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Review

Molecular Cancer Therapeutics

Picking the Point of Inhibition: A Comparative Review of PI3K/AKT/mTOR Pathway Inhibitors

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Abstract

The frequent activation of the PI3K/AKT/mTOR pathway in cancer, and its crucial role in cell growth and survival, has made it a much desired target for pharmacologic intervention. Following the regulatory approval of the rapamycin analogs everolimus and temsirolimus, recent years have seen an explosion in the number of phosphoinositide 3-kinase (PI3K) pathway inhibitors under clinical investigation. These include: ATP-competitive, dual inhibitors of class I PI3K and mTORC1/2; "pan-PI3K" inhibitors, which inhibit all four isoforms of class I PI3K (α, β, δ, γ); isoform-specific inhibitors of the various PI3K isoforms; allosteric and catalytic inhibitors of AKT; and ATP-competitive inhibitors of mTOR only (and thus mTORC1 and mTORC2). With so many agents in development, clinicians are currently faced with a wide array of clinical trials investigating a multitude of inhibitors with different mechanisms of action, being used both as single agents and in combination with other therapies. Here, we provide a review of the literature, with the aim of differentiating the genomic contexts in which these various types of inhibitors may potentially have superior activity. *Mol Cancer Ther; 13(5); 1021–31. ©2014 AACR.*

Introduction

The phosphoinositide 3-kinase (PI3K)/AKT/mTOR pathway is one of the most frequently dysregulated signaling cascades in human malignancies and is implicated in a wide variety of different neoplasms (1). Oncogenic PI3K/AKT/mTOR signaling (summarized in Fig. 1), is relatively well characterized and has been reviewed in depth elsewhere (1). PI3K signaling is initiated by receptor tyrosine kinases (RTK) or G-protein–coupled receptors located at the cell surface, and some oncogenic proteins, such as RAS. Kinase interactions downstream of PI3K are complex; several different feedback loops exist, and the pathway is known to interact with other signaling cascades (Fig. 1). Oncogenic activation of the PI3K pathway can occur through a variety of mechanisms; this often includes mutation and/or amplification of genes encoding RTKs [e.g., EGFR (*ERBB1*) and HER2 (*ERBB2*)], subunits of PI3K (e.g., p110α, p110β, p85α, and p85β; encoded by *PIK3CA*, *PIK3CB*, *PIK3R1* and *PIK3R2*, respectively), AKT (*AKT1*), or activating isoforms of RAS. Loss-of-function or expression of PTEN, through mutations, deletions, or epigenetic silencing is also common.

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The frequent activation of the PI3K pathway in cancer and its crucial role in cell growth and survival has made it a much desired target for pharmacologic intervention. The first PI3K pathway-targeted agents approved for the treatment of cancer were the rapamycin analogs (or "rapalogs") everolimus and temsirolimus, which allosterically inhibit mTORC1. These inhibitors of mTORC1 have since been joined by a range of investigational agents that target other components of the PI3K pathway (Table 1). These include ATP-competitive, dual inhibitors of class I PI3K and mTOR (and thus mTORC1 and mTORC2), "pan-PI3K" inhibitors, which inhibit all 4 isoforms of class I PI3K (α, β, δ, γ), isoform-specific inhibitors of the various PI3K isoforms, allosteric and catalytic inhibitors of AKT, and ATP-competitive inhibitors of mTOR only (and thus mTORC1 and mTORC2).

The multitude of different agents under investigation presents several challenges for drug development. First, a rational approach must be developed to identify the optimal clinical and genomic contexts in which each class of inhibitor should be used. Inhibitors of the PI3K pathway have shown most promise when given in combination with other therapies, therefore, additional consideration must also be given to the ideal partners for each class of agent. Another important implication of evaluating PI3K pathway inhibitors is whether such agents will be capable of achieving sufficiently deep inhibition of the pathway (and thus antitumor activity) at doses that can be tolerated by patients. Beyond this question lies another challenge that can only be elucidated by further research in the field: definition of acquired resistance mechanisms that restore PI3K signaling or activate parallel pathways in the presence of inhibitors. Here, we provide a review of the

