Benefits of mTOR kinase targeting in oncology: pre-clinical evidence with AZD8055

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Abstract

AZD8055 is a small-molecule inhibitor of mTOR (mammalian target of rapamycin) kinase activity. The present review highlights molecular and phenotypic differences between AZD8055 and allosteric inhibitors of mTOR such as rapamycin. Biomarkers, some of which are applicable to clinical studies, as well as biological effects such as autophagy, growth inhibition and cell death are compared between AZD8055 and rapamycin. Potential ways to develop rational combinations with mTOR kinase inhibitors are also discussed. Overall, AZD8055 may provide a better therapeutic strategy than rapamycin and analogues.

Introduction

mTOR (mammalian target of rapamycin) has been recognized as an essential component of tumour development and progression [1]. It functions as a sensor of mitogen, energy and nutrient levels, and is a central controller of cell growth and a negative regulator of autophagy. Mitogenic signals are transmitted to mTOR via PI3K (phosphoinositide 3-kinase) and Akt. mTOR kinase forms two distinct multiprotein complexes called mTORC (mTOR complex) 1 [containing raptor (regulatory associated protein of mTOR) and PRAS40 (proline-rich Akt substrate of 40 kDa)] and mTORC2 [containing rictor (rapamycin-insensitive companion of mTOR) and protor (protein observed with rictor)]. mTORC1, the molecular target of rapamycin, phosphorylates downstream proteins S6K (p70 S6 kinase) and 4E-BP1 (eukaryotic initiation factor 4E-binding protein 1), both involved in protein translation. mTORC2 phosphorylates Akt on Ser473, increasing its enzymatic activity ~5–10-fold. In normal physiology, mTOR activity is tightly regulated: phosphorylation of S6K by mTOR induces the degradation of IRS1 (insulin receptor substrate 1), decreasing insulin-driven Akt activity, and therefore mTOR activity.

Rapamycin and analogues

Rapamycin is an allosteric inhibitor of mTORC1 and does not affect mTORC2, except in some cell lines after prolonged exposure to rapamycin. Inhibition of mTORC1 by rapamycin and analogues results in the release of the negative-feedback loop between S6K and IRS1, leading to hyperactivation of Akt and mTORC2. There is evidence that hyperactivation of Akt is detrimental to cancer patients; Cloughesy et al. [2] showed that, in PTEN (phosphatase and tensin homologue deleted on chromosome 10)-negative patients with glioblastoma, hyperactivation of Akt after rapamycin treatment was associated with a shorter time to progression, suggesting that the absence of Akt inhibition through mTORC2 targeting limited anti-tumour activity. In most epithelial-derived tumour models, temsirolimus treatment results in cytostasis, with little induction of apoptosis. In contrast, in haemopoietic models of ALL (acute lymphoblastic leukaemia), AML (acute myeloid leukaemia) and multiple myeloma, temsirolimus achieved objective tumour regression. It is fair to say that preclinical data with rapalogues have been partly revisited based on their clinical activity. For example, temsirolimus had activity in patients with renal cancer with poor prognosis. The majority of patients in Phase III trials were clear-cell RCC (renal cell carcinoma) with VHL (von Hippel–Lindau protein) deficiency. Therefore the clinical activity in this subpopulation was suggested to be due to the effect of rapamycin on HIF1α (hypoxia-inducible factor 1α) and VEGF (vascular endothelial growth factor) production producing an anti-angiogenic effect. However, in the Phase III trial, patients with non-clear-cell carcinoma had similar or even better clinical benefit than those with clear-cell RCC. Similarly, temsirolimus showed activity in patients with in MCL (mantle cell lymphoma). Cyclin D1 is overexpressed in MCL due to a translocation t(11;14),(q23,q32). As mTORC1 regulates the translation of cyclin D1, this could be anticipated to be the main mechanism of action of temsirolimus on this.

Key words: Akt, AZD8055, mammalian target of rapamycin complex (mTORC), mammalian target of rapamycin kinase (mTOR kinase), oncology.

Abbreviations used: 4E-BP1, eukaryotic initiation factor 4E-binding protein 1; ERK, extracellular-signal-regulated kinase; ETV6, [F][FDG], [18 F]fluorodeoxyglucose; FOXO, forkhead box O; IRS1, insulin receptor substrate 1; LC3, light chain 3; MCL, mantle cell lymphoma; MEK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; mTORC, mammalian target of rapamycin complex (mTORC); NDRG1, N-myc down-regulated gene 1; PDGF, platelet derived growth factor; PI3K, phosphoinositide 3-kinase; PRAS40, proline-rich Akt substrate of 40 kDa; PTEN, phosphatase and tensin homologue deleted on chromosome 10; raptor, regulatory associated protein of mTOR; RCC, renal cell carcinoma; rictor, rapamycin-insensitive companion of mTOR; S6K, S6 kinase; ULK, unc-51-like kinase.

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disease. However, neither temsirolimus nor sirolimus reduced expression levels of cyclin D1 in MCL-cultured cell lines or primary cultures of cells derived from MCL patients in three independent studies [3].

