Linking Molecular Diagnostics to Molecular Therapeutics: Targeting the PI3K Pathway in Breast Cancer

Gordon B. Mills, Elise Kohn, Yiling Lu, Astrid Eder, Xianjun Fang, Hongwei Wang, Robert C. Bast, Joe Gray, Robert Jaffe, and Gabriel Hortobagyi

Modulation of the signaling pathways that are aberrant in cancer cells has the potential to provide an effective nontoxic approach to patient management in a broad range of cancers. This quest has taken a major leap forward with the demonstration that STI-571 (imatinib mesylate) induces clinical and molecular remissions in the majority of patients with interferon-refractory chronic myelogenous leukemia and gastrointestinal stromal tumors through inhibition of the Bcr/Abl fusion protein required for the initiation and progression of chronic myelogenous leukemia and inhibition of a mutant, activated c-*kit* present in gastrointestinal stromal tumors. Support for the concept of targeting products of fusion genes found in specific cancers was first provided by the efficacy of all-*trans* retinoic acid in acute promyelocytic leukemia where the RARα all-*trans* retinoic acid target is the target of multiple different chromosomal rearrangements. In breast cancer, trastuzumab, which alters the function of the HER2 protooncogene overexpressed in a portion of breast cancers, provides an additional example of targeting specific molecular aberrations present in cancer cells. Although the target for these signal transduction modulators is functional in normal cells, acceptable therapeutic indices sufficient to prevent tumor growth without unacceptable toxicities have been observed. Whether STI-571 and other signal transduction modulators also target the stroma, and specifically the neovasculature, in addition to the tumor remains an open question. The presence of the target in the cancer cells or in the surrounding stroma appears to be required but not sufficient for the action of molecular therapeutics. Thus, linking molecular diagnostics to identify patients where the target is amplified or activated and driving the pathophysiology of the patients' tumor to effective molecular therapeutics will be necessary to translate these concepts into approaches that will alter the outcome for breast cancer patients. This review will focus on the phosphatidylinositol 3-kinase pathway and novel molecules targeting this pathway to illustrate the questions and challenges underlying the implementation of molecular therapeutics in breast cancer.

Semin Oncol 30 (suppl 16):93-104.

BREAST CANCER is the most common malignancy in Western women.[1] It is predicted that there will be 211,300 new breast cancer cases in 2003 in the United States alone, representing about one third of all cancers in this country.[1] Despite continued progress, 39,800 women will die this year of breast cancer.[1] Thus, there is an urgent need to develop novel, more effective, and better-tolerated strategies for the treatment of breast cancer, particularly for metastatic breast cancer. The most likely source for these advances is the translation of our greatly improved understanding of the pathophysiology of breast cancer to novel therapeutics based on rational target identification. Understanding the expression, regulation, and function of critical signaling pathways that contribute to the initiation and progression of breast cancer will be an essential part of this process. Equally as important will be the identification of approaches to select appropriate patients and ensure that molecular therapeutics are delivered to the tumor in biologically relevant doses. As illustrated below, multiple components of the phosphatidylinositol 3-kinase (PI3K) pathway are aberrant and contribute to prognosis in breast cancer patients, suggesting that molecular therapeutics targeting this pathway, either alone or more likely

From the Departments of Molecular Therapeutics, Experimental Therapeutics, and Medical Breast Oncology, Division of Medicine, The University of Texas M. D. Anderson Cancer Center, Houston, TX; the Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, Bethesda, MD; the University of California San Francisco Cancer Center, San Francisco, CA; the Reproductive Endocrinology Center, University of California-San Francisco, San Francisco, CA.

Supported by National Institute of Health grant no. CA82716; DAMD17-99-1-962 and a Centers of Excellence Grant DAMD17-02-01-0694 from Department of Defense Congressionally Directed Medical Research Programs; and grant no. 9871 from the Komen Foundation (GBM).

All authors have received research grant support from Kiuetek, Eli Lilly and Company, Echelon Technologies, Celgene, and Abbott Pharmaceuticals. Dr Hortobagyi has received research grant support from Bristol-Myers Squibb and honoraria from Aventis, AstraZeneca, and Novartis. He has served as a consultant to Pfizer, Genentech, and Bristol-Myers Squibb.

Address reprint requests to Gordon B. Mills, MD, PhD, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd, Box 317, Houston, TX 77030.

0093-7754/03/3005-1611$30.00/0

doi:10.1053/S0093-7754(03)00444-5

in concert with other drugs, may contribute to an improved outcome in breast cancer.

