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Clinical Development of Phosphatidylinositol-3 Kinase Pathway Inhibitors

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Abstract

The PI3K pathway is the most commonly altered in human cancer. Several recent phase I studies with therapeutic inhibitors of this pathway have shown that pharmacological inhibition of PI3K in humans is feasible and overall well tolerated. Furthermore, there has already been clinical evidence of anti-tumor activity in patients with advanced cancer. The intensity and duration of PI3K inhibition required for an antitumor effect and the optimal pharmacodynamic biomarker(s) of pathway inactivation remain to be established. Preclinical and early clinical data support focusing on trials with PI3K inhibitors that are at a minimum enriched with patients with alterations in this signaling pathway. These inhibitors are likely to be more effective in combination with established and other novel molecular therapies.

1 Introduction

Abundant evidence indicate that the phosphatidylinositol-3 kinase (PI3K) signaling pathway is arguably the most commonly altered in human cancers (reviewed in chapters in this book). First, the p110 α catalytic subunit of PI3K is activated by mutation at a high frequency in multiple human tumors (Samuels et al. 2004). A recent review reported an overall frequency of mutations in the *PIK3CA* gene, which encodes p110 α , of 15% across all cancer types (Karakas et al. 2006). Second, the phosphatase PTEN (phosphatase and tensin homologue deleted in chromosome 10), which antagonizes PI3K signaling by dephosphorylating the second messenger phosphatidylinositol-3,4,5 trisphosphate (PIP3), is a tumor suppressor gene frequently inactivated by mutation, gene deletion, targeting by micro-RNA, and promoter methylation (Keniry and Parsons 2008; Salmena et al. 2008). Further, PI3K is potently activated by oncogenes such as mutant Ras (REF) and many tyrosine kinases that potently activate PI3K, such as Bcr-Abl, HER2 (ErbB2), MET, KIT, etc., which themselves are the target of mutational activation and/or gene amplification (Engelman et al. 2006). The serine/threonine kinase Akt is a key downstream effector of PI3K signaling output. Following growth factor-induced stimulation of PI3K, Akt is recruited to the plasma membrane where it is phosphorylated by PDK-1 in Thr308 and by TORC2 in Ser473 (Manning and Cantley 2007), respectively, resulting in its full enzymatic activation. Several human tumors, such as ovarian, pancreatic, breast, and gastric cancer, harbor Akt1 or Akt2 gene amplification. A transforming mutation in the pleckstrin homology (PH) domain of Akt1 (E17K), which results in its constitutive localization at the plasma membrane and activation, is present in a small percentage of breast, colorectal, and ovarian cancers (Carpten et al. 2007). Other components of the pathway, such as PDK-1, PIK3R1, PIK3CB, and P70S6K, are found to be amplified in human cancers (Thomas et al. 2007). All these abnormalities together identify a large repertoire of tumors with molecular

alterations in the PI3K network that are potentially targetable with specific pathway inhibitors.

At this time, there is significant clinical research addressing the role of inhibition of the PI3K pathway in human cancers. In this chapter, I will review the current status of clinical investigation in this field with different types of antagonists of the PI3K network, mechanistic and preclinical considerations that are of relevance to clinical development, the rationale for combinatorial therapies that will include inhibitors of the PI3K pathway, and finally propose some clinical trial designs that may streamline the pathway to FDA approval for PI3K-targeted agents.

2 Pharmacological Approaches

Several types of compounds to block multiple levels in the PI3K signaling network have been designed and are in variable stages of clinical development. The first group comprises inhibitors of class IA PI3K isoforms. These enzymes are heterodimeric lipid kinases that consist of a p110 catalytic subunit and a regulatory subunit, which mediates the receptor or adaptor binding, activation, and localization of the PI3K dimer. There are three genes, *PIK3CA*, *PIK3CB*, and *PIK3CD*, which encode the highly homologous p110 catalytic isoforms, p110 α , p110 β , and p110 δ , respectively (Cantley 2002; Engelman et al. 2006). The expression of p110 δ is largely restricted to immune and hematopoietic cells whereas p110 α and p110 β are expressed ubiquitously (Vanhaesebroeck et al. 1997). p110 α is essential for signaling and growth of tumors driven by *PIK3CA* mutations and/or oncogenic tyrosine kinases or mutant RAS, whereas p110 β responds to G protein-coupled receptors (GPCRs) and is the main isoform mediating tumorigenesis in PTEN-deficient cells [reviewed in (Jia et al. 2009)].

