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**Effects of PI3K Pathway Inhibition and
Biomarker Development
- Short report -**

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As an Australian born and Australian trained medical oncologist, the opportunity to broaden my professional and personal horizons has led me to seek work in Europe. I gained employment in the oncology unit at the Vall d'Hebron University Hospital, Barcelona, Spain. In 2008, I was awarded an ESMO fellowship - focussing on the PI3K pathway and its inhibition - a privilege that has helped fulfil personal goals and gain invaluable experience in this highly reputable European oncology centre.

The PI3K (phosphatidylinositol 3-kinase) cellular signalling cascade plays a central role in a multitude of physiological processes, including growth and proliferation, cell cycle, survival, metabolism, motility and angiogenesis. Under normal conditions, ligand bound receptor tyrosine kinases activates the previously quiescent PI3K heterodimer. p110, the catalytic subunit of PI3K, in turn phosphorylates PIP2 to form PIP3, with subsequent activation of the key effector of the cascade, the serine/threonine protein kinase Akt (otherwise known as PKB). Akt exerts its influence on a variety of downstream molecules, including mTOR, FOXO, GSK3 and BAD, which are ultimately responsible for the diverse functions of the pathway. The critical negative regulator of the system is the tumour-suppressor gene PTEN, whose action opposes PI3K in that it dephosphorylates PIP3 back to PIP2.

Through a host of genetic aberrations the PI3K signalling cascade is amongst the most deregulated systems in human cancer. Described with varying frequency are mutations and amplifications of p110 α , loss of heterozygosity and mutations of PTEN, and amplifications and mutations of Akt.

In view of its central role in myriad cellular functions, combined with its frequent oncogenic deregulation contributing to tumourigenesis, rational development of new therapeutic agents designed to target this pathway has been the focus of much research and development. My project has focussed on a selection of such small molecule inhibitors.

The most extensively studied in the pre-clinical setting is NVP-BEZ235 (Novartis), a dual inhibitor of both PI3K and mTOR. We have demonstrated PI3K pathway inhibition using this agent in a panel of human cell lines, including lineages harbouring activating mutations of p110 α , those with functional loss of PTEN and those with wild-type pathway elements. This was evidenced by reductions in the amounts of the activated (phosphorylated) forms of Akt (pAkt, downstream of PI3K) and the S6 ribosomal protein (pS6, downstream of mTOR), amongst others. An interesting observation was a paradoxical increase in pAkt when low concentrations of NVP-BEZ235 were used. The dual nature of this agent may provide the explanation. Downregulation of a negative feedback loop via p70S6K-IRS1-PI3K consequent to mTOR inhibition may increase pAkt until adequate PI3K inhibition is achieved at higher NVP-BEZ235 concentrations. This phenomenon has previously been described with mTOR inhibitors in the clinic leading to legitimate concern that the observed increase in PI3K/Akt activity may limit efficacy. Targeting elements upstream of mTOR, such as PI3K, is therefore considered a logical means to overcome this problem.

We next showed that cellular proliferation is reduced following exposure to NVP-BEZ235 (to a consistently greater extent than with the mTOR inhibitor everolimus). Cell growth was likewise reduced with NVP-BEZ235 in cells of varying tumor type and PI3K heritage. Although we observed a trend for reduced sensitivity in those cells harbouring mutations affecting other cellular pathways (Ras/Raf/MAPK and EGFR signalling), all cells tested were inhibited at low concentration. We also demonstrated cell cycle effects of NVP-BEZ235, in particular increased G1 arrest detected on flow cytometry, and on cell survival, with markers of apoptosis (cleaved PARP, cleaved caspase) demonstrable in cell lysates.

Subsequently, we were able to overexpress oncogenic PI3K mutations in Her2+ human breast cancer cells. Not only did this increase pAkt levels, it also rendered these cells resistant to the anti-Her2 antibody trastuzumab, indicating the importance of the PI3K pathway in this scenario. Critically, these cells remained sensitive to treatment with NVP-BEZ235. With primary and acquired resistance to trastuzumab affecting many women with Her2+ breast cancer this finding has important clinical implications.

Finally, we tested NVP-BEZ235 in xenograft models of breast cancer in nude mice. NVP-BEZ235 was able to slow tumor growth in the wild type PI3K models, and achieved tumor stasis where the cells contained oncogenic PI3K mutations therein.