Signal transduction pathways and networks have recently proven to be attractive targets for therapy in cell lineages with defined genetic aberrations. STI-571, all-*trans* retinoic acid, trastuzumab, IMC-C225, ZD1839, and CCI779 have all induced remarkable, nontoxic responses in a subset of patients where abnormalities in the appropriate signaling pathway are present in *bcr-abl* (STI-571), retinoid receptor fusion genes (all-*trans* retinoic acid), ErbB-2 or HER2/*neu* (trastuzumab), epidermal growth factor receptor (IMC-C225 and ZD1839), and the PI3K pathway (CCI779). However, despite expression of the therapeutic target in the patients' tumor, only a portion of cases achieve an acceptable response. Therefore, the effective development and utilization of novel molecular therapeutics will require a detailed understanding of the genetic aberrations present in tumors, how these aberrations alter signaling networks, and the identification of signaling nodes, which are likely to present high-quality targets.

Signal transduction is processed in complex networks. These networks are typically robust, such that a single perturbation may not have major effects. A large number of "knock-out" mice have particularly mild or absent phenotypes based on the robust nature of signaling pathways. However, there are key nodes in the network wherein perturbations may have profound effects both in terms of tumorigenesis and therapeutic targets. Indeed, this may be represented by genes that, when deleted or activated, either as experiments of nature or induced knockouts, result in marked phenotypic changes such as lethality or development of diseases such as cancer. Systematic analysis of protein function in yeast has shown that nodal points of convergence in signaling networks can be identified both by an analysis of "lethality" and by determining the number of interacting proteins.[2-5] With this paradigm in mind, it is particularly important to characterize signaling pathways in cancer cells as a series of "nodes" that integrate at critical convergence points to provide a robust mechanism to transmit signals from the external environment to functional outcomes.[2-5] To accomplish this, we will need to understand as completely as possible the relationships between sets of inputs and outputs in signaling in cells that vary both temporally and spatially. The same goal, stated from a slightly different perspective, is to understand fully how cancer cells interpret signals in the context of their microenvironment in the patient. This will require identification of the components that comprise a particular signaling network, the assessment of time-dependent information flow through the network, and finally, the reduction of the mass of detailed data into a set of testable theoretical models that describe information transfer through a cellular signaling network (see http://www.cellularsignaling.org/).

The best current manner to begin to understand cellular processes is to treat each protein in a signaling pathway as a "node."[2-5] Each node can then be defined based on its input (expression, regulation, localization, post-translational modification), binding partners, and output (targets). Linking several nodes begins to form an interactive network. Critical integrative nodes can be identified as convergence points due to the multiplicity and strength of interactions and inputs. A number of groups have begun to create databases that can be used to facilitate the process, and into which data can be entered to improve the rate of progress. For examples, see http://www3.oup.co.uk/nar/database/c/ for a listing of many of the databases; additional databases can be found through links in this database including: Database of Interacting Proteins (http://dip.doe-mbi.ucla.edu/), Signaling Pathway Database (http://www.grt.kyushu-u.ac.jp/spad/index.html), the Biomolecular Interaction Network Database (http://www.bind.ca/), and Kyoto Encyclopedia of Genes and Genomes (http://www.genome.ad.jp/kegg/). However, most of these databases are in their infancy with limited data and limited descriptions of protein interactions. Much of the current difficulty in generating the databases arises from a need to curate data so that the informational input is valid. It is important, however, to note that predictive models developed in yeast appear to be sufficiently robust as to function with high fidelity and predictive values despite gaps and errors in the input.[2]

An understanding of the pathophysiology of tumor progression in specific cell lineages, and potentially in the individual patient, may be required to determine the optimal point to target signal transduction pathways in particular cancer patients. Indeed, depending on the particular aberration present in the patient's tumor, different

molecular therapeutics targeting the same pathway may be required. For example, if a patient's tumor is driven by a downstream aberration in a particular pathway, it will be futile to target the pathway at a proximal site such as the growth factor or its cognate receptor at the cell membrane. In contrast, as intimated above, targeting the pathway at critical downstream integration points may prove particularly toxic. Targeting proximal events may prove ineffective because of redundant inputs to downstream convergence sites. Thus, it is impossible to predict a priori where to target a specific pathway. Rather, the outcome of a treatment with a particular molecular therapeutic will be dependent on the particular genetic aberration in the pathway, the requirement for the event in the pathophysiology of the cancer at the time of presentation, and the therapeutic index for a particular target. Thus, linking molecular diagnostics capable of identifying the lesion in the tumors of specific patients to approaches to assess mechanism-dependent efficacy and toxicity along with a method to determine that patients are receiving biologically relevant doses will be required to translate signal transduction modulators into effective drugs.