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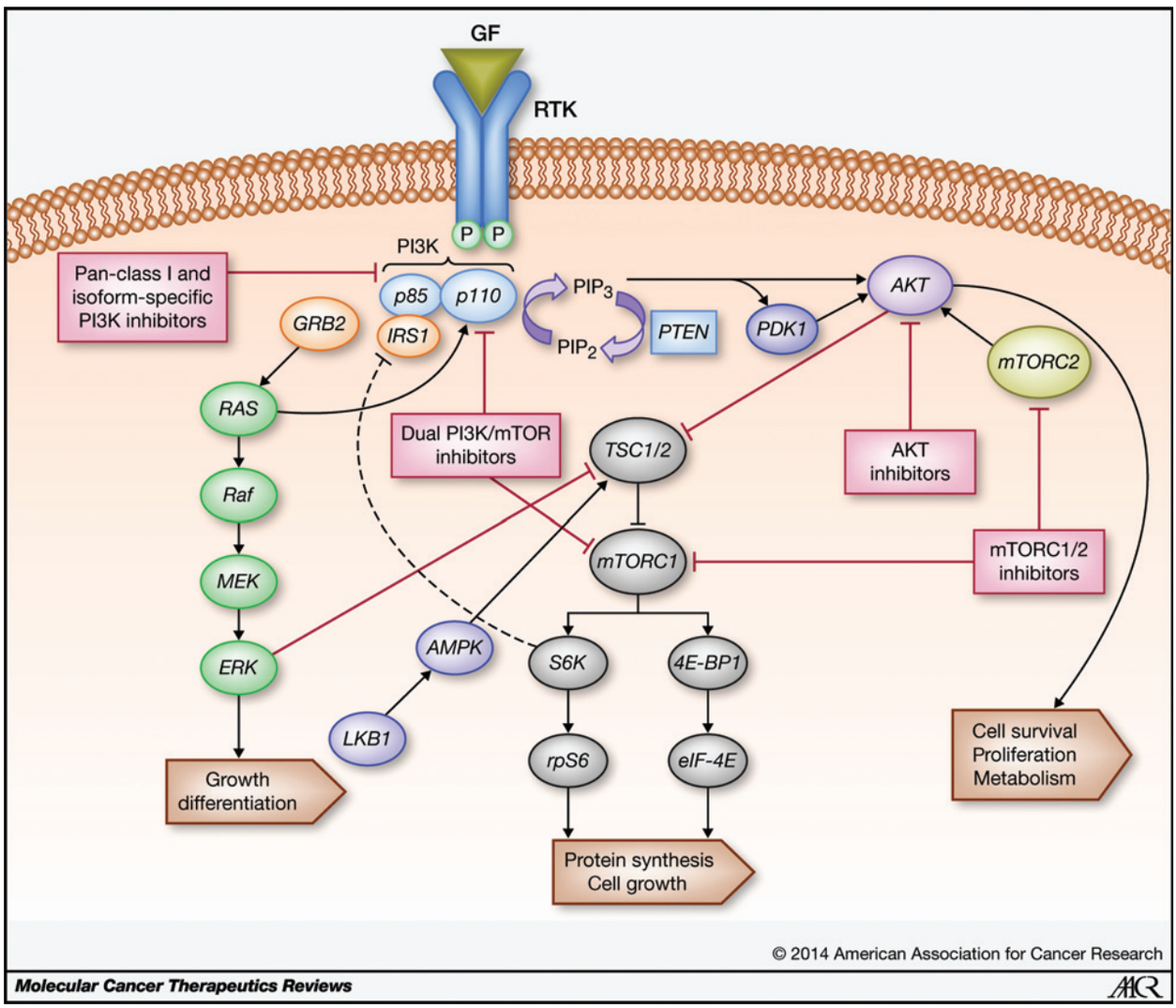

Figure 1. Overview of the PI3K/AKT/mTOR pathway and drug targets. Activating nodes (PI3K, AKT, PDK1, mTORC1 and mTORC2) and negative regulators (PTEN, TSC complex) are highlighted. Interaction with RAS and LKB1/AMPK pathways is also displayed. AMPK, AMP-dependent protein kinase; GF, growth factor; GRB2, growth factor receptor-bound protein 2; IRS1, insulin receptor substrate 1; PDK1, phosphoinositide-dependent kinase 1; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate.

literature, with the aim of categorizing the genomic contexts in which the various types of PI3K pathway inhibitors may have superior activity, with a view to establishing the best approach for subsequent investigations.

Dual PI3K/mTOR Inhibitors

PI3K and mTOR both belong to the PI3K-related kinases (PIKK) superfamily and share structural domains, and as a consequence, certain inhibitory compounds target both kinases (2). Dual inhibitors of PI3K and mTOR target the active sites of both holoenzymes, inhibiting the pathway both upstream and downstream of AKT, thus avoiding the problem of AKT activation following abolition of the mTORC1–S6K–IRS1 negative feedback loop, which is known to occur with rapalogs (3). Preclinical *in vitro* cell screenings with dual PI3K/mTOR inhibitors suggest a broader efficacy across more genotypes compared with agents targeting only one component of the pathway, with proapoptotic effects identified in a wider range of cell lineages than rapalogs (3, 4). Despite their lack of specificity for oncogenic p110α, there are several contexts in which dual PI3K/mTOR inhibitors may provide an advantage over more specific PI3K pathway inhibitors. Pan-PI3K inhibitors may fail to fully suppress tumors with alterations downstream of PI3K but upstream of mTOR (e.g., PTEN or TSC1/2; ref. 5). Such cancers may be more vulnerable to dual PI3K/mTOR inhibitors.

