

Neuroendocrine tumors resistant to mammalian target of rapamycin inhibitors: A difficult conversion from biology to the clinic

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Author contributions: Fazio N conceived the issue which formed the content of the manuscript and wrote the manuscript.

Conflict-of-interest statement: Nicola Fazio has received fees for serving as an advisory board member for Novartis, Ipsen and Lexicon.

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Received: June 29, 2015
Peer-review started: July 3, 2015
First decision: July 30, 2015
Revised: September 7, 2015
Accepted: September 25, 2015
Article in press: September 28, 2015
Published online: December 10, 2015

Abstract

Deregulation of the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) - mammalian target of rapamycin (mTOR) signaling pathway is one of the most commonly-

involved pathways in tumorigenesis. It has also been reported as altered in neuroendocrine tumors (NETs). mTOR inhibitors used in clinical practice are derived from rapamycin, an anti-cancer agent also used as an immunosuppressor after organ transplantation. Everolimus and temsirolimus are the two rapamycin-derived mTOR inhibitors used in NETs. Notably everolimus has been approved in advanced progressive well/moderately-differentiated pancreatic NETs (pNETs). It inhibits specifically the mTORC1 subunit of mTOR, not interacting with mTORC2. Although everolimus produced a significant prolongation of progression-free survival a number of patients with pNETs do not benefit from the drug due to early or late progression. Two supposed mechanisms of resistance to mTOR inhibitors are Akt and PI3K activation, by means of mTORC2 and insulin growth factor (IGF) - IGF receptor signaling, respectively. BEZ235 is a multi-targeted inhibitor binding to PI3K, mTORC1 and mTORC2, therefore potentially turning off all the supposed molecular targets of resistance to everolimus. The two clinical trials designed in pNETs were stopped early due to unmet statistical endpoint and the global clinical development of BEZ235 was also halted. Tolerability of this drug was challenging and conditioned the feasibility of therapy. The BEZ experience is an example of the huge difference between the preclinical and clinical setting and prompts us to pay more attention to the phase I step of clinical development and the design of phase II clinical trials.

Key words: Everolimus; BEZ235; Mammalian target of rapamycin; Phosphoinositide 3-kinase; Mammalian target of rapamycin C; Resistance; Mammalian target of rapamycin inhibitor

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Core tip: Although everolimus significantly prolongs progression-free survival in patients with advanced

pancreatic neuroendocrine tumors (NETs), some patients are refractory or progress early after an initial response. Mammalian target of rapamycin (mTOR) C2 and insulin growth factor (IGF) - IGF receptor signaling can mediate two supposed mechanisms of resistance to everolimus. BEZ235 is a multitargeted inhibitor binding to phosphoinositide 3-kinase, mTORC1 and mTORC2, therefore potentially turning off all the supposed molecular targets of resistance to everolimus. The two clinical trials designed in pancreatic NETs were stopped early due to unmet statistical endpoint and the global clinical development of BEZ235 was halted. Challenging tolerability probably conditioned the results. The BEZ experience is an example of the huge difference between preclinical and clinical setting and prompts us to pay more attention to the phase I step of clinical development and the design of higher-phase trials.

Fazio N. Neuroendocrine tumors resistant to mammalian target of rapamycin inhibitors: A difficult conversion from biology to the clinic. *World J Clin Oncol* 2015; 6(6): 194-197 Available from: URL: <http://www.wjgnet.com/2218-4333/full/v6/i6/194.htm> DOI: <http://dx.doi.org/10.5306/wjco.v6.i6.194>

MAMMALIAN TARGET OF RAPAMYCIN PATHWAY

The mammalian target of rapamycin (mTOR) is a sort of intracellular metabolic switch, that physiologically regulates growth, proliferation and survival of normal cells integrating growth factors and nutrient signals^[1]. It is an intracellular serine/threonine kinase activated by two main upstream factors, namely phosphoinositide 3-kinase (PI3K) and protein kinase B (Akt). This in turn activates downstream factors, including the ribosomal protein S6K and eukaryotic translation initiation factor 4E binding protein (4EBP-1). Based on the interaction of mTOR with other proteins, two functionally distinct subunits exist, mTORC1 and mTORC2; among others mTORC1 includes the regulatory-associated protein of mTOR, whereas mTORC2 includes the rapamycin-insensitive companion of mTOR. Activated mTORC1 activates in turn p70^{S6K}, the kinase that phosphorylates the ribosomal protein S6, finally inducing protein synthesis. Activation of mTORC1 and S6K inhibits the tyrosine phosphorylation and signaling functions of insulin receptor substrates (IRS-1) through a negative feedback mechanism, resulting in the attenuation of PI3K-Akt signaling. Activated mTOR leads to protein synthesis also through 4EBP1 activation, inducing translation.

One of the main upstream stimulating factors of mTOR is the insulin-like growth factor (IGF) and its receptor (IGFR), activated by IRS-1; whereas phosphatase and tensin homolog, tuberous sclerosis complex and neurofibromatosis-1 factor are inhibitors of mTOR

signaling.