THE PHOSPHATIDYLINOSITOL 3-KINASE (PI3K) PATHWAY

PI3K is a member of a protein superfamily that includes FRAP/TOR, the ataxia telangiectasia mutated gene (ATM) and the related ATR protein, the PAF400 and TRRAP components of the histone acetylase complex, and the DNA-dependent protein kinase.[6-11] Members of the PI3K superfamily phosphorylate proteins on serine and threonine residues.[6-11] In addition to phosphorylating proteins, including the regulatory p85 subunit of PI3K on serine and threonine, PI3K exhibits the unique ability to phosphorylate the 3′ site on the inositol ring of membrane phosphatidylinositols (PtdIns) (Fig 1). This activity is reversed by the activity of the PTEN/MMAC/TEP tumor suppressor (Fig 1), providing support for the concept that the PI3K pathway is critical for tumor development in humans. Phosphorylation of membrane PtdIns recruits a subset of proteins with pleckstrin homology, Phox, FYVE, C1, and C2 domains to the membrane.[6-11] This translocation places these proteins in the context of their upstream regulators and downstream targets.

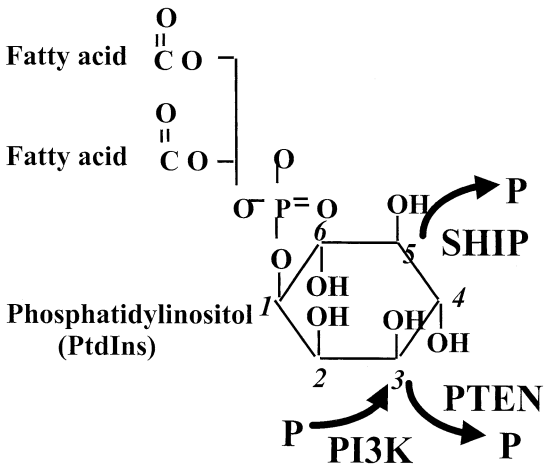

Fig 1. PI3K and PTEN target the same site in PtdIns.

Although phosphorylated membrane PtdIns have the potential to recruit and activate a large number of proteins at the cell membrane, genetic studies of *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, and mice strongly implicate AKT and its regulators PDK1 and PDK2, which phosphorylate threonine 308 and serine 473 in AKT (AKT1 numbering; highly homologous sites are present in AKT2 and AKT3), respectively, as major downstream targets of PI3K (Fig 2).[6-14] While PDK1 activity appears to be mediated by a single protein, there may be multiple enzymes able to mediate PDK2 function, including integrin-linked kinase and other less well-characterized moieties.[6-11,15-17] AKT, PDK1, and PDK2 are recruited to the cell membrane by 3-phosphorylated PtdIns[6-11] forming a complex where PDK1 and PDK2 have access to their targets on AKT. The number of identified downstream targets for AKT is rapidly increasing. Many of these may be direct substrates; however, a number may be indirect or caused by other components of the PI3K pathway. Identified substrates include BAD, forkhead, mdm2, CREB, NFκB, GSK3, p21, p27, TSC2, mTOR/FRAP, p70S6, 4E-BP1, and potentially caspase 9.[6-11,18-20] Phosphorylation of these proteins alters activity or localization contributing to the cellular consequences of activation of the PI3K pathway.[6-11,18-20] The PI3K pathway, at least in part through AKT, also regulates the expression of a number of proteins involved in cell function including p27, VEGF, BCL2, cyclin D1, myc, glucose transporters, hexokinase, and phosphofruc-

