Background:

There is an urgent need for new antibiotics with novel mechanisms of action to treat life-threatening infections caused by multi-drug resistant bacteria. Summit Therapeutics’s proprietary transposon-based platform technology (Discuva Platform) allows the identification of totally novel chemical optimisation allowing selection of the best clinical candidates. The Discuva Technology (Figure 3), which combines high density transposon mutant libraries and next generation sequencing, simultaneously assays all of the transposon mutants in a single pool. The genomic profile revealed by the Discuva Technology aligns very well with reported transcriptional impacts (Figure 4 and 5) of this antibiotic. Upregulation of murh (larger) is a strong driver of resistance (to 4µM), the analysis also identifies upregulation of a carbon-phosphorus lyase complex (phmG-I-P) as a potential driver for resistance through metabolism and inactivation of fosfomycin (Figure 4).

**Methods:**

**Transposon Library Generation**

- High density transposon mutant libraries were generated in identified bacterial strains (Figure 1). Tn5 or mariner transposition methodologies were adapted to ensure maximum efficiency in the target strain. Mutants pools were generated to ensure a frequency of insertion context the transposon can give rise to activation, downregulation or inactivation at a particular loci. The targeted pathogens capture the priority and urgent threat organisms recognised by CDC1 and WHO2.

**High density transposon mutant profiling to enable antibiotic drug discovery and development of novel antimicrobials**

Key disruption (inactivation) signals (Figure 5) reflect the mode of uptake and transport of fosfomycin; Disruption of the hexose phosphate transporter (phm,CA) enables a survival benefit by reducing uptake of fosfomycin. Additionally expression of ump is regulated by CAMP and disruption signals for cyoo and cyol reflect a mechanism to reduce CAMP and hence desensitize the transporter. Disruption at the pst loci (pstB,AC,S) also confers a mild survival benefit (below MIC) attributed to the function in phosphate sensing and transport.

The genomic profile revealed by the Discuva Technology aligns very well with reported in vitro and clinical resistance identified for fosfomycin1. Insertion site data is also used for comparative analysis across antibiotic classes and for benchmarking novel compounds (Fig. 6).

**Conclusions:**

There is a desperate need for differentiated antibiotics represented by new chemotypes associated with novel mechanisms of action that are devoid of pre-existing resistance liabilities. Our Discuva Platform brings together a proprietary transposon-based platform technology and bespoke bioinformatics software to revolutionise the antimicrobial discovery and development process. It empowers phenotypic screening by rapidly moving from a antimicrobial activity to a genome-wide interaction profile to inform on compound mechanism of action and resistance liabilities. The Discuva Platform not only provides a mechanism to prioritize compounds from HTS but also allows for a mechanistic understanding to be taken during lead optimisation. Ultimately, the Discuva Platform can select from within a chemical series or across different series the most optimal compound for clinical development.

**References:**

1. CDC Antimicrobial Resistance Threats Report 2013
2. WHO priority pathogens list for R&D of new antibiotics 2017