CARB-X
Combating Antibiotic Resistant Bacteria

Development Stages in Scope
Therapeutics

The earliest eligible stage for CARB-X funding is Hit-to-Lead, and CARB-X defines a "Hit" as meeting the following minimal entry criteria.

- Scaffold(s) demonstrated to act against 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, suggesting selectivity will be achievable (CC_{50}:MIC ratio ≥10).
- 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, e.g.: 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 (≥10 %F) in appropriate compartments when dosed orally in vivo. If permeability data are given, then transport in both directions (apical to basolateral and basolateral to apical), and assay control data (i. e. efflux control compounds), must be shared to underscore the extent to which efflux will be an optimization driver. Units must be given with any numerical data. 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.
- If an IV product is desired, physicochemical properties providing adequate aqueous solubility at a clinically acceptable pH should have been demonstrated.
- Suitable physicochemical properties to support formulation work in-line with anticipated route of administration.

Typical activities during 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 (minimum endpoint = stasis). 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 (minimum of rat, dog, and human).
- 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.

Typical activities during 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.
- Perform pre-formulation studies to identify an appropriate non-DMSO formulation in which the non-GLP toxicity and efficacy studies should be performed in.
- Profiling in *in vitro* safety and secondary pharmacology assays.
- Selection of candidate to progress into pre-clinical evaluation.

Typical activities during 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).
- 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 and material to support a first-in-human trial.
- 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.

Typical activities during 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.