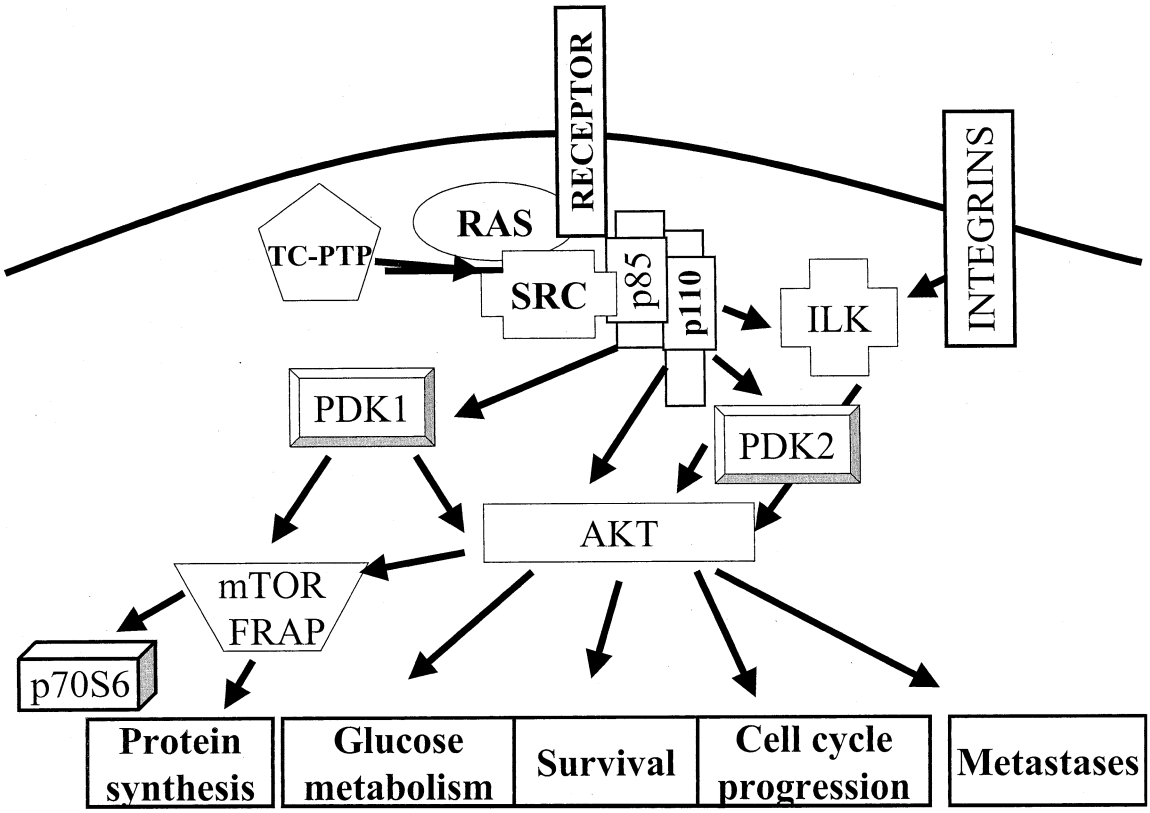

Fig 2. PI3K pathway.

tokinase.[6-11,21-23] These proteins are implicated in glucose metabolism, energy metabolism, cell size, cell cycle progression, apoptosis, metastases, and protein translation. The PI3K pathway bifurcates at multiple points. For example, p85 functions as both a regulator of the p110 catalytic subunit of PI3K and contributes to cell cycle progression and motility through regulation of G proteins of the Rac/cdc42/Rho family (Fig 2).[24,25] Similarly, PDK1, in addition to regulating AKT activity, also contributes to regulation of FRAP and its downstream targets, p70S6 kinase and 4E-BP1.[26,27] Integrin-linked kinase, which under some circumstances can directly or indirectly regulate phosphorylation of the PDK2 site in AKT, can also regulate cellular motility and neovascularization through AKT-independent pathways.[15,16]

RELEVANCE OF THE PI3K PATHWAY TO TUMORIGENESIS

PI3K contributes to multiple events critical to cell growth regulation and tumorigenesis including cellular proliferation, differentiation, cytoskeletal organization, motility, metastases, invasion, angiogenesis, and cell survival.[7-25] Aberrations of components of the PI3K pathway (Fig 2) have been shown to be present in a majority of tumors, approaching the frequency seen for changes in the p53 pathway. In addition, an ability to activate PI3K and components of the PI3K pathway is obligatory for transformation by multiple oncogenes including v-*src*, v-*abl*, v-*ros*, *ras*, and polyoma middle T antigen (PyT).[7-11] For example, the transforming activity of PyT in transgenic breast model systems requires a functional p85 interaction motif in PyT,[28] and the ability of Ras to mediate transformation requires an ability to bind and activate the p110 catalytic subunit of PI3K.[29] Although mammalian PI3Ks have not been shown to exhibit transforming activity, membrane targeting of a catalytically active avian PI3K p110 catalytic subunit ortholog is sufficient to transform avian embryonic fibroblasts.[30,31] Inhibition of the mTOR/FRAP component of the PI3K pathway

(Fig 2) reverses the transformation of chicken embryo fibroblasts induced by activated PI3K or AKT, but not by multiple other oncogenes including *src* and *ras*.[32] The p85 subunit of PI3K has recently been directly implicated in cancer development, with both deletions and gene fusions being found in solid and hematopoietic tumors.[33-36] These mutations appear to activate PI3K by releasing the inhibitory effects of a "cancer-associated negative regulatory domain" in p85 on the catalytic domain of p110.[25] We have shown that Src family kinases can mediate similar effects by phosphorylating tyrosine 688 in p85.[37] We have also shown that multiple components of the PI3K pathway are amplified at the genomic, RNA, and protein levels in ovarian cancer,[38] contributing to the growth and survival of ovarian cancer cells in vitro and in vivo.[39,40] All three AKT homologs exhibit abnormalities in multiple different cancer lineages. Aberrations of AKT2 are frequently present in ovarian and pancreatic cancer and less commonly in breast cancer.[41,42] In hormone-independent breast and prostate cancer cells, the AKT3 isoform is selectively amplified or activated.[43] AKT1 and AKT2 exhibit transforming activity.[41-46] Further, AKT1 is sufficient to result in the development of lymphomas in mice (H. Wang and G. Mills, unpublished data)[44] and contribute to tumorigenesis in other lineages.[45,46] However, AKT activation is not sufficient for tumorigenesis in several cell lineages.[45-48] Thus, multiple components of the PI3K pathway are targets for tumorigenic activation in multiple cell lineages.

