



HAL
open science

Are we missing the mTOR target in breast cancer?

Stephen R. D. Johnston

► **To cite this version:**

Stephen R. D. Johnston. Are we missing the mTOR target in breast cancer?. *Breast Cancer Research and Treatment*, 2010, 128 (3), pp.607-611. <10.1007/s10549-010-1207-2>. <hal-00615382>

HAL Id: hal-00615382

<https://hal.science/hal-00615382v1>

Submitted on 19 Aug 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Commentary for BCRT 2010

“Are we missing the mTOR target in breast cancer?”

Stephen RD Johnston

Address for correspondence:

Stephen RD Johnston, MA, FRCP, PhD

Professor of Breast Cancer Medicine

Royal Marsden Hospital, Fulham Road, Chelsea, London, SW3 6JJ, UK.

E-mail: stephen.johnston@rmh.nhs.uk

Tel: +44(0)20-7808-2748

Abnormalities in the PI3K/Akt/mTOR intracellular signalling pathway have been implicated in several forms of human cancer. The central component of the pathway is the phosphatidylinositol 3-kinase (PI3K) heterodimer which comprises the p85 regulatory and p110 catalytic subunit, and mutations in the catalytic domain of PI3K have been identified in 20-25% of breast cancers [1, 2]. A further 15-35% of breast cancer patients demonstrate reduced expression of PTEN (phosphatase and tensin homolog deleted on chromosome ten), an endogenous inhibitor of the PI3K/AKT pathway [3]. Akt is the ultimate effector of the pathway and directs a multitude of intracellular effects related to growth, glucose metabolism, protein synthesis and cell survival. Activation of the PI3K/Akt pathway provides a cell with unrestricted growth and survival signals, and one important downstream consequence is the alleviation of the suppression by the tuberous sclerosis protein complex (TSC1/2) of the mammalian target of rapamycin (mTOR) [4]. There are at least two groups of mTOR proteins, and the key protein involved in transmitting signals from PI3K-Akt is the mTORC1 complex. Within cells mTORC1 plays a critical role in the transduction of proliferative signals through phosphorylation of the translational regulator 4E-BP1 (eukaryotic initiation factor 4E-binding protein) and the ribosomal protein p70^{S6k} (the 70-kDa S6 kinase) that facilitate translation of mRNAs into proteins [5, 6]. In recent years our understanding of this pathway in breast cancer has been exploited by the development of a number of targeted anti-cancer therapeutics, with mTOR inhibitors being the most clinically developed to date.

Rapamycin is the original inhibitor of mTOR that was found to be a potent fungicide with immunosuppressive properties, and therapeutics were developed for clinical use in transplantation to prevent graft rejection. Subsequently rapamycin was found to have anti-proliferative effects in a range of experimental tumours [7], and rapamycin analogues with a more favourable pharmacological profile were synthesised including CCI-779 (temsirolimus), RAD-001 (everolimus), and AP23573 (ridaforolimus). In various breast cancer cell lines with constitutive activation of the PI3K-Akt pathway due to either HER2 amplification, ER activation or PTEN deletions temsirolimus showed specific sensitivity [8], while everolimus (an oral hydroxyethyl ether of rapamycin) demonstrated impressive anti-proliferative activity against a wide variety of tumour models in vivo [9]. In the clinic mTOR antagonists were found to be relatively well tolerated in early phase I/II trials [10, 11], and

development programmes were established in various solid malignancies, in particular renal cell cancer and breast cancer.

