Molecular targeting: PI3 kinase pathway

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Introduction

In recent years, cancer therapeutics development has emphasized the identification and evaluation of ‘targeted’ drugs directed at specific molecular aspects in signaling pathways of cancer cells. Targets of particular interest for therapeutics development are those involved in signaling pathways controlling cell cycle progression, gene transcription, motility, apoptosis and cell metabolism [1, 2]. Among these pathways, the phosphoinositide 3-kinase (PI3K)–Akt pathway is considered highly relevant. Activation of this pathway plays a pivotal role in essential cellular functions such as survival, proliferation, migration and differentiation that underlie the biology of human cancer. Aberrant activation contributes to tumorigenesis, tumor metastasis and resistance to standard cancer therapy. Non-clinical studies suggest that specific inhibitors may impede tumor cell growth or induce apoptosis, and combining inhibitors of the PI3K–Akt pathway with standard chemotherapy can attenuate chemotherapeutic resistance. Because the PI3K pathway has been implicated in a wide array of biological and pathophysiological responses and inhibition of the pathway induces antitumor effects in non-clinical models, small molecules designed to specifically target components of the pathway are now being developed for clinical use as single agents and in combination with standard cytotoxic drugs and radiation to overcome therapeutic resistance. In this review, the biology of the pathway and clinically relevant strategies to inhibit it will be described.

The PI3 kinase–Akt pathway in normal and cancer cells

PI3-kinases fall into three classes based on their primary structure and substrate specificity [2]. However, all PI3Ks phosphorylate phosphoinositides (PIPs) at the 3-hydroxy of the inositol ring. These phosphorylated phospholipids (PI3Ps), act as membrane tethers for many PI3K downstream effector proteins with pleckstrin homology (PH) regions. Of relevance to this discussion, effector proteins phospholipid-dependent kinase 1 (PDK1) and Akt are activated through the binding of their PH domains to lipid products of PI3K on the plasma membrane. PDK1 is a serine/threonine protein kinase that phosphorylates several members of the conserved AGC kinase superfamily [comprising the prototype protein kinases A (PKA), G (PKG) and C (PKC)] [3]. Three mammalian isoforms of the serine/threonine kinase Akt have been identified: Akt1, Akt2 and Akt3, which differ in tissue distribution and substrate specificity. Binding of the PH domain of Akt to membrane PI3Ps causes the translocation of Akt to the plasma membrane bringing it into contact with PDK1, which is responsible for at least one of two phosphorylation events necessary to activate Akt. PDK1 also activates other protein kinases related to Akt, including isoforms of p70 ribosomal S6 kinase (S6K), serum- and glucocorticoid-responsive kinases and p90 ribosomal S6K. The tumor suppressor phosphatase known as PTEN (for phosphatase and tensin homologue deleted on chromosome 10) dephosphorylates phosphoinositol-3,4,5-triphosphate at the 3-position of the inositol ring and, therefore, is a negative regulator of Akt and PDK1 activation. PI3K, Akt and PDK1 play important roles in the regulation of many cellular processes including proliferation, survival, carbohydrate metabolism and motility [2]. In addition, there is emerging evidence that these kinases are important components of the molecular mechanisms of diseases such as diabetes and chronic inflammation, as well as cancer [4].

Multiple components of this pathway are involved in oncogenesis [2, 5]. Growth factor receptor protein tyrosine kinases, integrin-dependent cell adhesion and G-protein-coupled receptors activate PI3K either directly or indirectly through activation of Ras. Losses of PTEN, PI3K amplification and Akt overexpression have been described in many malignancies [5]. In addition, persistent signaling through the PI3K–Akt pathway has been described as a mechanism of resistance to cytotoxic agents [6], radiation [7], epidermal growth factor receptor inhibitors [8] and trastuzumab [9]. Thus, PI3K and Akt are attractive targets for drugs as such agents might inhibit proliferation, and reverse the repression of apoptosis and resistance to cytotoxic therapy in cancer cells.