Tumors with alterations in PTEN

Neither of the two most studied pan-PI3K inhibitors, BKM120 and GDC-0941, has been shown to have preferential activity *in vitro* in tumor cells with PTEN alterations

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Table 1. A summary of PI3K/AKT/mTOR pathway inhibitors in clinical development

Agent (company)	Target	Phase[a]	Tumor types currently under investigation[a]
Everolimus (Novartis)	mTORC1	Approved	Approved for the treatment of renal cell carcinoma, subependymal giant cell astrocytoma associated with tuberous sclerosis, pancreatic neuroendocrine tumors, and ER+ breast cancer (in combination with exemestane)
Temsirolimus (Pfizer)	mTORC1	Approved	Approved for the treatment of renal cell carcinoma
BEZ235 (Novartis)	PI3K/mTOR	Phase II	Advanced solid tumors, breast cancer, castration-resistant prostate cancer, renal cell carcinoma, leukemias, pancreatic neuroendocrine tumors, urothelial transitional cell carcinoma
GDC-0980 (Genentech)	PI3K/mTOR	Phase II	Solid cancers, non-Hodgkin lymphoma, breast cancer, prostate cancer
PF-05212384 (Pfizer)	PI3K/mTOR	Phase I/II	Advanced solid tumors, colorectal cancer, endometrial neoplasms
SAR245409 (XL-765; Sanofi/Exelixis)	PI3K/mTOR	Phase II	Advanced solid tumors, CLL, indolent non-Hodgkin lymphoma, mantle cell lymphoma, ovarian cancer
BAY80-6946 (Bayer)	Pan-class I PI3K	Phase II	Advanced solid tumors, non-Hodgkin lymphoma
Buparlisib (BKM120; Novartis)	Pan-class I PI3K	Phase IV	Advanced solid tumors, breast cancer (ER+, HER2+, and HER2−), cervical cancer, colorectal cancer, endometrial cancer, esophageal cancer, GIST, glioblastoma, head & neck neoplasms, leukemias and lymphomas, melanoma, NSCLC, ovarian cancer, prostate cancer, renal cell carcinoma, urothelial transitional cell cancer
Pictilisib (GDC-0941; Genentech)	Pan-class I PI3K	Phase II	Breast cancer, NSCLC
PX-866 (Oncothyreon)	Pan-class I PI3K	Phase II	Advanced *BRAF*-mutant cancers, NSCLC, prostate cancer
SAR245408 (XL-147; Sanofi/Exelixis)	Pan-class I PI3K	Phase I/II	Advanced solid tumors
ZSTK474 (Zenyaku Kogyo)	Pan-class I PI3K	Phase I/II	Advanced solid tumors
BYL719 (Novartis)	PI3K p110α	Phase II	Advanced solid tumors (including those with *PIK3CA* alteration), breast cancer, colorectal cancer, esophageal cancer, gastrointestinal cancer, GIST, head & neck squamous cell cancer
GDC-0032 (Genentech)	PI3K p110α, δ, and γ inhibitor	Phase I	Advanced solid tumors and metastatic breast cancer (ER+)
MLN01117 (INK1117; Intellikine)	PI3K p110α	Phase I	Advanced solid tumors with *PIK3CA* mutation
GSK2636771 (GSK)	PI3K p110β	Phase I	Advanced solid tumors with PTEN deficiency
SAR260301 (Sanofi)	PI3K p110β	Phase I	Advanced solid tumors
Idelalisib (CAL-101; GS-1101; Gilead/Calistoga)	PI3K p110δ	Phase III	CLL, lymphomas

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Table 1. A summary of PI3K/AKT/mTOR pathway inhibitors in clinical development (Cont'd)

Agent (company)	Target	Phase[a]	Tumor types currently under investigation[a]
AMG319 (Amgen)	PI3K p110δ	Phase I	Hematologic malignancies
Perifosine (KRX-0401; Keryx)	AKT	Phase I/II	Advanced solid tumors, multiple myeloma
MK2206 (Merck)	AKT	Phase II	Advanced solid tumors, breast cancer, colorectal cancer, endometrial cancer, head & neck cancer, lung cancer, lymphomas, pancreatic cancer, prostate cancer
GDC-0068 (Genentech)	AKT	Phase II	Advanced solid tumors, gastric cancer, prostate cancer
GSK2110183 (GSK)	AKT	Phase II	Advanced solid tumors, CLL, multiple myeloma, ovarian cancer
GSK2141795 (GSK)	AKT	Phase II	Advanced solid tumors, breast cancer, cervical cancer, endometrial cancer, leukemias, melanoma, multiple myeloma
ARQ 092 (ArQule/Daiichi Sankyo)	AKT	Phase I	Advanced solid tumors
AZD5363 (AstraZeneca)	AKT	Phase I/II	Advanced solid tumors, breast cancer, prostate cancer
AZD2014 (AstraZeneca)	mTORC1/2	Phase II	Advanced solid tumors, breast cancer, renal cell carcinoma
MLN0128 (INK128; Intellikine)	mTORC1/2	Phase I	Advanced solid tumors, hematologic malignancies
CC-223 (Celgene)	mTORC1/2	Phase I/II	Breast cancer, glioblastoma, hematologic malignancies, liver cancer, NSCLC, neuroendocrine tumors

Abbreviations: CLL, chronic lymphocytic leukemia; ER+, estrogen receptor–positive; GIST, gastrointestinal stromal tumor; NSCLC, non–small cell lung cancer.

[a]Phase of development, based on trials that were listed in ClinicalTrials.gov as enrolling patients, or yet to be enrolling patients, as of November 2013.

