Resistance mechanisms to PI3K-mTOR inhibition in NSCLC

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Introduction

Non-small cell lung cancer (NSCLC) is a leading cause of cancer mortality globally, having a 5 year survival rate of less than 15%. PI3K-mTOR signalling has been implicated in various hallmarks of cancer and this pathway is often dysregulated in different cancers, including NSCLC. Efforts to therapeutically target the PI3K-mTOR pathway have shown limited clinical efficacy, however the inevitable emergence of drug resistance inhibits a durable response to treatment. In this study a cell line model of acquired resistance to a phase II PI3K-mTOR inhibitor GDC-0980 was established following several months of chronic treatments with IC50 drug concentrations. The sensitivity of the established GDC-0980 resistant cells (H1975GR) to other PI3K-mTOR inhibitors was interrogated using another clinically relevant PI3K-mTOR dual targeting inhibitor, BEZ235. IL-6/STAT3 overexpression in cancer stimulates angiogenesis, migration, invasiveness, cytokine signalling and notably drug resistance. The IL-6/STAT3 pathway has been shown to contribute to PI3K/ mTOR signalling and in this study a potential role in drug resistance mechanisms to PI3K-mTOR blockade are investigated. Additionally, a comparative study investigating short term and long term chronic exposure of BEZ235 and IBL-301 (a novel PIM/PI3K/mTOR inhibitor) for effects on cell viability/proliferation and downstream signalling pathways is ongoing.

Methods

- The sensitivity of GDC-0980 resistant cells , H1975GR, to PI3K-mTOR inhibitor BEZ235 following a 72 hour treatment was compared to the sensitivity of the age-matched parent cells (H1975P) using a Cell Titre Blue cell viability assay (n=3).
- Alterations to the mRNA expression profile of GDC-0980 resistant cells, H1975GR versus age-matched parent cells H1975P were examined using an IL-6/STAT3 signalling-specific RT2 gene profiler array (n=1). This array contains 84 key genes involved in the activation and downstream effects of IL6/STAT3 signalling. Selected genes from the array were validated by SYBR-based qPCR and western blot analysis (n=3-4).
- BEZ235 and IBL-301 drug dose responses were measured in two wild-type NSCLC cell lines (H1975 and H1838) by CellTitre Blue assay.
- Alterations to protein expression of H1975 and H1838 cells were measured by Western blotting analysis.

Development of PI3K/mTOR inhibitor-resistant cell line model:

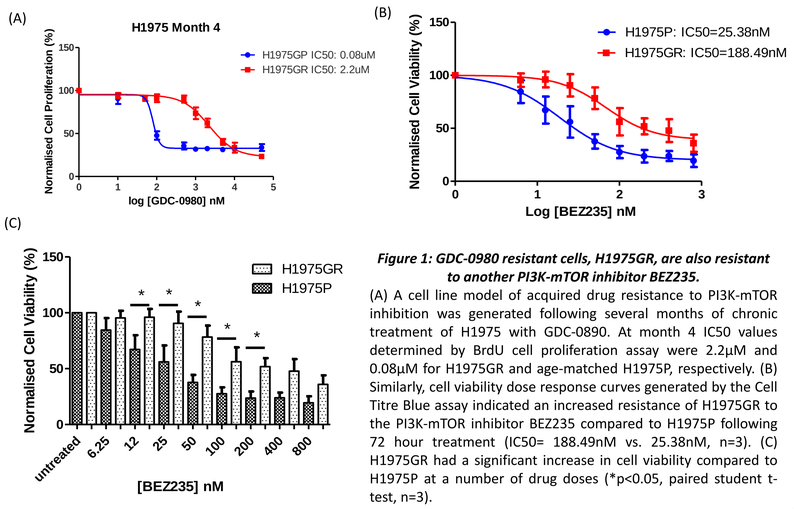


Figure 1: GDC-0980 resistant cells, H1975GR, are also resistant to another PI3K-mTOR inhibitor BEZ235.

