

The phosphatidyl-inositol 3-kinase/Akt/mammalian target of rapamycin pathway as a therapeutic target in head and neck cancer

Phosphatidyl-inositol 3-kinase/Akt/mammalian target of rapamycin pathway (PI3K/Akt/mTOR) is a critical signal transduction pathway in mammalian cellular biology, and is important to energy homeostasis, growth and survival. Activation of the pathway is an early event in head and neck squamous cell carcinoma (HNSCC) carcinogenesis. Moreover, PI3K/Akt/mTOR is the most commonly mutated pathway in HNSCC, with striking prevalence in human papillomavirus (HPV) associated disease. Genomic gain of function can also be conferred by *PIK3CA* gene amplification or loss of phosphatase and tensin homolog, the negative regulator of PI3K. Given the importance of PI3K/Akt/mTOR signaling across the neoplastic spectrum, pathway inhibitors are of unique interest in HNSCC. This review will summarize current knowledge, highlight differences in HPV(+) and HPV(-) disease and discuss therapeutic agents in various phases of clinical development.

Keywords: Akt • head and neck cancer • human papillomavirus • mTOR

Head and neck squamous cell carcinoma (HNSCC) will likely affect 55,000 patients in the USA in 2014, and 550,000 patients worldwide [1,2]. Although the past three decades have brought significant surgical, radiotherapeutic and pharmaceutical advances, the 5-year overall survival (OS) rate for HNSCC is 40–60% and has increased only incrementally. Despite histologic convergence, HNSCCs are genetically and etiologically diverse. In addition to the classic risk factors of tobacco and alcohol, human papillomaviruses (HPVs), predominantly HPV genotype 16, now represent a primary cause of HNSCC in North America and Europe and are recognized as the source of an emerging epidemic [3,4]. There is significant variation in both etiology and outcome for patients diagnosed throughout the world. In the USA, HPV infection is associated with 60–70% of oropharyngeal cancers, compared with less than 10% in less economically developed countries [5]. Because HPV(+) HNSCC is associated with improved outcomes after conventional treatment, the proportion of virally driven cancers in a population influ-

ences OS. In the USA, for example, the mortality for African Americans with laryngeal and pharyngeal cancer is higher than for whites, partially due to lower HPV prevalence [6]. In contrast to the rapid pace of molecular discovery in HNSCC, only two new systemic therapies have been approved by the US Federal Drug Administration in the past 40 years: docetaxel and cetuximab.

Notwithstanding successes, clearly much more work is needed to identify the critical biologic pathways and optimal treatment strategies for both HPV(+) and HPV(-) HNSCC. Although two distinct causes of HNSCC exist, environmental carcinogenesis or transformation by HPV oncogenes, both etiologies are associated with aberrant signaling along the phosphatidyl-inositol 3-kinase/Akt/mammalian target of rapamycin pathway (PI3K/Akt/mTOR). Overall, PI3K/Akt/mTOR is the most commonly mutated pathway in HNSCC (~30%) [7], and is more frequently mutated in HPV(+) than HPV(-) disease, even though HPV(+) HNSCC harbors fewer mutations in general [8,9]. The recognition

Saad A Khan^{*1}
& Julie E Bauman^{*,‡,2}

¹Department of Internal Medicine, Division of Hematology/Oncology, University of Texas Southwestern Medical Center, Dallas, TX, 75390, USA

²Department of Internal Medicine, Division of Hematology/Oncology, University of Pittsburgh Cancer Institute, 5150 Centre Ave, 5th floor, Pittsburgh, PA, 15232, USA

*Author for correspondence:

Tel.: +1 412 623 7833

Fax: +1 412 648 6579

baumanje@upmc.edu

[‡]Authors contributed equally

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that this pathway is genomically activated in HNSCC and many different epithelial cancers has led to the development and testing of agents targeting the various components. This review summarizes the current knowledge about the PI3K/Akt/mTOR pathway in HNSCC, highlighting differences in HPV(+) and HPV(-) disease, as well as therapeutic agents currently in various phases of clinical development.

Biologic importance of the PI3K/Akt/mTOR pathway

PI3K is a conserved intracellular signal transducer in mammalian biology which is critical for normal cellular processes including glucose homeostasis, proliferation and survival. The PI3Ks comprise dimeric proteins with differing structures and function, which are closely regulated in the nonmalignant cell. They are often stimulated by receptor tyrosine kinases (RTKs) or G-coupled protein receptors (GPCRs), and have historically been thought important in cell signaling and membrane trafficking. Ongoing research highlights their significant role in many different cancers, contributing to cell survival, growth, proliferation, invasion and metastasis of the malignant cell. Emerging data also suggest that autophagy, the mechanism whereby cells digest and recycle unnecessary cellular components to conserve energy during starvation, is closely regulated by PI3K signaling [10].

Activated PI3kinases lead to a complex cascade of intracellular events including the upregulation and activation of the oncoprotein Akt. This process inhibits apoptosis and also leads to the formation of an activated mTOR complex (Figure 1). In turn, mTOR guides protein synthesis and thus cellular growth, and inhibits the pathway via negative feedback. Aberrant activation of the PI3K/Akt/mTOR pathway has been described in many cancers. PI3Kinase/Akt/mTOR activation in malignant cells can occur by multiple mechanisms including: activation of upstream RTKs, such as the EGFR, alternate HER family RTKs or IGFR; gain-of-function mutations in the genes encoding Ras, PI3K or Akt; genetic or epigenetic loss-of-function of negative regulatory proteins, such as phosphatase and tensin homolog (PTEN).

PI3K/Akt/mTOR pathway activation in HNSCC carcinogenesis

Activation of the PI3K/Akt/mTOR pathway is an early event in carcinogen-induced HNSCC, and is associated with the progression of dysplasia into invasive carcinoma in human specimens as well as murine models [11,12]. In oral epithelial dysplasia and early-stage invasive HNSCC specimens, class III PI3K gene expression was elevated and phosphorylated-Akt was overexpressed in

contrast to adjacent, normal epithelium [13]. In another analysis of both dysplastic and invasive lesions, 1/6 low-moderate grade dysplasias, 7/9 high-grade dysplasias and 11/11 invasive carcinomas demonstrated copy number gain in *PIK3CA*, the gene encoding the α -catalytic subunit of PI3K, with increased p110- α protein expression also seen [12]. The oncologic importance is also suggested by the finding that patients with HNSCC are at higher risk for recurrence when pathway activation is identified at the surgical margins [14].

