review

Status of PI3K inhibition and biomarker development in cancer therapeutics

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The phosphatidylinositol 3-kinase (PI3K) signalling pathway is integral to diverse cellular functions, including cellular proliferation, differentiation and survival. The ‘phosphate and tensin homologue deleted from chromosome 10’ (PTEN) tumor suppressor gene plays a critical role as a negative regulator of this pathway. An array of genetic mutations and amplifications has been described affecting key components of this pathway, with implications not only for tumorigenesis but also for resistance to some classic cytotoxics and targeted agents. Emerging preclinical research has significantly advanced our understanding of the PI3K pathway and its complex machinations and interactions. This knowledge has enabled the evolution of rationally designed drugs targeting elements of this pathway. It is important that the development of suitable biomarkers continues in parallel to optimize use of these agents. A new generation of PI3K inhibitors is now entering early clinical trials, with much anticipation that they will add to the growing armamentarium of targeted cancer therapeutics.

Key words: biomarker, clinical trials, mTOR inhibitor, PI3K inhibitor, PI3K pathway, PTEN

introduction

The phosphatidylinositol 3-kinases (PI3Ks) are a family of lipid kinases whose primary biochemical function is to phosphorylate the 3-hydroxyl group of phosphoinositides [1]. Phosphorylation results in activation of second messenger molecules with consequent signal transduction that sets in motion a variety of physiological cellular metabolic and survival functions.

PI3Ks are grouped into three classes (I–III) with varying structure and substrate preference. Class IA molecules are heterodimers comprising a regulatory subunit (referred to as p85) and a catalytic subunit (p110). Three PIK3R genes give rise to five p85 isoforms (p85α, p55α, p50α, p85β and p55γ) as a consequence of splice variants. Each p85 isoform can associate with any of the three p110 isoforms (p110α, p110β and p110δ), which are the product of three separate PIK3C genes. Class IB molecules form a heterodimer between p110γ, a catalytic subunit similar to the class IA p110, and with a distinct regulatory subunit, p101 [2]. Class II PI3Ks comprises three monomeric catalytic isoforms (C2α, C2β and C2γ) with no regulatory proteins. The sole class III PI3K is composed of a regulatory subunit (p150) and a catalytic subunit (Vps34).

The functions of class I PI3Ks relate to glucose homeostasis, metabolism, growth, proliferation and survival. Isoform-specific roles are described, albeit with degree of overlap, with potential implications for toxicity and efficacy of novel inhibitors of class I PI3Ks [3]. In broad terms, the ubiquitously expressed p110α and p110β influence cellular proliferation and insulin signalling, respectively, whereas p110γ and p110δ, primarily expressed in leukocytes, appear involved in immune function and inflammation. The p110α isoform is widely mutated or amplified in human cancer. Interestingly, non-α p110 isoforms can induce oncogenic transformation in cultured cells as the wild-type protein, possibly explaining why they are not mutated in human cancer [4]. Class II PI3Ks are involved in the regulation of membrane trafficking, whereas class III PI3Ks have been implicated in the regulation of autophagy [5].

Activation of the class IA PI3Ks is initiated when a growth factor or ligand binds to its cognate receptor tyrosine kinase (RTK). These receptors include members of the human epidermal growth factor receptor family, platelet-derived growth factor receptor and the insulin and insulin-like growth factor 1 (IGF-1) receptors. Subsequent RTK dimerization and autophosphorylation enable the PI3K heterodimer to interact with its intracellular portion via p85. This is particularly the case for HER3, which carries multiple docking sites for p85, as opposed to HER2, which is unable to directly bind PI3K, thus stressing the importance of HER2–HER3 heterodimer formation for transmission of tumorigenic signals via PI3K [6]. Alternatively, an adapter molecule may act as an intermediary between an RTK and p85, such as occurs with the insulin receptor substrate 1 (IRS1) downstream of IGF-1R. Binding reduces the inhibitory effect of p85 on p110,
resulting in full activation of PI3K. The activated kinase catalyzes the phosphorylation of phosphatidylinositol-4,5-biphosphate (PI(4,5)P2) to phosphatidylinositol-3,4,5-triphosphate (PI(3,4,5)P3), which acts as a docking site. Akt, coded for by the human homologue of the viral oncogene v-akt (also known as protein kinase B, PKKβ), and phosphoinositide-dependent kinase 1 (PDK1) co-localize at this site where PDK1 phosphorylates Akt at threonine-308 in its kinase domain. A second phosphorylation event at serine-473 in the helical domain of Akt by the mTOR-rictor complex (mTORC2) is necessary for full Akt activity [7]. Akt, a serine/threonine kinase, is the central mediator of the PI3K pathway.