Agents targeting the PI3K–PDK1–Akt pathway

Agents in pre-clinical evaluation

Considerable effort has been extended to identify molecules that inhibit PI3K, PDK1 and Akt; however, to date there are no specific inhibitors of these kinases in clinical development. Agents evaluated in non-clinical studies can broadly be divided into lipid analogues that impede membrane binding through PH domains and those that inhibit kinase activation by competing with adenosine triphosphate (ATP). Foremost amongst the agents used in non-clinical studies are wortmannin [10], which was originally isolated from soil bacteria, and LY294002, a morpholino derivative of the broad-spectrum
kinase inhibitor quercetin [11]. Wortmannin is an irreversible inhibitor of all PI3K isoforms whereas LY294002 is a competitive inhibitor of ATP. Both compounds inhibit growth in concentrations that would be expected to inhibit class Ia PI3Ks and induce apoptosis in greater concentrations in vitro [11, 12] and in vivo [13, 14]. The lack of selectivity of these compounds, together with the instability of wortmannin in aqueous solution, and the insolubility of LY294002, have limited their development as cancer therapeutics although they are used extensively as pharmacological probes of the PI3K pathway in non-clinical studies.

Lipid analogues that inhibit PI3K, PDK1 and Akt have been extensively evaluated. Either lipid analogues of inositol phosphates [15, 16] andinositol polyphosphates [17] inhibit PI3K and are likely to act at the lipid binding site of the kinase. Among the most extensively studied PDK1 and Akt inhibitors, these molecules bind to the PH domains of the proteins, inhibiting their translocation to the plasma membrane and activation. While a number of molecules have been identified which inhibit PI3K, PDK1, Akt and tumor cell growth in vitro in micromolar concentrations, significant issues regarding target selectivity, pharmacology and toxicology need to be addressed before their clinical development.

Agents in clinical development

Although specific inhibitors of PI3K, PDK1 and Akt have not yet reached the clinic, agents that inhibit the downstream protein kinase mammalian target of rapamycin (mTOR) as well as agents that inhibit multiple kinases including components of the PI3K–Akt pathway are under clinical evaluation. Among the former are the kinase inhibitor UCN-01, heat-shock protein 90 (Hsp90) inhibitor, 17-(allylamino)-17-demethoxygeldanamycin (17AAG) and alkylphospholipid, perifosine. Among the latter group are rapamycin derivatives CCI-779, RAD001 and AP23573.

Protein kinase inhibitor UCN-01

UCN-01 (7-hydroxy-staurosporine) inhibits a number of serine/threonine kinases, including the Ca\(^{2+}\)- and phospholipid-dependent protein kinase C isoforms [18], cyclin-dependent kinases (CDK) 2, 4 and 6 [19], chk1 kinase [20] and, most recently, PDK1 [21]. Sato and colleagues found that UCN-01 directly suppressed upstream PDK1 with an IC\(_{50}\) value of <33 nM in vitro and in vivo and that inhibition of PDK resulted in inhibition of Akt and cell growth. UCN-01 mediates four distinct effects in vitro depending on the cell model: cell cycle arrest in G\(_1\), loss of the G\(_2\) compartment, induction of apoptosis [22] and potentiation of DNA-directed cytotoxicity of standard chemotherapeutic agents [23]. UCN-01 was selected for clinical development because of its potent anti-proliferative activity in vitro against several cell lines and its anti-neoplastic activity in several xenograft systems [24].

Phase I studies evaluating schedules of 72h and 3h intravenous (i.v.) infusions have been completed. Pharmacokinetic data from the first patients administered UCN-01 revealed that the agent binds tightly to human-1 acid glycoprotein (hAGP) resulting in a long half-life of several weeks, small volume of distribution at steady state and low systemic clearance [25, 26]. To prevent drug accumulation, doses were reduced by half after the first cycle with both schedules. The recommended phase II dose of UCN-01 is 42.5 mg/m\(^2\)/day for 3 days in cycle 1 and the same dose/day for 1.5 days for subsequent cycles and 90 mg/m\(^2\) over 3h in cycle 1 with half this dose subsequently given every 3–4 weeks [27]. Significant toxic effects seen with this agent include hyperglycemia with metabolic acidosis, pulmonary dysfunction, nausea, vomiting and hypotension. The mean total salivary concentration, which was evaluated as a surrogate measure of ‘free’ drug, was 111 nmol/l of UCN-01, within the range predicted to inhibit PDK1 and chk1 [27].

