#500 Co-targeting PIM kinase to overcome MET amplified resistance to EGFR TKIs in NSCLC

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Introduction

Currently there are five EGFR tyrosine kinase inhibitors (TKIs) (erlotinib, gefitinib, afatinib, dacomitinib, and osimertinib) available for treatment of EGFR-mutated non-small cell lung cancer (NSCLC). However for virtually all patients, resistance is inevitable, and disease progression occurs within 1 to 2 years of starting a TKI. Efforts to overcome resistance define the landscape of TKI research resulting in the development of second-generation and now third-generation agents and combination regimens. Third-generation agents, such as osimertinib, show improved response rates and extended median overall survival (OS), with potential to overcome previously untreatable resistance mechanisms. However acquired resistance mutations and activation of bypass RTK signalling mechanisms such as MET can mediate primary and secondary resistance to all EGFR TKIs. MET amplification has been observed after prolonged exposure of HCC827 cell lines to third-generation EGFR-TKIs (osimertinib or CNX-2006). We have pinpointed a novel strategic downstream target that plays a key role in MET regulation, cancer progression, drug resistance and immune evasion namely PIM kinase (PIM). The PIM family of serine/threonine kinases constitute three major isoforms namely PIM-1, 2 and 3 and have been shown to synergise with c-Myc. Here we show that PIM-1, PIM-3 and c-Myc are activated in the erlotinib resistant MET amplified HCC827ER cells compared to the erlotinib sensitive HCC827P cells. Further characterisation of MET amplified HCC827ER subclone 3 shows elevated c-Myc and PIM-1 expression and EMT HCC827ER subclone 10 shows PIM-1 overexpression but not c-Myc. We hypothesise that co-targeting PIM kinase and EGFR may provide a more durable response to treatment and overcome EGFR TKI resistance.

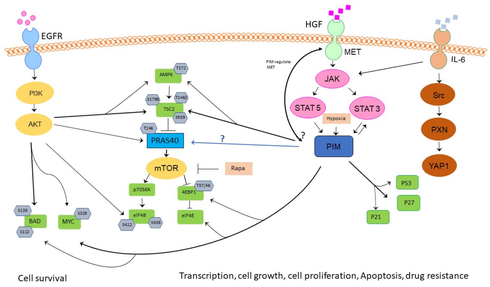

Figure 1: Pathway crosstalk driving resistance mechanisms to EGFR TKIs
The cross-talk between EGFR , HGF and IL-6 signalling pathways results in the emergence of acquired drug resistance to EGFR TKIs. PIM kinases share a common node within these signalling pathways and their activation has emerged as a significant mechanism of resistance to several pathway inhibitors.

Methods

- HCC827ER cells developed resistance to erlotinib through continuous exposure of the erlotinib sensitive parent cell line HCC827P to increasing concentrations (10nmM - 5μM) of erlotinib over 4 months.
- Clone 3 (MET amplified) and Clone 10 (EMT) were then isolated & characterised from the total population of resistant cells (HCC827ER) (1).
- MET, c-Myc and PIM-1 expression were examined by Western blot analysis in EGFR TKI sensitive (HCC827P) and resistant (HCC827ER) cell lines and selected resistant subclones (HCC827ER clone 3, HCC827ER clone 10).
- Quantification & localisation of MET & PIM-1 was examined by immunofluorescence.
- Efficacy of novel PIM/ PI3K/mTOR inhibitor (AUM302) were quantified using the CellTiter-Blue, cell viability assay, in all cell lines.
- Efficacy of AZD1208 & IBL-301 in gefitinib resistant HCC827GR & Clone 3 cell line were quantified (Crown Bioscience Inc)
- The Effect of PIM inhibitors on protein kinase phosphorylation was quantified by Proteome Profiler Arrays® (R&D Biosystems).

Development of Erlotinib-resistant HCC827 cell line models:

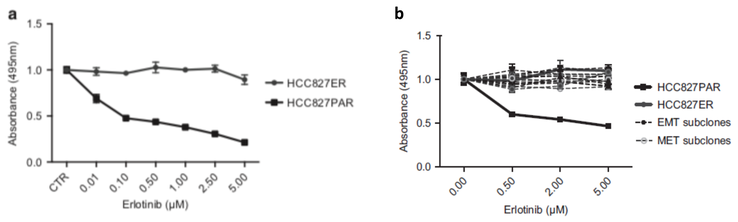

Figure 2: Development of the erlotinib resistant HCC827ER cell line and subclones Clone 3 and Clone 10
(a) Erlotinib resistance was developed over 4 months via the addition of increasing concentrations of erlotinib (10nM - 5μM) until a final concentration of 5μM (1). (b) To further characterise the different resistant clones present in the HCC827ER cell population HCC827ER cells were diluted to approximately 5 cells/ml and grown in erlotinib. 14 subclones were isolated and characterised, 6 clones were MET amplified (including Clone 3) and 8 clones were more EMT-like (including Clone 10).

