors have also demonstrated sensitivity to BEZ235, particularly those harboring *PIK3CA* mutations [29]. BEZ235 has been investigated in a Phase I/II open-label study patients with advanced solid malignancies (NCT00620594). The most common side effects associated with BEZ235 include mild to moderate nausea, vomiting, diarrhea, fatigue/asthenia, anemia and anorexia [74]. PF-04691502 (Pfizer, New York City, NY) is another dual inhibitor that targets PI3K and mTOR, and has been shown in a preclinical study to enhance TP53/p73 expression and significantly inhibit tumor growth alone or when combined with RT in SCCHN with wild-type *TP53* [31]. A Phase I study of PF-04691502 in patients with solid tumors has been completed (NCT00927823), revealing that PF-04691502 was tolerable across multiple dose levels and had a favorable safety profile, with the most common treatment-related AEs being fatigue, nausea, vomiting, reduced appetite and rash [75]. Multiple patients derived clinical benefit; however, this compound does not currently appear to be in clinical trials in SCCHN. Other PI3K/mTOR inhibitors in clinical development in a range of solid tumors include XL765 (Sanofi-Aventis, Paris, France), GDC0980 (apitolisib; Genentech, San Francisco, CA), GSK2126458 (GlaxoSmithKline, Brentford, UK), and PF-05212384 (Pfizer, New York City, NY). Their efficacy in SCCHN remains to be determined.

An examination of the potential value of PI3K/mTOR inhibitors alone or in combination with histone deacetylase (HDAC) inhibitors in an *in vitro* model of SCCHN revealed marked enhancement of HDAC inhibitor-induced cytotoxicity by PI3K, AKT, and dual PI3K-mTOR inhibitors [49]. This effect correlated with AKT inhibition and was attenuated by expression of constitutively active AKT. Intratumoral HDAC inhibition and PI3K inhibition was observed, as assessed by histone H3 acetylation status and phosphorylated AKT staining, respectively. However, there was no evidence of improved efficacy with HDAC/PI3K inhibitor combination.

Blocking PI3K pathway and other pathways concurrently

While conventional therapies continue to play an important role in treating recurrent or metastatic SCCHN, many patients develop intolerable adverse events, or become CT- or RT-resistant due to cross-talk and feedback inhibition along parallel intracellular signaling pathways. In addition, experience with other successful targeted agents in cancer suggests that clinical resistance to PI3K pathway inhibitors may reduce the durability of clinical

benefit [76]. A recent study using reverse-phase protein arrays indicated that the activation of RTKs, including EGFR and ERK, commonly occurred following PI3K inhibition in SCCHN cells resistant to PI3K pathway inhibitors [77]. In addition, combined inhibition of PI3K and EGFR or MEK pathways showed synergistic activity. Therefore, the success of SCCHN treatment may depend on the combination of PI3K pathway inhibitors with conventional therapy or indeed with other targeted therapies.

In addition to the EGFR–PI3K pathway, molecular alterations in other intracellular signaling pathways have been observed in patients with SCCHN, including, but not limited to, janus kinase/signal transducers and activators of transcription, mitogen-activated protein kinase (MAPK), NOTCH [39], VEGF [78], MET [79], and IGF [79]. Recently, inhibition of poly ADP ribose polymerase [81] and S100A4 signaling pathways have also been suggested as possible new target strategies for SCCHN [82]. Thus, PI3K inhibition in combination with targeted inhibition of other intracellular signaling pathways may provide a promising therapeutic strategy in SCCHN.

Numerous studies involving other molecular-targeted therapies are being, or have been, conducted either alone or in combination with other treatment modalities in SCCHN. These include inhibitors of VEGFR (bevacizumab, sorafenib, sunitinib, vandetanib), MET (foretinib/XL880, INC280, ARQ197/tivantinib, AMG337), and heat shock protein 90 (AUY922, STA-9090, 17DMAG). Numerous studies investigating EGFR inhibitors, including afatinib, erlotinib, and panitumumab (e.g. CONCERT-1; NCT00547157), are also underway. High expression of fibroblast growth factor receptors (FGFRs) have been identified in SCCHN [83,84], and demonstrated to be associated with poor clinical outcome [84], thus identifying FGFR as another potential therapeutic target in patients with SCCHN. Targeting AKT and MEK, downstream effectors of the PI3K/AKT and MAPK pathways, respectively, in combination may provide a molecular therapeutic target for RAS-driven tumorigenesis in oral cancer [85].