A number of pan-specific or isoform-specific PI3K antagonists have entered phase I clinical development and have the subject of several recent reviews (Garcia-Echeverria and Sellers 2008; Maira et al. 2008b). These include NVP-BEZ235, NVP-BGT226, GDC-0941, XL-765, XL-147, SF1126, CAL-101, and GSK1059615. These compounds are ATP-mimetics that bind competitively and reversibly in the ATP-binding pocket of kinase domain in p110. With the exception of CAL-101, which specifically inhibits the p110 δ kinase, the other small molecules are active against all p110 isoforms including oncogenic mutant forms of p110 α (Folkes et al. 2008; Garlich et al. 2008; Maira et al. 2008a). Some of these also have inhibitory activity against phosphatidylinositol-3 kinase-related kinases (PIKKs), such as the mTOR serine/threonine kinase (i.e., NVP-BEZ235, NVP-BGT226, XL-765, and SF1126).

Following the p110 antagonists are inhibitors of Akt isoforms. These compounds have shown antitumor activity against human xenografts and have been reviewed recently (Garcia-Echeverria and Sellers 2008). A-443654 and GSK690693 are ATP-competitive pan-Akt kinase inhibitors. They have shown antitumor activity in preclinical models and have recently entered phase I trials (Davies et al. 2007; Rhodes et al. 2008). Allosteric inhibitors of Akt that interact with its PH domain and/or hinge region thus promoting an inactive conformation of the enzyme, are also in development (Toral-Barza et al. 2007). MK-2206 is a highly selective non-ATP-competitive, allosteric inhibitor of Akt1, Akt2, and Akt3. This compound effectively inhibited the Akt kinase and its downstream effectors in vivo and caused marked suppression of growth of breast cancer xenografts with PI3K mutations and HER2 gene amplification (She et al. 2008). Early phase I clinical data in patients with advanced solid tumors have shown inhibition of P-Akt in peripheral blood mononuclear cells and good tolerability (Tolcher et al. 2009). Because of the high sequence identity

among the kinase domain of Akt1, Akt2, and Akt3, it is anticipated that the development of potent isoform-selective modulators will be difficult.

A third group of compounds designed to interrupt the PI3K pathway are inhibitors of the mTOR (mammalian target of rapamycin) serine/threonine kinase (Fasolo and Sessa 2008). This kinase regulates protein translation and functions within two multiprotein complexes which share mTOR itself: TORC1 associated with RAPTOR and TORC2 associated with RICTOR (Guertin and Sabatini 2007). Rapamycin and its analogs (see below) preferentially target TORC1. mTOR is an important component of PI3K-driven oncogenesis at different levels. TORC1 regulates protein translation and is downstream and positively modulated by Akt. On the other hand, TORC2 functions upstream where it phosphorylates and activates the Akt kinase (Sarbasov et al. 2005). The macrolide rapamycin inhibits mTOR by forming a complex with the FK506-binding protein (FKBP12), which binds to a region in the C-terminus of mTOR termed FRB (FKBP12 rapamycin-binding). The formation of this complex interferes with the kinase activity of the TORC1 but not the TORC2 complex (Sarbasov et al. 2004). The limited pharmacological properties of rapamycin prompted the development of analogs (so called “rapalogs”) such as CCI-779 (temsirolimus), RAD001 (everolimus), and AP-23573 (deferolimus). These rapalogs have already shown cytostatic activity in preclinical models and clinical trials particularly in patients with renal cell cancer and patients with mutations in TSC who harbor renal angioliomas. Compounds that target the ATP-binding cleft of mTOR (i.e., OSI-027 and AZD8055) and are thus active against both TORC1 and TORC2 have recently entered phase I clinical trials (Fasolo and Sessa 2008).

3 Preclinical Considerations for Drug Development

The somatic DNA alterations identified above (i.e., *PIK3CA* and *AKT1* activating mutations, PTEN deletion, PI3K-activating oncogene amplification) potentially mark tumor types as well as individual cancers with aberrant activation of the PI3K pathway. This is an important consideration for the purpose of selection of patients into trials with PI3K inhibitors. In the past decade, a number of examples have shown that mutations in somatic DNA identify gene products or pathways that are critical for tumor survival and progression and that, therefore, when interrupted by pharmacological means result in a clinically important antitumor effect. Examples include the effect of imatinib and dasatinib against Philadelphia chromosome-positive chronic myelogenous leukemia (CML) harboring the *BCR-ABL* oncogene, the EGF receptor tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib against tumors with *EGFR* gene activating mutations, the anti-HER2 antibody trastuzumab and the HER2 TKI lapatinib against breast cancers with *HER2* gene amplification, and, more recently, small molecule Raf inhibitors against metastatic melanomas containing *B-RAF* activating mutations [reviewed in (Stuart and Sellers 2009)].

