MICROBIOLOGY SUPPORT FOR PHAGE THERAPY TRIALS:

THE DEVILS ARE IN THE DETAILS

Summary of Bacteriophage Therapy Summit presentation

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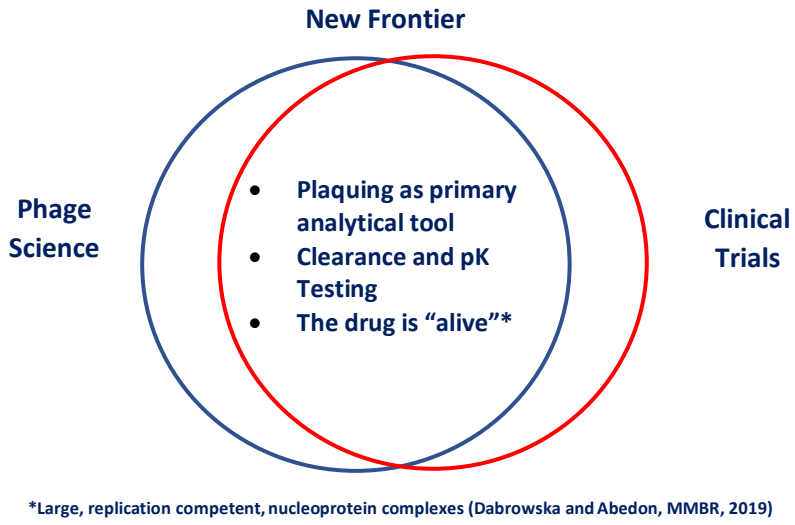
The "Devils" – How does phage science translate to regulatory approval and clinical trials?



*Large, replication competent, nucleoprotein complexes (Dabrowska and Abedon, MMBR, 2019)

Phage science is new to the clinical trial arena, and for that reason, brings with it a set of challenges since phage science does not seamlessly translate to the FDA's current drug development model. Although guidance documents from regulatory bodies are available for small molecules, none currently exist for bacteriophage clinical studies. There are some key points that challenge the navigation and execution of phage product development through clinical trials:

(1) Plaquing continues to be the primary analytical tool but is notably labor intensive.

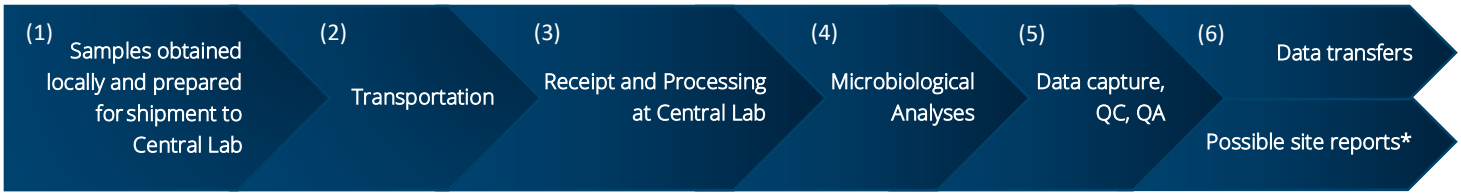
(2) Plaquing is used to not only measure bacterial susceptibility to phage, but also phage clearance and pK analysis.

(3) Phage are biologically active and, as such, transportation conditions, timing and processing methods can affect their viability and titers. The extent to which these factors affect phage cocktails must be understood long before any clinical protocol is finalized.

This document provides insights as to how these challenges can and should be addressed prior to beginning clinical trials.

What do "typical" small molecule antibacterial clinical trials look like?

The overall design between the sponsor and regulatory agency is study specific, but certain processes are common across many trials, as illustrated in the following timeline.



(1) Samples are obtained locally and prepared for shipment to a Central Laboratory. Samples can be specimens (e.g. sputa, urine, stool, etc.) or bacterial isolates from specimens processed locally.

(2) Details of the samples' transportation are determined. This includes the frequency, temperature, and speed at which the samples will be shipped. This can also include the length of time samples will be sent to the Central Laboratory (i.e. First-Patient First-Visit, Last-Patient Last-Visit).

(3) The Central Laboratory will receive and process samples accordingly (e.g. filter, centrifuge, etc.).

(4) The Central Laboratory will perform designated microbiological analyses which can include organism culture, identification, susceptibility testing, and molecular analysis.

(5) Data capture, quality control (QC), quality assurance (QA)

(6) Data transfers to CRO or sponsor and possible reports to clinical sites.

***** *Conventional microbiological considerations have been highly validated and standardized for some time.*

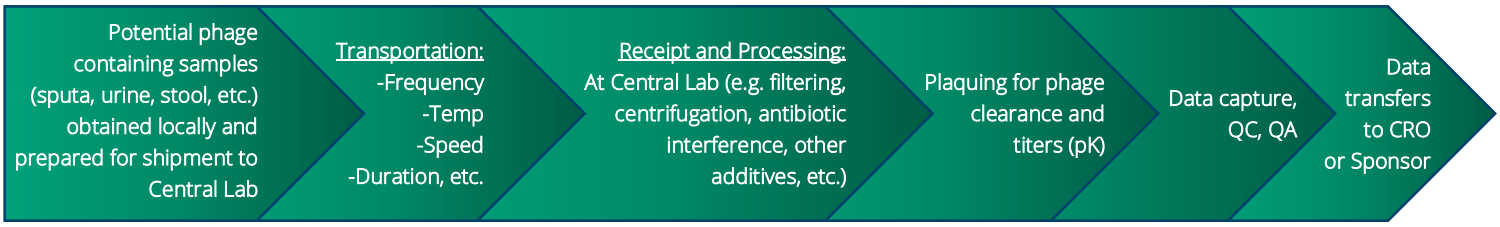
How does a central laboratory test phage effectiveness?

