

Drug Development Spotlight: The mTOR's New Clothes?



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In 1837, Hans Christian Andersen authored a short tale titled *The Emperor's New Clothes*. The main character, so enamored by his appearance and his clothing that he had a different suit for every hour of the day, was swindled by a pair of weavers purporting that they could create clothing from a magical fabric that would only be visible to those who were completely pure in heart and spirit. However, when the Emperor parades before his subjects in the new outfit, a child cries out "But he isn't wearing anything at all!" The Emperor had no clothes.

The tale seems fitting to illustrate the evolution of drugs that target the phosphatidylinositol 3-kinase [PI3K] pathway [see Table 2 for a listing of compounds in clinical development]. Despite ample evidence that pan-PI3K inhibitors and dual PI3K/mTOR inhibi-

tors might offer a therapeutic advantage, tailors continue to weave new compounds targeting individual components of the pathway with presumably superior properties. But does the "mTOR" really have new clothes?

Pathway Layout

The PI3K pathway regulates cell growth, survival, proliferation, migration, and the process of angiogenesis and is frequently deregulated in cancer, which makes it one of the most attractive targets for anticancer therapy. Big pharma's interest in the target is evidenced in part by Sanofi-aventis' (SNY) licensing of two early-stage PI3K inhibitor programs [XL147 and XL765] from Exelixis, Inc. (EXEL) in May 2009 that could result in development, regulatory and commercial milestone payments to the company that total over

\$1 billion in the aggregate [including \$140 million in cash upfront], as well as royalties on sales of any products commercialized under the license.

In general, the pathway comprises the following three components starting near the cell membrane and continuing towards the nuclear machinery at the heart of cellular processes:

1. PI3K

- Held in check by the phosphatase PTEN, PI3K can be activated by upstream tyrosine kinase receptors
- Four class I isoforms of PI3K [α , β , γ , δ , or alpha, beta, gamma, delta]

2. Akt

- Gets recruited to the proper location in the cell needed for activity [cell membrane] and is changed into the required active conformational state by phosphorylation of T308 by the action of PI3K

3. mTOR

- Promotes increased protein synthesis in part driven by activated Akt
- Forms complexes called mTORC1 and mTORC2, of which mTORC2 directly increases Akt by phosphorylation on S473

Dysfunction of PI3K, Akt, and/or mTOR is associated with cancer and while cellular signaling becomes more complex on almost a daily basis, much has been discovered about the best way to effectively block the pathway in cancer cells. Accordingly, the purpose of this article is to highlight some of the latest advances in our understanding of the PI3K pathway along with the leading companies working in this market segment.

Good, Better, and Best

Pfizer, Inc.'s (PFE) Torisel® [temsirolimus] and Novartis AG's (NVS) Afinitor® [everolimus], both for the treatment of renal cell carcinoma, were among the first PI3K pathway inhibitors [via inhibition of mTORC1] to reach the market – Torisel in May 2007 and Afinitor in March 2009. While inhibition of mTORC1 through rapamycin or the rapalogs ["Good"] demonstrated

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Table 1. Adverse Event Profiles as Reported at 2010 ASCO Annual Meeting

Drug	SF1126	XL765	XL765	GDC-0980	BEZ235	XL147	GDC-0941*	PX-866	BKM120	CAL-101
Target	PI3K/mTOR	PI3K/mTOR	PI3K/mTOR	PI3K/mTOR	PI3K/mTOR	PI3K	PI3K	PI3K	PI3K	PI3K delta
Route	IV	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral
Freq.	BIW	BID	QD	QD	QD	QD	QD	QD*	QD	BID/QD
Nausea	+	++	+	+	+	+	+	+	+	n/r
Diarrhea	++	+	+	+	+	+	+	++	+	n/r
Fatigue	+		++	+	+	+	+	+		n/r
Vomiting	+	++	+		+	+	+	+		n/r
Rash		++	++	+		++			+	n/r
Elevated AST/ALT		++	+				+	++		++

