Blocking the mTOR pathway: a drug discovery perspective

Carlos Garcia-Echeverria¹
Oncology Drug Discovery and Preclinical Research, Sanofi-Aventis, Vitry-sur-Seine, France

Abstract
Substantial drug discovery efforts have been devoted, over the last few years, to identifying and developing mTOR (mammalian target of rapamycin) kinase modulators. This has resulted in a number of mTOR inhibitors with different mechanisms of action and/or distinct protein and lipid kinase selectivity profiles. As briefly reviewed in the present paper, these compounds have provided us with a better understanding of the roles of mTOR and other phosphoinositide 3-kinase/mTOR pathway components in human cancer biology, and a few of them have already demonstrated clinical benefit in cancer patients.

Introduction
The PI3K (phosphoinositide 3-kinase)/mTOR (mammalian target of rapamycin) pathway plays an important role in controlling the growth, proliferation and survival of cells. Through various mechanisms, this signalling cascade is often dysregulated in human cancers, suggesting the use of pathway modulators as novel targeted anticancer agents [1]. To this end, substantial drug discovery efforts have been devoted, both in pharmaceutical companies and in academia, to identifying and developing agents to specifically down-regulate the serine/threonine kinase activity of mTOR [2]. This protein is found in two structurally and functionally distinct multiprotein complexes known as mTORC (mTOR complex) 1 and 2. The two complexes have different subunit composition, downstream substrates and biological effects [3]. mTORC1 is composed of mTOR, PRAS40 (proline-rich Akt substrate of 40 kDa; Akt is also known as protein kinase B), raptor (regulatory associated protein of mTOR) and mLST8 (also known as GβL), whereas mTORC2 consists of mTOR, rictor (rapamycin-insensitive companion of mTOR), Sin1 [SAPK (stress-activated protein kinase) interacting protein 1] and mLST8 [4].

Compounds with different mechanism of action and/or distinct protein and lipid kinase selectivity profiles have been exploited to inhibit the mTORCs, and some of these drugs have already provided proof-of-concept in cancer clinical settings and received marketing approval [5,6]. The present paper reviews the remarkable progress that has been achieved over the last few years in mTOR drug discovery, with an emphasis on compounds currently undergoing clinical trials in oncology. Potential resistance mechanisms to these new therapeutic agents and combination strategies are also briefly discussed.

Rapamycin and derivatives thereof: the first kids on the block
Rapamycin (sirolimus), which was originally isolated from the Rapa Nui Easter Island soil, was the first compound shown to inhibit mTOR kinase activity by an unusual allosteric mechanism. This natural product forms a complex with the immunophilin FKBP12 (FK506-binding protein of 12 kDa), a protein that interacts with a region at the C-terminus of mTOR that is in close proximity to its catalytic kinase site [7]. The formation of this protein–protein interaction interferes exclusively with the kinase activity of mTORC1, but it does not inhibit all the functions of mTOR; nor does it block the kinase activity of mTORC2 [8], although the last point is still controversial. Several studies have found that, although mTORC2 is not directly inhibited by rapamycin and derivatives thereof, prolonged exposure may reduce the levels of mTORC2 below those needed to activate Akt in cancer cells [9] (for recent publications on the role of mTORC2 in malignant transformation, see [10–12]).

The limited pharmacological properties of rapamycin prompted the preparation of synthetic analogues, which are also known as rapalogues [e.g. CCI-779 (temsirolimus), RAD001 (everolimus) and AP-23573 (deferolimus)]. Preclinical studies have shown that the sensitivity of tumour cell lines to these compounds correlates with specific genetic alterations of the PI3K/mTOR pathway [e.g. loss of heterozygosity and sporadic mutations of TSC (tuberous sclerosis complex) 1 and TSC2] [13]. Overall, these clinical agents are relatively well tolerated in cancer patients, and proof of pharmacological inhibition has been obtained in well-designed phase I tumour pharmacodynamic studies [14]. Consistent with the reported mechanism-based primary drug activity, induction of prolonged stable disease and

Key words: cancer, mammalian target of rapamycin inhibitor (mTOR inhibitor), phosphoinositide 3-kinase (PI3K), rapalogue, targeted therapy.

Abbreviations used: eIF4E, eukaryotic initiation factor 4E; 4EBP, eIF4E-binding protein; ERK, extracellular-signal-regulated kinase; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; mTOR, mammalian target of rapamycin; mTORC, mTOR complex; PI3K, phosphoinositide 3-kinase; PIKK, PI3K-related kinase; TSC, tuberous sclerosis complex.

