TEMPR (Targeted Endogenous Mapping of Pharmacological Resistance) v2: a CRISPR-based strategy to identify mutations in the MET kinase domain conferring resistance to Savolitinib

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Lung cancer is the most common cause of cancer worldwide, with NSCLC accounting for 85% of cases and a minority driven by EGFR signalling. Effective therapies for EGFR driven NSCLC include EGFR tyrosine kinase inhibitors (TKIs). Whilst these are initially very effective, patients relapse due to emergence of the EGFR T790M mutation (60% of cases) or amplification of the receptor tyrosine kinase MET (20% of cases). MET TKIs, such as Savolitinib (AZD6094, HMPL504, volitinib), in combination with EGFR TKIs hold great promise for treatment of NSCLC. However, recent clinical evidence has shown emergence of additional MET mutations conferring resistance to MET TKIs. Thus, a method to confidently predict pre-clinically the onset of acquired mutations in a targeted fashion is an attractive prospect, which would enable to evaluate the resistance "potential" of new drug candidates.

The aim of this project is to combine a saturation mutagenesis approach with the CRISPR-Cas9 technology to endogenously mutate each amino acid position across the MET kinase domain to every other possible amino acid. The pool of cells harbouring MET mutants will be treated with Savolitinib to identify mutational 'hot-spots' that drive resistance to MET-TKIs. This endogenous, on-target approach has been termed TEMPR v2. As starting point, we will target those amino acid positions that from structural analysis result in close proximity to known MET ligands.

As a proof of concept for the feasibility of this approach, we show that it is possible to introduce known mutations in EGFR in PC9 cells using a long dsDNA oligo (~2Kb) as a template. We then identified a suitable MET amplified cell line amenable to efficient transfection with genome editing reagents as well as exhibiting a high degree of sensitivity to savolitinib. To this end, we tested the efficacy of savolitinib in 3 MET amplified cell lines and optimised the delivery method to obtain the best overall editing efficiency. Consequently, we identified the best guide RNA that in complex with Cas9 would introduce genome editing in the MET exons of interest. A DNA donor library containing all the possible mutations for each target amino acid was designed, synthesised at TWIST and will be delivered to the cells together with CRISPR/Cas9. After treatment with Savolitinib, cell pools will be analysed using amplicon sequencing to identify resistance mutations.