Deregulation of PI3K-Akt-mTOR signaling pathway is one of the most common mechanisms of tumorigenesis^[2]. It has been reported as dysregulated also in neuroendocrine tumors (NETs), familial and sporadic^[3,4].

mTOR INHIBITORS

The term mTOR derives from rapamycin, which is a macrolide, initially studied as an antifungal and antibiotic agent, known for its immunosuppressant activity, which has also demonstrated antitumor properties. Two derivatives of rapamycin have been used in NETs, everolimus and temsirolimus. Everolimus (RAD001) was approved by the FDA and EMA in progressing well-moderately differentiated pancreatic NETs (pNETs), based on the results of a randomized phase III study comparing it with placebo (RADIANT-3 trial).

RESISTANCE TO mTOR INHIBITION

Some patients with pNETs show primary or secondary (acquired) resistance to everolimus. The precise mechanism is unknown, but some hypotheses have been postulated, including Akt activation by means of mTORC2 and IGF1/IGFR signaling activation due to inhibition of the S6K negative feedback^[5,6]. On this basis, drugs inhibiting these supposed targets of resistance were preclinically studied.

DUAL INHIBITOR BEZ235

BEZ235 is a potent oral multitargeted inhibitor of all four class I PI3K isoforms and the downstream effectors, mTORC1 and mTORC2^[7]. In preclinical studies BEZ235 showed clearly higher activity than everolimus in NETs^[8-10] and BEZ/everolimus combination was suggested as synergistic^[8,9]. Furthermore BEZ235 reversed resistance to other anti-cancer therapies in a variety of tumor cell line^[11-13]. However, conducting phase I studies with this agent has proved challenging. In the more than 200 patients treated, both by single agent and in combination, in several phase I / I b studies with different types of tumor, the formulation was changed moving from gelatine capsule to sachet^[14]. Furthermore, the schedule was moved from QD (once per day) to BID (twice per day). High intra- and inter-patient pharmacokinetic variability was observed. In spite of these troubling premises, given the impressive preclinical activity, a world BEZ235 clinical development plan was launched for several types of tumor, including prostate, breast, renal cancers and pNETs. In pNETs two trials were designed with BEZ235 as single agent: One small multicentre phase II trial in pNETs resistant to everolimus and a large randomized phase II vs everolimus in pNETs not previously treated with mTOR inhibitors. Both were prematurely halted, the former after completion of the first stage with 30 patients

enrolled, due to unmet statistical endpoint, and the latter after randomization of 62 out of the 140 foreseen patients, due to unlikely superiority to everolimus at a first interim analysis of 35 patients^[15]. In the phase II trial beyond everolimus the initial BEZ235 dose of 400 mg was amended to 300 mg due to intolerable toxicity. This agrees with a recently published phase I study of 33 patients with different types of malignancies who received BEZ235 administered twice daily as an oral sachet, where 300 mg BID resulted the recommended dose^[14].

The poor tolerability of BEZ235 negatively influenced both studies. Although its toxicity profile was confirmed without evidence of new toxicities, BEZ235 was less well tolerated than everolimus in the randomized study; furthermore in both studies a high percentage of adverse events led to frequent treatment discontinuation, in particular 39% in the BEZ arm of the randomized study (vs 16% for everolimus) and 36% in the phase II stage I study. Based on this experience, further clinical investigation of BEZ235 in cancer was halted.

CONCLUSION

This is an example of the huge difference that sometimes exists between bench and bedside. Why would a drug such as BEZ235, which binds to the potentially correct targets for overcoming mTOR inhibition resistance and which is highly effective in preclinical investigations, meet with failure in clinical trials? A number of reasons may be advanced, both tumor-related and drug-related. Of course it is possible that the PI3K pathway is not the sole driver of resistance in pNETs and/or that the targets of BEZ235 do not represent the mechanism of activation of the PI3K pathway in some pNETs. However, the difficulty in managing the BEZ235 changeable toxicity in trials beyond phase I / I b strongly suggests that it is not only a matter of level of dose and therefore that the maximum tolerated dose concept is not suitable for all drugs. Other areas, including transportomics and metabolomics, which can strictly influence the tolerability of a drug, should be investigated. On the one hand, BEZ235 has a particular oral formulation susceptible to variable absorption while on the other hand its metabolism depends on CYP3A4, CYP1A2 and aldehyde oxidase activity.

Finally, the BEZ235 experience has taught that multidisciplinary could be useful for planning an anti-cancer agent clinical investigation, with clinicians dedicated to the specific tumor area and with pharmacologists who should work in close collaboration with phase I trial clinical researchers.

ACKNOWLEDGMENTS

The author would like to thank William Russell for English revision.

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P- Reviewer: Boy C, Hopfner M, Moschetta M
S- Editor: Qiu S **L- Editor:** A **E- Editor:** Li D





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