Akt stimulates protein synthesis and cell growth by activating mTOR through effects on the intermediary tuberous sclerosis (TSC) 1/2 complex. Subsequently, mTOR phosphorylates the ribosomal protein S6 kinases (S6K1 and S6K2) and the eukaryotic initiation factor 4E (eIF4E)-binding protein 1, components involved in ribosomal biogenesis and messenger RNA translation, and thus determinants of growth and cell size. Further, the ability of TSC2 to inhibit S6K has been shown to correlate with its function as a tumor suppressor [8].

Akt influences cellular proliferation by inactivating cell cycle inhibitors, such as p27Kip1 and p21, through its action on the forkhead box transcription factors (FOXO) [9]. This, together with an effect on cell cycle proteins e-Myc and cyclin D1 via the substrate GS3, promotes progression through the G1-S cell cycle checkpoint [10]. The PI3K pathway is also critical in cell survival, through Akt mediated inhibition of pro-apoptotic genes (Fas ligand, Bim and BAD) and degradation of the tumor suppressor protein p53; the net result is limiting programmed cell death [5]. PI3K also features in cellular metabolism and insulin signalling by enhancing glucose uptake and glycogen synthase activity in muscle and fat and by inhibiting gluconeogenesis in the liver [11]. Taken together, these actions demonstrate the diverse and critical cellular functions influenced by the PI3K/Akt pathway (see Figure 1).

Figure 1. Signalling through class I phosphatidylinositol 3-kinases (PI3Ks): a ligand engaged receptor tyrosine kinase binds PI3K, either directly or indirectly via adapter molecules such as insulin receptor substrate 1 (IRS1), removing the inhibitory action of p85 subunit on the catalytic p110 subunit. The active kinase generates PIP3 at the lipid membrane. PIP3 facilitates the phosphorylation of Akt by phosphoinositide-dependent kinase 1, while the mTOR-rictor complex contributes a second phosphate residue to Akt. As the central effector of the PI3K pathway, Akt transmits signal to a host of downstream substrates, thus orchestrating a variety of key cellular functions, including growth, metabolism, proliferation and survival. Pathway activity is negatively regulated by phosphate and tensin homologue deleted from chromosome 10, opposing the action of PI3K by converting PIP3 back into PIP2, and the S6 kinase (S6K)-IRS1 feedback loop. The Ras/Raf/mitogen-activated protein kinase cascade also influences signalling through PI3K at various levels, with the small guanosine triphosphatase RAS able to activate the p110 subunit directly, while downstream extracellular signal-regulated kinase negatively affects tuberous sclerosis 2.
downregulation of the PI3K/Akt pathway

The Src-homology 2 (SH2)-containing phosphatases (SHIP1 and SHIP2) abrogate signalling through PI3K by converting PI(3,4,5)P3 to PI(3,4)P2. However, a second mechanism exists to downregulate pathway activity, involving the ‘phosphate and tensin homologue deleted from chromosome 10’ (PTEN). PTEN was originally identified as a tumor suppressor gene and mapped to chromosome 10q23. Also known as MMAC1 and TEP1, PTEN is a dual-specificity phosphatase that dephosphorylates both protein and lipid substrates. Importantly, PTEN dephosphorylates cytoplasmic PI(3,4,5)P3, forming the inactive PI(3,4)P2. Thus, PTEN antagonizes PI3K function and negatively regulates Akt activities. PTEN functions as a tumor suppressor through an ability to control cellular differentiation [12]. PTEN also acts in the nucleus in a phosphatase-independent manner to further impact cell cycle, apoptosis and chromosomal integrity [13]. Germline and somatic deregulations of this critical protein both occur, the latter occurring frequently in human cancer.