Although the PKC family has long been thought to be a major target of UCN-01, it is unlikely that PKC inhibition is involved in its antitumor activity. Certainly, the occurrence of hyperglycemia as a dose-limiting toxicity indicates that modulation of the insulin signaling pathway through inhibition of PDK1 may be occurring in patients. UCN-01 enhances the antitumor effects of several chemotherapeutic cancer agents including anti-metabolites, camptothecins and cisplatin, in vitro and in vivo [28–30]. Both abrogation of the G\(_2\) checkpoint, which prevents repair of DNA damage, and inhibition of PDK1 survival signaling, may be relevant to the mechanism of enhanced cytotoxicity seen when UCN-01 is combined with cytotoxic agents. Ongoing clinical trials are assessing the appropriate dose and antitumor activity of UCN-01 in combination with a variety of cytotoxic agents.

Perifosine

Alkylphospholipids (APLs) represent a new class of lipid-related compounds that exhibit a different spectrum of antinecancer activity from conventional therapies. Whereas malignant cells are sensitive to the lethal action of APLs, normal cells remain relatively unaffected, suggesting these agents have selective antitumor properties [31]. Miltefosine (hexadecylphosphocholine), the best-characterized representative of this group, displays cytostatic and cytotoxic effects towards a variety of tumor cell lines in vitro and in vivo [32, 33]. European clinical studies with oral miltefosine were terminated at the phase II stage of development due to cumulative gastrointestinal side-effects. However, miltefosine at lower oral doses is an effective treatment of leishmaniasis [34] and topical formulations are used to treat cutaneous breast cancer metastases. After screening other potential APL molecules, perifosine was chosen for further development because it was found to be more active and better tolerated than miltefosine in preclinical models [35, 36].

The exact mechanism of action of alkylphospholipids remains unclear. Modulation of cell surface receptors and inhibition of lipid-mediated signal transduction pathways appear to play key roles in the observed antitumorigenic effects. Recently published non-clinical studies indicate that perifosine and other APLs causes dose-dependent inhibition of Akt.
phosphorylation [37, 38]. Interestingly, introduction of the
myristoylated form of Akt into sensitive cells, which bypasses
the requirement for PH domain-mediated membrane recruit-
ment, abrogated perifosine-mediated decrease in Akt phos-
phorylation and cell growth inhibition. Thus perifosine may
decrease the plasma membrane localization of Akt. However,
ALPs can have multiple effects in addition to inhibiting Akt
phosphorylation including inhibiting mitogen-activated protein
kinase (MAPK) activation [39], activation of jun-kinase path-
way [40] and release of intracellular calcium [41].

In the phase I trial, patients received perifosine at doses ran-
ing from 50 to 350 mg/day for 3 weeks, followed by 1 week
of rest [42]. Toxicity consisted of nausea, vomiting, diarrhea
and fatigue; however, hematological toxicity was not
observed. The recommended dose was 200 mg/day. Because
of its prolonged terminal half-life (100–200 h) additional
phase I studies evaluating loading dose/maintenance dose
schemas to rapidly achieve plasma concentrations predicted to
be active in pre-clinical models are nearing completion.
Whether perifosine can modulate Akt in human cancers
remains an unresolved question.

17-(Allylamino)-17-Demethoxygeldanamycin (17AAG)
The molecular chaperone heat-shock protein 90 (Hsp90) is
responsible for the correct folding, function and stability of
multiple oncogenic client proteins including HER2, Raf-1,
Erk, Akt, CDK4, Met, steroid hormone receptors and mutant
p53. 17AAG is an analogue of the benzoquinone ansamycin
geldanamycin. By binding Hsp90, the geldanamycins destabi-
lize client proteins that require Hsp90 for proper conformation
[43, 44]. Client proteins are depleted through the ubiquitin–
proteasome pathway. Therefore, 17AAG is an indirect modu-
lator of a number of kinase pathways including PI3K–Akt.
The agent may induce cell-type-dependent cell cycle arrest in
either G1 and G2/M phases or apoptosis [45]. Other characteris-
tics of the malignant phenotype, including differentiation,
invasion and angiogenesis are also affected by 17AAG
[43, 44]. 17AAG-induced cell cycle effects may be retinoblas-
toma (RB) dependent as RB expressing breast cancer cells
undergo G1 arrest, differentiation and apoptosis, while RB-
negative cells arrest in mitosis and undergo apoptosis [46].