But has the development of mTOR antagonists for breast cancer moved ahead too fast before it was really appreciated how best to utilise these drugs as targeted therapeutics? As a single agent temsirolimus showed only modest activity in the advanced breast cancer setting [12], and subsequently both temsirolimus and everolimus were developed in combination with endocrine therapy following encouraging evidence for additive or synergistic effects from several pre-clinical models of endocrine resistant breast cancer [13, 14]. However, results from randomised clinical trials of mTOR antagonists in combination with endocrine therapy were mixed and failed to show substantial clinical benefit for the combination [15-17]. It became clear that as a targeted therapeutic, mTOR antagonists were being developed in unselected patients without understanding whether mTOR activation was indeed critical for all breast cancers, or indeed what the molecular consequence of mTOR inhibition would be. As such, it is now imperative that we refine the clinical development programme for mTOR antagonists by developing biomarkers that might select breast cancers most likely to respond to mTOR inhibition, and by understanding the evolving complexity of the PI3K/AkT/mTOR signalling pathway and applying that knowledge to design better therapeutic strategies.

In this month's edition of Breast Cancer Research and Treatment, three articles are published that contribute to a better understanding of mTOR as a target in breast cancer. In particular they relate to an evaluation of downstream mTOR targets and biomarkers for clinical response [18, 19], and review how best we might combine mTOR antagonists either with conventional cytotoxics, endocrine agents or other biological therapies for breast cancer [20]. Neo-adjuvant or short-term pre-operative studies can provide predictive biomarker data that may help select appropriate patients for future clinical trials. Macaskill et al evaluated the effects of just 14 days exposure to everolimus (5mg once daily) in 31 post-menopausal women with unselected early stage breast cancer [18]. Paired biopsies taken at diagnosis and at surgery after drug exposure allowed an immuno-histochemical (IHC) assessment of changes in cell proliferation (Ki67), apoptosis (active caspase-3), ER and PgR, in addition to key activated components of the pathway (pAkt, p-S6 kinase, p-mTOR).

Increasingly, these type of studies are using Ki-67 as the primary endpoint and key biological readout for drug exposure in this setting [21]. Furthermore, short-term changes in Ki-67 can be correlated with both the tumour's molecular profile and changes in downstream signalling that in turn might identify predictive biomarkers for clinical benefit from the drug. The authors showed that reduction in proliferation was greatest in HER2+ tumours and/or those tumours with the highest pre-treatment Ki67 scores, and that this also correlated with inhibition of signalling demonstrated by associated reduction in p-Akt levels. In addition, a number of tumours showed no biological response to mTOR antagonism suggesting that some harbour innate de-novo resistance. As such these data would suggest tumour profiles can be selected which may be more or less likely to benefit from mTOR inhibition, and in turn this could form key selection criteria for future clinical trials.

However, there are limitations to this short-term exposure in only a small group of patients in being able to correlate biological changes with clinical outcome, and to evaluating resistance mechanisms that might quickly develop and limit the drug's effectiveness. Larger neo-adjuvant studies that treat patients for 3-4 months prior to surgery can start to correlate biomarker data with traditional clinical response features. A recent randomised phase II study in 270 postmenopausal women with ER+ primary operable breast cancer evaluated the benefit of adding everolimus (10mg/day) or placebo to neo-adjuvant letrozole (2.5mg/day) for 16 weeks pre-operatively [22]. The primary endpoint of the study was clinical response, with a greater tumour shrinkage being observed for the combination (68.1% vs 59.1%). A significantly greater reduction in cell proliferation (Ki67) was seen in the letrozole/everolimus combination arm compared to letrozole. Associative correlative studies in pre- and post-treatment biopsies demonstrated expected down-regulation in progesterone receptor (PgR) and cyclin D1 in both treatment arms, with reductions in phosphorylated ribosomal protein S6 (mTOR downstream target) being observed only in the everolimus arm. Interestingly, in this study specific mutations in exon 9 of PIK3CA were found to be associated with a greater likelihood of an anti-proliferative response to the combination of letrozole plus everolimus compared to letrozole alone – this was not observed in the study of Macaskill et al (18), although they acknowledge that numbers in their study were too small. This particular PIK3CA mutation has been associated with a worse prognosis in breast cancer [23], and a

greater likelihood of response to the combination implicates the PI3K/Akt/mTOR pathway in endocrine resistance.