+ = Adverse event listed among the top five most frequent Grade 1 or 2 in the trial

++ = Dose limiting toxicities

* = For GDC-0941, results are from GDC4254g study; for PX-866, AST/ALT toxicity is only in the continuous daily dosing arm

n/r = Grade 1 and 2 data has not been reported

sufficient clinical activity for U.S. Food and Drug Administration [FDA] approval, there is clear evidence that blocking only mTORC1 activity paradoxically leads to activation of the PI3K pathway through redundant or alternative signaling mechanisms. For example, mTORC2 can activate Akt by phosphorylation on the S473 position. This led to the design of mTOR complex catalytic site inhibitors [“Better”] that block the activity of both mTORC1 and mTORC2. While effective in shutting down mTOR activity, this approach still provides for partial activation of Akt on T308 by PI3K. Therefore, simultaneous inhibition of both PI3K and mTOR kinase activity with a dual PI3K/mTOR inhibitor [“Best”] would be expected to more effectively shut down PI3K-Akt-mTOR signaling and such an agent could remain effective in situations where the activity of mTOR inhibition has been circumvented.

Isoform Selectivity: Is Less Really More?

In addition to the benefits of dual PI3K/mTOR inhibition as described in the prior section, there is also compelling biological rationale for inhibiting all four of the class 1A PI3K isoforms [α , β , γ , δ , or alpha, beta, gamma, delta] rather than inhibiting only a subset. The most compelling support for pan-PI3K inhibition is the recent disclosure that the activity of any class 1A PI3K isoform [alpha, beta, or delta] can sustain cell proliferation and survival [ref 1]. Additionally, both in vitro and in vivo studies indicated that for PTEN-negative tumors inhibition of the beta isoform is needed [ref 2]. Moreover, evolving analyses of cancer tissues provides additional rationale for inhibiting the various isoforms as for example the recent finding that the gamma isoform has tumor-specific overexpression in pancreatic cancer [ref 3].

The role of PI3K in a wide range of normal biologic processes raised potential toxicity concerns about pan-PI3K inhibitors and dual PI3K/mTOR inhibitors, which led to the development of isoform-selective inhibitors. However, clinical data presented at the 2010 American Society of Clinical Oncology [ASCO] annual meeting demonstrated relatively consistent toxicity profiles among pan-PI3K, dual PI3K/mTOR, and isoform-selective PI3K inhibitors, with no discernable safety advantage among the class [see Table 1 below]. The most common side effects reported with these inhibitors included diarrhea, nausea, vomiting, and fatigue [ref 8]. Liver damage, as evidenced by elevated aspartate aminotransferase [AST] and alanine aminotransferase [ALT] levels, were reported only with orally administered pan-PI3K, dual PI3K/mTOR, and PI3K delta isoform-specific inhibitors and were dose limiting in some cases. Interestingly, insulin resistance [hyper-



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insulinaemia or hyperglycaemia] was originally predicted to be one of the most likely toxicities resulting from on-target effects of PI3K inhibitors, but has not been widely observed in clinical trials to date.

Helping explain the lack of variation between pan-PI3K, dual PI3K/mTOR, and isoform-selective PI3K inhibitor toxicity profiles is the translation of data from in vitro potency to in vivo settings. For example, while Calistoga Pharmaceuticals' (private) CAL-101 product candidate demonstrates relative selectivity for the PI3K delta isoform using traditional two-dimensional [2D] monolayers of cancer cells, the significant blood levels seen clinically suggest that all isoforms may be inhibited at least part of the time. In addition, in a PTEN-null PC3 xenograft model Roche Holding AG's (RHHBY.PK) PI3K inhibitor GDC-0941 at 75mg/kg daily [ref 4] showed similar inhibition of about 80% of tumor growth as Semafore Pharmaceuticals' (private) dual PI3K/mTOR inhibitor SF1126 at 20mg/kg three times per week [ref 5] even though SF1126 is reported to be at least 10-times less potent on all PI3K isoforms. Two recent publications help further support the dramatic differences between 2D and 3D cell cultures and perhaps shed some light on how potency translates, or fails to translate, into in vivo models [refs 6,7].