Biomarker (BM) discovery is a hot topic in developmental therapeutics for oncology and has been one aim of my project. BMs are measurable characteristics of physiological or pathological states. BMs fall into two broad groups – pharmacodynamic (PD) and predictive biomarkers. In the former, a change in the level of the PD endpoint is assessed pre- and post-treatment with a given therapy. In the latter, the given BM aims to assess the likelihood of a given therapy working prior to its administration. These two situations are not mutually exclusive, and a BM can be both PD and predictive. Thus, BMs aim to serve a variety of functions. They serve to provide proof-of-principle, evaluate response or lack thereof, assist in refined patient selection, and determine the optimal biologic dose of targeted agents.

Our work with NVP-BEZ235 included biomarker evaluation, especially in tumor tissue and skin. Using immunohistochemistry and western blot analysis, we have demonstrated significant reductions in pAkt and pS6 levels in tumor specimens post-treatment compared to baseline (pre-treatment). Similar findings were seen in skin biopsies taken at equivalent time points, suggesting that skin may prove a useful surrogate tissue for assessing PI3K and mTOR inhibition. Skin has the advantage of being more accessible for repeat evaluations, thus avoiding potential risk or discomfort of tumor biopsies in a clinical setting.

Peripheral blood is one of the most attractive as a compartment for BM study. With minimal risk and invasiveness, it lends itself to repeat sampling. IGFBP2 is a secreted protein with a regulatory role in insulin / insulin-like growth factor signalling. Others have previously established a gene expression signature representative of PTEN loss, amongst which IGFBP2 was found. An inverse relation was shown between PTEN and

IGFBP2 expression, implying that serum IGFBP2 may serve as a BM of PTEN status and PI3K pathway activation status.

We have done preliminary work with IGFBP2. Increased levels of secreted IGFBP2 have been seen in conditioned medium of cells over expressing oncogenic PI3K mutations. Further, treatment with NVP-BEZ235 was associated with a reduction in the medium from wild-type and PI3K mutated cells alike leading us to hypothesise that this may be a reflection of decreased signalling through PI3K.

Concurrently with preclinical investigations, we have been participating in ongoing phase I clinical trials of PI3K inhibitors in patients with solid tumors. These are open label, single agent, dose escalation studies with the primary objectives of defining the dose limiting toxicities (DLT), establishing the maximum tolerated dose (MTD) and determining the recommended phase II dose (RP2D). The agents involved are NVP-BGT226 (Novartis), and XL765 and XL147 (both from Exelixis). The first two target both PI3K and mTOR, the latter a 'pure' PI3K inhibitor.

To date, the MTD and RP2D of each agent is either not determined or published. Some preliminary data has been presented for the Exelixis compounds. Following accrual of the first 30 patients on the XL147 protocol, DLTs of skin rash were observed, whereas the most common adverse events have been skin rash and nausea. Prolonged stable disease (from ≥ 3 -14 months) has been observed in 8 patients. Data from the first 29 patients enrolled on the XL765 protocol has also been presented. DLTs of skin rash, nausea / vomiting and elevated hepatic transaminases have been noted, with nausea, diarrhea and raised transaminases the most frequent adverse events. Prolonged stable disease (from 3.7-9.6 months) has been documented in 5 patients.

Each of these protocols have a strong pharmacodynamic / biomarker component. Retrospective tumor genotyping looking for PI3K or PTEN mutations is underway. In addition, some PD data from the Exelixis trials has been presented. PI3K signalling influences glucose metabolism. Thus, pre- and post-treatment glucose and insulin levels are tested. With both XL765 and XL147 there has been a trend for plasma insulin to increase with drug exposure, although plasma glucose levels are minimally impacted. This suggests that compensatory mechanisms to prevent hyperglycaemia are invoked, whereas insulin levels may find utility as a circulating BM reflecting pathway modulation.

More direct PI3K pathway endpoints, such as Akt, PRAS40, 4EBP1 and S6 are being measured in multiple tissues, in particular peripheral blood mononuclear cells, skin, hair follicles and tumour tissue. Despite its accessibility, PBMC analysis of PI3K pathway readouts remains challenging. Due to its convenience, serial hair follicle specimens have been obtained, where progressive inhibition of pathway elements has been evident, with suggestions of dose response. Work is continuing to correlate changes observed in surrogate tissues to effects in serial tumour biopsies (where available) and pharmacokinetic compartment parameters.

Both my pre-clinical and clinical investigation is ongoing for all of the compounds mentioned in this report. Future laboratory work is focussed on characterising BGT226, unravelling possible feedback loops and further biomarker evaluation. The phase I clinical trials should near completion towards the end of 2009, with combination therapy trials involving PI3K with other targeted agents or traditional cytotoxic therapies already underway.