PI3 kinases

Classification

The PI3 kinases are traditionally divided into three classes on the basis of their structure and *in vitro* lipid substrate specificity [15]. This classification is relevant to identify recurrent mutations, as well the differential effect of drugs on specific PI3K subtypes.

Class IA PI3Ks have been most studied in human cancer, and are the focus of the current review [16]. Class IA PI3Ks are heterodimers composed of both regulatory and catalytic subunits. The regulatory subunits are referred to as p85 and comprise p85 α , p85 β and p50 α isoforms, encoded by *PIK3R1*, *PIK3R2* and *PIK3R3*, respectively [17–19], and mediate the activation of Class IA PI3Ks by growth factor receptor tyrosine kinases. The catalytic subunits comprise the isoforms p110 α , p110 β and p110 δ , produced by *PIK3CA*, *PIK3CB* and *PIK3CD*, respectively [17,19]. p110 α is encoded by *PIK3CA*, the most commonly mutated or amplified PI3K pathway gene in epithelial cancer, which confers an oncogenic gain-of-function. Class IB PI3Ks comprise the p101 regulatory subunit and the p110 γ catalytic subunit encoded by *PIK3CG*. While class IB PI3Ks have been found overexpressed in pancreatic cancer compared with normal pancreatic duct epithelium [20], their role in HNSCC is less clear; *PIK3CG* is rarely mutated in HNSCC.

Class II PI3Ks comprise three catalytic subunits involved with receptor signaling and endocytosis. They are products of the *PIK3C2A*, *PIK3C2B* and *PIK3C2G* genes [21]. Though less well studied, cell line analyses suggest *PI3KC2A* and *PI3KC2B* may be involved in cancer as well [22].

Class III PI3Ks comprise an enzyme identified as Vps34, encoded by *PIK3C3*. The regulatory subunit is Vps15, and is involved with membrane trafficking, including endosomal protein sorting, autophagy and cytokinesis [21,23–26].

Activation

Class I PI3Ks are activated by upstream growth factor receptors or the Ras signaling protein. In a regulated fashion, cell surface signals are transmitted through

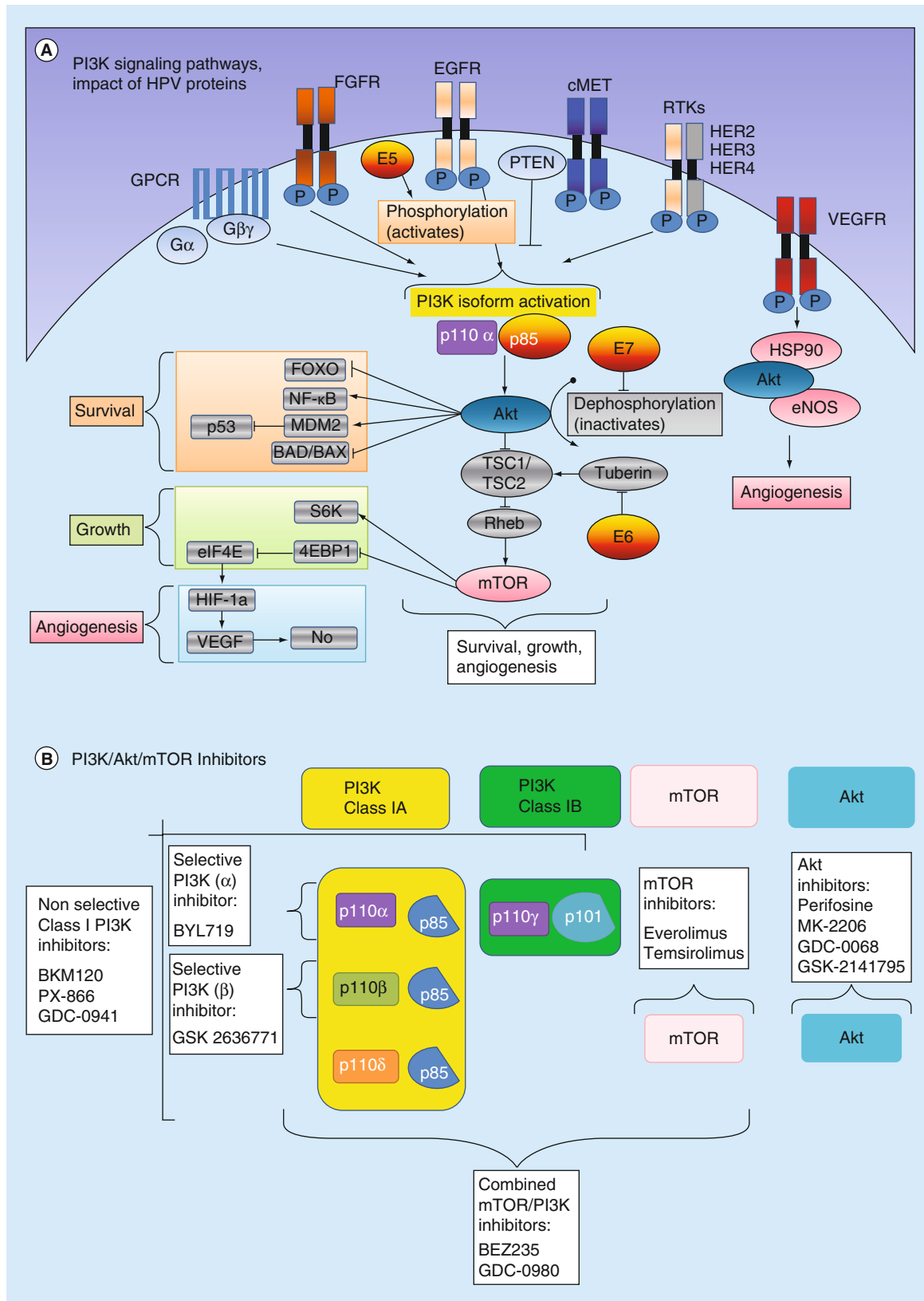


Figure 1. (A) The PI3k/Akt/mTOR signalling pathway, with impact of human papillomavirus-associated proteins and (B) important agents in the PI3K/Akt/mTOR pathway and their mechanism of action.