CREDENTIALING THE PI3K PATHWAY AS A TARGET IN BREAST CANCER

The PTEN (also known as MMAC1 or TEP)[49-51] tumor suppressor gene on 10q23, which dephosphorylates the same 3′ site on PtdIns phosphorylated by PI3K, is aberrant at the level of loss of heterozygosity (> 40%),[52-55] mutation (2% to 20%),[56-61] methylation, protein stability, or function in a significant number of breast and other cancers[62-66] (G.B. Mills and Y. Lu, unpublished data). Germ-line mutations in PTEN are responsible for the Cowden's breast cancer predisposition syndrome,[67-69] validating the PI3K pathway as a critical mediator of breast tumorigenesis. In addition to decreased mRNA expression, which can be the consequence of mutation, promoter methylation, and protein phosphorylation,[49-69] PTEN protein is present and nonfunctional in a number of tumor cell lines (G.B. Mills and Y. Lu, unpublished data). PTEN mutations occur at a higher frequency in advanced breast cancers.

We have shown that loss of PTEN function in breast cancer cells results in an increase in basal levels of phosphorylation of multiple components of the PI3K signaling cascade, as well as an increase in duration of ligand-induced signaling through the PI3K cascade.[22] These alterations are reversed by wild-type but not phosphatase-inactive PTEN. In the presence of high concentrations of serum, enforced expression of PTEN induces a predominant G_1 arrest consistent with the capacity of PTEN to evoke increases in the expression of the p27Kipl cyclin-dependent kinase inhibitor.[22] The ability of activated AKT to phosphorylate both p21 and p27, resulting in their sequestration in the cytosol,[19,20] likely also contributes to cell cycle arrest. In the presence of low concentrations of serum, enforced PTEN expression results in a marked increase in cellular apoptosis, a finding consistent with the capacity of PTEN to alter the phosphorylation, and presumably function, of the apoptosis regulators AKT, mdm2, NFκB, BAD, p70S6 kinase, TSC2, and GSK3α.[6-11,18-22] Under anchorage-independent conditions, PTEN also induces anoikis, a form of apoptosis that occurs when cells are dissociated from the extracellular matrix, which is enhanced under low-serum culture conditions.[22] Together, these data suggest that PTEN effects on the PI3K signaling cascade are influenced by the extracellular environment and that, depending on the exposure to growth factors and other exogenous stimuli such as integrin ligation, PTEN can induce cell cycle arrest, apoptosis, or anoikis in breast cancer cells.

The PI3K pathway is activated by multiple cell surface tyrosine kinases including members of the epidermal growth factor receptor family, which is frequently aberrant in breast cancer.[70,71] Further, Src, which has been proposed to play a role in breast cancer development or progression including that in transgenic mice, interacts with the PI3K pathway at multiple levels.[37,72-74] Protein kinase C (PKC) activation is aberrant in breast cancer,[75,76] and we have shown that specific members of the protein kinase C family interact with the PI3K pathway at multiple levels.[37,77] Furthermore, the SH2 domain containing tyrosine

phosphatase, SHP1, binds and regulates both the p85 subunit of PI3K and PTEN[72] (unpublished data). Strikingly, heterozygous knockout SHP1 mice appear to have a propensity to develop breast cancer at an early age (K. Siminovitch, personal communication, July 2000) in addition to lymphomas.[78] The AKT2 isoform is genomically amplified in a portion of breast cancers,[15] and AKT3 is overexpressed in breast cancers lacking the estrogen receptor.[42,43] The PI3K pathway is both regulated by the estrogen receptor with the receptor binding p85[79] and can activate the estrogen receptor with AKT phosphorylating the estrogen receptor, thus bypassing ligand dependence for estrogen receptor signaling.[80] The p70S6 kinase, which is downstream of PI3K, is genomically amplified and increased at the RNA and protein level in a significant portion of breast cancers.[81] The tuberous sclerosis tumor suppressor gene isoform 2, which inhibits p70S6 kinase,[18] is located at a point of loss of heterozygosity in breast cancer. Further, the p21-activated kinase, PAK1, which is a target for several members of the PI3K pathway,[6-10] exhibits increased activity in invasive breast tumors and plays a critical role in the development and metastases of breast cancer.[82] Thus, germline and somatic genetic and epigenetic events along with post-translational modifications affecting the function of the PI3K pathway have the ability to contribute to the pathogenesis of breast cancer. A complete understanding of the regulation of the PI3K pathway could thus lead to improved management approaches.