(6, 7), and no clinical responses with these compounds have been observed in patients with PTEN-altered tumors in single-agent phase I trials (8, 9). In contrast, 1 of the 2 patients who experienced a partial response in the phase I single-agent trial of BEZ235 had non–small cell lung cancer (NSCLC) with *PTEN* mutation (10). Although these preliminary data are few in number, it can be hypothesized that mTOR inhibition may be required for optimum cell growth inhibition in cells with PTEN loss. A pilot study of sirolimus (rapamycin) for the treatment of Cowden syndrome, a rare genetic disorder associated with mutations in *PTEN*, demonstrated improvements in symptoms and reductions in skin/gastrointestinal tumor lesions, thus validating the efficacy of mTOR inhibition in PTEN-deficient contexts (11).

Malignancies arising from *TSC1/2* alterations

Tuberous sclerosis complex (TSC) is a rare inherited disorder caused by mutations in *TSC1/2* and characterized by multisystemic lesions (12). Everolimus was approved by the U.S. Food and Drug Administration in 2012 for the treatment of subependymal giant cell astrocytoma associated with tuberous sclerosis (13). Since dual PI3K/mTOR inhibitors also target mTORC1, which lies downstream of TSC, efficacy might also be expected from such agents.

Deletions of *TSC1* or *TSC2* are generally rare in the cancer population as a whole, but are reported in certain isolated tumor types. More than 50% of transitional cell carcinomas of the bladder show loss of heterozygosity in a region spanning the *TSC1* locus, and missense mutations have been identified in 15% of these tumors (14). In a small analysis of 14 patients with metastatic bladder cancer who were treated with everolimus as part of a phase II trial, nonsense mutations in *TSC1* were found to be associated with longer duration of treatment (7.7 months vs. 2.0 months for TSC wild-type) and significant improvement in time to recurrence (4.1 vs. 1.8 months; ref. 15). This suggests that TSC mutations may potentially help predict sensitivity to agents targeting mTOR in certain settings.

Tumors with *STK11/LKB1* alterations

Nearly one-third of NSCLC samples harbor inactivating alterations of *STK11/LKB1* (16), which are known to frequently coexist with *KRAS* mutations, and are thought to play an important role in progression to metastasis (17, 18). Coexisting *STK11/LKB1* and *KRAS* mutations sensitize NSCLC cells to either mTOR or MEK inhibition (18), and evidence from xenograft experiments also supports the use of dual PI3K/mTOR inhibition in this context (4). Mouse models have demonstrated that LKB1 loss can also cooperate with PTEN loss to drive the tumorigenesis of a

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range of malignancies, including intestinal polyps, lymphoma, prostate cancer, and breast cancer (19). Pan-PI3K inhibitors and mTORC1/2 inhibitors have each been shown to instigate temporary tumor regression in mice with concurrent PTEN/LKB1 deficiencies; however, the effect of combining PI3K and mTOR inhibition, either together, or with other forms of inhibition, has yet to be investigated (20).

Pan-PI3K Inhibitors

Since the majority of PI3K pathway inhibitors are in early clinical development, there is a lack of clinical data comparing the efficacy and toxicity of pan-PI3K and dual PI3K/mTOR inhibitors. Initial data from phase I trials suggest that pan-PI3K and dual PI3K/mTOR inhibitors share similar toxicity profiles, with the exception of pneumonitis and mucositis/stomatitis, which are known class effects of rapalogs that have been most frequently reported with dual PI3K/mTOR inhibitors (8, 9, 21, 22). It may be possible that the greater specificity of pan-PI3K inhibitors (and their presumably wider therapeutic window) may make them more amenable to being combined with certain targeted or cytotoxic therapies, relative to those that also target mTORC1/2. As evidence of this, at the time of writing, the ClinicalTrials.gov database contains 9 combination trials of PI3K/mTOR inhibitors with chemotherapy, compared with 19 combination trials with chemotherapy for pan-PI3K inhibitors.

Pan-PI3K inhibitors may show improved potential for synergy with antimitotic therapies versus mTOR inhibitors

As allosteric mTOR inhibitors target the pathway downstream of AKT, they do not have a strong proapoptotic effect, and may therefore be less likely to synergize with agents such as paclitaxel. Although clinical trials of everolimus plus paclitaxel have not met expected endpoints (23), it is not yet certain whether pan-PI3K inhibitors will demonstrate improved synergy with antimitotic agents, compared with agents that target mTOR. Encouraging clinical data suggest that addition of BKM120 to paclitaxel may reverse resistance to this chemotherapeutic agent (24). In mouse xenograft models, combined treatment with GDC-0941 and docetaxel has demonstrated that pan-PI3K and antimitotic agents show synergy *in vivo* (25).

The efficacy of pan-PI3K inhibitors may not be restricted to tumors harboring *PIK3CA* mutations

The four different catalytic isoforms of class I PI3K (p110α, p110β, p110δ, and p110γ) are known to preferentially mediate signal transduction and tumor cell survival, depending upon the type of malignancy and the genetic or epigenetic aberrations it harbors (26). For example, p110α is essential for the signaling and growth of tumors driven by *PIK3CA* mutations and/or oncogenic receptor tyrosine kinases and RAS, whereas p110β is thought to be the major isoform mediating tumorigenesis arising from PTEN loss (26). By inhibiting all four PI3K isoforms, pan-PI3K and dual PI3K/mTOR inhibitors may be better suited to treating PI3K pathway–activated tumors associated with heterogeneous molecular alterations. Examples include triple-negative breast cancer, prostate, and endometrial cancer, which may harbor multiple simultaneous PI3K alterations (27, 28).