PI3K, Akt and mTOR to the nucleus where they exert their effect. This pathway is complex and results in a negative feedback loop mediated by S6 kinase and the adaptor protein IRS-1 [16].

Receptor tyrosine kinases

Stimulation of RTKs on the cell surface, including EGFR and other HER family RTKs, cMET and FGFR, leads to the regulatory subunit p85 binding to RTK phosphotyrosine residues [27]. This results in the p110 catalytic subunit becoming active and the localization of PI3K to the plasma membrane [16]. Of note, upon binding of insulin to the insulin receptor, its kinase activity increases causing downstream activation of this pathway as well [28]. The importance of PI3K in mediating insulin response results in the on-target toxicity of hyperglycemia from clinical PI3K inhibitors.

GTPase Ras

Ras can activate all four members of Class I PI3K via a Ras-binding domain on the p110 isoform [29–31]. The interaction of Ras with p110 α has been implicated in tumorigenesis in a *KRAS* mutant murine model [31].

G-protein-coupled receptors

Class IB PI3Ks are activated by GPCRs, either directly via G β activating p110 γ or indirectly via the GPCR's effects on the other PI3K activators Ras and the RTKs [19,32,33].

Function of activated PI3 kinases

Upon activation, the PI3 kinase pathway's downstream effects include survival, proliferation, cell growth, metabolism, proliferation, autophagy and angiogenesis.

Survival

PI3K activation leads to phosphorylation of Akt, which in turn de-activates multiple apoptosis proteins by phosphorylation. The prosurvival effect is mediated by inhibition of the Bcl-2 family of anti-apoptotic proteins, the forkhead family of transcription factors, and NF- κ B [34,35]. Akt activation also leads to phosphorylation of Mdm2, promoting nuclear localization and antagonizing p-53-mediated apoptosis [34].

The importance of the PI3K pathway in survival of HNSCC has been demonstrated in cells with *PIK3CA* abnormalities [7]. When both canonical and novel mutations in *PIK3CA* were isolated and transfected into HNSCC cell lines with previously wild-type *PIK3CA*, cells exhibited enhanced growth and the ability to survive in serum-deprived conditions.

Proliferation

The effect of PI3K on proliferation is mediated by phosphorylated Akt. Progression through cell cycle checkpoints depends upon activated cyclin-dependent kinases (CDK)/cyclin complexes. Phosphorylated Akt dissociates the CDK inhibitor, p21, from CDK/cyclin complexes resulting in their sustained activation [36]. Thus PI3K activation releases cells from the G1 and G2 checkpoints, leading to cell cycle progression in both normal and malignant cells.

Cell growth & metabolism

PI3K regulates cell growth through the interactions between Akt and mTOR. Akt inhibits the rheb GTPase activity of the TSC1/TSC2 dimer via phosphorylation of TSC2 [16], releasing MTORC1 from potent inhibition and leading to higher S6 kinase activity and increased protein synthesis [17]. Higher S6 kinase activity does lead to diminished PI3K activation through inhibition of insulin RTK activity, including the IGFR scaffolding protein, IRS-1 [37].

Autophagy

Autophagy is the controlled process by which all eukaryotic cells under nutrient stress sequester and degrade cellular proteins and structures. These auto-phagosomes then fuse with lysosomes and the action of lysosomal hydrolases leads to these products being recycled [38]. Autophagy has been demonstrated to be regulated by the activity of PI3K and impacts the response of cancer cells to PI3K/Akt/mTOR inhibitors [39,40]. Accumulation of the pleiotropic protein p62/SQSTM1 which is consumed during autophagy, was associated with increased antioxidant response and cell survival as well as resistance to PI3K inhibitors in HNSCC cell lines [10].

Angiogenesis

The PI3K pathway acts through hypoxia-inducible factor-1 (HIF-1) and VEGF to increase angiogenesis. PI3K pathway activation leads to upregulation of HIF-1 α and increased VEGF expression [41]. HIF-1 is a heterodimer that activates transcription of VEGF [42]. PI3K also regulates endothelial secretion of nitric oxide (NO) signaling [43]. PI3K inhibitors lead to attenuation of VEGF-induced NO production [44]. The importance of PI3K for angiogenesis has also been demonstrated by the fact that dual PI3K/mTOR inhibitors such as BEZ235 and GDC-0980 block neovascularization in syngeneic and xenograft murine models, whereas mTOR inhibitors alone are ineffective [45,46].

PIK3CA mutations in cancer

Gain-of-function mutations of *PIK3CA* are commonly observed in human malignancies, and are most frequent

in brain, head and neck, breast, colon and lung cancers. Canonical or 'hotspot' missense mutations occur in exons 9 and 20 [47]. Exon 9 encodes the helical domain of p110 α , and mutations lead to diminished inhibition by the regulatory subunit [48,49]. Exon 20 encodes the catalytic domain of p110 α , and mutations confer constitutive catalytic activity. While the canonical mutations are most common in HNSCC, multiple novel *PIK3CA* mutations have been identified and appear to confer increased function in cell line models [7].

Akt

Classification

Akt is the cellular homolog of the transforming oncogene of the AKT8 retrovirus, characterized by a pleckstrin homology (PH) domain in the N-terminus which is followed by a catalytic domain and a C-terminal regulatory domain [50]. The Akt family of proteins contains a central kinase domain that has specificity for serine/threonine in substrate proteins.

Akt comprises three isoforms: Akt1, Akt2 and Akt3. These isoforms have 87–90% identical amino acids in their catalytic domains [51]. Akt1 is expressed in most tissues, while Akt2 is found in insulin-responsive organs such as liver, skeletal muscle and adipose tissue. Akt3 is expressed at high levels in the brain and testes but at lower levels compared with the other isoforms in all other tissues.

Activation & deactivation

The best understood mechanism of activation of Akt is by accumulation of PIP₃ as a consequence of phosphorylation by PI3K. PIP₃ binds phosphoinositide-dependent protein kinase 1 (PDK1) and Akt, recruiting them to the plasma membrane where PDK1 phosphorylates and activates Akt at threonine 308 [52]. PTEN is the tumor suppressor that counteracts the kinase function of PI3K by de-phosphorylating PIP₃ to PIP₂, thereby suppressing phosphorylation of Akt [53].