¹email echeverria@sanofi-aventis

increased time to progression has been observed in a subset of non-stratified clinical trials. So far, promising clinical activity has been reported in patients with advanced renal cell carcinomas, tuberosclerosis and mantle cell lymphomas, and temsirolimus and everolimus have received marketing approval for the treatment of some of these diseases [15,16]. The observed clinical responses in the preceding malignancies appear to confirm the compelling scientific rationale linking PI3K/mTOR activation with von Hippel–Lindau mutations [17] in the case of renal cancer carcinomas or TSC1/2 loss in sporadic angiomylipomas [18]. However, and in spite of the clinical results obtained so far, the development of rapamycin-based therapies may be impaired by the intrinsic molecular activity of this class of mTORC1 modulators. Thus a negative-feedback loop mechanism identified in preclinical tumour models and clinical settings can lead to activation of Akt through up-regulation of receptor tyrosine kinase or downstream effectors, such as IRS-1 (insulin receptor substrate-1), and contribute to cancer cell survival [19]. The relief of this negative-feedback loop is not the only way mTORC1 inhibition can affect pro-survival signals. Analyses of tumour samples from cancer patients with metastatic disease have shown a dose- and schedule-dependent increase in ERK (extracellular-signal-regulated kinase) phosphorylation after treatment with RAD001 [20]. Moreover, investigation of the molecular activity of the rapalogues in cancer cells has shown that these molecules cannot effectively block the phosphorylation of the mTORC1 substrate 4EBP1 [eIF4E (eukaryotic initiation factor 4E)-binding protein 1] and suppress protein synthesis at levels required for antitumour activity. The potential therapeutic limitations in rapamycin-based therapies has led to the initiation of combination trials to encompass resistance mechanisms or pathway redundancies (e.g. IGF-1R (insulin-like growth factor 1 receptor) or MEK [MAPK (mitogen-activated protein kinase)/ERK kinase] inhibitors) [21] and the development of new drugs with alternative mechanism of action or selectivity profiles. To this last point, medicinal chemistry activities have been directed to identifying and optimizing compounds with inhibitory activity against single or multiple levels in the PI3K/mTOR signalling cascade. Many of these compounds are in clinical development and the most advanced are able to concomitantly target PI3K and mTOR (e.g. NVP-BEZ235, XL765/SAR245409, GDC-0980 and GSK2126458) [22,23].

New inhibitors of mTOR: from allosteric to ATP-competitive modulators

Early reported ATP-competitive kinase inhibitors of mTOR also block the enzymatic activity of PI3K and related PIKKs (PI3K-related kinase), but demonstrate good selectivity over the rest of the human kinome. The mTOR activity of these lipid kinase modulators was found retrospectively, and their polypharmacological profile was probably not part of the original medicinal chemistry optimization strategy [24]. One reason behind the late identification of mTOR inhibitory activity for these PI3K compounds is that, contrary to the relatively early availability of a broad range of biochemical and cellular assays for assessing the selectivity profile of compounds against protein kinases, suitable biochemical or cellular systems for determining the activity profile of compounds against PIKK family members have been established only recently. The dual PI3K/mTOR inhibitory profile of these compounds is consistent with the high sequence homology and identity in the ATP-catalytic cleft of these kinases, especially with the p110γ isoform.

The identification of the dual PI3K/mTOR kinase inhibitory activity of some of these early molecularly designed PI3K compounds has triggered preclinical efforts to differentiate in cellular and in vivo animal models the potential competitive advantages of this polypharmacological profile. According to the published results, the dual PI3K/mTOR modulators potently inhibit the proliferation of a broad panel of tumour cell lines by specifically blocking the biological function of PI3K/mTOR signalling components, inducing G1 arrest or apoptosis. Overall, the biological activity of these clinical candidates translates well in in vivo models of human cancer, in particular erbB2-amplified breast cancers or tumours with PI3KCA (PI3K catalytic subunit α) mutations [25]. Compound treatment results in disease stasis or tumour regression when administered orally, and ex vivo pharmacokinetic/pharmacodynamic analyses of tumour tissues showed a time-dependent correlation between compound concentration and inhibition of Akt phosphorylation and downstream effectors. In some cases, these agents enhanced the efficacy of other targeted or standard-of-care anticancer drugs when used in in vivo combination studies [6]. Parallel to the identification of genetically defined cancers that are sensitive to these dual inhibitors as single agents, there is also growing preclinical evidence that some cancer subtypes may be insensitive. A remarkable piece of evidence of lack of sensitivity to PI3K/mTOR blockade was obtained in K-Ras mutant models. In spite of achieving significant and sustained pathway modulation, the dual PI3K/mTOR inhibitor NVP-BEZ235 failed to alter the growth of established K-Ras mutant tumours and in vivo experiments performed with some of these dual PI3K/mTOR inhibitors have demonstrated...
marked, compound-mediated alterations in microvessel permeability and significantly reduced tumour interstitial fluid pressure reflecting vascular permeability changes in preclinical in vivo models [28,29]. The possibility to target tumour growth indirectly, by interacting with the maintenance of endothelial cells and pericytes that are required for tumour angiogenesis, may provide additional, albeit challenging, opportunities for the clinical development of these inhibitors.