A number of preclinical tumor models including transgenic mice bearing cancers engineered to lack PTEN or overexpress *PIK3CA* activating mutations have already shown tumor dependence on PI3K in that administration of pharmacological inhibitors of PI3K resulted in an antitumor effect (Eichhorn et al. 2008; Engelman et al. 2008). However, in several phase I clinical trials with PI3K pathway inhibitors in progress, there have been no reports yet of major tumor reductions in patients treated with such compounds. Two previous reports using cancer cell lines with PTEN deletions suggested that PTEN-deficient cancers would be highly sensitive to mTOR inhibitors (Neshat et al. 2001; Podsypanina et al. 2001). Again, despite the extensive clinical use of “rapalogs” and the relative frequency of PTEN loss in cancers at large, significant clinical responses to mTOR inhibitors have not been observed. Thus, although it might still be early, the dramatic clinical responses that were observed

during the early clinical development of other now approved molecule-targeted inhibitors have not yet been observed with therapeutic antagonists of the PI3K pathway.

The potential dependence of some cancers over that of normal host tissues on an oncogenic pathway suggests that the possibility of a “therapeutic window” that can be exploited in the drug development process. This would allow delivery of an oncogene-directed therapy at an optimal biological dose (OBD) that would inhibit its molecular target and exert a biological effect on the tumor. This dose would be less than a maximally tolerated dose (MTD) of the inhibitor which would likely induce toxicity against normal host tissues. Imatinib and trastuzumab are examples of molecule-targeted therapies where such therapeutic window was present. Because of the role of PI3K in normal physiological processes, it is not clear whether therapy-induced toxicities will be entirely avoidable. One special concern with these therapies is the induction of insulin resistance. Under normal physiological conditions, the PI3K pathway, predominantly p110 α and less so p110 β , mediates insulin action (Foukas et al. 2006; Knight et al. 2006). Therefore, PI3K antagonists are likely to perturb glucose homeostasis and/or aggravate states of insulin resistance. Preclinical data with Akt inhibitors have already shown the induction of hyperglycemia in experimental mice (Crouthamel et al. 2009; Rhodes et al. 2008). Interestingly, mice treated with NVP-BEZ235 did not exhibit significant changes in blood glucose levels (Maira et al. 2008a). In any case, an important question in the clinical development of PI3K inhibitors is whether clinical efficacy and tolerability can be achieved without the induction of insulin resistance.

Genetically engineered mice lacking p110 α exhibit defective endothelial cell migration during vascular development (Graupera et al. 2008). Consistent with this, mice lacking PI3K regulatory subunits (p85 α , p85 β , p55 α , and p50 α) also exhibit localized vascular abnormalities (Yuan et al. 2008). Interestingly, mice expressing a p110 α mutant allele incapable of interacting with endogenous Ras display defective VEGF-C signaling to PI3K in lymphatic endothelial cells and impaired development of the lymphatic vasculature (Gupta et al. 2007). Consistent with these results, PI3K inhibitors have been shown to inhibit tumor blood vessels when administered to mice bearing human xenografts (Garlich et al. 2008; Schnell et al. 2008). These data suggest that in addition to tumor cell-autonomous effects, PI3K inhibitors could exert an additional antimetastatic effect by blocking angiogenesis and lymphangiogenesis. They also suggest that the possibility of side effects (i.e., bleeding, defective wound healing, etc.) as a result of impairment of endothelial cell function.

It has been shown that genes encoding most glycolytic enzymes are under dominant transcriptional control by Akt activation (Majumder et al. 2004). Thus, a rapid downregulation of [18F]-fluorodeoxy-D-glucose positron emission tomography (FDG-PET) intensity might be a reliable surrogate marker of inactivation of the PI3K/Akt pathway that can be used as a noninvasive approach to predict the outcome of therapy. This also implies that tumors that are FDG-PET negative contain low glycolytic activity and, thus, are not ideal candidates for therapy with PI3K inhibitors. At this time, FDG-PET is being widely used as a pharmacodynamic biomarker of drug action in investigational trials with inhibitors of PI3K.

4 Clinical Trials

At this time, several PI3K pathway inhibitors are in phase I clinical development. This phase of the clinical development process is aimed at defining the effective dose of these compounds as well as their tolerability and toxicity profile. Preliminary results have been communicated for phase I trials with XL-147, XL-765, GDC-0941, PX-866, and CAL-101 in patients with solid tumors and hematological neoplasias (Flinn et al. 2009; Jimeno et al.