A further elegant level of control exists within the PI3K system. S6K, one of the key effectors of mTOR, can feedback to downregulate IRS1, the adapter molecule linking the IGF-1R and PI3K. This effect appears to be direct and to impede the ability of IRS1 to associate with the insulin receptor. The outcome is to dampen further input into the PI3K pathway in the presence of ongoing stimulation of the insulin/IGF receptor [14].

In addition to the complexity of the PI3K pathway, extensive crosstalk exists with other cellular signalling networks. For example, mTOR exerts influence on PI3K signalling via the S6K-IRS1 feedback loop and via mTOR-riCTOR-mediated Akt-Ser473 phosphorylation [7, 15]. Activation of the tumor suppressor p53 causes both increased PTEN and decreased p110 expression. Further, p53 degradation is reduced indirectly by PTEN via its antagonism of PI3K [16, 17]. These actions safeguard the cell in times of genotoxic strain against ongoing DNA replication, though the interplay between p53 and PTEN requires further elucidation. Finally, activated guanosine triphosphate-bound RAS proteins are capable of activating the PI3K pathway by binding directly to p110 [18]. Downstream of RAS, in the mitogen-activated protein kinase (MAPK) pathway, extracellular signal-regulated kinase has been shown to negatively regulate TSC2 [19]. Finally, MAPK pathway activation has been identified as a consequence of mTORC1 inhibition, further intercalating these two important cascades [20].

mutations affecting the PI3K/Akt pathway

Genetic mutations and aberrations affecting various components of the PI3K pathway have been described. The consequences of each alteration depend on a host of factors such as which pathway element is affected and whether other tumor genes or proteins are concomitantly deregulated.

A group of overlapping clinical syndromes is described in association with germline mutations affecting the PTEN gene locus with hamartoma formation being the common clinical feature—Cowden syndrome (CS), Lhermitte-Duclos disease, Bannayan-Riley-Ruvalcaba syndrome (BRRS), Proteus syndrome (PS) and Proteus-like syndrome (PLS). In addition to hamartomas in the skin, breast, thyroid and intestine, the autosomal dominant CS is associated with a predisposition to cancer, especially breast (30%–50%), thyroid (10%) and endometrial cancers [21, 22]. PTEN mutations are described in 80% of those affected by CD, 60% of BRRS cases, 20% with PS and approximately 50% with PLS [23]. It has been indicated that any individual with a germline PTEN mutation, regardless of the clinical presentation, should be classified as having a PTEN hamartoma tumor syndrome (PHTS). Further, though an increased risk of malignancy is only documented in CS, cancer surveillance is warranted for such PHTS cases [24, 25].

Other tumor suppressor genes exert inhibitory effects on PI3K signalling, in particular restricting mTOR activity, by acting through the TSC complex. Germline mutations, with consequent loss of function of NF1, LKB1 and TSC1/2 tumor suppressor genes, give rise to neurofibromatosis, Peutz-Jeghers syndrome and tuberous sclerosis, respectively, characterized by hamartomas and the increased risk of certain malignancies. Together with CD, these entities result in chronic activation of mTOR signalling. However, the potential for overt malignancy is considerable in CD yet rare in the other conditions, reflecting the constitutively active Akt in CD but attenuated Akt activity courtesy of feedback inhibition in the other syndromes [26].

somatic mutations

With the exception of the p53 tumor suppressor pathway, the PI3K pathway is the most highly mutated in human cancer. Deregulation of this cascade can be due a host of genetic aberrations, resulting in either decreased expression of PTEN, amplification or mutation of PIK3CA, amplification or mutation of AKT and other less frequent events (see Table 1). Not only is pathway activation prevalent in myriad human cancers but also its status has prognostic and predictive relevance.