**mTOR kinase inhibitors**

The discovery of mTOR kinase inhibitors has uncovered novel areas of mTOR biology and clarified the relative importance of mTORC1 and mTORC2 in normal physiology, and also pathology. For example, the probe compound KU-0063794 was used to demonstrate that NDRG1 (N-myc down-regulated gene 1) was a substrate of mTORC2 [4]. Similarly, the small-molecule inhibitor torin 1 was used to demonstrate that inhibition of mTOR kinase activity was a more potent inducer of autophagy compared with rapamycin [5]. More recently, the mTORC1 and mTORC2 regulator deptor was also shown to be influenced by torin 1 [5]. Finally, evidence is now emerging in several areas of physiology that mTORC2 exerts specific functions independently of mTORC1. However, the effects observed with rapamycin may be attributed to either mTORC1 inhibition or to either the hyperactivation of Akt or lack of inhibition of the mTORC2 complex. Using the mTOR inhibitor PP242 showed clearly that mTORC2 inhibition abrogates insulin-induced activation of mTOR in fat, skeletal muscle and liver, whereas rapamycin induces an increase in phospho-Akt in these organs [6].

AZD8055 is an ATP-competitive inhibitor of mTOR kinase activity, with an IC50 against mTOR of 0.8 nM and an excellent selectivity profile. AZD8055 inhibits mTORC2 and decreases the phosphorylation of the mTORC2 substrates phospho-NDRG1 and phospho-Akt on Ser473. AZD8055 also decreases the phosphorylation of Akt substrates such as phospho-PRAS40 or FOXO1 (forkhead box O1). AZD8055 has also provided critical evidence of the partial effect of rapamycin on cap-dependent translation in that AZD8055 decreases the phosphorylation of 4E-BP1 at positions 37 and 46, sites which are resistant to rapamycin. This leads to a greater inhibition of cap-dependent translation compared with rapamycin [7].

**AZD8055 and autophagy**

Recent evidence suggests that S6K and 4E-BP1 play different roles, with S6K controlling cell growth, whereas 4E-BP1 may control proliferation [8]. This may be related to two biological functions controlled by mTOR: autophagy and cell-cycle progression. Perhaps surprisingly considering its major effect on cell growth and autophagy in yeast, in cancer cell lines, rapamycin is a poor inducer of autophagy. mTORC1 is a negative regulator of autophagy, directly by phosphorylating ULK (unc51-like kinase) 1 and preventing ULK1–Atg13–FIP200 (focal adhesion kinase family-interacting protein of 200 kDa) complex formation, and indirectly by phosphorylating S6K and 4E-BP1. Under stress conditions (low energy, low amino acids, hypoxia or endoplasmic reticulum stress), mTORC1 activation is rapidly switched off by multiple upstream mechanisms. For example, low glucose or a high AMP/ATP ratio activates the energy sensor LKB1–AMPK (AMP-activated protein kinase) pathway which decreases mTORC1 activity, both indirectly via TSC2 (tuberous sclerosis complex 2) and directly via phosphorylation of raptor. Similarly, hypoxia and endoplasmic reticulum stress lead to the activation of REDD1 (regulated in development and DNA damage-responses 1) reducing mTORC1 activity, and a decrease in amino acids inhibits MAP4K3 (mitogen-activated protein kinase kinase kinase kinase 3) and Rag GTPases, acting upstream of mTORC1 [9].

More recently, rictor has also been shown to regulate autophagy, independently of mTORC1 in muscle cells under fasting conditions, where a decrease in Akt activity allowed FOXO transcription factors to enter the nucleus and transcriptionally regulate some key autophagy genes such as LC3b (light chain 3b), GABARAP (γ-aminobutyric acid receptor-associated protein), ULK2 and beclin1 [10]. mTOR kinase inhibitors, by providing a more complete inhibition of mTORC1 and inhibition of mTORC2 would therefore be expected to have a greater impact on autophagy, compared with rapamycin. Indeed, rapamycin was unable to induce autophagy in muscle cells, in which autophagy is mainly controlled by mTORC2. In the H383 LKB1-deficient lung cancer cell line, AZD8055 induces the formation of punctate acidic vesicles in the cytoplasm detected by Acridine Orange indicative of an increase in autophagy [11]. Furthermore, immunostaining with antibodies against LC3 revealed a concentration-dependent increase in the punctate staining pattern, consistent with LC3 being localized to these acidic vesicles. Finally, AZD8055 increases LC3-II levels and this increase is more pronounced in the presence of E64d and leupeptin, consistent with an increase in autophagosome formation.