2009; LoRusso et al. 2009; Shapiro et al. 2009; Wagner et al. 2009). Overall, these compounds seem to be well tolerated with modest grade 3 and grade 4 toxicity. Main side effects have been nausea, vomiting, diarrhea, anorexia, fatigue, and rash with minimal hyperglycemia. Dose escalations are still proceeding, although pharmacodynamic evidence of drug action in skin and hair follicles has already been reported. This has been assessed by measuring levels of T308 P-Akt, S473 P-Akt, T246 P-PRAS40, T70 P-4EBP1, and S240/244 P-S6 by immunohistochemistry (IHC) using site specific antibodies in tissue sections obtained on days 21–28 after initiation of treatment.

There is significantly more clinical experience with the mTOR inhibitors temsirolimus (CCI-779), everolimus (RAD001), and deferolimus (AP23573). These drugs exhibit a comparable toxicity profile, spectrum of antitumor activity, pharmacokinetic features, and profile of biomarkers they inhibit in situ [recently reviewed in (Fasolo and Sessa 2008)]. Main side effects include mucositis, rash, fatigue, neutropenia, anorexia, edema, hyperglycemia, and gastrointestinal toxicities. These three compounds inhibit mainly TORC1. The TORC1 complex activates S6K which, in turn, inhibits IRS-1 through phosphorylation in Ser102 (Harrington et al. 2004). Consistent with this, in a recent paper, O'Reilly et al. demonstrated feedback activation of Akt following pharmacological inhibition of TORC1 in patients with breast cancer treated with everolimus (O'Reilly et al. 2006).

A recent phase III trial compared single-agent temsirolimus vs. interferon vs. the combination in 626 patients with poor-prognosis metastatic renal cell carcinoma. Patients receiving temsirolimus alone achieved a significantly longer overall survival (OS) and progression-free survival (PFS) than patients treated with interferon alone. In the group treated with the combination, the OS was comparable of that exhibited by patients in the single-agent interferon arm. Rash, peripheral edema, anemia, dyspnea, diarrhea, hyperglycemia, and hyperlipidemia were more common in patients treated with the mTOR inhibitor whereas asthenia was more common in the interferon group. Grade 3 and grade 4 toxicities were more common in the combination group, resulting in more delays and reductions in the dose of temsirolimus potentially explaining the lack of advantage of the combination over interferon alone. Median OS in the interferon, temsirolimus, and combination therapy groups was 7.3, 10.9, and 8.4 months, respectively (Hudes et al. 2007). Based on these results, temsirolimus was approved by the FDA for the initial treatment of patients with advanced poor-prognosis renal cell cancer.

A double-blind, multicenter phase III trial in patients with renal cell cancer who have progressed on primary therapy for metastatic disease was recently completed (Motzer et al. 2008). In this study, 400 patients were randomized to everolimus 10 mg/day vs. placebo, both with the best supportive care. Everolimus produced a significant extension in PFS of 4 vs. 1.9 months, with an overall favorable safety profile. Stomatitis, anemia, and asthenia were the most common grade 3 and grade 4 toxicities (Motzer et al. 2008). Finally, Baselga et al. (2009) just reported the results of a neoadjuvant randomized phase II study of the aromatase inhibitor letrozole vs. letrozole plus everolimus in postmenopausal patients with newly diagnosed ER-positive breast cancer. Clinical response rate and inhibition of tumor cell proliferation as measured by Ki67 IHC were higher in the combination arm compared to the group treated with single-agent letrozole (Baselga et al. 2009).

Promising clinical activity in single-arm phase II studies with temsirolimus and everolimus has been reported in endometrial cancer and relapsed mantle cell lymphoma (Oza et al. 2005; Slomovitz et al. 2008; Witzig et al. 2005). Because of their ability to inhibit TORC1 and TORC2 and thus, potentially bypass feedback activation of Akt, higher single-agent clinical activity compared to everolimus, temsirolimus, and deferolimus is anticipated for

AZD8055 and OSI-027. Up to now, however, the original concept that dysregulation of PI3K signaling predicts sensitivity to mTOR inhibitors has not been verified in clinical practice. In fairness though, most of these therapeutic studies have not actively explored a correlation between clinical benefit and detectable genetic alterations in the PI3K pathway by profiling a meaningful number of tumors from patients enrolled in these trials. At the time of this writing, combination studies of mTOR inhibitors with EGFR, VEGF, PI3K, and IGF-IR inhibitors are in development.