Future clinical perspectives

Although improvements in survival have been observed with recent advancements in treatment, the prognosis for patients presenting with advanced SCCHN remains poor. As such, there is a strong unmet need for novel therapies in these patients. Genetic abnormalities are associated with the development and progression of many of these tumors, thus the use of genomic and proteomic biomarkers may enable targeted therapeutic interventions to be developed for patients with cancer, particularly those with SCCHN. Identification and validation of biomarkers that can help to predict which patients are most likely to respond to targeted therapies is an essential unmet need in SCCHN [29,31,86–88].

While there are many preclinical data implicating PI3K/AKT/mTOR pathway activation in response to PI3K/AKT/mTOR pathway inhibitors, the predictive nature of PI3K pathway mutations and clinical response to PI3K/AKT/mTOR pathway inhibitors is controversial. Analyses have shown that patients with *PIK3CA* mutations had a higher rate of partial responses, while coexisting *PIK3CA* and *KRAS* mutations were associated with a lack of response [55,89]. However, early evidence from the pan-PI3K inhibitor buparlisib has not shown a correlation between clinical response and PI3K/AKT/mTOR pathway activation status [50].

Predicting response to pathway inhibition in various cancer types and clinical validation of these findings is complicated by the impracticalities of tumor sampling. Recent research suggests a possible role of circulating tumor cells (CTCs) in determining different types of cancer [90]. These CTCs have been used to identify molecular alterations of *PIK3CA* and *PTEN* in patients with SCCHN.

This highlights the possibility that analysis of CTCs may enable the identification and validation of biomarkers predictive of response to PI3K/AKT/mTOR pathway inhibition in SCCHN.

Combination studies with RT are regarded as an important therapeutic strategy in SCCHN. It is unknown whether biomarkers are required to preselect patients for certain combination treatment strategies, but data suggest that inhibition of the α or β isoform of PI3K may enhance sensitivity to RT. Further exploration of this concept would be interesting and could guide the clinical development of PI3K pathway inhibitors. The recent identification of improved prognosis among patients with SCCHN who have HPV-induced tumors may also allow better treatment selection for these patients [91] and it will be important to stratify patients by HPV status in future clinical trials. As *PIK3CA* alterations are more common in this population, combining standard therapy with a PI3K inhibitor that selectively targets the α isoform could be effective. A growing body of data suggests that EGFR may not be as relevant a target in HPV-positive tumors [92], and new treatment combinations will be needed. As *PIK3CA* alterations are more common in this population, combining standard therapy with an α isoform-selective PI3K inhibitor could be effective.

Conclusions

Despite the encouraging results achieved with anti-EGFR therapy, particularly with cetuximab, resistance to EGFR therapy remains a major obstacle to achieving a positive clinical outcome. The existence of PI3K/AKT/mTOR pathway alterations in SCCHN, combined with evidence for the involvement of this pathway in the development of RT- or EGFR-resistant disease, has led to the investigation of PI3K/AKT/mTOR pathway inhibitors in SCCHN. Several PI3K/AKT/mTOR pathway inhibitors have demonstrated antitumor activity in preclinical models, but this has not always translated into clinical activity. Clinical trials investigating PI3K/AKT/mTOR pathway inhibitors as single agents and in combination with conventional therapies are ongoing in SCCHN. It is doubtful that targeting one receptor will provide meaningful benefits to patients; accordingly, agents that target multiple receptors alone or in combination will likely provide the most therapeutic benefit for patients with SCCHN. Clinical trials investigating tumor HPV status and new combinations of existing treatment modalities (e.g. CT, RT, cetuximab) with molecularly targeted agents may potentially pave the way towards more personalized therapeutic approaches.

Conflict of interest statement

Dr. Cohen reports personal fees from Novartis, outside the submitted work.

Dr. Mell reports grants from Genelux Inc., and grants, personal fees and non-financial support from Varian Medical Systems, outside the submitted work.

Dr. Simpson has nothing to disclose.

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