PIK3CA, encoding p110α, is commonly mutated or amplified in several human cancers. Mutations occur with highest frequency in breast, colon, endometrial and hepatocellular cancers and in glioblastomas, while amplifications are most often encountered in cervical, lung, gastric, ovarian and head and neck cancers [5, 27–34]. Approximately 80% of the mutations have been localized to three ‘hot spots,’ resulting in single amino acid substitutions; E545K and E542K in the helical domain (exon 9) and H1047R in the kinase domain (exon 20). The nonrandom distribution of these changes in highly conserved residues points to a vital functional role. Indeed, they increase enzymatic function, enhance downstream signalling elements including Akt and promote oncogenic transformation in vitro and in vivo with high efficiency [35–37]. The precise mechanism inducing a gain of function remains elusive. The helical domain mutations may affect interactions with regulatory proteins, including p85. The kinase domain mutation may affect specificity or affinity of p110α toward its substrates [38]. What is established is that to induce transformation, H1047R mutants depend on p85.
binding, whereas E545K and E542K mutants depend on RAS binding [39]. Mutations affecting p85α have been found in <5% of colon and ovarian cancers [40].

Amplification of Akt is seen most often in head and neck, gastric, pancreatic and ovarian cancers [5, 31, 41–44]. A missense mutation at E17K of Akt1 with transforming potential has recently been identified in a small proportion of breast, colorectal and ovarian cancers [45].

Decreased expression of PTEN occurs through several mechanisms. Loss of heterozygosity (LOH) 10q occurs when the remaining copy of PTEN is haploinsufficient and thus unable to prevent the malignant phenotype. LOH rates are highest in glioblastoma; endometrial, gastric, prostate and breast cancers and melanoma, whereas PTEN mutation is most prevalent in endometrial cancers (especially the endometroid subtype in 80%–90%) and glioblastomas [5, 46–56]. LOH occurs with greater frequency than mutation alone due the presence of alternate mechanisms such as homozygous deletion and epigenetic silencing via promoter methylation. Functional PTEN loss appears to be important in cancer progression [57–59]. PTEN mutation is associated with earlier stage and a more favorable survival in endometrial cancers [55, 60]. In prostate cancer, loss of PTEN expression is correlated with high Gleason grade, advanced stage and biochemical recurrence [61, 62]. In gliomas, PTEN mutation is more frequent in primary high-grade than in low-grade gliomas or secondary glioblastomas [59, 63] and is associated with reduced survival [64, 65]. Alteration in PTEN expression is associated with an increased Breslow thickness and ulceration in melanomas [66, 67] and with lymph node metastasis, loss of estrogen receptor expression and poor outcome in breast cancers [66, 68].

Interestingly, largely mutually exclusive mutations of PTEN and RAS family members are described in tumor types including endometrial cancer and glioblastoma [69, 70]. A similar relationship between PI3K and RAS pathway mutations and between PTEN and TP53 mutations has been noted in breast cancers [71, 72]. These findings indicate that mutation of either RAS or TP53 may often provide sufficient drive for tumorigenesis so as to remove any selective pressure for additional PTEN mutations to occur. On the other hand, some studies indicate that functional PTEN loss and PIK3CA mutations can coexist in breast, endometrial and colon cancers, implying a level of nonredundancy.

### Therapeutic Relevance of PI3K Pathway Activation

A substantial body of evidence exists in support of the notion that not only does PI3K pathway activation promote cell survival and tumor progression but also it can predict for therapeutic resistance to a broad range of anticancer therapies. Importantly, preclinical models have demonstrated that use of inhibitors against the PI3K pathway can restore sensitivity in many such instances.

The most extensively studied agents that target the PI3K signalling cascade are Wortmannin and LY294002. Wortmannin is a fungal metabolite initially isolated from Penicillium wortmanni in 1957. LY294002, about 500 times less potent and first produced about 25 years ago, is a synthetic compound derived from quercetin, a broad-spectrum kinase inhibitor [73]. Both of these inhibitors are characterized by the ability to target the ATP-binding site of PI3K, albeit with differing mechanisms, and to achieve significant growth inhibition across a broad spectrum of cancer cell lines when administered as single agents, especially in circumstances of excess PI3K activity. However, neither Wortmannin nor LY294002 have progressed to clinical trials. Wortmannin shows poor stability and targets many other PI3K-related molecules and thus showed considerably toxicity in animal models at low doses. LY294002 has superior stability, but is poorly soluble and shares the same undesirable characteristic of poor selectivity [74]. Regardless, invaluable knowledge of the PI3K/Akt pathway has been gained through their use, adding momentum to the pursuit of more specific and tolerable agents.