Results

MET, PIM-1, PIM-3 & c-Myc expression in HCC827P/ER, Clone 3 & Clone 10 cell lines

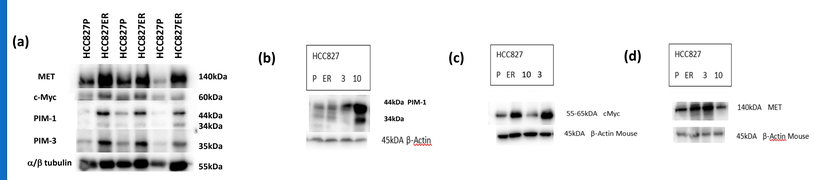

Figure 3: MET, PIM-1, PIM-3 & c-Myc expression in HCC827P/ER, Clone 3 & Clone 10 cell lines
(a) MET, PIM-1, PIM-3 & c-Myc protein expression is activated in the erlotinib resistant HCC827ER cells compared to erlotinib sensitive HCC827P cells.
(b) PIM-1 protein expression is activated in HCC827ER, Clone 3 cells and Clone 10 cells compared to HCC827P cells.
(c) c-Myc protein expression is significantly increased in HCC827ER and Clone 3 cells compared to HCC827P cells (P<0.05, n=3)
(d) Met protein expression is activated in HCC827ER and Clone 3 cells compared to HCC827P and Clone 10 cells.

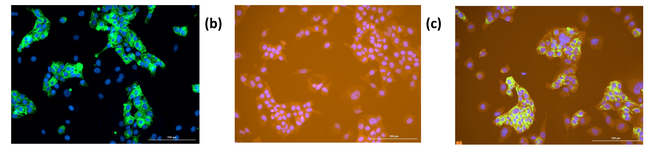

Figure 4: Immunofluorescence staining of PIM-1 , MET & PIM-1/MET expression in HCC827ER cells
(a) MET is expressed in some clones and not others in the mixed population of erlotinib resistant cells (HCC827ER)
(b) PIM-1 is expressed in all cell populations in the HCC827ER cell line
(c) Dual staining of MET and PIM in the HCC827ER cell line

Efficacy of PI3K/mTOR/PIM inhibitor AUM302 in HCC827P/ER, Clone 3 & Clone 10 cell lines

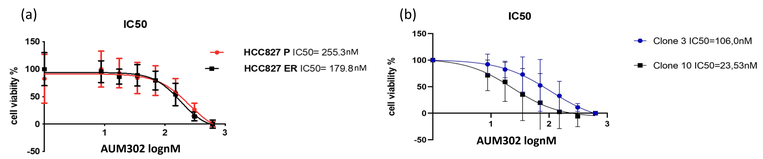

Figure 5: Efficacy of PI3K/mTOR/PIM inhibitor AUM302 in HCC827P/ER, Clone 3 & Clone 10 cell lines
(a) Drug dose response curves examined the efficacy of AUM302 in HCC827P versus HCC827ER cells using the Cell Titre Blue assay (Promega). Cell viability was measured after drug treatment for 72hr (IC50=255.3nM vs. 179.8nM, n=3)
(b) Drug dose response curves, examined the efficacy of AUM302 in HCC827ER Clone 3 (MET amplified) versus HCC827ER Clone 10 (EMT amplified). Cell viability was measured after drug treatment for 72hr (IC50=106nM vs. 23.53nM, n=3).

Efficacy of AZD1208 & IBL-301 in gefitinib resistant HCC827GR & Clone 3 cell lines

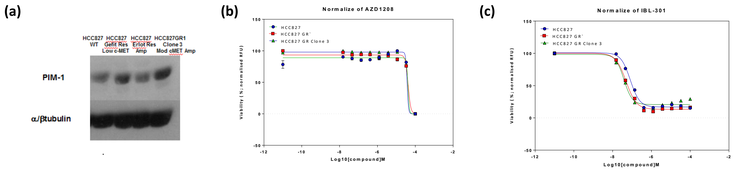

Figure 6: PIM-1 expression & Efficacy of AZD1208 & IBL-301 in HCC827GR & Clone 3 cell lines (Crown Bio)
(a) PIM-1 expression was examined in gefitinib sensitive HCC827 cells, gefitinib resistant cells with low MET amp, erlotinib resistant cells & gefitinib resistant Clone 3 with moderate MET amp (Resistant cell line models developed by Crown Bioscience)
(b) Drug dose response curves examined the efficacy of AZD1208 in gefitinib resistant HCC827GR and HCC827GR Clone 3 (MET amplified) versus HCC827P cells using the Cell Titre Blue assay (Promega). Cell viability was measured after drug treatment for 72hr (IC50s: 48.2nM vs 44.9nM vs 47.1nM respectively)
(c) Drug dose response curves examined the efficacy of IBL-301 in gefitinib resistant HCC827GR and HCC827GR Clone 3 (MET amplified) versus HCC827P cells. Cell viability was measured after drug treatment for 72hr (IC50s: 111nM vs 83nM vs 141nM respectively)