Function

Akt activation has effects both on mTOR and other substrates outside of the pathway. In addition to the cellular functions listed as part of PI3K activation in Section 3.3, specific functions have been ascribed to the various isoforms of Akt.

Akt1 is involved in apoptotic inhibition, survival and angiogenesis and is expressed in most tissues. Akt1 null mice have slower growth, and show an increased sensitivity to apoptosis caused by gamma and UV radiation, as well as TNF and anti-Fas [54,55]. Akt1 deficiency has been associated with delayed tumor progression, invasion and metastasis in murine thyroid cancer, and was associated with marked reduction in phos-

phorylated Akt even in the presence of adequate Akt2/Akt3 [56]. In PTEN-deficient mice, tumor formation is suppressed when Akt1 levels are lowered [57].

Loss of Akt2 function has mostly been associated with derangements in insulin metabolism. Akt2 overexpression leads to the upregulation of β -1 integrins, as well as increased invasion and metastatic potential in human breast and ovarian cells [58]. Akt2 downregulates PTEN and cells expressing higher levels of Akt2 are more resistant to hypoxia than cells with high Akt1/Akt3 [59]. This mechanism involves upregulation of microRNA 21 only in Akt2-expressing cells.

Although Akt3 has more limited tissue distribution and thus there is a less clear understanding of its specific function [60,61], it appears to play a role in mitochondrial biogenesis and autophagy, through regulation of the major nuclear export protein CRM-1 [62].

Akt & PTEN activation & mutation in cancer

The expression of activated Akt is much higher in malignant cells than in their normal counterparts across the spectrum of human cancers. In HNSCC specifically, phosphorylated Akt was expressed in 12 out of 21 (57%) pharyngeal cancers, and 13 out of 16 (81%) laryngeal tumors, but not in normal tissue [63]. Isolated Akt mutations without concomitant PI3K abnormalities are rare, though have been described in 1% of squamous cell lung cancer [64].

A rare gain-of-function mutation, E17K, has been identified in the pleckstrin homology domain of the Akt1 gene in less than 1% of non-small-cell lung (squamous), breast, ovarian and colorectal cancer [65,66]. Although *AKT1* mutations have been recently identified in HNSCC, these events are also rare, and superseded by gene copy number gains in up to 14% [67].

Akt3 abnormalities have been reported in triple-negative breast cancer, including amplification (11%) or deletions (13%) [60]. Downregulation of Akt3 inhibits growth in triple-negative breast cancer cell lines and murine xenografts, while depleting Akt3 sensitized them to the pan-Akt inhibitor, MK-2206 [68]. MAGI3-Akt3 is a fusion protein that has been identified in the PI3K pathway in breast cancer, and the resulting protein was constitutively phosphorylated [69]. This fusion protein was found to be resistant to MK-2206. These intriguing findings suggest that further genomic analysis of Akt in different tumors may illuminate a greater role in the development of malignancy than currently appreciated.

Loss of phosphatase activity by the PTEN tumor suppressor is a common mechanism of PI3K/Akt/mTOR pathway activation in cancer [70,71]. Abrogation of PTEN function by mutation or deletion

has been described in 8–23% of HNSCC specimens [7,9,72]. Silencing of PTEN also occurs by gene methylation in HNSCC [73]. Loss of PTEN heterozygosity at chromosome 10 is associated with poorer prognosis in HNSCC, again underlining the functional importance of this pathway [74].

mTOR

Classification

mTOR is 250 kDa protein which was originally identified as the target of rapamycin in yeast and mammalian cells. mTOR is an atypical serine threonine kinase which forms the nucleus of two multi-protein complexes that integrate signals of cell stress and nutrient status, subsequently inducing the translation of pro-growth, proliferation and anti-apoptotic proteins.

mTORC1

This complex is formed with regulatory associated protein of mTOR (RAPTOR), mLST8 and proline-rich Akt Substrate 40 kDa (PRAS40) [75]. RAPTOR positively regulates and PRAS40 negatively regulates the mTOR activity. mTORC1 is sensitive to nutrient levels and rapamycin.

mTORC2

This complex is formed with protein associated with RICTOR (PROTOR) and mLST8, but contains RICTOR instead of RAPTOR and PRAS40, and is involved with cell survival and morphology [76]. mTORC2 is sensitive to growth factors and nutrients but not to rapamycin.

Activation & deactivation

mTORC1

The mTORC1 complex is activated as a consequence of two stimuli: localization of mTORC1 to the lysosomal membrane in response to the presence of amino acids, where it contacts rheb-GTPase, its direct activator; inactivation of the TSC1/TSC2 dimer, the negative regulator of rheb-GTPase, via phosphorylation of TSC2 by growth-factor activated Akt [16,77]. Activation of rheb stimulates mTORC1 and leads to higher S6 kinase activity and increased protein synthesis, proliferation, ribosome biogenesis, cell survival and invasion [17]. Inhibition of mTORC1 can be caused by signals of cellular stress including hypoxia, WNT-GSK3 signaling and glucocorticoids via TSC1/TSC2 activation.

mTORC2

mTOR also forms the nucleus of a second protein complex called mTORC2, whose mechanism of activation is less clear. Insulin-stimulated PI3K signaling

results in mTORC2-ribosome binding, a requirement for subsequent mTORC2 signaling [76]. mTORC2 directly phosphorylates and activates Akt, establishing a feed-forward loop potentiating cell growth.