Preclinical PTEN null glioblastoma models demonstrate resistance to ionizing radiation and the alkylating agent temozolomide; radiosensitivity can be restored with the use of LY294002 [75–77]. Constitutively active Akt contributes to

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**Table 1.** Types and frequencies of genetic aberrations of the phosphatidylinositol 3-kinase pathway affecting different tumor types

<table>
<thead>
<tr>
<th>Genetic aberration</th>
<th>Tumor type</th>
<th>Frequency (%)</th>
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<tbody>
<tr>
<td><strong>PIK3CA mutations</strong></td>
<td>Breast</td>
<td>21–40</td>
</tr>
<tr>
<td></td>
<td>Colorectal</td>
<td>13–32</td>
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<tr>
<td></td>
<td>Glioblastoma</td>
<td>5–8</td>
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<tr>
<td></td>
<td>Endometrial</td>
<td>24–32</td>
</tr>
<tr>
<td></td>
<td>Hepatocellular</td>
<td>6–36</td>
</tr>
<tr>
<td><strong>PIK3CA amplifications</strong></td>
<td>Cervix</td>
<td>69</td>
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<tr>
<td></td>
<td>Gastric</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Lung (squamous)</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>H&amp;N</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Ovary</td>
<td>25</td>
</tr>
<tr>
<td><strong>PTEN LOH</strong></td>
<td>Glioblastoma</td>
<td>54–74</td>
</tr>
<tr>
<td></td>
<td>Endometrial</td>
<td>32–83</td>
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<tr>
<td></td>
<td>Gastric</td>
<td>47</td>
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<tr>
<td></td>
<td>Prostate</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Breast</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Melanoma</td>
<td>44–57</td>
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<tr>
<td><strong>PTEN mutation</strong></td>
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<td>17–44</td>
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<tr>
<td></td>
<td>Endometrial</td>
<td>36–50</td>
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<tr>
<td></td>
<td>Colorectal</td>
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<tr>
<td></td>
<td>Prostate</td>
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<tr>
<td></td>
<td>Breast</td>
<td>0–4</td>
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<tr>
<td></td>
<td>Melanoma</td>
<td>7</td>
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<tr>
<td><strong>AKT amplification</strong></td>
<td>H&amp;N</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Gastric</td>
<td>20</td>
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<tr>
<td></td>
<td>Pancreas</td>
<td>20</td>
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<tr>
<td></td>
<td>Ovary</td>
<td>12</td>
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<tr>
<td><strong>AKT1 mutation</strong></td>
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<td>1.8–8</td>
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<tr>
<td></td>
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<td>6</td>
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<tr>
<td></td>
<td>Ovary</td>
<td>2</td>
</tr>
<tr>
<td><strong>p85α mutation</strong></td>
<td>Colorectal</td>
<td>&lt;5</td>
</tr>
<tr>
<td></td>
<td>Ovary</td>
<td>&lt;5</td>
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</tbody>
</table>

LOH, loss of heterozygosity; H&N, head and neck; PTEN, phosphate and tensin homologue deleted from chromosome 10.
tumor cell survival and therapeutic resistance to a range of cytotoxics and radiation in lung cancer, to carboplatin and paclitaxel in ovarian cancer and to doxorubicin, etoposide, tamoxifen and trastuzumab in breast cancer. In each case, use of LY294002 or Wortmannin increases the apoptotic effects of these therapies when used in combination, thus laying the foundation of possible future treatment strategies [78–84].