While these pre-operative studies have helped identify some tumour profiles that might predict for benefit from mTOR inhibition based on activation and/or dependence on the pathway (ie. high proliferation rate, HER2 amplification, ER+ve tumours with certain PI3KCA mutations), these biomarker profiles need to be validated prospectively in subsequent studies. What about the molecular downstream consequences of mTOR antagonism that may modify or nullify target inhibition? Emerging evidence suggests an important negative feedback loop, whereby the mTOR activated kinase S6K1 phosphorylates and destabilizes the IRS1 and IRS2 proteins in insulin-like growth factor (IGF) responsive cells [24]. In these cells, mTOR inhibition can lead to a reduction in S6K1 activity, which in turn allows IRS1/2 expression to be increased with associated enhanced activation of IGFR1-dependent Akt activity – as such, concern has been raised that loss of this negative feedback loop may overcome the anti-tumour effectiveness of mTOR blockade and limit their effectiveness [25, 26]. This has supported by the clinical observation that phosphorylated Akt is upregulated in both tumour and skin biopsies of patients treated with everolimus [27], although again Macaskill et al observed no change in pAkt in their short-term exposure study [18]. While technical difference in detection of activated Akt can make such studies difficult, they have raised concern that loss of negative feedback may in time overcome the anti-tumour effectiveness of mTOR blockade alone, resulting in short term clinical remissions and perhaps rebound growth at the time of disease progression. As discussed below, this has driven the rationale either for better mTOR inhibitors, for drugs that provide dual blockade with PI3K, or indeed mTOR inhibition combined with blockade of upstream growth factors (HER2, IGFR) to block this compensatory feedback loop.

In addition to the role that the mTOR substrate S6 kinase plays in regulating IRS1 and IRS2, the *S6K1* and *S6K2* genes which code for these substrate proteins are amplified in breast cancer, which in theory could abrogate the effects of mTOR inhibition. Perez-Tenorio et al. in this edition of BCRT have studied S6K1 and S6K2 gene / protein expression in postmenopausal patients with early breast cancer followed up over many years within the previously reported Stockholm adjuvant trials

of adjuvant tamoxifen in low-risk patients, and of radiotherapy versus chemotherapy in high risk patients [19]. They report that amplification / gain of these genes occurs in over 20% of breast cancer, and this is perhaps not surprising given that the loci sit within commonly amplified regions in 17q21-23 and 11q13, which may explain the association of *S6K1* amplification with HER2 status. They report that *S6K2* amplification correlates with ER status and Cyclin D1 amplification, and that in ER+ PgR-ve tumours nuclear expression of the *S6K2* protein may be associated with a worse outcome from tamoxifen. While these data are clearly of interest, no studies to date have looked at amplification of these mTOR substrates in terms of responsiveness to mTOR inhibition – for example, amplification of a downstream target could make blockade of mTOR ineffective if endogenous drive to protein translation is already occurring via S6Kinase activation. It will now be imperative to include this biomarker in subsequent studies of mTOR inhibitors.

So how can we improve upon the current mTOR antagonists that we have in the clinic, and should we be exploring a different strategy to utilise these drugs, namely combining them with other inhibitors to provide more effective blockade of the key components of intra-cellular signalling networks? In the third article in this edition of BCRT Margariti et al review the current progress that we have made with mTOR inhibition, in particular the attempts to reverse resistance to current clinical therapeutics including cytotoxics, endocrine therapy, and HER2 blockade with trastuzumab [20]. They conclude that while the pathway is a logical one to target and pre-clinical data have been especially encouraging, clinical progress with these drugs has been very modest to date. Heterogeneity of breast cancer and inability to target the right tumours may explain some of these results, although the most promising areas to develop further remain HER2+ tumours that may be resistant to trastuzumab, and endocrine resistant tumours – several key studies with existing mTOR antagonists in combination with trastuzumab and endocrine therapies are ongoing.