As with other signaling inhibitors, 17AAG modulates the
cytotoxic effect of chemotherapeutic agents. However, inter-
actions between 17AAG and cytotoxic agents appear to be
drug, cell line and sequence specific [47, 48]. Concentration-
dependent synergy of 17AAG combined with paclitaxel was
seen in two ovarian cell lines, SKOV3 and A2780, with strong
antagonism at low drug concentrations and additivity to
synergy at higher drug concentrations [47]. In the same cell
lines, 17AAG and doxorubicin were additive, whereas 17AAG
and etoposide were antagonistic in A2780 cells and moder-
ately synergic in SKOV3 cells [47]. The effects of cisplatin
and 17AAG in colon carcinoma p53 wild-type cell line
HCT116 were additive but antagonistic in p53-deficient colon
carcinoma cell line HT29 [49]. In breast cancer cells with
intact RB, exposure to 17-AAG before paclitaxel resulted in
G1 arrest and abrogated apoptosis but such schedule depen-
dence was not seen in cells with mutated RB [46]. These find-
ings suggest that inhibition of Hsp90 function by 17-AAG
enhances the apoptotic effects of cytotoxic agents, justifying
the evaluation of the agent in combination with cytotoxics;
however, the sequence of drug administration and the tumor
molecular profile significantly influence efficacy [46].

Interestingly, the combination of UCN-01 and 17AAG
caused enhanced cytotoxicity that was associated with dimin-
ished Akt activation and marked down-regulation of Raf-1,
and MAPK [50]. Co-administration of 17AAG and UCN-01
diminished expression of Bcl-2, Mcl-1 and XIAP. In addition,
detectable expression of constitutively active MEK1/2 or myr-
istemated Akt constructs, which overcame inhibition of ERK
and Akt activation, respectively, significantly attenuated
17-AAG/UCN-01-mediated lethality. Together, these findings
indicate that the Hsp90 antagonist 17-AAG potentiates
UCN-01 cytotoxicity and suggest that interference with both
the Akt and MAPK cytoprotective signaling pathways contrib-
ute to this phenomenon [50].

Phase I studies evaluating weekly, daily ×5 days and daily
×3 days schedules of 17AAG are under way. Pharmacokinetic
studies demonstrated the achievement of plasma concen-
trations predicted to be active based on pre-clinical studies.
Pharmacodynamic end points showing target modulation will
provide proof of principle for Hsp90 inhibition in peripheral
blood lymphocytes and tumor biopsies. Whether 17AAG can
modulate Akt signaling will be of particular interest. Elevation
of liver enzymes is the main dose-limiting side-effect of the
agent.

In summary, perifosine, UCN-01 and 17AAG modulate
specific components of the PI3K–Akt pathway. However,
these agents inhibit multiple additional targets, thus antitumor
effects in non-clinical and clinical studies cannot be viewed as
providing information on the effects of pathway-specific inhi-
bition. In addition, successful modulation of the pathway in
human tumors has not been reported in clinical trials to date.
Such data would be of interest and could support combining
these agents to optimize inhibition of the pathway.

Inhibitors of the mammalian target of rapamycin
In mammalian cells, mTOR is a large polypeptide kinase that
acts as a nutrient sensor and regulator of translation [51].
Among its many functions, mTOR regulates the translation of
a specific subset of mRNA transcripts that encode proteins
involved in regulating the G1 to S phase transition. Although
mutations of mTOR have not been reported in human cancers,
mTOR is a downstream component in the PI3K–Akt
pathway.

