Written responses to open questions of the webinar 'From discovery to IND: Roadmap to a successful antibacterial project' by Patricia Bradford and Alita Miller, originally broadcast on 25 February 2021. See webinar recording here: https://revive.gardp.org/from-discovery-to-the-pre-clinical-antimicrobial-candidate/

Question asked	Response from the speakers
How would the approach be different when trying to discover inhibitors of virulence factors that wouldn't kill bacteria?	The key difference is that the *in vitro* assay to measure relative potency of analogs would not be inhibition of bacterial growth (as measured by MIC) but some other phenotypic assay that had been proven to strongly correlate to in vivo efficacy. Therefore, from a practical standpoint, this approach might be quite challenging for both development and clinical use.
Thank you for nice webinar. I am a PhD scholar. In my lab I test the intracellular killing activity of Ikarugamycin compound against intracellular S. aureus. The compound has shown a promising result against both intracellular S. aureus and Extracellular. I did toxicity assay toward mammalian epithelial cell, the cells line tolerance the compound even 4 time thank bactericidal concentration. So, my question what you suggest to me? Do I can continuous to test this compound or what is the problem of Ikarugamycin?	Ikarugamycin is a known toxin, although the mechanisms of toxicity are not well-defined. See https://www.caymanchem.com/msdss/15386m.pdf
It is straightforward to check, but what is the practical clinical relevance if drug is static or cidal?	The differentiation between bacteriostatic and bactericidal drugs is a useful piece of information in deciding on treatments for serious infections like endocarditis or in immunocompromised patients. In these cases, a bacteriostatic drug may not provide optimal results. For most infection types, either mechanism is acceptable.
Q for Dr. Miller: If one has a good candidate hit that inhibits in the low/mid uM range. What is the best next step to begin hit optimization to lower the MIC. At what point does one want to start talking to chemists and who should one approach in the first place (i.e., colleagues in academia vs. commercial services)?	It is best to engage a medicinal chemist (which is not necessarily the same thing as an organic chemist) as early as possible so s/he can help you select analogs to establish SAR. This is more straightforward if the molecular target is known and even easier to do (in theory) if there is a crystal structure. In that case, a computational chemist could also help. If your academic chemistry colleagues are not medicinal chemists, they still should be able to tell you where to find this type of commercial resource.

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Dr. Bradford: Please, could you discuss how much Fasted State Simulated Intestinal Fluid is important in the early stage of drug development process? Thanks!	This is used in formulation studies for oral drugs. It is not important in early phases of drug development
How is a "CFU-count in the thigh" performed?	At the desired time point, the animal is sacrified and the thigh is harvested, weighed and ground up with buffer into a slurry which is then plated on agar in serial dilutions (and incubated overnight at 37^{o}C). This allows for the quantification of the number of bacteria per gram of tissue. Often this agar contains selective media such that only the pathogen of interest is isolated (to minimize contamination).
Can you recommend how people judge the can you recommend how people decide on the duration of dosing in early clinical studies?	The duration of dosing for early clinical studies is based on the severity of the disease and standard of care. Most clinical trials require treatment for either 5 or 7 days as the lower limit and up to 14 days for the upper limit. PK/PD will determine the length of infusion time. For example, drugs that have a PK driver of *f*T>MIC may provide increased efficacy with a prolonged infusion.
Would you recommend a textbook that covers today's content?	Unfortunately, I am not aware that such a textbook exists.
What are the requirements for anti-virulence agents?	This is covered in a recent, excellent review by Drs. Theuretzbacher and Piddock in Cell Host & Microbe 2019 (26) 61-72 https://doi.org/10.1016/j.chom.2019.06.004.
If one is targeting an inhaled antibiotic, what PK/PD models are available.	There are animal models that can be done with inhaled delivery of antibiotics. However, doing any kind of PK to go along with the PD is difficult, because it is never certain how much drug the animal receives. The FDA currently views inhaled antibiotics as topical agents. Therefore, PK/PD requirements are substantially reduced.

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What kind of cell line is more suitable to predict the in vivo toxicity	There are multiple eukaryotic cell lines routinely used as a first pass for in vitro toxicity, such as HeLa, HEK293, HT29 etc. However, results from a single cell line will not be sufficient to predict in vivo toxicity. Rather, it will likely require evaluation in several counterscreens depending on the mechanism of action, route(s) of metabolism and likely off-target effects. For example, scaffolds with known nephrotoxicity issues (like polymyxins and aminoglycosides) should be screened in primary kidney cell lines.
How to check mutation frequency if compounds not working well in agar plates	It is also possible to raise resistant mutants in liquid culture using a method called serial passage. The method used for daptomycin in S. aureus is a good example, see: https://aac.asm.org/content/45/6/1799
Can the in vivo efficacy studies be done in rats instead of mouse?	Yes, they can and some important models are done in rats or even rabbits such as endocarditis models. For most discovery efforts, the amount of compound available is an issue and this is much lower in mice. Also mice are less expensive.
Dr. Miller or Dr. Bradford, regarding the use of in vitro models does the mechanisms of resistance development temper your enthusiasm for use of these models (i.e., beta-lactamase producers and rapid development of beta-lactam resistance)?	Sometimes yes. Compounds that have a single gene target often result in rapid resistance development in the target gene. These are then considered to be poor candidates for advancement.
What issues related to PK parameters are needed to the repurposing of a non-antimicrobial drug (e.g., antiepileptic) that shows antibacterial activity (low MIC against bacteria) as an antimicrobial agent?	Repurposing drugs that were designed for other diseases is tricky because they already have built in side effects. With antibacterials, there is very little tolerance for any toxicity or tolerability issues. To date, this approach has not yielded any useful candidates for further development as an antibacterial agent.
Dr Miller. How does one contend with safety data if high inhibition/activation values are observed?	The only path forward would be if the SAR for the mechanism of toxicity is clearly distinct from the SAR of target inhibition (i.e. mechanism of bacterial killing). The sooner this can be ruled in/out, the more quickly the decision to stop or move forward can be made.
Would you substitute in vitro micronucleus for Ames?	Both of those assays must be done.

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Is it recommended to perform in-vitro cytotoxicity studies in serum or protein deficient media	In vitro cytotoxicity studies should be performed using the same amount of protein as the MIC so a direct comparison can be made. Ideally, both of these assays should be tested with and without the presence of serum.
Can Patricia further comment on duration of GLP tox studies and minimal exposure margin needed to enable FIH?	The duration of GLP tox studies is set- two weeks in two species prior to IND filing. The optimal exposure margin between efficacy and toxicity should be at least 5X. A safety margin is less than that should then be discussed with the regulatory authorities to determine if the project can proceed into FIH studies.
Dr Miller: For P1 studies, are there strategies to get a read out of "efficacy" via in-vitro studies. E.g. screening urine samples for bacterial kill.	Phase 1 is for safety only. However, as a part of this, parameters like urine levels will be measured. Testing in vitro activity in various bodily fluids such as urine, or synovial fluid is done as a part of the preclinical data package.