Ultimately we need to develop more complete mTOR antagonists, as well understand how best to use them in smarter targeted combinations. The current rapamycin analogues in development only block the mTORC1 complex, with no effect on the mTORC2 complex that independently activates Akt, thus potentially limiting their ability to totally block this pathway [28, 29]. Several novel targeted

agents are in development against various components of the PI3K/Akt/mTOR pathway including mTOR inhibitors that target both mTORC1 and mTORC2, Akt inhibitors, PI3K inhibitors and dual mTOR/PI3K inhibitors (Table 1). For example the dual mTOR/PI3K inhibitor NVP-BEZ235 has previously demonstrated superior anti-proliferative activity to everolimus in cell lines [30], suggesting that greater blockade of the pathway can be achieved than just targeting mTOR alone. Likewise, a greater understanding of the pathway's molecular biology will allow partnering of key signal transduction inhibitors together to provide maximal "vertical" or "horizontal" blockade. For example dual vertical inhibition of IGF-1R signalling using either monoclonal antibodies or tyrosine kinase inhibitors could be used to overcome the rapamycin induced Akt activation loop. Preliminary evidence to support has come from some promising results in neuroendocrine tumours where octreotide (which targets IGF-1R signalling) was combined with everolimus [31]. More recently the mTOR inhibitor ridaforolimus has been combined with the IGF-1R antibody dalotuzumab in patients with advanced solid tumours [32]. The most frequent adverse events were stomatitis, fatigue and hyperglycaemia which were mostly grade 1-2. Interestingly there were signs of activity in 5 heavily pre-treated breast cancer patients with 2 achieving partial responses, 1 stable disease and a further 2 demonstrating partial metabolic responses on PET scan. All 5 of these patients were ER+ and 4 had high expression of Ki67, consistent with data above relating to tumour profiles most likely to respond to mTOR inhibition.

So in conclusion, have we missed the mTOR target in breast cancer? Probably not completely is the answer, but we can certainly do better – perhaps the key lesson to remember is that development of a targeted agent in breast cancer requires a full understanding of the signalling pathway, and recognition that not all cancers will respond. Predictive biomarkers and understanding the consequences of target inhibition in-vivo allow identification of who best to treat, and how best to use the drug. Signalling networks are complex and sophisticated, and elucidating which ones are key oncogenic drivers and moreover what regulates them is central to getting the best out of modern targeted therapeutics. We should consider the current mTOR antagonists as first generation probes, and exploit the emerging science and clinical data to develop superior drugs against this pathway in smarter, better designed clinical trials.

Table 1: Novel PI3K and mTOR inhibitors in development (adapted from ref 33) (33).

Agent	Company	Molecular targets	Disease
AZD8055	AstraZeneca	mTORC1 and mTORC2	Phase I – solid cancers
SF1126	Semaphore Pharmaceuticals	PI3K, mTOR, DNA-PK	Phase I – solid tumours, myeloma
XL-147	Exelixis	PI3K	Phase I – solid tumours, NSCLC
XL-765	Exelixis	PI3K / mTOR	Phase I – solid tumours, NSCLC, glioma
NVP-BEZ-235	Novartis	PI3K / mTOR	Phase I – solid tumours, Breast Ca
NVP-BGT-226	Novartis	PI3K / mTOR	Phase I – solid tumours, Breast Ca
GDC-0941	Genentech / Piramed Pharmaceuticals	PI3K / mTOR (weak)	Phase I – solid tumours
GSK1059615	GlaxoSmithKline	PI3K	Phase I – solid tumours, Breast Ca

References:

1. Bachman KE, Argani P, Samuels Y, Silliman N, Ptak J, Szabo S, et al. The PIK3CA gene is mutated with high frequency in human breast cancers. *Cancer Biol Ther* 2004;3(8):772-5.
2. Saal LH, Holm K, Maurer M, Memeo L, Su T, Wang X, et al. PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. *Cancer Res* 2005;65(7):2554-9.
3. Shoman N, Klassen S, McFadden A, Bickis MG, Torlakovic E, Chibbar R. Reduced PTEN expression predicts relapse in patients with breast carcinoma treated by tamoxifen. *Mod Pathol* 2005;18(2):250-9.
4. Inoki K, Corradetti MN, Guan KL. Dysregulation of the TSC-mTOR pathway in human disease. *Nat Genet* 2005;37(1):19-24.
5. Hara K, Yonezawa K, Kozlowski MT, Sugimoto T, Andrabi K, Weng QP, et al. Regulation of eIF-4E BP1 phosphorylation by mTOR. *J Biol Chem* 1997;272(42):26457-63.
6. Chung J, Grammer TC, Lemon KP, Kazlauskas A, Blenis J. PDGF- and insulin-dependent pp70S6k activation mediated by phosphatidylinositol-3-OH kinase. *Nature* 1994;370(6484):71-5.
7. Eng CP, Sehgal SN, Vezina C. Activity of rapamycin (AY-22,989) against transplanted tumors. *J Antibiot (Tokyo)* 1984;37(10):1231-7.
8. Yu K, Toral-Barza L, Discafani C, Zhang WG, Skotnicki J, Frost P, et al. mTOR, a novel target in breast cancer: the effect of CCI-779, an mTOR inhibitor, in preclinical models of breast cancer. *Endocr Relat Cancer* 2001;8(3):249-58.
9. O'Reilly T, Vaxelaire J, Muller M, et al. In vivo activity of RAD-001, an orally active rapamycin derivative, in experimental tumor models. *Proc. Am. Assoc. Cancer Res* 2002;43(71): Abstract 359.
10. Raymond E, Alexandre J, Faivre S, Vera K, Materman E, Boni J, et al. Safety and pharmacokinetics of escalated doses of weekly intravenous infusion of CCI-779, a novel mTOR inhibitor, in patients with cancer. *J Clin Oncol* 2004;22(12):2336-47.
11. O'Donnell A, Faivre S, Burris HA, 3rd, Rea D, Papadimitrakopoulou V, Shand N, et al. Phase I pharmacokinetic and pharmacodynamic study of the oral mammalian target of rapamycin inhibitor everolimus in patients with advanced solid tumors. *J Clin Oncol* 2008;26(10):1588-95.
12. Chan S, Scheulen ME, Johnston S, Mross K, Cardoso F, Ditttrich C, et al. Phase II study of temsirolimus (CCI-779), a novel inhibitor of mTOR, in heavily pretreated patients with locally advanced or metastatic breast cancer. *J Clin Oncol* 2005;23(23):5314-22.
13. Boulay A, Rudloff J, Ye J, Zumstein-Mecker S, O'Reilly T, Evans DB, et al. Dual inhibition of mTOR and estrogen receptor signaling in vitro induces cell death in models of breast cancer. *Clin Cancer Res* 2005;11(14):5319-28.
14. deGraffenried LA, Friedrichs WE, Russell DH, Donzis EJ, Middleton AK, Silva JM, et al. Inhibition of mTOR activity restores tamoxifen response in breast cancer cells with aberrant Akt Activity. *Clin Cancer Res* 2004;10(23):8059-67.
15. Baselga J, Fumoleau P, Gil M, Colomer R, Roche H, Cortes-Funes H, et al. Phase II, 3-arm study of CCI-779 in combination with letrozole in postmenopausal women with locally advanced or metastatic breast cancer: preliminary results. *Proc. Am. Soc. Clin. Oncol.* 2004;23(13):A544.