The effect of AUM302, AZD1208 & AZD1208 + Erlotinib treatments on protein kinase phosphorylation in Clone 3 and Clone 10 :

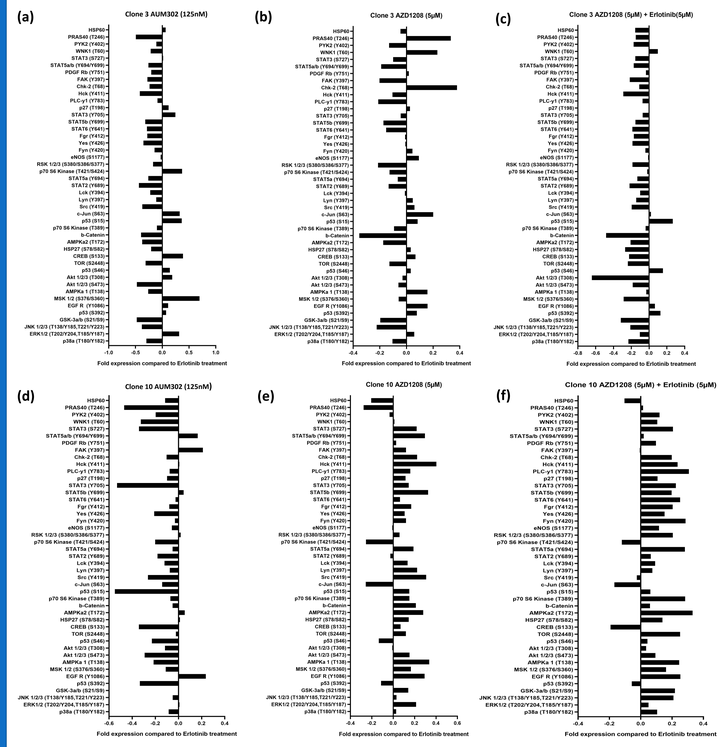

Figure 7: The effect of PIM inhibitors on protein kinase phosphorylation in Clone 3 and Clone 10
After 24 hours treatment with (a) AUM302 (125nM), (b) AZD1208 (5μM) and (c) a combination of AZD1208 (5μM) & erlotinib (5μM) protein kinase phosphorylation was decreased in HCC827ER Clone 3 when compared with HCC827ER Clone 3 treated with (5μM) erlotinib alone. After 24 hours treatment with (d) AUM302 (125nM), protein kinase phosphorylation was decreased in HCC827ER Clone 10 when compared with HCC827ER Clone 10 treated with (5μM) erlotinib alone however treatment with (e) AZD1208 (5μM) and (f) a combination of AZD1208 (5μM) & erlotinib (5μM) had little effect on protein kinase phosphorylation. Densitometry analysis was carried using Image J and the calculation and graphs were made using GraphPad Prism 8. All graphs are compared to Erlotinib treated cells.

Conclusion

Here we identified activated PIM kinase and c-Myc in MET amplified erlotinib resistant NSCLC cell line HCC827ER. Further characterisation of erlotinib resistant subclone 3 (MET amplified) and 10 (EMT) showed elevated PIM-1 kinase expression. C-Myc expression was elevated in subclone 3 cells but not subclone 10 cells. Erlotinib resistant HCC827ER cells and both subclones 3 (MET amplified) and 10 (EMT) were more sensitive to AUM302 than the erlotinib sensitive cells HCC827P. Gefitinib resistant HCC827GR cells and HCC827GR clone 3 had similar IC50 values to AZD1208 as gefitinib sensitive HCC827P cells while gefitinib resistant cells were more sensitive to IBl-301 than gefitinib sensitive cells. Exposure to pan-PIM inhibitor (AZD1208) alone and in combination with erlotinib resulted in a decrease in protein kinase phosphorylation in Clone 3 but had little effect on Clone 10 cells. AUM302 was effective at decreasing protein kinase phosphorylation in both Clone 3 and Clone 10 cells. Co-targeting treatment strategies (either in combination or sequentially) with a PIM kinase inhibitor and EGFR TKI may provide a more durable response for patients with an EGFR mutation and overcome EGFR TKI resistance.

References

(1) Jakobsen KR et al. MET amplification and epithelial-to-mesenchymal transition exist as parallel resistance mechanisms in erlotinib-resistant, EGFR-mutated, NSCLC HCC827 cells. Oncogenesis. 2017 Apr 3;6(4):e307.

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