CARB-X
Combating Antibiotic Resistant Bacteria

Development Stages in Scope
Therapeutics

What CARB-X considers a "Hit" when identifying your project/program in "Hit-to-Lead".

- Scaffold(s) demonstrated to inhibit a defined target in at least in one relevant assay, has ample chemical space to explore, shows indications of Structure-Activity-Relationships that can be optimized
- Demonstration of expected cell-based antibacterial mechanism of action
- Active upon resynthesis; >90% purity
- Low cytotoxicity against a relevant human cell line which suggests selectivity will be achievable
- Demonstration of activity against a wild-type pathogen(s) that is relevant to the desired target indication in a biologically appropriate assay (e.g. MIC assay for direct-acting therapeutics)
- If an oral product is proposed, molecular properties that portend good oral bioavailability (medium-to-high Caco-2-cell (or equivalent) permeability or – if in a class where transport or efflux liabilities are a consideration or if in a later stage of development – positive baseline exposure (at least >10 %F) in appropriate compartments when dosed orally *in vivo*). Any proposed prodrug strategy must be explicit and well-defined, with baseline demonstration of conversion to the parent drug at a rate consistent with pharmacological efficacy.

Hit-to-Lead (Lead Generation)

- Initial structure-activity relationship established and chemical design across a number of lead scaffolds have been explored and areas to improve on *in vitro* potency, selectivity, chemical stability and synthetic tractability, cytotoxicity, and other drug-like properties have been identified.
- Demonstrate *in vitro* activity against wild-type representatives of all TPP-targeted pathogen(s)
- Generate preliminary *in vivo* proof-of-concept efficacy data in relevant infection model showing statistically-significant efficacy versus untreated control group. Where a positive control is available, demonstration of model validation.
- *In vitro* ADME suggests no obvious liabilities with respect to microsomal stability, plasma stability, and protein binding across relevant species
- Synthetic route suggests compound scale-up will be possible to support LO and IND enabling studies and source of relevant starting materials/intermediate(s) identified
- If an oral product is proposed, positive baseline exposure (>10 %F) in appropriate compartments when dosed orally *in vivo*, plus evidence of efficacy in a relevant infection model via the oral route

Lead Optimization

- Synthesize compounds from selected lead scaffold(s) - new analogs with improved potency, reduced off-target activities, and physiochemical/metabolic properties suggestive of reasonable in vivo pharmacokinetics compatible with human dose
- Expand microbiological understanding of lead molecules (spontaneous resistance frequency, mechanisms of resistance, killing kinetics, population MIC determination, including testing against contemporary, molecularly-characterized antibiotic-resistant pathogens); confirmation of antimicrobial mechanism of action in targeted pathogens
- Identify suitable toxicological species and conduct non-GLP *in vivo* toxicity (MTD and repeat dosing) in accordance with the product's intended use
- Initiate experiments to identify correlate of efficacy (e.g. PK-PD driver) and suitable endpoints for further pre-clinical studies
- Demonstrate *in vivo* activity and potential for efficacy consistent with the product's intended use (i.e. dose, schedule, duration, route of administration) against multiple TPP pathogens (or multiple isolates for narrow-

spectrum agents), covering the range of activity seen in vitro. Efficacy endpoint should be comparable to or better than standard-of-care.
- Profiling in *in vitro* safety and secondary pharmacology assays
- Selection of candidate to progress into pre-clinical evaluation

Pre-Clinical (IND Enabling)
- Demonstrate acceptable Absorption, Distribution, Metabolism, and Elimination (ADME) characteristics in non-GLP animal studies as necessary for IND filing
- Completion of suitable microbiological IND package demonstrating appropriate differentiation
- Conduct GLP non-clinical studies for toxicology, safety pharmacology, genotoxicity, and immunogenicity (as appropriate) to demonstrate that an acceptable therapeutic index (TI) is achievable
- Continue evaluation in animal models or *in vitro* systems with efficacy and dose-ranging studies to understand the range of PK/PD driver magnitudes required across a panel of isolates to provide confidence for clinical success
- Develop a scalable and reproducible manufacturing process amenable to GMP. Manufacture GMP-compliant pilot lots
- Initiate development of in-process assays and analytical methods for product characterization and release, including assessments of potency, purity, identity, strength, sterility, and quality as appropriate
- Prepare a defined strategy for interaction with regulatory agencies
- Prepare and submit Investigational New Drug (IND) package to FDA or appropriate documentation to other relevant regulatory authorities

Phase 1
- First-in-Human single ascending dose (SAD) and multiple ascending dose (MAD) clinical trial(s) to determine the safety and pharmacokinetics of the clinical test article in healthy volunteers (in certain circumstances, patients)
- Determination of microbiological QC ranges and manufacture and testing of AST assays to support Phase II studies
- Expansion of PK/PD understanding, refinement of population PK models and Monte Carlo simulation to refine Phase II dose selection
- Additional CMC, formulation, and analytical activities required to support further clinical development