In HER2 overexpressing breast cancer, trastuzumab requires intact PTEN for its therapeutic response and PTEN loss predicts for trastuzumab resistance [85]. On the other hand, it has been indicated that the small-molecule tyrosine kinase inhibitor lapatinib is not dependent on PTEN for its activity, although there is evidence to the contrary in some models [86, 87]. Similarly in colorectal cancer, PIK3CA mutation and PTEN loss renders cells resistant to the anti-EGFR monoclonal antibody cetuximab [88]. In these scenarios, inhibition of PI3K may provide one alternate avenue for treatment in cases of trastuzumab or cetuximab resistance due to abnormal PTEN/PI3K status.

clinical trials

Significant investment into rational drug development has been underway in recent times due to enormous potential therapeutic benefit by targeting the PI3K pathway. Several novel compounds have been developed with the intention of improving pharmacokinetic profiles, achieving superior target specificity and thus minimizing toxicity. Some of these agents are pure inhibitors of PI3K, others are dual inhibitors of both PI3K and mTOR and isoform-specific agents are also emerging. The varying target specificity of these agents lead to expectation of differing safety and efficacy profiles. Consequent to the extensive and intensive efforts of the pharmaceutical industry and scientific community alike, new-generation PI3K inhibitors are ready for clinical evaluation, though to date the number achieving active use in the clinic is limited [74] (see Table 2).

SF1126 (Semafore Pharmaceuticals, Indianapolis, IN) initiated a phase I trial for patients with solid tumors in 2007. It is administered intravenously (unlike most other PI3K inhibitors currently in clinical use) and has been well tolerated over the first three cohorts [89]. An additional phase I trial for multiple myeloma patients was initiated in early 2008. SF1126 is a pan-class IA PI3K inhibitor and also targets mTOR and DNA-PK. It is a small-molecule prodrug, a conjugate of LY294002 linked to an integrin-binding component designed to enhance delivery to the tumor and its associated vasculature, where cleavage leads to release of the active drug. It has shown significant antitumor effects in a variety of in vitro and in vivo settings, including glioblastoma, breast and prostate cancer xenograft models in nude mice. Further, potent antiangiogenic activity has been observed, felt at least partly to be due to a reduction in HIF-1α levels [90].

NVP-BEZ235 (Novartis, Basel, Switzerland) is a novel orally available product belonging to the class of imidazoquinolines that potently inhibits class I PI3Ks in an ATP-competitive manner [91]. In preclinical studies, it has demonstrated antiproliferative activity against a wide range of cancer cell lines, including HER2-overexpressing breast cancer models of trastuzumab and lapatinib resistance. Further, tumor growth suppression has been shown in PI3K-mutated xenograft models of human cancer [87, 92]. A phase I trial of NVP-BEZ235 has been underway since early 2007. NVP-BGT226 is the second PI3K inhibitor from Novartis to have entered clinical trials, having enrolled the first patient into a dose escalation trial in

<table>
<thead>
<tr>
<th>Agent</th>
<th>Company</th>
<th>Molecular targets</th>
<th>Monotherapy/combination</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF1126</td>
<td>Semaphore Pharmaceuticals</td>
<td>PI3K, mTOR, DNA-PK</td>
<td>Monotherapy</td>
<td>Solid tumors</td>
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<td>Multiple myeloma</td>
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<td>Exelixis</td>
<td>PI3K</td>
<td>1. Monotherapy</td>
<td>1. Solid tumors</td>
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<td></td>
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<td>2. Combined with erlotinib</td>
<td>2. Solid tumors, NSCLC</td>
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<td>3. Combined with carboplatin/paclitaxel</td>
<td>3. Solid tumors, NSCLC, ovary, endometrial</td>
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<tr>
<td>XL765</td>
<td>Exelixis</td>
<td>PI3K/mTOR</td>
<td>1. Monotherapy</td>
<td>1. Solid tumors</td>
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<tr>
<td></td>
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<td></td>
<td>2. Combined with erlotinib</td>
<td>2. Solid tumors, NSCLC</td>
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<tr>
<td></td>
<td></td>
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<td>3. Combined with temozolomide</td>
<td>3. Anaplastic gliomas, GBM</td>
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<td>PI3K/mTOR</td>
<td>Monotherapy</td>
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<td>Monotherapy</td>
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<td>GlaxoSmithKline</td>
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<td>CAL-101</td>
<td>Calistoga Pharmaceuticals</td>
<td>PI3K—p110δ specific</td>
<td>Monotherapy</td>
<td>CLL, NHL, AML</td>
</tr>
</tbody>
</table>