**AZD8055 and inhibition of proliferation**

Another major difference between rapamycin and AZD8055 is their respective effects on cancer cell proliferation. Rapamycin induces typically a partial inhibition of proliferation *in vitro* in cancer cell lines. This has been related to its limited effect on cyclins, allowing a proportion of cancer cell lines to continue to progress through the cell cycle. Conversely, AZD8055 affects the level of the major cyclins as well as the CDK inhibitors p27 and p21, resulting in a profound G1-phase arrest. This translates into a much greater inhibition of proliferation compared with rapamycin. In some cell lines, AZD8055 also induces cell death. This is in contrast with the effects of rapamycin and analogues *in vivo*, where rapamycin induces a greater anti-tumour effect in some models than the *in vitro* activity would have predicted. This seems to be particularly prominent when dosing is only started once the xenograft tumours are well-established (>200 mm³). The major effect observed *in vivo* may therefore be related to the impact of rapamycin on the vasculature rather than
AZD8055 biomarkers

One of the key elements of drug development is whether the mechanism of action observed in vitro is achieved in vivo and drives the intended biological effect. Normally, $[^{18}F]$FDG ($[^{18}F]$fluorodeoxyglucose)-PET (positron emission tomography) measures the metabolic activity of the tumour and is a surrogate for tumour burden. However, as a consequence of Akt inhibition, AZD8055 decreases glucose uptake in cancer cells. This was exploited as a non-invasive way to show modulation of mTORC2 in tumour tissue [12]. In pre-clinical models, after a single dose of AZD8055 administered by oral gavage, a decrease in $[^{18}F]$FDG uptake was observed by PET scanning of tumour xenografts (Figure 1). This was correlated with the decrease in phospho-Akt and phospho-S6 in tumour xenografts, confirming that the change in $[^{18}F]$FDG uptake was a surrogate for Akt modulation. This non-invasive measure of mTORC2 modulation has been incorporated in the clinical trial with AZD8055 as ‘proof-of-mechanism’ biomarker. A strong relationship between biomarker modulation and anti-tumour effect was observed.

Alteration of the pharmacokinetics of the compound or its schedule of administration had significant consequences on the anti-tumour activity, ultimately allowing the optimization of the doses and schedules of administration to achieve the best therapeutic index.

Rational combinations with AZD8055

Tumour cells have devised a number of strategies to increase the output of mTOR signalling; for example, enhanced upstream signalling such as mutations in receptor tyrosine kinases, activating mutations of PI3K, PTEN loss of function, or mutations of LKB1. The complexity of genetic abnormalities and dependency on growth factors and nutrients led to the suggestion that mutations in PI3K or loss of PTEN would render cancer cells hypersensitive to mTOR inhibition. Similarly, KRas mutation may be a marker of resistance. Our data so far with AZD8055 show that such genetic abnormalities do not alter the sensitivity profile of AZD8055, suggesting that it may have a broad spectrum of activity. However, it may also suggest that mTOR-dependency in cancer is a more complex process than previously thought. Cancer cells develop multiple strategies to proliferate and grow, activating sometimes multiple nodes of the same pathway, sometimes parallel pathways. Oncogenic activation can activate not only the mTOR pathway, but also other signalling pathways such as MEK.
[mitogen-activated protein kinase/ERK (extracellular-signal-regulated kinase) kinase/ERK or STAT (signal transducer and activator of transcription)]. Oncogenic activation is also associated with strong feedback inhibition to maintain a sustainable output of the pathway. mTOR inhibition may relieve some of these feedback loops (such as feedback inhibition of IRS1 by S6K), resulting in upstream activation or, in some cases, activation of a parallel pathway. However, feedback activation may not always correspond to an increased reliance on the activated pathway. Understanding these phenomena may be key to developing rational combinations.

Conclusions may aim at maximizing the inhibition of the pathway, for example by targeting the oncogenic drive and its downstream cascade. Exposure of some ErbB2-amplified breast cancer cells to AZD8055 increases phospho-ERB3. The combination of the ErbB2 inhibitor lapatinib with AZD8055 in this context is synergistic, such that, in vitro, the combination results in a phenotypic switch, where either agent alone induces growth inhibition, but no cell death, whereas the combination induces significant cell death. The biomarker profile shows that the combination, by abrogating the feedback activation of phospho-ERB3, results in a greater inhibition of the mTOR pathway, especially against Akt substrates such as phospho-PRAS40 [13].

Combinations may also aim to target parallel pathways that both contribute to proliferation. For example, the occurrence of both MEK/ERK and mTOR activation is observed in a significant proportion of cancers. Notably, a transient increase in phospho-ERK is observed after rapamycin in AZD8055. This would suggest that the combination with MEK inhibition should produce an additive or even synergistic effect. Indeed, either AZD8055 or selumetinib (AZD6244; ARRY-142886), an allosteric MEK inhibitor, alone produced tumour growth inhibition in non-small-cell lung xenograft models, whereas the combination of selumetinib with AZD8055 led to cell death and tumour regression in vivo. Interestingly, however, in some breast models in which selumetinib is inactive, the transient increase in phospho-ERK observed after exposure to AZD8055 did not result in a greater anti-tumour efficacy when combined with selumetinib. This would suggest that the functionality of feedback activation is dependent on the cellular context and that the measure of single biomarkers is unlikely to be representative of the pathway output. In this respect, dynamic changes in gene expression are more likely to be informative [14].

AZD8055 is currently in Phase I clinical development in oncology. AZD8055 has also been instrumental to understand the respective contribution of mTORC1 and mTORC2 in tumour growth, suggesting the superiority of mTOR kinase inhibition compared with mTORC1 allosteric inhibition as a therapeutic strategy in oncology. This is likely to also provide better treatment opportunities in other disciplines.

References


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