(A) A cell line model of acquired drug resistance to PI3K-mTOR inhibition was generated following several months of chronic treatment of H1975 with GDC-0890. At month 4 IC50 values determined by BrdU cell proliferation assay were 2.2μM and 0.08μM for H1975GR and age-matched H1975P, respectively. (B) Similarly, cell viability dose response curves generated by the Cell Titre Blue assay indicated an increased resistance of H1975GR to the PI3K-mTOR inhibitor BEZ235 compared to H1975P following 72 hour treatment (IC50= 188.49nM vs. 25.38nM, n=3). (C) H1975GR had a significant increase in cell viability compared to H1975P at a number of drug doses (*p<0.05, paired student t-test, n=3).

Interrogation of IL-6/STAT3 signalling in PI3K/mTOR inhibitor resistance:

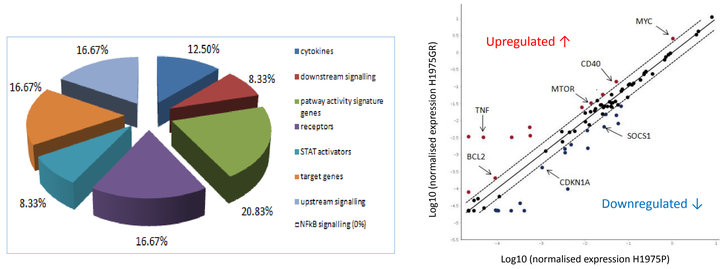


Figure 2: The gene expression profile of H1975GR was analysed using an IL-6/STAT3 pathway array.

mRNA expression of IL-6/STAT3 pathway-related genes was compared between H1975GR and age-matched H1975P. 22 genes involved in molecular functions of IL-6/STAT3 signalling were found to be differentially expressed between the two cell lines. As indicated on scatter plot, a number of genes altered by ≥2-fold in the array were chosen for further validation by qPCR .

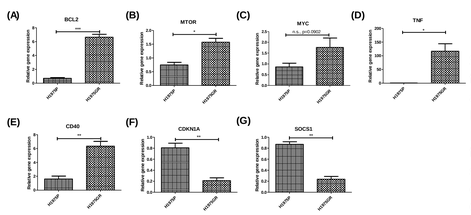


Figure 3: GDC-0980 resistant cells, H1975GR, overexpress pro-survival and pro-inflammatory genes and under express negative regulators of cell cycle and protein synthesis.

Seven genes were chosen from the array for further validation by SYBR-based qPCR. There was a significant upregulation of (A) anti-apoptotic BCL2 (10.6-fold, p<0.001), (B) mTOR (2.5-fold, p<0.05), (C) MYC (2.04-fold, p=0.09) (D) TNF (>100-fold, p<0.05) and (E) the TNF receptor co-stimulatory molecule CD40 (3.6-fold, p<0.01), In contrast there was a downregulation of (F) CDKN1A (3.7-fold, P<0.01) and (G) SOCS1 (3.8-fold, P<0.01). *p<0.05, **p<0.01, ***p<0.001, paired student t-test, n=4.

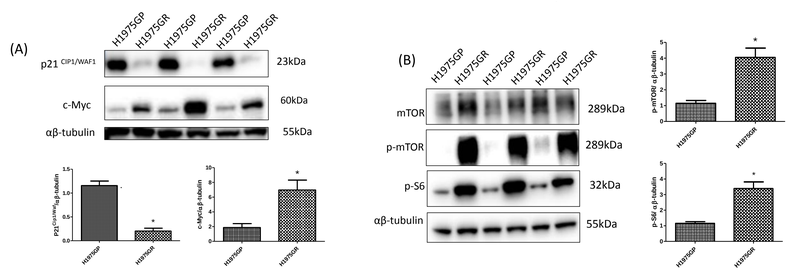


Figure 4: Protein levels of p21 are downregulated in H1975GR, while the levels of activated/phosphorylated PI3K-mTOR signalling molecules are upregulated compared to age-matched H1975P.