Cell lines with *PIK3CA* mutation (but not PTEN loss) demonstrate increased sensitivity to pan-PI3K inhibitors *in vitro*; however, these biomarkers have not been strongly confirmed in the clinic (6, 7). In early-phase studies with pan-PI3K inhibitors, responses and prolonged stable disease have been observed in patients both with and without *PIK3CA* mutation (8, 9). Tumors lacking *PIK3CA* mutation may still exhibit pathway activation driven by molecular alterations in other pathway components (e.g *HER2* amplification, *PIK3R1* or *PIK3R2* mutations; ref. 29), which may also sensitize tumors to treatment. Despite being described as pan-PI3K inhibitors, most agents in this class demonstrate far greater inhibitory potency against p110α than p110β *in vitro* (Table 2). Observations of clinical efficacy with pan-PI3K inhibitors in *PIK3CA*-mutant cancer, but not in tumors with PTEN loss (8, 9), could therefore be directly linked to the reduced affinity of these agents for p110β.

Isoform-Specific PI3K Inhibitors

Isoform-specific PI3K inhibitors have been developed with the aim of targeting specific alterations in the PI3K pathway, while avoiding the cumulative toxicity of inhibiting multiple isoforms. The availability of isoform-specific agents presents the interesting possibility of combining these agents with inhibitors of other pathway components. Theoretically, inhibiting multiple pathway components with a combination of separate agents may offer greater opportunities to customize therapy than single agents with multiple targets (e.g., dual PI3K/mTOR inhibitors). For example, using individual inhibitors would enable optimal dosing and scheduling of each agent.

The selectivity of isoform-specific PI3K inhibitors, coupled with the high doses at which they can be given, has two implications. First, the high specificity of these agents implies that they may be particularly active in tumors with certain types of molecular alterations. Second, profound inhibition of a single isoform of PI3K is likely to change the toxicity profile relative to what has been observed with pan-isoform inhibition.

p110α inhibitors: higher activity in *PIK3CA*-mutant tumors but reduced efficacy in PTEN-null and *PIK3CA* plus *KRAS*-mutant tumors?

Experimental characterization of p110α inhibitors has revealed distinct patterns of sensitivity and resistance. In a large panel of cancer cell lines exposed to BYL719, cells with *PIK3CA* alterations (mutation or amplification) or *HER2* amplification were found to be more likely to be sensitive, whereas *PTEN* and *BRAF* mutation, and concurrent *PIK3CA* and *KRAS* mutations were

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Table 2. Reported potencies of dual PI3K/mTOR and pan-PI3K inhibitors against the 4 class I isoforms of PI3K

	IC_{50} (nmol/L)				
Inhibitor (ref.)	**PI3Kα**	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**mTOR**
BEZ235 (78)	4	75	5	7	21
GDC-0980 (4)	5	27	14	7	17
GSK2126458 (79)	0.019	0.13	0.6	0.0024	mTORC1: 0.18 mTORC2: 0.3
PF-05212384 (80)	0.4	6	6 (81)	8	1.6
SAR245409 (XL-765) (81)	39	113	9	43	mTORC1: 190 mTORC2: 908
Buparlisib (BKM120) (82)	52	166	262	116	4610
SAR245408 (XL-147) (83)	39	383	23	36	>15,000
Pictilisib (GDC-0941) (84)	3	33	75	3	580
BAY80-6946 (85)	0.5	3.7	6.4	0.7	>1,000
PX-866 (86)	39	88	183	124	>30,000

Abbreviations: IC_{50}, half maximal inhibitory concentration; mTORC, mammalian target of rapamycin complex.

associated with resistance (30). Disregarding genotype, sensitive cell lines included HER2-positive and luminal breast cancer. In similar experiments with the novel inhibitor INK1402, selective p110α inhibition was also found to be significantly more effective in *PIK3CA*-mutated cell lines, compared with those with mutated or absent PTEN (80%–100% growth inhibition vs. 50%–60%, respectively; ref. 31).

Clinical trials with BYL719 and GDC-0032 (a p110β-sparing inhibitor) have confirmed the potential activity of these agents in tumors with *PIK3CA* mutations. In the phase I trial of BYL719, which only recruited patients with advanced *PIK3CA*-mutant cancers ($N = 75$), 7 partial responses were reported in the multiple tumor types, including estrogen receptor-positive (ER^+) breast cancer, gynecologic malignancies, and head and neck cancers (32). Enrollment of patients into the phase I trial of GDC-0032 was not dependent upon *PIK3CA* status; however, among the 34 patients treated, confirmed partial responses were reported exclusively in patients with *PIK3CA*-mutant breast cancer and NSCLC (33).

p110β inhibitors: necessary and sufficient for controlling PTEN-null tumors?