Genetic aberrations in tumors can contribute to tumor initiation or tumor progression. Signaling pathways that contribute to progression as indicated by influencing patient outcome are likely to provide important targets for therapy. The mutational and activation status of the PI3K pathway appears to contribute to patient outcome, at least as indicated by altering prognosis. Phosphorylation of regulatory sites on downstream products of the PI3K pathway provides a sensitive measure of the activation status of the pathway. Indeed, in breast cancer cells with mutant PTEN, multiple components of the pathway are constitutively phosphorylated.[22] Activation of AKT, as indicated by phosphorylation, is associated with a worsened outcome in patients treated with endocrine therapy.[83] Consistent with this observation, AKT phosphorylates the estrogen receptor, bypassing ligand dependence for estrogen receptor signaling.[80] The AKT2 isoform is genomically amplified in a portion of breast cancers[42] and AKT3 is overexpressed in breast cancers lacking the estrogen receptor,[43] potentially contributing to ligand-independent activation of the estrogen receptor and resistance to hormonal modulation. Further, the PI3K pathway is regulated by the estrogen receptor through binding p85.[79] Similarly, loss of heterozygosity of the PTEN locus at 10q23 is strongly correlated with increased tumor invasiveness, poor differentiation, and loss of estrogen receptor expression compatible with PTEN loss being a late event contributing to tumor pathogenesis at patient presentation.[53,55] Loss or decreased expression of PTEN or potential mislocalization of PTEN is associated with a worsened prognosis or poor prognostic outcomes in breast cancer,[65,66] emphasizing the importance of this pathway in the pathophysiology of breast cancer.

PRECLINICAL AND EARLY CLINICAL STUDIES OF PI3K PATHWAY INHIBITORS

Taken together, the studies suggest that multiple components of the PI3K pathway could be targets for therapy in breast and other cancers. Indeed, multiple companies are developing drugs targeting the PI3K pathway (Fig 3). These include novel small-molecule compounds in preclinical studies targeting PI3K (Lilly [Indianapolis, IN], Iconix [Mountain View, CA], Echelon-ComGenex [Salt Lake City, UT]), AKT (Kinetek [Vancouver, Canada], Celgene [San Diego, CA], Abbott [Abbott Park, IL], Kinacia [Box Hill, Australia]), integrin-linked kinase (Kinetek), and T-cell phosphotyrosine phosphatase (Kinetek).

Clinical trials are currently under way with the rapamycin analogs, CCI779 and RAD001, both of which target FRAP/mTOR (Fig 3). Several important concepts in targeting the PI3K pathway and, indeed, use of signal transduction mediators in general are illustrated by the preclinical and early clinical studies with these drugs. FRAP/mTOR inhibitors interfere with the functional interaction of FRAP/mTOR with its binding partner, Raptor.[84] FRAP/mTOR is a member of the PI3K superfamily, which phosphorylates p70S6 kinase and 4E-BP1/PHAS1 on serine and threonine residues.[85] p70S6 kinase and 4E-BP1, in turn, regulate translation of mRNAs with a 5′-terminal oligopolypyrimidine tract and CAP-dependent translation,