Early clinical results for the preceding compounds have been disclosed at several clinical oncology meetings. Overall, the dual PI3K/mTOR clinical candidates are, to date, well tolerated. Interestingly and contrary to other modulators of the PI3K/mTOR pathway, such as the ATP-competitive Akt inhibitors (e.g. GSK2141795 and MK-2206), the available clinical results indicate the feasibility of effectively blocking the pathway with manageable effects on glucose regulation [30]. In this context, mechanistic proof-of-concept (e.g. significant inhibition of phospho-Akt or reduced uptake of [18F]deoxyglucose as determined by positron emission tomography) has been observed in surrogate (e.g. hair follicles or activated peripheral blood mononuclear cells) or tumour tissue. However, it is unclear whether the levels of pathway inhibition reported so far for these agents at peak plasma concentrations are going to be sufficient to induce tumour shrinkage and clinical benefit in potentially responsive tumours, or whether more complete and sustained target modulation as reported in preclinical efficacy studies will be required. Interestingly, concomitant and significant inhibition of the MAPK pathway as measured by phospho-ERK levels in tumour tissue has been reported for some compounds (e.g. XL765/SAR245409), although the mechanism(s) behind this experimental observation has not yet been revealed [31]. Partial responses and stable diseases in non-pre-selected patients have been reported at well-tolerated doses for some of these agents, but it is still too early to determine whether these early hints of clinical efficacy are going to translate into effective agents with acceptable safety profiles as single agents or in combination. Similarly, it is too early to determine whether PI3K and/or mTOR drug-resistance mutations will emerge in cancer patients treated with these agents as it does for protein kinase inhibitors. Preclinical studies have already identified potential p110α mutations that can confer insensitivity to PI3K-based anticancer agents [32], but it seems that the dual PI3K/mTOR inhibitors may be less susceptible to such mechanisms of resistance owing to their preserved activity against mTOR.

The uncertainty about the clinical outcome with the current allosteric mTOR inhibitors and the dual PI3K/mTOR modulators, together with the availability of structure–activity relationship data to decipher key molecular interactions to modulate activity and selectivity, has spurred the development of ATP-competitive mTOR inhibitors with more stringent protein and lipid kinase selectivity profiles. Recently, several selective ATP-competitive mTOR kinase inhibitors arising from different chemical scaffolds have been reported [2].

The preclinical characterization of these molecules has demonstrated that this new generation of mTOR inhibitors can block the phosphorylation of the mTORC1 substrate 4EBP1 and cap-dependent RNA translation more effectively than rapalogues. Surprisingly, these biological effects are independent of inhibition of mTORC2 kinase activity and are instead due to suppression of rapamycin-insensitive functions of mTORC1 [33]. This experimental finding could be explained by a model in which rapalogues block access to a specific and limited subset of mTORC1 substrates, while the ATP-competitive modulators, owing to their mechanism of action and smaller size, could block the phosphorylation of a broader set of downstream effectors. These ATP-competitive inhibitors are more potent inhibitors of tumour cell proliferation than rapamycin and derivatives thereof, causing G1 cell-cycle arrest, and in some cases, apoptosis [33–37]. To this point, it has been shown that the apoptotic effect is dependent, to a large extent, on eIF4E hyperactivation and that the 4EBP–eIF4E axis plays a major role in Akt-mediated tumorigenesis [38].

As in the case of the dual PI3K/mTOR inhibitors mentioned above, the current clinical ATP-competitive mTOR inhibitors (e.g. AZD8055, AZD2014, OSI-027 and INK-128) [39] have demonstrated broad preclinical antitumour cytostatic activity against a range of solid tumour types and haematological malignancies. Oral administration of these agents inhibited tumour growth in multiple preclinical models at tolerated doses and with predicted tumour exposure–pharmacodynamic relationships. Potent antiproliferative activity was also observed in tumour cell lines insensitive to rapamycin. All of these clinical candidates are still at an early stage of development and yet none of them has demonstrated significant target modulation or clinical benefit.

### Conclusions and outlook

Although temsirolimus and everolimus provide clinical benefit in a limited set of individualized tumour types, the introduction of mTOR inhibitors as potential targeted anticancer agents is still in an early stage and, despite the lack of complete target specificity, several interesting new compounds from different chemical classes have entered clinical trials. The differences in therapeutic response and toxicity observed with the currently available mTOR inhibitors in preclinical models suggest that blocking the PI3K/mTOR pathway at different nodes or by exploiting different mechanisms of action (allosteric compared with ATP-competitive modulators) might yield different antitumour activities and safety profiles. Moreover, there is growing preclinical evidence that the up-to-down linear cascade representation of this pathway is inappropriate, as some of its components are feedback-regulated and cross-talk to other signalling cascades [40]. The need to block integrated survival processes, cross-talk and/or feedback mechanisms will require combination treatments and a careful assessment of suitable molecular and safety biomarkers for optimal dose
and schedule. Further experimentation will be required to determine where and how to target mTOR and other relevant signalling cascade nodes within or outside the PI3K/mTOR pathway in order to achieve maximal clinical efficacy and therapeutic window in cancer patients.

References


© The Authors Journal compilation © 2011 Biochemical Society