(A) Protein expression of $p21^{CIP1/WAF1}$ (i.e. CDKN1A) was largely suppressed in H1975GR compared to H1975P, while c-MYC was upregulated (both p<0.05). (B) Total mTOR protein was not significantly altered in H1975GR however an overexpression of phospho-mTOR was found (p<0.05). Additionally acquired resistance to PI3K-mTOR blockade resulted in increased phosphorylation of the PI3K/Akt/mTOR downstream signalling molecule S6 riboprotein (p<0.05). *p<0.05, paired student t-test, n=3.

Effect of triple targeting PI3K/mTOR/PIM kinase inhibitor IBL-301 on NSCLC *in vitro*:

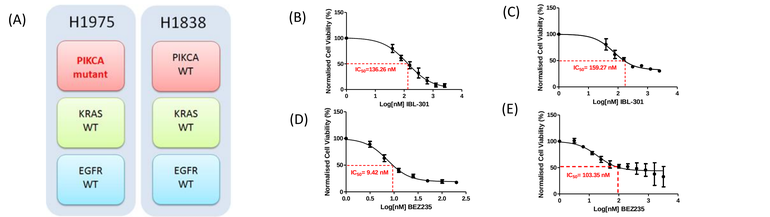
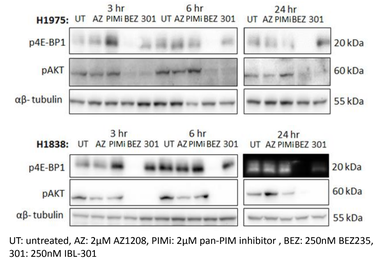


Figure 5: PI3K/mTOR inhibitor BEZ235 and triple targeting PI3K/mTOR/PIM inhibitor IBL-301 demonstrate dose dependent effects on cell viability in NSCLC cell line H1975 and H1838.

The cell viability of two NSCLC cell lines; H1975 and H1838 were measured by cell titre blue assay, following 72 hour treatment with BEZ235 or IBL-301. (A) H1975 have a PIKCA mutant while H1838 have a wild type PI3KCA genotype. Cell viability IC50 doses were determined for both drugs by non-linear regression (N=3). (B)-(C) BEZ235 had a IC50 dose of 9.42nM and 103.35nM for H1975 and H1838 cells respectively. (D)-(E) IBL-301 had similar effects to viability in the two cell line lines with IC50 values of 136.26nM and 159.27nM in the H1975 and H1838 cells respectively.



UT: untreated, AZ: 2μM AZ1208, PIMi: 2μM pan-PIM inhibitor , BEZ: 250nM BEZ235, 301: 250nM IBL-301

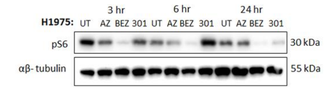


Figure 6: BEZ235 and IBL-301 decrease PI3K-Akt-mTOR signalling and cap-dependent translation.

H1975 and H1838 cells were treated with drugs at the indicated dose for 3, 6 and 24 hours. PI3K/mTOR signalling and downstream proteins were measured by western blotting. BEZ235 and IBL-301 displayed similar suppression of phospho-AKT across all time points in both cell lines. BEZ235 was more effective at reducing 4E-BP1 and S6 phosphorylation at the single dose tested.

Conclusion

Our group has developed a PI3K-mTOR inhibitor resistant NSCLC cell line model that demonstrates acquired resistance to both GDC-0980 and BEZ235. This indicates the utility of this model to interrograte resistance mechanisms to other PI3K-mTOR inhbitors and is not limited to just GDC-0980. This study identifies alterations in the IL-6/STAT3 signalling pathway contributing to resistance to PI3K-mTOR inhibition and these data may provide novel effective multi-targeted therapeutic strategies for lung cancer patients. A novel PI3K/mTOR/PIM inhibitor IBL-301 has shown promising in vitro data that warrant further investigation as a therapeutic strategy for NSCLC.

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