Function

The mTOR complexes fundamentally control cell growth. mTORC1 coordinates protein and nucleotide synthesis, glycolysis and autophagy while mTORC2 regulates lipogenesis, glucose metabolism, actin cytoskeletal conformation and apoptosis [77].

mTOR controls protein synthesis by phosphorylation of important regulators of eukaryotic translation including p70-S6 kinase, which in turn phosphorylates the ribosomal protein S6 and 4E-BP1 [78]. 4E-BP1 represses eukaryotic initiation factor 4E (eIF4E) [79], part of the RNA-helicase complex binding the cap of mRNA, which unwinds the 5' untranslated region of mRNA and allows the ribosome subunits to initiate protein synthesis [80].

mTOR activation & mutation in cancer

Given its integrative role in cell growth, proliferation and survival, mTOR is predictably activated in multiple human cancers [81]. mTOR pathway activation, as manifest by eIF4E gene amplification and protein overexpression, is commonly observed in HNSCC. When eIF4E is overexpressed at tumor margins, it is associated with malignant progression of HNSCC [14]. Aberrant accumulation of phosphorylated S6 has been found in both HNSCC cell lines and patient specimens [82]. mTOR activation has also been described in patient tumors, independent of Akt activation status [83]. mTOR mutations have been identified in 2% of HNSCC in the TCGA; functional consequences are still under study.

The PI3K/Akt/mTOR pathway & viral infection

HPV infection

The PI3K pathway in the HPV epidemic era will likely take on great therapeutic significance due to the association between HPV neoplasia and PI3K/Akt/mTOR signaling. At the earliest infection step, even prior to oncoprotein expression, extracellular binding by HPV activates the PI3k/Akt/mTOR pathway, inhibits autophagy, stimulates PTEN phosphorylation and facilitates viral entry into the cell [45].

Nongenomic activation of the PI3K/Akt/mTOR pathway is a nearly universal aspect of mammalian viral infection, and is of particular importance for dsDNA viruses such as HPV relying upon 5' cap-dependent protein translation [52]. The HPV early oncoproteins E5, E6 and E7 have direct roles in pathway activation

(Figure 1A). E5 enhances ligand-dependent phosphorylation of EGFR, which results in activation of PI3K/Akt signaling [84]. E6 binds tuberlin, a component of the TSC1/TSC2 complex which negatively regulates mTORC1. Consequent upregulation of Rheg-GTP activates mTORC1 and maintains the phosphorylation states of 4E-BP and S6 [85]. Under conditions of nutrient deprivation, HPV16 E6 activates PDK1 and mTORC2, which leads to Akt activation [86]. This may occur even in the absence of TSC2 level change in the cell.

E7 prevents de-phosphorylation of Akt following activation by PI3K, by suppressing PP2A activity [87]. In cervical cancer, universally caused by oncogenic HPV infection, E7-induced degradation of retinoblastoma upregulates Akt activity [88]. HPV type 16 positive high-grade cervical squamous intraepithelial lesions also demonstrated Akt activation in association with loss of Rb expression, in contrast to normal cervical epithelium, suggesting that E7-related Akt activation may be an early event in HPV neoplasia. Ultimately, the convergent activities of E5, E6 and E7 induce net protein synthesis and increased translation of viral oncoproteins, resulting in a positive feedback loop.

Epstein–Barr Virus infection

Nasopharyngeal cancer is a subtype of HNSCC associated with Epstein–Barr Virus (EBV), which is endemic in southern Chinese, Southeast Asian, North African and Arctic native populations [89]. EBV infection of cells leads to production of Viral Latent Membrane Proteins 1 and 2 (LMP1 and LMP2). LMP1 is required for oncogenic transformation of human cells by EBV, and can directly activate PI3K [90]. LMP2A is a transmembrane protein which inhibits cell signal transduction by mimicking the B-cell receptor [91]. LMP2A has an aminoterminal domain that constitutively associates with the Src family kinases and Syk. LMP2A directly activates PI3K leading to downstream activation of the Akt/mTOR pathway [91]. Constitutive activation of the PI3k/Akt/mTOR pathway by LMP2A in EBV-infected HNSCC is a possible mechanism of transformation [92,93]. Genomic analysis of a nasopharyngeal cancer cohort demonstrated *PIK3CA* mutations in 4.9% [94].

Differences in genomic PI3K pathway activation between HPV (+) & HPV(-) HNSCC

Mutations of *PIK3CA* affect the p110 α catalytic subunit of PI3K, and confer an oncogenic gain-of-function. Activating *PIK3CA* mutations have been reported in 5–10% of all HNSCC [9,95,96], however when the entire PI3K pathway was assessed for mutations, includ-

ing *AKT* and *MTOR*, up to 30% of all HNSCC tumor specimens showed mutations in a largely HPV(-) cohort where 136 of 151 (90%) cases, were HPV(-) [7]. In general, HPV(+) HNSCC tumors harbor significantly fewer mutations compared with HPV(-) tumors [9,95]. Yet, HPV(+) tumors demonstrate a disproportionately high prevalence of genomic PI3K pathway alterations. These include activating *PIK3CA* mutations (27–31%), *PIK3CA* amplification (20%) and *PTEN* deletion or mutation (22–33%) [7–9,95,97,98]. Unlike HPV(-) disease, where activating *PIK3CA* mutations are associated with genomic instability and multiple other genetic lesions, in HPV(+) HNSCC, *PIK3CA* mutation is frequently the solitary mutational event [7]. Overall genomic events hypothesized to result in PI3K pathway activation are present in approximately 45–60% of HPV-transformed HNSCC [8,98], and are associated with efficacy of PI3K inhibitors in preclinical models [7]. Thus, the etiologic, demographic and clinical differences between HPV(+) and HPV(-) HNSCC are underscored by significant differences in genomic biology. While targeting the PI3K/Akt/mTOR pathway is a lead developmental strategy in HNSCC, it may be of particular therapeutic relevance for patients with HPV(+) HNSCC, a population naturally biomarker enriched for genomic pathway activation.

Aspirin & the PI3K pathway

Cyclo-oxygenase 2 (COX2), an enzyme which synthesizes the inflammatory GPCR ligand PGE2, is upregulated in HNSCC and has been linked to tumorigenesis [99,100]. As such, COX2 has been proposed as a target for both chemoprevention as well as the treatment of established HNSCC. Of interest to this review, PI3K/Akt signaling upregulates PGE2 in preclinical models of colorectal cancer pathway [101,102], and *PIK3CA* mutations have been associated with tertiary chemopreventive benefit from aspirin following colon cancer surgery *PIK3CA* [103]. A similar relationship between *PIK3CA* mutation status and efficacy of COX targeting has not been documented in HNSCC, although only one small posthoc analysis has been published [104]. However, given PGE2 stimulates GPCR-dependent transactivation of EGFR and downstream PI3K/Akt signaling, this potential feed-forward loop may be exploitable with dual COX-PI3K targeting in HNSCC.