5 Patient Selection and Role of Presurgical Trials

As with other targeted therapies, it is likely that only a fraction of patients treated with PI3K inhibitors will benefit from these drugs. Because of this, there is an expectation that the clinical development of a molecule-targeted therapy will also include the deployment of a diagnostic test(s) that will identify patients that are likely to respond to and thus be offered such therapy. Examples include fluorescent in situ hybridization (FISH) and IHC for HER2 which identify patients with breast cancer for whom trastuzumab and lapatinib are approved (Press et al. 2008); and *EGFR* activating mutations which identify patients with nonsmall-cell lung cancer (NSCLC) with a high likelihood of response to EGFR TKIs (Lynch et al. 2004; Paez et al. 2004), among others. An example of a negative predictor of response is the presence of mutant K-RAS, which identifies patients with colon cancer that do not benefit from therapy with the neutralizing EGFR antibodies panitumumab or cetuximab (Amado et al. 2008; Benvenuti et al. 2007).

There is an agreement that early therapeutic studies should be enriched with patients harboring known detectable abnormalities in the PI3K pathway. However, it is not clear whether clinical responses will be limited to these patients. Testing the possible selectivity of PI3K inhibitors against cancers with PI3K pathway alterations and/or another molecular signature in single-arm phase II trials in patients with metastatic disease is intrinsically problematic because of (1) the difficulty in obtaining biopsies from metastatic sites and (2) the limitations of assessment of tumor response as a meaningful clinical endpoint in the absence of a placebo control arm.

There are, however, examples of short-term, tissue-based pharmacodynamic novel trial designs which could provide information that can be later used for patient selection or exclusion into early trials with novel targeted therapies such as PI3K antagonists. For example, administration of antiestrogens for a period of 1–3 weeks has been shown to induce a significant antiproliferative effect, as measured by Ki67 IHC (Assersohn et al. 2003), in ER-positive but not ER-negative breast cancers (DeFriend et al. 1994; Dowsett et al. 2000, 2001). Treatment-induced tumor cell apoptosis, as measured by cleaved caspase-3 IHC 1 week after administration of single-agent trastuzumab correlated with clinical response of HER2-overexpressing breast cancers to trastuzumab plus chemotherapy (Mohsin et al. 2005). The neoadjuvant IMPACT trial compared the aromatase inhibitor anastrozole vs. tamoxifen vs. the combination of both drugs. Drug-induced inhibition of cancer cell proliferation in situ as measured by Ki67 IHC in a tumor biopsy obtained after 2 weeks of therapy was better in anastrozole-treated patients compared to patients in the other two arms (Dowsett et al. 2005). Interestingly, this change in proliferation (Ki67) after only 2 weeks of therapy mirrors the results of the adjuvant ATAC trial where >9,000 patients with ER + tumors were randomized to the same three arms as in the IMPACT study following surgical resection of the primary tumor. In this large study, relapse-free survival was also better in patients treated with anastrozole compared to the other two treatment arms (Howell et al. 2005). In terms of PI3K pathway-targeted drugs, Cloughesy and colleagues demonstrated a dramatic effect of rapamycin on the Ki67 index in a group of patients with recurrent glioblastoma. Tumors were surgically-resected after 7 days of therapy with the

mTOR inhibitor. Interestingly, the reduction in Ki67 after short-term rapamycin was limited to PTEN-deficient tumors and correlated with an improved PFS in patients treated with the mTOR inhibitor following surgery (Cloughesy et al. 2008).

The above mentioned examples suggest that the use of presurgical nontherapeutic trials with PI3K pathway inhibitors to ensure that critical endpoints in their clinical development are met. For example, after a safe dose of the inhibitor has been defined in a conventional phase I study, patients with *operable* breast cancer (or another tumor known to exhibit PI3K alterations where this approach is ethical and feasible) that are not candidates for neoadjuvant therapy can be treated with the inhibitor for 2 weeks, which is likely a period of time adequate for the drug to achieve steady-state levels in plasma. Effects on cell proliferation (Ki67), apoptosis (TUNEL, cleaved caspase-3 IHC), and inhibition of the drug target in situ (i.e., with P-Akt, P-PRAS40, P-S6, etc. antibodies) can be easily assessed in formalin-fixed tumor cores from the surgical specimen. A gene expression signature indicative to kinase inactivation can be generated from fixed or frozen tumor material that is not further required for clinical purposes. Evidence of inhibition of the molecular target of the inhibitor will validate the therapeutic dose selected by the early drug development (phase I) process. Lack of inhibition of the target in situ would suggest that the drug is not reaching its target despite adequate drug levels or another pharmacological limitation. This possibility can then be studied by measuring drug levels in tumor homogenates. Addressing these questions would be critically important before engaging in larger and (potentially) uninformative efficacy trials. Evidence of inhibition of cell proliferation (Ki67) and/or induction of apoptosis (TUNEL, etc.) can be correlated with *PIK3CA* or *AKT1* mutations, PTEN deletion, etc. as well as other routine clinical markers, such as ER, PR, and HER2 levels in the case of breast cancer, to determine if the drug has or has not activity against an obvious cancer subtype. In turn, this can potentially identify cancer subtypes in which the clinical development should be focused and/or subtypes that can be enriched for in early phase II studies. A flow diagram of this presurgical approach using Ki67, pathway activation markers, and FDG-PET for the testing of novel PI3K inhibitors during the preapproval process of clinical development is shown below in Fig. 1.