Although p110α inhibitors are likely to show benefit in *PIK3CA*-mutant malignancies, tumors with PTEN loss may be better suited to treatment with p110β-specific inhibitors, agents that also target mTOR (as discussed above), or AKT inhibitors (see below). Specific inhibitors of p110β typically show far greater potency against PTEN-deficient cell lines than *PIK3CA*-mutant lines, both *in vitro* and *in vivo* (34, 35). However, *in vitro* experiments comparing the various classes of PI3K inhibitor suggest that simultaneous p110α and p110β inhibition (or in some circumstances complete blockade of all class I PI3K activity) may be required for optimal growth inhibition in certain PTEN-deficient malignancies, including endometrial cancer and lymphoblastic leukemia (36–38). Such observations may point to a redundancy in isoform-specific signaling, which suggests that in certain contexts, inhibition of one PI3K isoform may be offset by the increased activity of others.

Two p110β inhibitors are currently in early clinical development, GSK2636771 and SAR260301 (39). Information on this class of inhibitor is only just starting to emerge, so it may be some time before it becomes clear whether p110β-specific inhibitors show greater or equivalent efficacy to other PI3K/mTOR inhibitors or AKT inhibitors in the treatment of PTEN-negative tumors.

p110δ inhibitors versus pan-PI3K and dual PI3K/mTOR inhibition in the treatment of hematologic malignancies

The PI3K/AKT/mTOR pathway is a validated target for inhibition in hematologic malignancies and the mTOR inhibitor temsirolimus has been approved in Europe for the treatment of relapsed/refractory mantle cell lymphoma since 2009 (40). Novel agents that target the PI3K pathway are being pursued in the hope of improving on the response rates already demonstrated with rapalogs. Since the p110δ and p110γ catalytic isoforms of PI3K are highly enriched in leukocytes, they are particularly desirable targets for inhibition in the treatment of hematologic malignancies.

The p110δ inhibitor GS-1101 (CAL-101) is the most extensively studied isoform-specific PI3K inhibitor and is currently being investigated in phase III trials for non-Hodgkin lymphoma (NHL) and chronic lymphocytic leukemia (CLL). Objective response rates were seen in about 50% of the patients with relapsed or refractory B-cell malignancies (41, 42). Although GS-1101 shows great

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potential as a candidate therapy for CLL and NHL, some recent studies with experimental models have suggested that pan-class I PI3K inhibition could offer broader activity in a variety of hematologic malignancies (43–45). Such observations should be treated with caution: although GS-1101 demonstrated moderate activity during preclinical development, it has shown impressive activity in the clinical setting, which has been attributed to its action on the tumor microenvironment, as opposed to its activity within leukemia and lymphoma cells themselves (46). Moreover, while the *in vivo* and *in vitro* activity of p110α- and p110β-targeting inhibitors has been clearly associated with cell lines harboring specific genomic alterations, hematologic malignancies typically acquire PI3K activation by other means.

Inhibitors that target all four isoforms of PI3K remain relatively untested in hematologic malignancies. Early-phase studies with the pan-PI3K inhibitor SAR245408 (relapsed/refractory CLL and lymphoma) and PI3K/mTOR inhibitor SAR245409 (relapsed/refractory lymphoma) are ongoing, but early data suggest modest efficacy, compared with GS-1101 (47, 48). Additional clinical data are needed to compare the safety and efficacy of pan-PI3K/mTOR inhibitors and p110δ-specific inhibitors in hematologic malignancies.

Toxicity profile: hyperglycemia and immunologic side effects vary according to the specific PI3K isoform being targeted

As p110δ is predominantly expressed in the immune system, agents lacking specificity for this isoform may avoid immunomodulatory side effects. Experimental evidence supporting this hypothesis stems from investigations with p110δ-deficient mice, which demonstrate a variety of impaired immune mechanisms (49). An early-phase trial of GS-1101 in CLL reported immune-related side effects, including grade ≥3 pneumonia (24% of patients), neutropenia (24%), and neutropenic fever (7%; ref. 50). In contrast, early results from the phase I trials of BYL719 and GDC-0032 support the hypothesis that p110α inhibitors are less likely to exhibit immunomodulatory effects, with less than 10% of patients reporting any form of immune-related adverse event of any grade (33, 51). Clinical data also show that immunologic adverse events occur infrequently with pan-PI3K inhibitors, although individual cases of grade 3 neutropenia and grade 3 thrombocytopenia were reported (8, 9).

The safety profile of single-agent p110α inhibitors appears to be consistent with that of pan-class I inhibitors; however, high rates of hyperglycemia have been reported (33, 51). Nearly 50% of patients treated with BYL719 in the phase I trial experienced at least one hyperglycemic event; however, these events were manageable and reversible (51). Of the four PI3K isoforms, p110α is known to be the primary intracellular mediator of insulin response via its interactions with insulin receptor substrate (IRS), an adaptor protein that facilitates insulin-like growth factor 1 receptor and leptin action (52). Although p110β has also been associated with insulin signaling, pharmacologic inhibition of the catalytic function of p110β does not appear to affect glucose metabolism in mouse models (53).