EGFR-independent PI3K pathway signaling as a resistance mechanism to EGFR inhibitors: the rationale for co-targeting

EGFR (HER-1) is a member of the erythroblastic leukemia viral oncogene homolog family of transmembrane RTKs. Aberrant activation of EGFR and other RTKs is a major upstream source of PI3K pathway activation

in epithelial malignancies, as RTKs initiate convergent proliferative signaling via PI3K/Akt, Ras/MAPK and STAT3. Overexpression of EGFR and its ligands is observed in more than 90% cases of HNSCC [105]. There is an association between higher EGFR expression or *EGFR* copy number and shorter overall and progression-free survival [106,107]. This finding compelled the initial development of cetuximab, a murine-human monoclonal antibody against EGFR, in HNSCC. However, despite target overexpression in the majority, only a minority of patients realizes clinical benefit from cetuximab treatment [108]. In EGFR-overexpressing cancer cell lines, independent activation of Akt predicts for resistance to EGFR inhibitors [109]. Akt activation in HNSCC does not correlate well with phosphorylated EGFR, suggesting Akt activation may be independent of EGFR. Potential mechanisms include accessory activation of alternate RTKs, activating *PIK3CA* mutations and EGFR-independent STAT3 signaling [96,110,111]. These laboratory findings have resulted in developmental clinical strategies aimed at overcoming resistance to EGFR inhibition by co-targeting the PI3K/Akt/mTOR downstream resistance node [112]. Dual inhibition of EGFR and PI3K or mTOR has been synergistic in preclinical studies in HNSCC and colorectal cancer [113–115]. However, two trials evaluating EGFR-mTOR inhibitor combinations in HNSCC were terminated early due to excess toxicity [116]. Clinical trials of more tolerable combinations of EGFR and PI3K/Akt/mTOR blockade are proceeding (Table 1).

Co-targeting the PI3K pathway with conventional chemotherapeutics: focus on taxanes

The taxane chemotherapies, docetaxel and paclitaxel, demonstrate significant clinical activity in HNSCC and are frequently used in recurrent/metastatic disease for palliation. Taxanes bind reversibly to microtubules, promoting stability and preventing disassembly. Accumulation of polymerized microtubules ultimately results in failure of mitotic division, triggering apoptosis. Due to the antiapoptotic properties of PI3K signaling, the combination of taxanes and PI3K pathway inhibitors has been investigated. In HNSCC models, tumor matrix proteins mediate resistance to paclitaxel via PI3K signaling [117]. In breast cancer, GDC-0941 enhances cell death from docetaxel *in vitro* and *in vivo* [118].

Drugs targeting the PI3K/Akt/mTOR pathway

PI3K inhibitors

Pan-isoform PI3K inhibitors

BKM120 is an orally bioavailable inhibitor of all four class I isoforms of PI3K, lacking significant activity

against mTOR. It also demonstrates preclinical activity against common somatic *PIK3CA* mutations [119]. A Phase I study in biomarker-unselected patients with advanced solid tumors demonstrated one partial response and seven patients with stable disease, out of 35 treated [120]. Currently, BKM120 is in patient trials for HNSCC as monotherapy (NCT01737450) and also in combination with cetuximab (NCT01816984). For patients with recurrent/metastatic HNSCC who have not received taxane therapy, a randomized trial of paclitaxel with or without BKM120 (buparlisib) is also underway (NCT01852292). Adverse effects of BKM120 include changes to mood, gastrointestinal upset, rash, asthenia, LFT abnormalities and hyperglycemia [120].

PX-866 is an orally bioavailable, nonspecific PI3K inhibitor which blocks the kinase activity of all four Class I isoforms. PX-866 has been studied in two randomized, Phase II trials in biomarker-unselected, recurrent/metastatic HNSCC: docetaxel with or without PX-866 and cetuximab with or without PX-866. Results are awaited with interest.

GDC-0941 is an oral class I selective PI3K inhibitor which demonstrates preclinical activity in PI3K pathway-activated solid tumors including lung and breast cancers [121,122], and demonstrates preclinical synergy with taxanes, EGFR inhibitors and MAPK inhibitors. GDC-0941 is under Phase II/III study in combination with carboplatin, paclitaxel and bevacizumab in lung cancer, and paclitaxel or fulvestrant in breast cancer. No HNSCC-specific trials have been initiated.

Isoform-specific PI3K inhibitors

BYL719 is an orally bioavailable selective inhibitor of the Class I p110 α -isoform of PI3K. This has been tested in *PIK3CA* mutant breast cancer with promising results [123]. There is preclinical evidence that some mutant tumors may be resistant to BYL719 due to the high levels of pS6 in the setting of unrestricted mTORC1 activity [124]. In these resistant cell lines and xenografts, the combination of the mTOR inhibitor everolimus and BYL719 was successful in overcoming resistance. Adverse effects of BYL719 include class toxicities such as gastrointestinal upset, rash, asthenia, LFT abnormalities and hyperglycemia [125]. Due to p110 α selectivity, BYL719 may cause less immune suppression than pan-isoform inhibitors, and psychiatric toxicity has not been observed.

Dual PI3K/mTOR inhibitors

The separate mechanisms of action and relative structural similarity of the active sites of PI3K and mTOR have encouraged dual inhibitor development. Vertical blockade of PI3K and mTOR has been synergistic in preclinical lung cancer models, and is hypothesized

to overcome Akt activation induced by sustained mTOR inhibition [126]. BEZ235 is the first-in-class, and has been tested in several tumor types with common adverse effects including anemia, gastrointestinal upset, rash and asthenia [127]. Development has recently been halted in favor of BKM120 and BYL719.

Table 1. PI3K/Akt/mTOR pathway inhibitors in clinical development.