NSCLC, non-small-cell lung cancer; GBM, glioblastoma multiforme; CLL, chronic lymphocytic leukemia; NHL, non-Hodgkin’s lymphoma; AML, acute myeloid leukemia; PI3K, phosphatidylinositol 3-kinase.
early 2008. NVP-BKM120, also from Novartis, commenced phase I clinical development in late 2008. While NVP-BEZ235 and NVP-BGT226 are dual class I PI3K and mTOR inhibitors, NVP-BKM120 is a selective pan-class I PI3K inhibitor without mTOR inhibition capacity (C. Garcia-Echeverria, personal communication). All are orally administered therapies.

XL765 and XL147 (Exelixis, San Francisco, CA) both commenced phase I clinical trials in 2007. XL765 is a dual inhibitor of class I PI3K isoforms and of mTOR, whereas XL147 is a pure PI3K inhibitor. Target binding for each compound is potent, specific, ATP competitive and reversible. Tumor stabilization or shrinkage has been observed with XL765 in a variety of mouse xenograft models of human cancer, including breast, ovary, lung, prostate and brain cancers. Similar observations have been reported from breast, lung and prostate cancer models treated with XL147. Further, enhanced antitumor effects have been demonstrated when XL147 was combined with cytotoxic chemotherapy (paclitaxel or carboplatin) or with rapamycin. Preliminary data from the phase I trials have reported prolonged disease stabilization in patients with solid tumors that have been treated with these orally available compounds [93–95]. In addition, studies exploring these agents in combination with cytotoxic or other targeted agents have started recruiting patients.

PI103 (Piramated Pharma, Slough, UK) was one of a new generation of PI3K inhibitors that showed proof-of-concept whereby targeting members of the PI3K family with high selectivity was able to achieve target modulation with resultant in vivo antitumor activity [96]. Its rapid metabolism precluded clinical development, but proved a valuable tool that ultimately led to development of GDC-0941. GDC-0941 (Genentech/Piramated Pharma) is a selective and potent inhibitor of class I PI3Ks with weaker activity against mTOR [97]. This derivative of thieno[3,2-d]pyrimidine has demonstrated tumor growth inhibition in xenograft models when administered either as monotherapy or in combination with other agents [98]. A dose escalation study is underway, with favorable pharmacokinetics and safety observed in the early cohorts [99].

PX-866 (ProlX Pharmaceuticals, Tuscon, AZ), a semisynthetic agent with a mechanism similar to Wortmannin, is another pan-class I PI3K inhibitor, although it acts in an irreversible manner. Recently published in vivo studies demonstrate that PIK3CA-mutant or PTEN-null xenografts were sensitive to treatment with PX-866. However, mutant oncogenic RAS acts as a dominant predictor of resistance to this agent [100]. Phase I data are accruing.

Other phase I studies of PI3K inhibitors are also underway for GSK1059615 (GlaxoSmithKline, Brentford, London) and CAL101 (Callitoga Pharmaceuticals, Seattle, WA), an isoform-specific agent targeting p110α enrolling patients with hematological malignancies, in particular chronic lymphocytic leukemia, non-Hodgkin’s lymphoma and acute myeloid leukemia.

**biomarker development**

Biomarkers (BMs) are measurable characteristics of physiological or pathological states. Thus, BMs aim to serve a variety of functions during drug development including provision of proof-of-principle, refinement of patient selection, assessment of responders from nonresponders early in treatment and facilitation of optimal biological dose determination [101, 102]. Pertinent examples include the assessment of Her2 overexpression in breast cancers to predict for sensitivity to trastuzumab and the evaluation of KRAS mutations in colorectal carcinomas to predict resistance to anti-EGFR antibodies [103, 104].

To date, BMs reflecting PI3K and mTOR pathway activity or inhibition have been investigated in both preclinical and clinical settings. Although they do not yet have a defined role in the clinic, emerging data are showing promise.