6 Rationale for Combination Therapies

The PI3K pathway is highly interconnected with multiple negative feedback loops and with complex cross-talk with other signaling networks. The redundancy with the MAPK pathway and with the LKB1/AMPK energy-sensing pathway has been reviewed in chapters in this book. Much of this network is conserved back to flies and worms and this cross-talk and negative autoregulation has apparently evolved to ensure homeostatic control of cell growth in response to mitogenic factors, and to prevent inappropriate growth under conditions of energy stress. The mutations that involve the PI3K network in human cancers invariably circumvent one or more of the negative feedback pathways that provide homeostatic control to the network (Shaw and Cantley 2006). Nonetheless, interruption of single nodes within the PI3K network can suppress this negative feedback auto-regulation and endow tumor cells with compensatory molecular signals that counteract drug action. Moreover, the prior experience with other molecule-targeted drugs (Arteaga 2007) strongly suggest that, even in patients who initially respond to these drugs, single-agent PI3K inhibitors will be insufficient to cure patients with advanced disease.

The existence of a TORC1-PI3K/Akt negative feedback loop has been well documented in studies with cells in culture (reviewed in chapter). Recently, however, two clinical studies elegantly documented that pharmacological inhibition of TORC1 (with rapamycin or everolimus) led to Akt activation as measured by tumor levels of Ser473 P-Akt in patients with breast cancer and glioblastoma (Cloughesy et al. 2008; O'Reilly et al. 2006). These

findings have important therapeutic implications as they imply that the limited efficacy of TORC1 inhibitors might be due to their intrinsic capacity to abrogate this negative feedback to Akt. Indeed, in the study by O'Reilly et al., inhibition of TORC1 with everolimus led to insulin-like growth factor (IGF)-I receptor/IRS-1-dependent activation of Akt. IGF-IR inhibition with small molecule TKIs prevented RAD001-induced Akt phosphorylation and sensitized tumor cells to the TORC1 inhibitor (O'Reilly et al. 2006). Based, in part, on these data, at this time, clinical trials testing combinations of mTOR inhibitors with neutralizing IGF-IR monoclonal antibodies are in progress.

In another relevant example, inhibition of TORC1 with rapalogs in primary breast tumors and in xenografts induced a dose-dependent increase in MAPK activation which was dependent on an S6K-PI3K-RAS pathway (Carracedo et al. 2008). Supporting the notion that this compensation limits the therapeutic inhibition of a single pathway, the combined inhibition of mTOR and MEK has shown synergistic activity against several cancer xenografts (Carracedo et al. 2008; Kinkade et al. 2008; Legrier et al. 2007). Therefore, although PI3K inhibitors have not yet been shown to induce upregulation of MEK (or an upstream activator of MEK), it is not unreasonable to expect they will do so in cells where PI3K inhibitors downregulate TORC1 activity downstream. Based in part on these data, combinations of TORC1/TORC2 inhibitors with MEK inhibitors and Akt inhibitors with MEK inhibitors are under early planning. Furthermore, since activation of mTOR downregulates PDGF receptor signaling (Zhang et al. 2007), it is likely that inhibition of mTOR will also lead to PDGFR activation in some cancers. In tumors where this receptor is overexpressed, this response would limit the action of mTOR inhibitors and potentially inform the use of novel therapeutic combinations aimed at blocking such compensatory response.