Drug (company)	Target	Highest phase of development in HNSCC	Dosing	Ongoing trials in HNSCC
Nonselective PI3K inhibitors				
BKM-120 (Buparlisib) (Novartis)	Nonselective PI3K: four isoforms, class IA and IB. No significant mTOR activity	Phase II	Oral	Recurrent/metastatic NCT01816984; BKM120 + cetuximab NCT01737450, NCT01527877; BKM120 monotherapy NCT01852292; Paclitaxel ± BKM120
PX-866 (Oncothyreon)	Nonselective PI3K; four isoforms class IA and IB. No significant mTOR activity	Phase II	Oral	Recurrent/metastatic NCT01204099: Docetaxel ± PX-866 NCT01252628: Cetuximab ± PX-866
GDC-0941 (Genentech)	Class I P3K	Phase I	Oral	None
Selective PI3K inhibitors				
BYL-719 (Novartis)	Selective PI3K α (p110 α). No significant mTOR activity	Phase II	Oral	Recurrent/metastatic disease NCT01602315: Cetuximab ± BYL719 NCT02051751: BYL719 + Paclitaxel
Combined PI3K and mTOR inhibitors				
BEZ-235 (Novartis)	Combined PI3K and mTOR	Phase II	Oral	Development halted.
GDC-0980 (Genentech)	Combined PI3K and mTOR	Phase I	Oral	None
Akt inhibitors				
Perifosine (Æterna Zentaris)	Allosteric Akt inhibitor	Phase II	Oral	Recurrent/metastatic NCT00062387: Perifosine monotherapy
MK-2206 (Merck)	Allosteric Akt inhibitor	Phase II	Oral	Recurrent/metastatic NCT01349933: MK-2206 monotherapy.
GDC-0068 (Genentech)	ATP-competitive pan-isoform Akt inhibitor	Phase I	Oral	None
GSK-2141795	ATP-competitive pan-isoform Akt inhibitor	Phase I	Oral	None
Drug (Company)	Target	Maximal HNSCC phase of development	Dosing	HNSCC ongoing trials
mTOR inhibitors				
Rapamycin (Pfizer)	mTORC1 inhibitor, limited on mTORC2	Phase II	Oral	Operable HNSCC NCT01195922: Rapamycin monotherapy for 21 days prior to definitive surgery
Everolimus (Novartis)	mTORC1 inhibitor	Phase II	Oral	Induction chemotherapy NCT00935961: Docetaxel, Cisplatin, Everolimus [135] recurrent/metastatic NCT01283334: Carboplatin, cetuximab, everolimus NCT01111058, NCT01133678: placebo vs everolimus Nasopharyngeal NCT01341834: Panobinostat + Everolimus
Temsirolimus (Pfizer)	mTORC1 inhibitor	Phase II	IV	Recurrent/metastatic NCT01256385: Temsirolimus ± Cetuximab

HNSCC: Head and neck squamous cell carcinoma.

GDC-0980 is a pan-PI3K and mTOR inhibitor with significant preclinical activity in breast, prostate and lung cancers but lower activity in melanoma and pancreatic cancer where frequent driver mutations in *BRAF* and *KRAS* serve as resistance pathways [118]. Phase II development is proceeding in breast cancer, in combination with fulvestrant, and in prostate cancer, in combination with abiraterone.

Akt inhibitors

Perifosine is a novel oral alkylphospholipid which inhibits Akt activation by preventing its localization to the plasma membrane [128]. Perifosine was ineffective as monotherapy in a Phase II trial in HNSCC, although candidate biomarker analysis indicated improved survival in patients with baseline overexpression of Akt [129]. Toxicities included gastrointestinal adverse events (nausea, vomiting and constipation) and fatigue. Perifosine remains of interest in biomarker-selected or combination strategies, however is not undergoing current development in HNSCC.

MK-2206 is an oral, highly selective allosteric inhibitor of all three Akt isoforms. MK-2206 binds at the pleckstrin-homology (PH) domain, resulting in a conformational change preventing localization of Akt to the plasma membrane and associated activation [130]. Phase I combinations of MK-2206 and paclitaxel, docetaxel, cisplatin or erlotinib have been evaluated in patients with advanced solid tumors. Two patients with HNSCC demonstrated response to the combination of paclitaxel and MK-2206 [131]. MK-2206 is under development across multiple solid and hematologic malignancies, with a monotherapy trial in recurrent/metastatic HNSCC actively recruiting (NCT01349933).

GDC-0068 is an oral, highly selective, pan-isoform Akt inhibitor that demonstrates preclinical activity against cell lines with endogenous Akt activation secondary to *PIK3CA* mutation, homozygous *PTEN* deletion and HER2 amplification [132]. GDC-0068 has been combined safely with 5-fluorouracil-oxaliplatin, docetaxel, paclitaxel and abiraterone and is now under investigation in Phase II clinical trials in multiple solid tumors, excluding HNSCC.

GSK-2141795 is an oral, ATP-competitive, pan-isoform kinase inhibitor of Akt and is under Phase I/II development in multiple solid tumor types including cervical cancer, an HPV-driven malignancy. In Phase I monotherapeutic investigation, prolonged stable disease was observed in two patients with genomic PI3K activation [133]. An HNSCC-specific trial has not been initiated.

mTOR inhibitors

Temsirolimus and everolimus are rapamycin analogs currently FDA approved and widely used for the treat-

ment of renal cell cancer. This class of drugs binds to FKBP-12, and the protein–drug complex inhibits mTOR activation. Everolimus was evaluated as a single agent in recurrent/metastatic HNSCC, however the trial closed after the first stage due to limited preliminary activity [134]. Everolimus may have more promise in combination therapy, including induction therapy in combination with cisplatin and docetaxel for regimen in locally advanced HNSCC [135]. Temsirolimus has been studied in recurrent/metastatic HNSCC in combination with the EGFR inhibitors, erlotinib or cetuximab, due to preclinical synergy. The Phase II combination with erlotinib was closed prematurely after 12 patients enrolled due to toxicity of the combination; preliminary activity was observed in a patient with a *PIK3CA* mutation [116]. The randomized, Phase II trial of temsirolimus with or without cetuximab in patients with acquired cetuximab resistance was negative, however 12.5% demonstrated prolonged responses to the combination suggesting the importance of this resistance pathway in a subset [136].