AKT Inhibitors

Advances in drug design have seen the development of allosteric and catalytic AKT inhibitors, which are currently being investigated in clinical studies. Early-phase single-agent trials with these agents have generally shown antiproliferative, rather than antitumor activity, with stable disease identified as the best overall response (54, 55). However, data from combination trials with chemotherapy suggest that tumor shrinkage can be achieved at tolerable dose levels (56).

AKT-specific inhibitors may show greater potency in tumors with PTEN alterations than PI3K-specific inhibitors

Experimental models strongly suggest that sensitivity to AKT-specific inhibitors is dependent upon activation of the PI3K/AKT/mTOR pathway (57). Interestingly, catalytic AKT inhibitors (e.g., GDC-0068 and AZD5363) have inhibitory effects in cell lines with *AKT1* mutations (E17K) and *AKT3* fusions, whereas allosteric inhibitors (e.g., MK-2206) do not (58, 59). MK-2206, AZD5363, and GDC-0068 have all demonstrated increased activity in cell lines with *PIK3CA* or PTEN alterations (60–62). This may imply that there is a stronger rationale for AKT inhibitors in tumors with PTEN alterations than pan-PI3K inhibitors.

Early-phase trials with MK-2206 and GDC-0068 support the hypothesis that AKT inhibitors will be at their most potent in tumors with PTEN deficiency. Tumor shrinkage was reported in PTEN-deficient pancreatic and colorectal cancer (54, 56). Although PTEN loss appears to be the strongest indicator of sensitivity to AKT inhibitors, clinical evidence suggests that AKT inhibitors could also benefit patients with *PIK3CA*-mutant tumors. For example, in the phase I trial of single-agent GDC-0068, the patient with greatest benefit had *PIK3CA*-mutant colorectal cancer (55). Most recently, in phase I trials of single-agent AZD5363, clinical responses were observed in *PIK3CA*-mutated cervical and endometrial cancer (63). In conclusion, this may suggest that AKT inhibitors could be indicated in tumors with either PTEN loss or *PIK3CA* mutation.

mTORC1/2 Inhibitors

The clinical development of catalytic mTORC1/2 inhibitors follows that of their rapalog counterparts, with agents from this class being investigated in metastatic breast cancer, renal cell carcinoma, and lymphomas (Table 1). Dual mTORC1/2 inhibitors aim to improve on rapalogs by inhibiting both mTORC complexes, thus eliminating the activation of AKT by mTORC2 (Fig. 1). The differential antiproliferative and proapoptotic effect seen in preclinical experiments with mTORC1/2 inhibitors compared with allosteric mTORC1 inhibitors has

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been partly attributed to the profound inhibition of 4E-BP1, which is well known to be resistant to rapamycin inhibition, and could be an important differentiating property between these two types of inhibitors (64, 65).

As dual mTORC1/2 inhibitors are catalytic (as opposed to allosteric) inhibitors of mTORC1, they are likely to have differing inhibitory potency compared with rapalogs. For example, the dual mTORC1/2 inhibitor AZD2014 demonstrates greater inhibitory activity against mTORC1 than rapamycin (66). Nonetheless, MLN0128 and rapamycin had equivalent therapeutic benefit in a mouse model of tuberous sclerosis; both agents were only capable of instigating transient inhibition of tumor development, which could imply that these agents are susceptible to similar resistance mechanisms in certain malignancies (67). Importantly, first-in-man trials with mTORC1/2 inhibitors have identified single-agent activity apparently higher than that seen previously with rapalogs. Confirmed partial responses with AZD2014 and CC-223 were seen in patients with NSCLC, hepatocellular carcinoma, and ER+ breast cancer (68–70). Emerging clinical data also suggest that CC-223 may have clinical relevance in diffuse large B-cell lymphoma; 3 of 17 patients achieved a partial response in the single-agent trial (71). Genomic profiling of the tumors of responding patients has not yet been reported.

PI3K/AKT/mTOR Pathway Inhibitors as Combination Therapy

Experiments across multiple tumor types have found associations between maintained PI3K signaling and resistance to both targeted and cytotoxic therapies (72). A host of preclinical laboratory studies across a diverse range of cancer models also suggests that PI3K pathway inhibition can serve to reverse resistance to certain therapies, when given as part of combination therapy. The most convincing argument for using PI3K pathway inhibitors as part of combination therapy comes from phase III trials with everolimus. In the BOLERO-2 trial, the combination of everolimus and exemestane significantly improved median progression-free survival (PFS) to 7.8 months compared with 3.2 months with exemestane alone, in women with advanced ER+ breast cancer (73). In BOLERO-3, the combination of everolimus, trastuzumab, and vinorelbine, improved PFS to 7.0 months, compared with 5.8 months, in patients with $HER2^+$ advanced breast cancer (74). Ongoing trials with PI3K pathway inhibitors are investigating rational therapeutic combinations in resistant malignancies including: (i) hormonal therapies in castration-resistant prostate cancer and advanced ER+ breast cancer; (ii) HER family tyrosine kinase inhibitors in HER2+ breast cancer and EGFR-mutant NSCLC; (iii) PARP or MEK inhibitors in ovarian cancer and NSCLC; (iv) BRAF or MEK inhibitors in BRAF-mutant melanoma; and (v) cytotoxic chemotherapies in aggressive malignancies such as glioblastoma and triple-negative breast cancer (Table 1).