In a phase I study of the mTOR inhibitor RAD001 (Everolimus) a strong correlation was noted between the degree of mTOR inhibition in skin and tumor (through evaluation of pS6 and peIF-4G levels), indicating that skin might serve as a valuable surrogate tissue reflecting pathway modulation [105]. Another trial evaluated short-term neoadjuvant rapamycin in patients with PTEN-negative recurrent glioblastomas where the magnitude of mTOR inhibition, not intratumoral drug concentration, was associated with reduced proliferation. Further, those cases with Akt activation (secondary to reduced feedback inhibition) were associated with a shorter time to progression, providing the rationale for combining mTOR and PI3K inhibition [106].

Microarray analysis of human tumor samples identified an inverse relationship between PTEN status and the insulin growth factor-binding protein 2 in glioblastoma and prostate cancer [107]. In breast cancer, these techniques demonstrated the protein stathmin was found to be an accurate immunohistochemistry surrogate of PTEN loss; it also appeared prognostic [105]. Recently, a large-scale RNA interference ‘barcode’ screen found PTEN as a critical gene involved in conferring resistance to trastuzumab therapy [108]. The group also demonstrated that assessment of PI3K pathway activation, through the combined analysis of PTEN and PIK3CA status, is an independent prognostic factor. Further, evaluation of colorectal cancer samples found that PIK3CA mutations and PTEN loss independently predict for resistance to monoclonal antibodies targeting EGFR (cetuximab and panitumumab) [109, 110]. This held true even in KRAS wild-type tumors indicating that, in addition to KRAS status, PI3K pathway activity may need to be evaluated before administration of such therapies. Though assessment of PIK3CA and PTEN as a BM requires procurement of tumor tissue in these examples, a further technique involves measurement of free nucleic acids in plasma. Detectable mutations (such as PIK3CA, KRAS or TP53) in such circulating tumor DNA are highly tumor specific and have the potential to be used to monitor tumor burden with the obvious advantage of compartment accessibility [111]. Finally, positron emission tomography is also being employed as a BM when using PI3K inhibitors. Use of these strategies continues to evolve.

Effectively incorporating BM studies into clinical trials of new PI3K inhibitors is a further challenge for investigators. Exelixis has presented some early data from their XL765 and XL147 monotherapy studies where plasma glucose and insulin are being evaluated as BMs. Treatment has minimally impacted glucose levels but a trend to increased insulin levels has been observed in an exposure-dependent manner. Further,
modulation of PI3K pathway elements has been shown in tumor and nontumor tissue collected from study subjects. In particular, hair follicles appear to be a useful surrogate tissue not only because of sampling convenience but also because of high basal pathway activity [93, 94]. Many of the current trials will carry out retrospective analyses of the mutational status of enrolled patients including PI3K and PTEN. Implementing such evaluations prospectively in the future may achieve enriched patient populations and greater chance for success with PI3K pathway inhibitors.

**future directions**

As the first of the PI3K inhibitors enter phase I clinical trials, a number of challenges and questions remain. Will isoform-specific PI3K inhibitors have advantages over the pan-class I agents? Toxicity profiles may improve but redundancy between the isoforms could limit their utility. Will the pure inhibitors be sufficiently efficacious or will multitargeted agents or combination therapy be of greater value by circumventing avenues for resistance mediated by pathway crosstalk? The latter seems probable. For example, one group has shown that despite the presence of hot-spot PIK3CA mutations, mutant KRAS-driven xenograft tumors do not respond to PI3K inhibitors until combined with a MEK inhibitor [112]. And importantly, will the benefits of PI3K inhibition be restricted to patients whose tumors are driven by oncogenic mutations in PI3K or PTEN. Although preclinical data imply this will be the optimal setting, an open mind needs to be maintained.

The clinician and researcher need to continue a translational collaboration as we monitor for responses and toxic effects, to ensure we seek explanation of what transpires, success or failure, expected or unexpected. BM development needs to continue in order to assist patient selection, monitor for responses and further understanding. Molecular characterization of patients should remain a goal. Early clinical data with PI3K inhibitors indicate that these agents are generally well tolerated; trials of combination therapy with chemotherapy, radiation, hormones and other biological agents are following closely behind. The product of several decades of work investigating inhibition of the critical PI3K cellular signalling pathway is finally coming to fruition, with much reason for optimism.

**disclosures**

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