Future Directions

Prodrugs

One of the most widely studied PI3K inhibitors, LY294002 possesses a unique mechanism of drug action through dual inhibition of all Class 1 PI3K isoforms and mTOR, inhibition of additional cancer kinases such as PIM1, DNA-PK, and PLK1, and the molecule's ability to induce apoptosis and oxidative stress through other mechanisms. However, the strong hydrophobicity of LY294002 drastically limits its use in natural form. In addition, there is the aforementioned concern for toxicity through the non-specific, indiscriminate inhibition of the

PI3K pathway in normal cells. Therefore, Semafore Pharmaceuticals sought to improve the use of LY294002 by enhancing its solubility, selectivity and in vivo delivery by preparing a functional prodrug that selectively accumulates in tumor areas to maximize efficacy and minimize toxicity. The resulting new chemical entity, SF1126, has been tested in more than 50 patients in Phase I trials.

Disease Settings and Biomarkers

In general, the standard paradigm for early drug development is to test compounds in a broad range of cancers to identify those in which the compounds work, which then forms the basis for future clinical development and regulatory strategy. Such has been the case with development of PI3K inhibitors.

Across the nine mixed solid tumor Phase I studies reported at ASCO 2010, 114 out of 469 patients [24%] showed stable disease, prolonged in some cases, as the best response [ref 8]. Only 5 partial responses out of 469 patients [1%] of unknown duration were reported from the group in total. Of these 3 were in breast cancer patients, one was in non-small cell lung carcinoma [NSCLC], and one was in a patient with lung cancer/Cowden disease. On this basis, there is no clear direction for development of PI3K inhibitors in the solid tumor setting.

In contrast, significant responses in hematological cancers have been reported with PI3K inhibitors. For example, Calistoga Pharmaceuticals' delta selective PI3K inhibitor CAL-101 demonstrated overall response rates of 57%, 67%, and 30% in indolent non-Hodgkin's lymphoma [NHL], mantle cell lymphoma [MCL], and chronic lymphocytic leukemia [CLL], respectively. However, in acute myeloid leukemia [AML], multiple myeloma [MM] and diffuse large B-cell lymphoma [DLBCL] there were no responses and no stable disease. Accordingly, several pan-PI3K inhibitors and dual PI3K-mTOR inhibitors are advancing clinical development in the responsive B-cell malignancies both

alone and in combination with potentially synergistic agents. Updated clinical data from various PI3K inhibitor programs is expected at the upcoming American Society of Hematology [ASH] annual meeting held December 4-7, 2010, in Orlando, FL.

Instead of testing compounds in mixed patient populations, another strategy is to use the most compelling preclinical data to guide genotype-directed trials. For example, preclinical work suggests that cancers with PIK3CA mutations might be most sensitive [ref 9] and cancers with KRAS mutations might be difficult to treat with single agent PI3K inhibitors [refs 10,11].

Dual Pathway Inhibition – Better than Best?

Beyond the aforementioned PI3K pathway redundancies highlighting the potential benefits of dual PI3K/mTOR inhibition, recent data demonstrate crosstalk between the mitogen-activated protein kinase [MAPK] pathway and PI3K pathway. This can serve as a back-up pathway to survival, particularly in the case of mutations in the MAPK pathway such as KRAS mutations [ref 10].

This discovery has led to the unusual step of evaluating clinical combinations of unapproved PI3K and MAPK inhibitors. For example, Novartis' PI3K inhibitor BKM120 is being combined with GlaxoSmithKline plc's (GSK) MEK inhibitor GSK1120212 in a Phase I study focused on tumors with RAS/RAF mutations and triple negative breast cancer [ref 12]. In addition, Merck & Company, Inc.'s (MRK) allosteric Akt inhibitor MK-2206 is being combined with AstraZeneca plc's (AZN) MEK inhibitor AZD6244 [ref 13] and Roche Holding AG has a trial combining their PI3K inhibitor GDC-0941 and MEK inhibitor GDC-09773 [ref 14].