16. Baselga J, Roche H, Fumoleau P, Campone M, Colomer R, Cortes-Funes H, et al. Treatment of postmenopausal women with locally advanced or metastatic breast cancer with letrozole alone or in combinations with temsirolimus; a randomized 3-arm, phase 2 study. *Breast Cancer Res. Treat.* 2005;94(Suppl 1):A1068.
17. Chow LWC, Jassem J, Baselga J, Hayes DF, Woff AC, Hachemi S, et al. Phase 3 study of temsirolimus with letrozole or letrozole alone in postmenopausal women with locally advanced or metastatic breast cancer. *Breast Cancer Res. Treat.* 2006;97(Suppl 1):Abs 6091.
18. Macaskill JE, Sabine VS, Bartlett JMS, et al. The mammalian target of rapamycin (mTOR) inhibitor everolimus (RAD001) in early breast cancer; results of a pre-operative study. *Breast Cancer Res. Treat.* 2010;xxxx.
19. Perez-Tenorio G, Karlsson E, Waltersson MA, et al. Clinical potential of the mTOR targets S6K1 and S6K2 in breast cancer. *Breast Cancer Res. Treat.* 2010;xxxx.
20. Margariti N, Fox SB, Bottini A, Generali D. Overcoming drug resistance in breast cancer; could mTOR inhibitors be a myth or a real possibility for the future. *Breast Cancer Res. Treat.* 2010;xxxx.
21. Dowsett M, Smith IE, Ebbs SR, Dixon JM, Skene A, A'Hern R, et al. Prognostic value of Ki67 expression after short-term presurgical endocrine therapy for primary breast cancer. *J Natl Cancer Inst* 2007;99(2):167-70.
22. Baselga J, Semiglazov V, van Dam P, Manikhas A, Bellet M, Mayordomo J, et al. Phase II randomized study of neoadjuvant everolimus plus letrozole compared with placebo plus letrozole in patients with estrogen receptor-positive breast cancer. *J Clin Oncol* 2009;27(16):2630-7.
23. Barbareschi M, Buttitta F, Felicioni L, Cotrupi S, Barassi F, Del Grammastro M, et al. Different prognostic roles of mutations in the helical and kinase domains of the PIK3CA gene in breast carcinomas. *Clin Cancer Res* 2007;13(20):6064-9.
24. Sabatini DM. mTOR and cancer: insights into a complex relationship. *Nat Rev Cancer* 2006;6(9):729-34.
25. O'Reilly KE, Rojo F, She QB, Solit D, Mills GB, Smith D, et al. mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. *Cancer Res* 2006;66(3):1500-8.
26. Sun SY, Rosenberg LM, Wang X, Zhou Z, Yue P, Fu H, et al. Activation of Akt and eIF4E survival pathways by rapamycin-mediated mammalian target of rapamycin inhibition. *Cancer Res* 2005;65(16):7052-8.
27. Tabernero J, Rojo F, Calvo E, Burris H, Judson I, Hazell K, et al. Dose- and schedule-dependent inhibition of the mammalian target of rapamycin pathway with everolimus: a phase I tumor pharmacodynamic study in patients with advanced solid tumors. *J Clin Oncol* 2008;26(10):1603-10.
28. Jacinto E, Loewith R, Schmidt A, Lin S, Ruegg MA, Hall A, et al. Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat Cell Biol* 2004;6(11):1122-8.
29. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 2005;307(5712):1098-101.
30. Serra V, Markman B, Scaltriti M, Eichhorn PJ, Valero V, Guzman M, et al. NVP-BEZ235, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits the growth of cancer cells with activating PI3K mutations. *Cancer Res* 2008;68(19):8022-30.
31. Yao J, Phan A, Chang D, Jacobs C, Mares JE, Rashid A, et al. Phase II study of RAD001 (everolimus) and depot octreotide in patients with advanced low grade

neuro-endocrine carcinoma (LGNT). Proc. Am. Soc. Clin. Oncol. 2006;24:Abstract 4042.

32. Di Cosimo S, Bendall JC, Cervantes-Ruiperez A, Roda D, Prudkin L, Stein MN, et al. A phase I study of the oral mTOR inhibitor ridaforolimus in combination with the ICF-1R antibody in patients with advanced solid tumours. Proc. Am. Soc. Clin. Oncol. 2010;28(15S):Abstract 3008.

33. Markman B, Atzori F, Perez-Garcia J, Tabernero J, Baselga J. Status of PI3K inhibition and biomarker development in cancer therapeutics. Ann Oncol 2010;21(4):683-91.