Rapamycin (sirolimus, Rapamune), a macrocyclic lactone
produced by Streptomyces hygroscopicus, has fungicidal,
immunosuppressive and anti-proliferative properties [52].
Because of its ability to suppress lymphocyte activation, rapa-
mycin was developed and received regulatory approval as
an immunosuppressant for the prophylaxis of renal allograft rejection. However, the growth-inhibitory actions of rapamycin are not restricted to lymphoid cells. Three related compounds, RAD001 (everolimus, Certican™), CCI-779 and AP23573, are under development as cancer therapies.

Rapamycin and its derivatives disrupt the function of mTOR and downstream signaling pathways contributing to cellular proliferation [53]. The rapamycins bind to a ubiquitous intracellular protein, called the FK506-binding protein, of molecular mass 12 kDa (FKBP12). The rapamycin–FKBP12 complex interacts with TOR proteins to inhibit signaling to downstream targets. Among the downstream targets of mTOR are p70S6 kinase (S6K) and 4E-binding protein-1 (4EBP1). These two proteins are important to the translation of subsets of mRNAs of proteins involved in ribosomal genesis and cell proliferation [54]. Rapamycins inhibit proliferation by arresting cells in the G1 phase, induce apoptosis in a limited number of tumor models and have limited normal tissue toxicity.

Rapamycins have anti-proliferative activity in a variety of hematological and solid tumor systems as single agents [52, 55–60] and appear to augment the antitumor activity of certain cytotoxic agents such as cisplatin [61], topoisomerase I inhibitors [62] and doxorubicin [63]. In animal studies, intermittent dosing of rapamycin and its derivatives appears to retain antitumor activity while limiting immunosuppression.

Of the rapamycins under clinical development in oncology, CCI-779, a soluble ester of rapamycin, has progressed the furthest. Preliminary results from three phase I studies evaluating increasing doses of CCI-779 on different schedules have been reported [64, 65]. The first study evaluated the pharmacokinetics and biological effects of CCI-779 administered as a 30 min i.v. infusion daily ×5 days every 2 weeks to patients with solid neoplasms [64]. In heavily pretreated patients, the recommended phase II dose was 15 mg/m²/day as thrombocytopenia limited repeated dosing at 19.1 mg/m²/day. In the second study, CCI-779 was given as a weekly 30-min infusion over a dose range of 7.5–220 mg/m²/week [65]. The maximum tolerated dose (MTD) of CCI-779 on this schedule has not been reported. In a preliminary report from a phase I study evaluating the safety/tolerability of CCI-779 administered orally daily ×5 days every 2 weeks, the recommended phase II dose was 75 mg orally daily for 5 days in patients with solid tumors [66].

Toxic effects across the phase I studies have been fairly consistent. Grade 3 toxic effects were hypocalcemia, elevation of hepatic transaminases, vomiting, mucositis and thrombocytopenia. Other toxic effects were mild–moderate and included neutropenia, mucositis, diarrhea, asthenia, fever, hyperlipidemia and hyperglycemia. Skin toxicity, such as dryness with mild pruritis, eczema-like lesions, urticaria and aseptic folliculitis have also been reported. Hypersensitivity reactions described as chest discomfort, dyspnea, flushing and urticaria during CCI-779 infusions also were observed. Objective tumor responses and prolonged stable disease were reported across all studies and occurred at multiple dose levels.