Two papers have recently shown that inhibition of MEK with a small molecule inhibitor, although partially effective, leads to feedback upregulation of PI3K/Akt in human breast cancer cells with a basal-like gene expression signature (Hoeflich et al. 2009; Mirzoeva et al. 2009). This compensatory response upon therapeutic inhibition of MEK was enhanced in cells lacking PTEN (Hoeflich et al. 2009). Further, studies with human cancer cell lines and transgenic tumors that harbor both PI3K pathway and Ras mutations do not respond to PI3K inhibitors (Engelman et al. 2008; Ihle et al. 2009). One example of therapeutic synergy conferred by the addition of a PI3K pathway inhibitor to a MEK inhibitor was recently reported by Engelman et al. Transgenic mice harboring lung cancers driven by mutant K-RAS did not respond to the MEK inhibitor ARRY-142886 or to the PI3K/mTORC inhibitor NVP-BEZ235 when given alone. However, the combination was markedly synergistic in inducing tumor shrinkage (Engelman et al. 2008). This combined approach may be applicable to other tumors if we consider recent studies showing that cancers with mutant p110 α often possess mutations or alterations in other components of the PI3K pathway, such as Ras, HER2 (ErbB2), and PTEN (Oda et al. 2008; Perez-Tenorio et al. 2007; Stemke-Hale et al. 2008). In any case, these data suggest that basal-like breast cancers and NSCLC with K-Ras mutations are tumor types where combinations of PI3K and MEK inhibitors are worthy of clinical testing.

Aberrant PI3K activity has also been associated with resistance to multiple drugs, thus suggesting a role for PI3K pathway inhibitors with other established primary therapies. For example, presence of *PIK3CA* mutations and loss of PTEN in HER2-overexpressing cancers correlates with a lower response to the HER2 antibody trastuzumab (Berns et al. 2007; Nagata et al. 2004) and the HER2 TKI lapatinib (Eichhorn et al. 2008). Overexpression of constitutively active Akt renders HER2-overexpressing breast cancer cells insensitive to trastuzumab (Yakes et al. 2002). Treatment with the p110/TORC1 inhibitors NVP-BEZ235 or GDC-0941 has been shown to restore the action of trastuzumab and lapatinib against

HER2-overexpressing cells and xenografts that also harbor PTEN loss or *PIK3CA* activating mutations (Eichhorn et al. 2008; Junttila et al. 2009; Serra et al. 2008). EGFR TKIs are ineffective in high-grade gliomas that lack PTEN expression (Mellinghoff et al. 2005). Restoration of PTEN expression into *PTEN* mutant cancer cells sensitizes them to EGFR inhibitors (Bianco et al. 2003; She et al. 2003) and downregulation of PTEN using shRNAs dampens the apoptotic effect of EGFR TKIs against receptor-dependent tumor cells (She et al. 2003; Wang et al. 2006). Recently, *MET* gene amplification was shown to engage HER3 in order to activate PI3K/Akt and induce acquired resistance to gefitinib in lung cancer cells and primary NSCLC (Bean et al. 2007; Engelman et al. 2007). These data suggest that inhibitors of the PI3K pathway, currently in clinical development, can be used to potentially reverse acquired and de novo drug resistance.

7 Neoadjuvant Clinical Trials

Amplification of PI3K signaling has also been associated with resistance to endocrine therapy in breast cancer (Perez-Tenorio and Stal 2002; Tokunaga et al. 2006). Breast cancer cells with upregulated Akt signaling exhibit resistance to antiestrogens which can be abrogated by cotreatment with everolimus and other mTOR inhibitors (Beeram et al. 2007; Boulay et al. 2005; deGraffenried et al. 2004). Based on these data, Baselga et al. conducted an exploratory randomized phase II study of the aromatase inhibitor letrozole vs. letrozole plus everolimus administered over a 4-month period to 270 postmenopausal women with operable ER-positive breast cancer (Baselga et al. 2009). The primary endpoint was clinical response by palpation. Mandatory biopsies were obtained at baseline and after 2 weeks (day 15) of treatment. Specimens were assessed for presence of exon 9 (E545K, E542K) and exon 20 (H1047R) *PIK3CA* mutations, and for pharmacodynamic changes in Ki67, P-S6, P-Akt, cyclin D1, and progesterone receptor (PgR) by IHC. Response rate as assessed by clinical palpation was statistically higher in the everolimus-containing arm vs. single-agent letrozole. Consistent with target inhibition, a marked downregulation of P-S6 levels occurred only in the day 15 biopsy in patients receiving everolimus. A significant reduction in tumor cell proliferation as measured by Ki67 IHC was observed in 57% of patients in the everolimus arm vs. 30% of patients in the letrozole alone arm ($p < 0.01$) (Baselga et al. 2009). The results of this trial have important implications that could not have been arrived to in the absence of this elegant design. First, because of the better response rate to the combination, this result provides a signal that the combination should be explored further. Second, they suggest that early pharmacodynamic biomarkers (Ki67, noninvasive imaging) might identify tumors that benefit from the combination vs. not. Finally, this approach ensures the access to abundant tumor tissue in a large proportion of patients (since all are operated) where unbiased molecular profiling aimed at identifying a signature of response or lack thereof can be investigated.