Developing one investigational drug is challenging enough, but developing two investigational compounds simultaneously can be daunting. Complexities can arise from trying to match



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different administration schedules and differing pharmacokinetics [PK], distribution, and metabolism profiles between the combined agents. A single molecule that simultaneously inhibits both PI3K and MAPK would therefore be preferable and the following three companies are currently pursuing this single-molecule, dual pathway inhibition strategy with their respective pre-clinical product candidates:

1. **AEterna Zentaris, Inc. (AEZS):** pre-clinical molecule [AEZ132] that inhibits PI3K and Erk
2. **Progenics Pharmaceuticals, Inc. (PGNX):** preclinical molecule [PGNX-01/02] that inhibits mTOR/PI3K and MNK [downstream of Erk]
3. **Semafore Pharmaceuticals:** pre-clinical molecule [SF2626] that inhibits PI3K and MEK

Conclusion

Our understanding of the PI3K pathway has advanced significantly since the FDA approved the first mTORC1 inhibitors for the treatment of renal cell carcinoma in 2007/2009. Promising results have been demonstrated in the area of hematological malignancies with next-generation PI3K inhibitors and new insights into the pathway biology has led to the development of new molecules and combination approaches that will allow us to realize the ultimate potential of this pathway as a therapeutic target for a variety of diseases.

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Table 2: Select PI3K Pathway Inhibitors in Clinical Development

Company	Product	Status	Target(s)/Isoforms	Route
Novartis AG (NVS)	BEZ235	Phase I/II	Dual PI3K,mTOR	Oral
Bayer (BAYRY.PK)	BAY80-6946	Phase I	Dual PI3K,mTOR	IV
Novartis (NVS)	BGT226	Phase I/II	Dual PI3K,mTOR	Oral
Exelixis (EXEL)/Sanofi-aventis (SNY)	XL765	Phase I	Dual PI3K,mTOR	Oral
Semafore Pharma (private)	SF1126	Phase I	Dual PI3K,mTOR	IV
GlaxoSmithKline plc (GSK)	GSK2126458	Phase I	Dual PI3K,mTOR	Oral
Pfizer, Inc. (PFE)	PF-04691502	Phase I	Dual PI3K,mTOR	Oral
Roche Holding AG (RHHBY.PK)	GDC-0980	Phase I	Dual PI3K,mTOR	Oral
Pfizer, Inc. (PFE)	PKI-587	Phase I	Dual PI3K,mTOR	IV
Exelixis (EXEL)/Sanofi-aventis (SNY)	XL147	Phase I	Pan-PI3K	Oral
Oncothyreon, Inc. (ONTY)	PX-866	Phase I/II	Pan-PI3K	Oral
Roche Holding AG (RHHBY.PK)	GDC0941	Phase I	Pan-PI3K	Oral
Novartis AG (NVS)	BKM120	Phase I	Pan-PI3K	Oral
Calistoga Pharma (private)	CAL-101	Phase I/II	PI3K/delta isoform	Oral
Novartis AG (NVS)	BYL719	Phase I	PI3K/alpha isoform	Oral
Keryx Biopharmaceuticals (KERX)	Perifosine	Phase III	Akt	Oral
VioQuest Pharma (VOQP.PK)	Triciribine	Phase I	Akt	IV
Merck & Co. (MRK)	MK2206	Phase I/II	Akt	Oral
Astellas Pharma Inc.	OSI-027	Phase I	mTOR/catalytic site	Oral
AstraZeneca plc (AZN)	AZD8055	Phase I/II	mTOR/catalytic site	Oral
Intellikine (private)	INK128	Phase I	mTOR/catalytic site	Oral
Novartis AG (NVS)	Everolimus	Approved	mTORC1	Oral
Ariad (ARIA)/Merck & Co. (MRK)	Ridaforolimus	Phase III	mTORC1	Oral
Pfizer, Inc. (PFE)	Temsirolimus	Approved	mTORC1	IV