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, showed improved response rates and extended median overall survival (OS), with potential to overcome previously unattainable resistance mechanisms. However, acceptance of mechanisms and activation of bypass RTK signaling 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 line to third-generation EGFR TKIs (osimertinib or CNI-206). We have pinpointed a novel strategic downstream target that plays a key role in MET resistance. Here we show that MET, PIM-1 and p-Met are activated in the erlotinib-resistant MET amplified HCC827ER cells compared to the erlotinib-sensitive HCC827P cells. Furthermore, subclones of MET amplified and MET subclone 3 shows elevated c-Met and PIM-1 expression and MET HCC827ER subclone 10 shows MET PIM-1 overexpression but not c-Met. We hypothesise that co-targeting PIM kinase and EGFR may provide a more durable response to treatment and overcome EGFR TKI resistance.

Methods

• HCC827ER cells developed resistance to erlotinib through continuous exposure of the erlotinib-sensitive parent cell line HCC827 to increasing concentrations (20nM - 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.
• MET, c-Met and PIM-1 expression were examined by Western blot analysis in HCC827 sensitive (HCC827E7R) and resistant (HCC827ER) cell lines and selected subclones of HCC827ER under both MET and PIM-1 clone 10.
• Quantitation & localisation of Met & PIM-1 was examined by immunofluorescence.
• Efficacy of novel PIM inhibitor IBL (AUM302) was quantified using the CellTiter Blue, cell viability assay, in all cell lines.
• Efficacy of AZD1208 & IBL-301 in gefitinib resistant HCC827G7R & Clone 3 cell line were quantified (Crown Bioscience Inc).

The Effect of IBL on Protein Kinase Phosphorylation

• The Effect of PIM inhibitors on protein kinase phosphorylation was quantified by Proteome Profiler Arrays® (R&D Systems). In this in vitro approach, efficacy of novel PIM inhibitor IBL in decreasing protein kinase phosphorylation was evaluated in both Clone 3 and Clone 10 resistant subclones, and compared with a control (Clone 3) subclone.

Results

• MET, PIM-1, PIM-3 & c-Met expression in HCC827ER, Clone 3 & Clone 10 cell lines.

(a) MET, PIM-1, PIM-3 & c-Met protein expression 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-Met protein expression is significantly increased in HCC827ER and Clone 3 cells compared to HCC827P cells (P<0.05, n=2).
(d) Met protein expression is activated in HCC827P Clone 3 cells compared to HCC827P and Clone 10 cells.

Efficacy of IBL on TKI/PIM inhibitor AUM302 in HCC827ER, Clone 3 & Clone 10 cell lines.

(a) Drug dose response curves examined the efficacy of AUM302 in HCC827ER versus HCC827P cells using the CellTiter Blue assay. Cell viability was measured after drug treatment for 72h in (20nM-5μM) erlotinib and (0.5μM) AUM302.
(b) Drug dose response curves, the efficacy of AUM302 in HCC827ER Clone 3 (MET amplified) and HCC827ER Clone 10 (EMT amplified). Cell viability was measured after drug treatment for 72h in (20nM-5μM) erlotinib.

Efficacy of AZD1208 & IBL-301 in gefitinib resistant HCC827G7R & Clone 3 cell lines.

(a) PIM-1 expression in gefitinib-resistant HCC827G7R cells and gefitinib-resistant cells with low MET amp, erlotinib-resistant cells & gefitinib-resistant Clone 3 with moderate MET amp (Resistant cell lines models developed by Crown Bioscience Inc). In this drug response curves examined the efficacy of AZD1208 in gefitinib resistant HCC827ER and HCC827ER Clone 3 (MET amplified) versus HCC827P cells using the Cell Titer Blue assay. Cell viability was measured after drug treatment for 72h in (0.5-20μM) AZD1208 and (0-20μM) IBL-301.
(b) Drug dose response curves examined the efficacy of IBL-301 in gefitinib-resistant HCC827G7R and HCC827ER Clone 3 (MET amplified versus MET or HCC827ER cells. Cell viability was measured after drug treatment for 72h in (10nM-4μM vs 30nM-14μM) IBL-301 treatments.

Conclusion

Here we have identified activated PIM kinase and c-Met in MET amplified erlotinib resistant HCC827ER cell line. Further characterisation of erlotinib-resistant subclone 3 (MET amplified) and Clone 10 (EMT) showed elevated PIM-1 expression. C-Met expression was elevated in Clone 3 cells but not subclone 10 cells. Erlotinib-resistant HCC827ER cells and both subclones 3 (MET amplified) and Clone 10 (EMT) were more sensitive to AUM302 than the erlotinib-sensitive HCC827P. Gefitinib-resistant HCC827ER cells and HCC827ER Clone 3 had similar c-Met in gefitinib-sensitive HCC827G7R cells while gefitinib-resistant cells were more sensitive to IBL 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


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