Conclusion

The PI3K/Akt/mTOR pathway involves a complex interplay of cell surface receptors and cellular components critically involved in protein synthesis, growth and survival. Activation of this pathway is a common feature of human malignancy, and nearly universal in mammalian viral infection including HPV. It is the most commonly mutated oncogenic pathway in HNSCC, and is therefore of considerable interest for targeted therapeutics and companion biomarker development. Progressive insights into the genomic and viral context of pathway activation, the interactions of various pathway constituents and the functional role of each PI3K isoform may translate into more effective therapy for correctly selected patients. Biomarkers of interest include *PIK3CA* mutation or amplification, *PTEN* loss, HPV or EBV status and expression levels of various pathway constituents.

Future perspective

The PI3K/Akt/mTOR pathway is likely to take on even more importance due to the increasing incidence of HPV-associated cancer. As etiology shifts toward HPV-associated cancer, the proportion of cancers with PI3K/Akt/mTOR abnormalities is also increasing. Further work must be done to determine whether the malignant behavior of these cancer cells is critically dependent on this pathway.

The biological differences between HPV(+) and HPV(-)HNSCC in terms of the PI3k/Akt/mTOR pathway may be exploitable. In the HPV era, PI3K activation in early infection and the incidence of

PIK3CA alterations upon neoplastic transformation are of special therapeutic interest. Although HPV infection is associated with superior prognosis following conventional therapy, current multimodality paradigms may represent overtreatment in good-risk disease. An investigational priority is the identification of de-intensification paradigms which reduce long-term toxicity while preserving excellent survival. Currently, there are no HPV-selective therapies which explicitly target viral biology. Investigation of PI3K/Akt/mTOR pathway inhibitors as a component of de-intensification may be warranted, due to the high prevalence of genomic or viral pathway activation. Moreover, HPV status should be accounted for during randomization to recurrent/metastatic trials, and evaluated as a response biomarker.

Even if PI3K/Akt/mTOR agents do not have striking activity as single agents, these drugs may still play a major role in therapy. Identifying the mutational landscape of HNSCC, for example by the TCGA, will likely lead to rational combinations. Due to cumulative experience in other pathway-addicted tumors, such as EGFR-mutated lung cancer or BRAF-mutated melanoma, combinations targeting two nodes on the pathway may increase efficacy. This strategy may be pertinent for vertical blockade, such as dual blockade of PI3K and mTOR, or horizontal blockade, where a PI3K/Akt/mTOR inhibitor is paired with an agent indirectly impacting the pathway, such as agents influencing parallel signaling streams (RAS/RAF/MEK/MAPK; STAT;PARP), causing DNA damage, or influencing protein synthesis.

Executive summary

- HPV-associated and HPV-negative head and neck squamous cell cancer (HNSCC) are molecularly distinct diseases. Activating genetic alterations in the PI3K/Akt/mTOR pathway are more commonly found in HPV-associated cancer, which is increasing in incidence.
- Activation of the pathway regulates critical cellular processes. PI3 kinase is stimulated by receptor tyrosine kinases (RTK) or G-coupled protein receptors (GPCRs), which leads to Akt upregulation and an activated mTOR complex. Malignant cells show pathway activation by the following mechanisms: upstream RTK activation; gain-of-function mutations; and loss of regulatory controllers such as phosphatase and tensin homolog. In HNSCC PI3K/Akt/mTOR abnormalities are seen in dysplastic lesions as well as at surgical margins of resected tumors.
- PI3 kinases are divided into three classes with regulatory and catalytic subunits. PI3K functions include promoting survival by inhibiting apoptosis, cellular proliferation, growth, angiogenesis and regulation of autophagy. The combination of various subunits leads to the formation of different isoforms of Class I PI3Kinases. *PIK3CA* mutations are often found in exon 9 and 20 leading to increased activity.
- Akt activation by PI3K leads to formation of an activated mTOR complex. In HNSCC phosphatase and tensin homolog loss is often seen, leading to pathway activation.
- mTOR forms two multi-protein complexes that control cell growth, protein production and apoptosis. mTORC1 is activated directly by Rheb-GTPase, and leads to higher S6 kinase levels with ribosomal biogenesis, protein synthesis and proliferation. mTORC2 formation leads to Akt activation, resulting in a positive feed-forward loop.
- HPV infection leads to nongenomic activation of the PI3k-Akt/mTOR pathway. E5 stimulates EGFR phosphorylation and thus PI3K activation, E6 prevents negative regulation of mTORC1 by TSC1/TSC2 and E7 prevents Akt de-phosphorylation. The Epstein-Barr virus also produces proteins (LMP1/LMP2) that directly activate the PI3K pathway.
- Isolated *PIK3CA* mutations and amplification are more commonly seen in HPV-associated cancers (~30%) compared with HPV negative (~5%). When the entire PI3K/Akt/mTOR pathway is evaluated, abnormalities can be seen in half of HPV-associated, and 30% of HPV-negative cases.
- PI3K/Akt signaling upregulates PGE2, a GPCR ligand that also transactivates EGFR and is synthesized by COX2. Therefore COX2 inhibitors such as aspirin may have a synergistic effect when combined with PI3K inhibitors.
- In HNSCC, resistance to EGFR inhibitors can be seen as a result of independent Akt activation. To overcome this resistance mechanism, combinations of EGFR and PI3K/Akt/mTOR inhibitors are being evaluated.
- Taxanes and PI3K inhibitors have a synergistic effect due to their mechanism of action. Taxanes lead to cellular microtubule accumulation and mitotic failure, while PI3K inhibitors promote apoptosis.
- PI3K inhibitors can have an effect on all four class I PI3Kinases (pan-isoform, e.g., BKM120) or be selective (isoform specific, e.g., BYL719). Dual inhibitors such as GDC-0980 have an effect on both mTOR and PI3K. Selective Akt inhibitors such as GDC-0068 also are in clinical trials in multiple solid tumors. mTOR inhibitors such as temsirolimus show minimal monotherapeutic activity in HNSCC.
- PI3K/Akt/mTOR is the most commonly activated oncogenetic pathway in HNSCC, especially HPV associated. Combination therapy may yield greater efficacy and delay the development of resistance.

The pace of molecular insight into HNSCC is yielding exciting advances. Therapeutic approaches targeting the PI3K/Akt/mTOR pathway are of unique promise. The overarching hope is that novel compounds and combinations will improve the efficacy and/or toxicity profile of current multimodality treatments for locally advanced disease, and pave the way for new and effective treatments in recurrent/metastatic disease.

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