Assumption is that microbiology specimens are obtained prior to initiation of phage therapy.
If bacterial susceptibility to phage is needed for enrollment, rapid turn-around-time is needed.

What are the measures of phage effectiveness? There are more questions than answers at this point in time. The following are key considerations for measuring phage effectiveness:

- What phage "effectiveness" is to be measured?
    - Activity against target bacteria isolated? Susceptibility to phage – How is this defined?
        - There are a lack of standard definitions and measurement units. Examples of possible units include PFU/mL or ratio organisms compared to control.
    - Ability to reduce bacterial burden? How is reduction defined? How much is enough?
    - Phage effect on microbiomes? How much change is considered significant?
    - Others?
- If microbiology samples are taken to measure microbiological outcomes after therapy has been initiated; how is phage effect on culture to be managed?
- If spot titering and/or whole plate methods be used for plaquing assays, the number of replicates, or if different methods need to be developed and deployed.
    - Do you know how well target bacteria grow in the plaquing lawns? This must be validated with clinical bacterial species prior to study start up.
    - <u>There are currently no standard methods.</u> Effectiveness measurements will need to be validated in hands of the Central Lab prior to trial; especially for phage-bacteria interactions.
- Do you measure effectiveness of cocktail only? Or individual components as well? This can impact workload and costs for the plaque-based assay.
- What are the criteria for re-test or unacceptable results?
- Data recording and transmission (i.e. Data Management).

What are the challenges of clearance/pK testing of a "live drug"?



Take into consideration the following aspects to mitigate potential challenges.

- What is the durability of the cocktail and its components from study site collection through specimen processing at the Central Lab?
- How well is titer maintained?
    - In relevant specimen sources and condition of specimen (e.g. varying urine pH's).
    - Over anticipated time spans and transport/storage conditions during travel and at Central Lab.
    - Through lab processing (centrifugation, filtration, additives).
    - What validations need to be run by Sponsor or Central Lab to determine titer stability?
- Potential substance interference of phage titers
    - Contaminating bacterial flora
    - Other antimicrobial substances/processing supplements
    - Processes to neutralize the effect of such substances on phage titers need to be developed and validated.
- Design must consider workload and costs incurred by sample numbers
    - Plaquing assay is not a high throughput process.
    - Reading plaquing is more of an art form than an exact science.
    - Alternative less manual methods to plaquing are needed.
    - Central lab workflow adjustments will be needed:
        - Number of samples per patient and time milestones drive up cost and workload.
        - Number of replicates and controls, cocktail +/- components.
        - Keep in mind the throughput ability of the Central Lab. IHMA's throughput is approximately 25 samples/day/full-time employee (FTE).
        - *How is the frequency of pK sampling established, could it be optimized based on animal trials?*

Data Management Considerations – What to keep in mind

Data management pertains to both phage effectiveness and clearance/pK aspects of a trial. Below are some key points to remember when making decisions involving your study's data.

- For plaque-based assays; Identify what data needs to be recorded.
- What is considered "Raw" data vs "Final" data to be transferred?
    - All (plaque forming units) PFU per cocktail and components done by spot and whole plate, with controls, in triplicate, etc.
- Scalability can be very challenging in the Central Lab setting.
    - In IHMA's experience, phage sensitivity of target bacteria is approximately 20/day/FTE. Antimicrobial susceptibility testing (AST) by minimum inhibitory concentration (MIC) is approximately 200/day/FTE.
- What criteria need to be set for assigning a pass/fail or rejection of results? For example:
    - Unexpected results due to technical errors or inherent difficulties of the assay
    - What is the repeat policy for failures?
- For passes, what data needs to be sent across in the data transfers to the CRO or Sponsor?

The infrastructure to capture all the various data, replicates, controls, re-tests etc. can be complex and cumbersome and requires careful planning; especially for the volume of pK samples.

Summary

- Phage science is meeting the fast-paced expectations of clinical trial processes.
- Use of a "Live" drug presents many unique and untested challenges.
- Plaque assay as the current primary analytical method presents scalability and cost control challenges – what are the potential alternatives?
- Challenges can be met and overcome but requires early and often collaborations between sponsor and central microbiology laboratory (CML).
- Selection of a CML that is able to support both microbiology and phage testing reduces logistical, communication, and data coordination challenges.
- Early and frequent discussions with the regulatory agency is key to clinical trial design due to lack of regulatory guidance documents.
- The sooner a CML is selected and engaged, the better it is for all parties.

Support of Phage-Based Therapeutics – What can IHMA do to support your phage product?

In addition to our full suite of conventional and molecular microbiology capabilities IHMA has extended capabilities to now include the testing of bacteriophage. We have the ability to design and perform pre-clinical trial validations, perform phage plaquing assays with a goal of determining effectiveness of the client's phage cocktails against target bacterial species and determination of phage concentrations in human samples such as serum, sputa, stool, urine, etc. Other laboratory tests include pre-clinical profiling of phage activity against target species curated from the IHMA Bacterial Repository and evaluating *in vitro* effectiveness of phage products against bacterial species as part of a clinical trial.

If you require testing beyond plaquing, IHMA can adopt new methods such as liquid-based, automation, qPCR, etc.

Clinical trials will put your phage's stability to the test. At IHMA, we can help design and implement validation studies to determine the stability of the phage in biological samples over time, transport conditions, and specimen processing protocols.

About IHMA

IHMA is a premier provider of antimicrobial drug development studies, offering a full suite of technical capabilities and domain expertise in all phases of antimicrobial drug development: drug discovery, clinical development, regulatory approval and commercialization. With facilities in the USA, Europe, China and India, IHMA is able to collaborate with and support companies around the world in the pharmaceutical, biotechnology and diagnostic device industry.