Conclusion

Following this review of literature comparing the multitude of different inhibitors targeting the PI3K/AKT/mTOR pathway, several broad themes of differentiation emerge.

1. Dual PI3K/mTOR inhibitors appear to have the broadest activity profile, as they target the pathway at multiple points. Their downstream effects on mTOR appear to be important in some genomic contexts, such as loss-of-function in the negative regulators PTEN, TSC1/2, and STK11. However, multikinase blockade may lead to increased toxicity, suggesting that this class of agents may not be as well suited to combination therapy as other agents. This inhibitor class may therefore be the most appropriate choice for evaluation as a single-agent, especially in understudied malignancies, or in tumors associated with broad heterogeneous genetic abnormalities. Clinical validation is also underway to establish whether dual PI3K/mTOR inhibitors or mTORC1/2 inhibitors will have improved activity in tumor types that are known to respond to single-agent rapalog therapy.
2. Pan-PI3K inhibitors may be better suited to combination therapy than dual PI3K/mTOR inhibitors, but demonstrate a narrower activity profile. On the basis of early clinical evidence, agents that target all 4 isoforms of PI3K equally (i.e., either pan-PI3K inhibitors or dual PI3K/mTOR inhibitors) may show greater activity than isoform-specific inhibitors in tumor types that lack *PIK3CA* mutations.
3. Isoform-specific PI3K inhibitors have the narrowest activity profile among the agents described here and may require careful patient selection based upon potential biomarkers of sensitivity or resistance. For instance, tumor types characterized by high rates of either PTEN or KRAS alterations may be particularly unsuitable for evaluation with p110α inhibitors. Despite this potential limitation, response rates in phase I trials with p110α- and p110δ-specific inhibitors have exceeded those seen with pan-PI3K or dual PI3K/mTOR inhibitors. It remains to be determined whether the activity of p110α inhibitors will vary according to specific *PIK3CA* mutation (e.g., H1047R vs. E545K). Clinical data with p110β inhibitors in trials with PTEN-deficient tumors are eagerly awaited.
4. Preclinical evidence with second-generation AKT inhibitors suggests that these agents may show particularly interesting activity in tumors with PTEN loss. This is in contrast with experimental investigations with PI3K-targeted therapies, which have generally failed to identify an association between PTEN alterations and increased sensitivity. Initial data from early-phase trials support this

hypothesis, with clinical benefit observed in cancers with either PTEN loss or *PIK3CA* mutations.

5. Dual mTORC1/2 inhibitors have demonstrated apparently greater single-agent activity than rapalogs in early-phase trials in advanced solid cancers; however, genomic profiling of responsive tumors has not been reported to date, and it is currently unclear whether molecular correlates of response will be established.

Further evaluation of PI3K/AKT/mTOR pathway inhibitors is required to confirm whether the patterns of sensitivity observed in preclinical studies can be applied in the clinic. Although many early-phase trials have independently failed to identify a distinct association between clinical response and the most common alterations in the PI3K pathway (*PIK3CA* mutation and PTEN loss), a pooled analysis of 140 patients with various breast and gynecologic cancers treated with different PI3K/AKT/mTOR inhibitors identified an increased rate of RECIST-defined clinical responses among patients with *PIK3CA* mutations (75). A follow-up study pooling 1,012 patients with diverse cancers treated with PI3K/AKT/mTOR pathway inhibitors also identified an increased rate of response among tumors with *PIK3CA* H1047R mutation, but not those with other *PIK3CA* mutations or concurrent *PIK3CA* and *KRAS* mutations (76). The findings of these association studies have sparked interest in the research community; however, additional work is required to confirm predictive biomarkers of sensitivity and resistance to each agent in large scale trials with homogenous patient populations. Studies of biomarkers will have to cope with many challenges, such as observations of discordancy between primary and metastatic lesions, and intratumoral heterogeneity in molecular alterations (28). Thankfully, the emergence of noninvasive technologies, such the analysis of circulating free DNA and tumor cells (77), is set to improve the acquisition of samples both directly before and after treatment, which will hopefully help elucidate mechanisms of acquired resistance to these agents—a question that has yet to be answered. Future studies may also benefit from more comprehensive analyses of the entire PI3K/AKT/mTOR pathway, which could be achieved using high-throughput technologies such as next-generation sequencing and phosphoproteomic analyses.

In conclusion, the recent explosion in the number of PI3K/AKT/mTOR pathway inhibitors under clinical investigation is testimony to the key role of the pathway in cancer cell survival. New classes of PI3K pathway inhibitor are continuing to emerge (e.g., PDK1 inhibitors) and many of the compounds are now showing promise when used as part of combination therapy with other targeted agents. With so many therapies in development, a concerted effort to distinguish these agents, both in the laboratory and in the clinic, is warranted.

Disclosure of Potential Conflicts of Interest

J. Tabernero is a consultant/advisory board member for Novartis, Millennium, Genentech, Pfizer, Amgen, Merck-Serono, Roche, and Sanofi. No potential conflicts of interest were disclosed by the other authors.

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Picking the Point of Inhibition: A Comparative Review of PI3K/AKT/mTOR Pathway Inhibitors

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