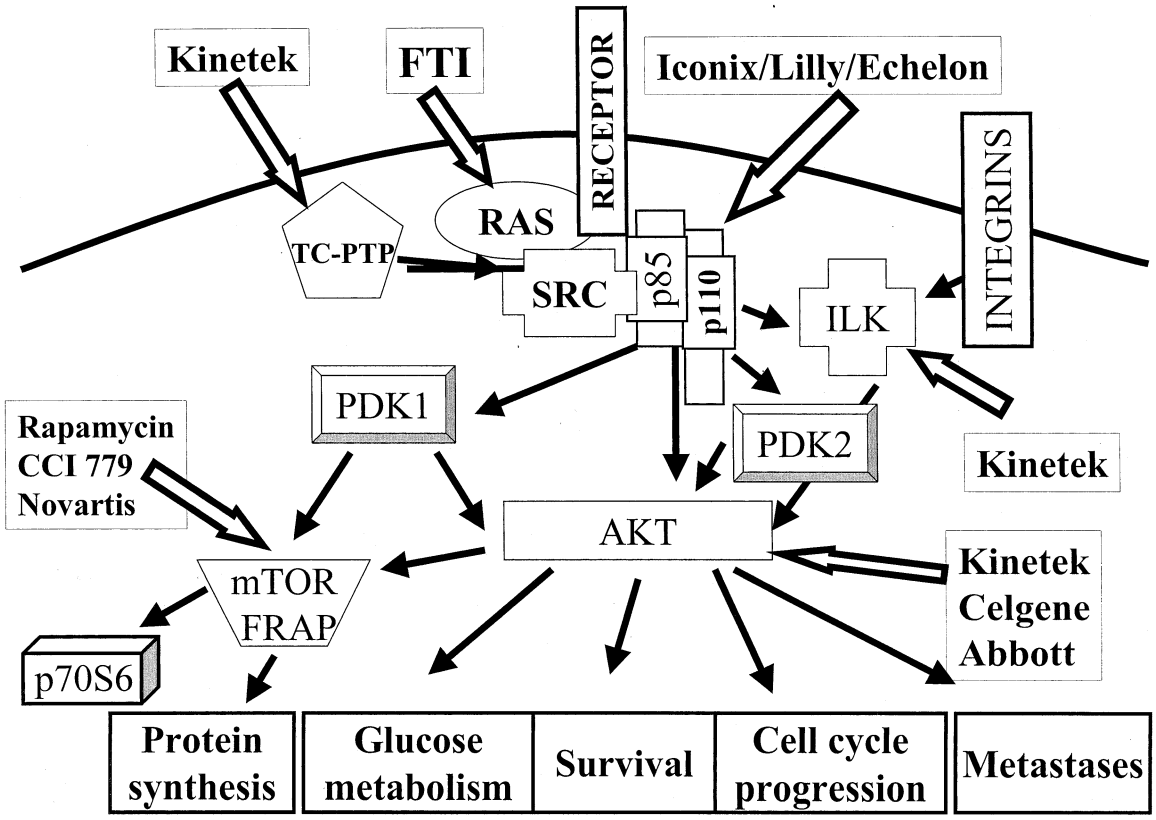

Fig 3. PI3K pathway drugs in development.

respectively.[85] In animal and in vitro models, aberrations in the PI3K pathway render cells particularly sensitive to the activity of inhibitors of FRAP/mTOR.[86-89] These aberrations, however, are neither required nor sufficient for the action of rapamycin or CCI779.[87,88,90] Thus, in terms of designing clinical trials, it appears preferable to design studies wherein the status of the components of the PI3K pathway and cell cycle regulators are known and be used, post hoc, to determine surrogate markers for responsiveness rather than to make detection of aberrations in PTEN or the PI3K pathway as a prerequisite for trial entry. Whether the activity of FRAP/mTOR inhibitors in vivo is restricted to the tumor cell or could affect the stroma and, in particular, the neovasculature is under investigation.[91,92] Indeed, at least some cell lines that are resistant to FRAP/mTOR inhibitors in vitro are responsive in vivo.[90] It has proven difficult to convert the cytostatic activity of FRAP/mTOR inhibitors, which induce a potent G_1 arrest, into cytotoxic activity in preclinical tumor models.[86-89] Thus, patient efficacy may require long-term dosing with FRAP/mTOR inhibitors. In early clinical trials there have been multiple dose-independent tumor regressions, perhaps emphasizing the need to use biologically relevant doses rather than maximally tolerated doses. Further, as mTOR inhibitors have primarily demonstrated cytostatic activity in preclinical models, the tumor regressions observed in patients suggest that the preclinical models are not particularly predictive.

THERAPEUTIC INDEX

The efficacy of drugs in the cancer patient is driven by the therapeutic index, which represents the ability to alter tumor progression relative to the toxicity towards normal tissues. In the case where the toxicity is mechanism-mediated, there may be limited ability to target the pathway or at least a particular component in the pathway. Given that the PI3K pathway plays a critical role in the physiology of multiple processes in normal

cells, it is important to consider possible mechanisms by which a therapeutic index may be achieved in the cancer patient. As indicated above, cell signaling tends to be robust with the complex network of signaling cascades providing the potential to bypass the effects of an inhibitor by the use of alternative signaling molecules. One of the underlying characteristics of tumorigenesis is genomic instability. Under circumstances where tumor growth is driven by an aberration in a particular signaling pathway, the alternative signaling pathways may, because of genomic instability and a lack of selective pressure, undergo mutational inactivation. Under these circumstances, the tumor cell may become "addicted" to a specific pathway and thus sensitized to the effects of inhibitors of signaling through that pathway. Normal cells, due to maintaining the alternative pathways, may prove resistant to the therapeutic agent. As an alternative model, normal cells driven to proliferate because of the action of growth factors are also sensitized to undergo programmed cell death.[93,94] Continued activation of cell surface receptors, with concomitant expression of survival molecules, is required to maintain cells in a viable state. Thus, termination of signaling through a pathway in activated cells, once they have fulfilled their functional role in response to stress or injury, induces the activated cells to die, thus maintaining homeostasis. In contrast, signaling pathways are frequently constitutively activated in a cancer cell, precluding the tumor cell from undergoing apoptosis. Thus, inhibition of a specific signaling pathway in a cancer cell may allow a normal consequence of cellular activation, cell death, to be manifest. This hypothesis is supported by the observation that many different cancer lineages express high levels of pro-apoptotic mediators. Multiple different experimental approaches support this concept. Strikingly, cellular cytokines and growth factors, including those implicated in maintaining cellular viability, predispose cells to undergo death if survival signals are inhibited.[93] In prototypical studies, overexpression of the *myc* proto-oncogene induces normal cells from multiple different lineages to undergo apoptosis.[94]