The neoadjuvant trial described above illustrates a clinical platform that can be utilized in breast and other cancers for testing of feasibility and identifying early signals for “go-no go” decisions to pursue combinations of PI3K inhibitors with the current standards of care (i.e., chemotherapy, endocrine therapy, other targeted agents). Obviously, these would have to be done after safety of the combinations has been documented in traditional phase I studies. A diagram of such generic approach in breast cancer is shown in Fig. 2 but can be modified to other tumor types where neoadjuvant therapy is used. Patients are randomized to the “standard” therapy with or without the PI3K pathway inhibitor. A “research” biopsy can be obtained after 2 weeks in order to document effects on tumor cell proliferation/apoptosis as well as pathway inactivation. Incorporation of noninvasive FDG-PET could identify early metabolic changes as a function of PI3K/Akt inhibition (or lack thereof). Clinical and pathological complete response can be evaluated after approximately 4 months of therapy. As designed, this approach asks three questions: (1) is there a difference in the cellular and

molecular response between the two treatment arms during the first 2 weeks? (2) is clinical and/or pathological complete response statistically better in the arm containing the PI3K pathway inhibitor, and (3) is there a tissue and/or noninvasive imaging pharmacodynamic biomarker in the pretherapy, the 2-week, and/or the surgical specimen that correlates with response or lack of response to the combination? A difference in favor of the combination of the “standard” therapy plus the PI3K inhibitor would support the further development of the combination.

8 Conclusions

The introduction of antagonists of the PI3K signaling pathway as a therapeutic anticancer strategy is still at a relatively early stage of development. Early clinical data, however, suggest that this strategy is clinically feasible and that these drugs, at least as single agents, will be well tolerated. Temsirolimus, an inhibitor of one element of this pathway, TORC1, has already been approved for treatment of high risk, metastatic renal cell cancer. A significant number of unknowns that apply to the wide clinical use of these inhibitors still remain. These include pharmacodynamic tissue and/or imaging biomarkers of drug action against its target(s), mid-term and long-term toxicities associated with their use, the need or not to develop isoform-specific p110 and Akt inhibitors, the combined inhibition of TORC1 and TORC2 with single agents, novel mechanisms of compensation (i.e., feedback) deployed upon therapeutic inhibition of this pathway, the development of rational combinations that will include PI3K pathways inhibitors, and perhaps more importantly, the use of an unbiased approach to determine the patients that will likely benefit from these drugs as well as the better combinatorial therapies to pursue. With the plethora of PI3K pathway inhibitors in development and the increased perception of the need to assess the effect of these drugs in tumor tissues in real time and link such assessment to clinical benefit, it is likely we will have answers to most of these questions in the next few years.

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Fig. 1.

Diagram of presurgical clinical trial with PI3K pathway inhibitor(s). Each of these three groups of patients with newly diagnosed operable breast cancer (*PIK3CA* mutant, *PIK3CA* wild-type/*PTEN* mutant, and *PIK3CA* wild-type/*PTEN* wild-type) will be treated with the PI3K pathway inhibitor for 2 weeks until the day before surgical resection of the primary tumor. All patients/tumors will be evaluated with the indicated immunohistochemical markers and FDG-PET at the start of the study and at the completion of therapy (in tumor sections from the surgical specimen and the day of surgery, respectively). An estimate of 90 patients, 30 per arm, will be required to achieve the study endpoints

Fig. 2.

Schema of neoadjuvant clinical trial with PI3K pathway inhibitor. Patients with breast cancers requiring neoadjuvant therapy prior to breast conserving surgery are randomized to the "standard" therapy $\pm$ the PI3K pathway inhibitor. Formalin-fixed and flash-frozen core biopsies are obtained after 2 weeks of therapy in order to document effects on tumor cell proliferation and/or apoptosis as well as pathway inactivation (i.e., downregulation of P-Akt, P-S6, P-PRAS40, etc., by IHC). Incorporation of noninvasive FDG-PET at 2 weeks could identify early metabolic changes (or lack thereof). Clinical response can be evaluated after approximately 4 months of therapy by measuring the tumor with calipers, ultrasound, and/or mammography. Absence of tumor in the surgical specimen would be scored as a path CR. Rate of breast conserving surgery is another endpoint that can be compared between both arms. No difference in terms of clinical and/or pathological response in favor of the "standard" therapy plus PI3K inhibitor arm would indicate the clinical development of the combination is not a priority.