Results from phase I studies showed that CCI-779 was not only tolerable but also that antitumor activity occurred in patients treated over broad dosing ranges. Without clear indication of the optimal dose to carry forward, phase II trials were designed to evaluate antitumor activity of CCI-779 at doses of 25, 75 and 250 mg i.v. weekly. Preliminary results from phase II trials in breast [67] and renal cell [68] carcinomas indicated that CCI-779 induced objective responses and prolonged progression-free survival compared with historical data and that higher doses were no more active than lower doses although they were more toxic. A randomized phase II study evaluating the activity of CCI-779 doses of 75 or 250 mg weekly among 106 metastatic breast cancer patients showed that the safety profile was better in the 75 mg arm with equivalent antitumor activity. Objective responses were seen in 8% of patients and 40% of patients had stable disease lasting longer than 8 weeks [67]. Similarly, the randomized, double-blind, phase II trial evaluating the safety and efficacy of 25, 75 or 250 mg of CCI-779 i.v. weekly in previously treated patients with advanced renal cell carcinoma (RCC) [68] reported equivalent toxicity and activity across the dose range. CCI-779 produced an objective response rate of 7% (one complete response and seven partial responses). Median time to tumor progression was 5.8 months and median survival was 15.0 months. The most frequently occurring CCI-779-related adverse events were maculopapular rash (76%), mucositis (70%), asthenia (50%) and nausea (43%). The most frequently occurring grade 3 or 4 adverse events were hyperglycemia (17%), hypophosphatemia (13%), anemia (9%) and hypertriglyceridemia (6%).

RAD001, an orally administered 40-O-(2-hydroxyethyl) derivative of rapamycin under development as an immunosuppressant and cancer therapy. Recently, a phase I study of RAD001 administered orally weekly was reported [69]. Among patients receiving doses of 5–30 mg, RAD001 was well tolerated with only mild–moderate anorexia, fatigue, rash, mucositis, headache, hyperlipidemia and gastrointestinal toxic effects. Pharmacokinetic results showed that exposure increased in proportion to dose, a plateau in peak plasma concentrations and sustained S6K1 inhibition equivalent to the pharmacokinetic and pharmacodynamic changes that correlate with antitumor effects in rodents treated with this schedule [69]. Based on this translational research, 20 mg once weekly was identified as the initial dosage for subsequent clinical investigation.

Both CCI-779 and RAD001 appear to be well tolerated and have antitumor activity among patients enrolled on trials reported to date. Questions remain regarding the optimal
dose/schedule and patient population for testing these agents. Based on the results of pre-clinical studies, tumors that rely on paracrine or autocrine stimulation of receptors that trigger the PI3K–Akt–mTOR pathway or tumors with mutations causing constitutive activation of the PI3K–Akt pathway may be sensitive to rapamycins [56, 57, 70, 71]. A number of studies have suggested that PTEN null human cancer cell lines were preferentially sensitive to CCI-779 [70, 72, 73]. Taken together, the data indicate that mTOR may be a good target for cancer therapy in tumors with PI3K–Akt activation resulting from either growth factor dependence, activating mutations or loss of PTEN function. Methods to select patients likely to benefit from the agent based on the profile of their tumors would facilitate the development of these compounds.

Conclusions and future directions

In summary, agents in clinical development that target the PI3K–Akt pathway are currently limited to the rapamycins, which inhibit the downstream kinase mTOR, and multi-targeted inhibitors UCN-01, perifosine and I7AAG. Whether modulating downstream targets such as mTOR rather than PI3K and Akt will provide a better therapeutic index or whether simultaneously modulating multiple signaling pathways will have greater therapeutic effect remain unanswered questions. Certainly, general principles can be applied to the eventual clinical development of agents that specifically inhibit PI3K and Akt. It would be preferable to have a robust method to identify PI3 kinase pathway activation and signaling that can be applied to pathological specimens to select the patient population to study these agents. Testing the effect of the agent on its target in patients is difficult but may be necessary to determine the appropriate dose if minimal toxicity is seen with these agents or to limit toxicity by selecting a biologically active dose based on target modulation that may be below the MTD. Clinical end points for assessing antitumor activity should be based on whether tumor regression or tumor stabilization is seen in pre-clinical models. Similarly, the schedule of administration of the single agent and of combinations with standard cytotoxics and other molecularly targeted agents should be driven by the results of pre-clinical studies.

Given the frequent implication of the PI3K pathway in the pathophysiology of human malignancy, there is every reason to be optimistic that inhibition of the pathway will induce antitumor effects in cancer patients. Small molecules designed to specifically target components of the pathway are now being developed for clinical testing. As with other signal transduction inhibitors, the major clinical development challenges will be efficiently identifying the appropriate dose, schedule and combination regimens for patients with susceptible malignancies.

References