As more direct proof of the ability to successfully target the PI3K pathway in cancer cells, we have assessed the effect of activating the PI3K pathway on sensitivity to drugs targeting the PI3K pathway. Introduction of an activated PI3K into ovarian cancer cells renders the cells sensitive to the effect of inhibitors of PI3K[40] and also to downstream pathway inhibitors (data not presented). Strikingly, while it might be expected that introduction of PI3K by transfection followed by inhibition of PI3K with a small-molecule inhibitor would simply return the transfected cell to the state of the parent cell, this is not the case. The PI3K-transfected cells were markedly more sensitive to the effects of inhibitors than were the parental cells.[40] Similar results have been observed in that deletion of PTEN (which activates the PI3K pathway) or the introduction of an activated AKT renders cells more sensitive to the effects of inhibitors of the downstream target FRAP/mTOR by CCI779.[86-89] Thus, tumor cells may become dependent on the continued activation of specific signaling pathways that drive pathophysiology, allowing molecular therapeutics to selectively target the tumor cell.

Inhibition of PI3K with LY294002 decreases the growth of the OVCAR3 tumor cell line in nude mouse models.[39] However, the doses of LY294002 required to achieve this effect induce wasting and a patchy dermatitis.[39] This suggests that, similar to chemotherapy agents, combinations of molecular therapeutics or of molecular therapeutics and chemotherapy drugs may be required to provide an adequate therapeutic index.[40,95,96] Inhibition of different signaling pathways or the same pathway at multiple sites may allow the development of an acceptable therapeutic index in the cancer patient. Activation of the PI3K pathway contributes to resistance to multiple different chemotherapy agents including paclitaxel, one of the most active drugs in ovarian cancer. Compatible with this observation, introduction of an activated PI3K into ovarian cancer cells renders them resistant to the effects of paclitaxel, particularly at the doses achieved in the cancer patient.[40] As predicted, inhibition of PI3K reconstitutes the sensitivity to paclitaxel.[40] Indeed, the effects of paclitaxel and PI3K inhibitors show additivity and potentially synergy. These in vitro effects of paclitaxel and PI3K inhibitors are recapitulated in vivo, where the effects of paclitaxel and LY294002 on tumor growth are complementary.[40] Further, the addition of paclitaxel to the regimen allowed a dose reduction of LY294002, alleviating the toxic effects of LY294002. Taken together, these studies suggest that in cancer patients where the PI3K pathway is

Table 1. Underlying Principles for Implementation of Molecular Therapeutics

1. Target is likely to transcend disease boundaries (not tissue or histotype specific)
   Drugs may have broad applicability
2. Only a portion of patients with each cancer lineage may be sensitive
3. Aberrations at different points in the pathway may require different targets and drugs
4. Presence of the target is required but not sufficient for activity
   If the target is not driving tumor progression at time of presentation, it will not be an effective target
5. Activation state of target and pathway likely more important than protein levels
6. Effective molecular diagnostics will be required to identify appropriate patients for each drug
7. Target should be required for persistence or metastases at the time of presentation
   Is the aberration prognostic?
8. Combinations of signal transduction modifiers or signal transduction modifiers and chemotherapy may be required for optimal efficacy
9. Target is likely present and important in normal tissues
10. Target is unlikely to be more sensitive to the effects of the inhibitor and the target or activity is higher than normal cells. This should render cells resistant to signal transduction modifiers
11. Where to target the pathway is dependent on the balance between efficacy and toxicity
    Impossible to determine a priori
12. Biologically relevant dose must be achieved in patients
    Whether continuous or intermittent inhibition of pathway may be more effective is unknown
    Surrogate markers, molecular imaging, and tissue acquisition critical
13. Combinations of multiple signal transduction inhibitors or with chemotherapy likely to be required to attain efficacy
14. **THERAPEUTIC INDEX will drive ability to target a specific molecule in the patient**
    Balance of mechanism-based toxicity and mechanism-based efficacy
    Do not discard effective drugs or good targets
15. Novel trial designs linking molecular diagnostics to molecular therapeutics combined with assessments of the biologically relevant dose, efficacy, and toxicity of signal transduction modifiers required

activated and driving tumor growth, it may be possible to target the PI3K pathway, achieving efficacy in the absence of unacceptable toxicity.

SUMMARY

While molecular therapeutics targeting the PI3K pathway in breast cancer hold great promise, additional preclinical and clinical studies will be required to determine whether such drugs will improve patient outcome. Taken together, the studies described above suggest a number of questions (summarized in Table 1) that will need to be answered before the introduction of these agents into patient care.

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