charles river

DISCOVERY

Infectious Disease Drug Discovery Pharmacology Models and *In Vitro* Capabilities

Charles River has a strong drug discovery background in infectious disease, for the assessment of novel antimicrobials, antivirals and vaccines, using established *in vitro* assays and *in vivo* models. Our in-house expertise enables us to work with drug discoverers in designing experiments, optimally, to determine the efficacy of anti-infectives and vaccines, supporting clients in early proof-of-concept and the selection of key candidate drugs.

In addition to our *in vitro* services and *in vivo* pharmacology models, we offer the *Galleria mellonella* model for early screening. For bacterial studies, we use both a range of Gram-negative and Gram-positive strains which include clinical isolates, resistant strains, as well as bioluminescent strains enabling in-life analysis and temporal monitoring of infection using the IVIS® *in vivo* imaging system.

Services and Tools for Infectious Disease Drug Discovery:

- *In vitro* bacterial assays
- *In vitro* viral assays
- ELISA/Luminex®/FACS
- In-life imaging (IVIS®)
- Additional tools and assays

- *In vivo* bacterial models:
  - *Galleria mellonella* model
  - Neutropenic thigh model
  - Bacterial peritonitis model
  - Intravenous infection model of sepsis
  - Bacterial lung infection models
  - Deep wound and topical skin infection models
  - Urinary tract infection model

- *In vivo* viral infection models
  - Influenza models
  - Herpes simplex virus models

EVERY STEP OF THE WAY

In Vitro Bacterial Assays

In vitro testing of antimicrobial compounds can determine potential efficacy, in bacterial cultures or cell-based assays, to provide important data to determine optimal dosing regimen. Our *in vitro* offering includes MIC, MBC, time-to-kill, *in vitro* pharmacokinetic and pharmacodynamic assays, cell-based, and immune modulation assays.

In Vitro Viral Assays

Cell-based viral assays include plaque assays, $TCID_{50}$, EC_{50}, and HAI assays (hemagllutination inhibition). Virus can be propagated in cells or eggs (influenza virus) and EID_{50} determined.

ELISA/Luminex®/FACS

Charles River can provide assessment of immune responses by *in vitro* analysis of blood (serum/plasma) for specific antibodies or cytokines. Cellular responses in spleen and/or lymph nodes may be determined using flow cytometry (FACS).

In-life Imaging (IVIS®)

Charles River's *in vivo* imaging system (IVIS) utilizes bioluminescent bacteria such as *P. aeruginosa* Xen5 which stably expresses the *Photorhabdus luminescens* lux operon on the bacterial chromosome. Measuring bioluminescence allows the progression of an infection to be monitored in-life, reducing the need for satellite groups. Bioluminescence, which has a linear relationship with bacterial load, can be compared directly to clinical observations. Growth of bacteria in untreated/vehicle treated groups can readily be compared with control/test treatment groups to demonstrate reduction in bacterial growth.

Additional Tools and Assays

Additional readouts include clinical disease (disease scores, body weight, temperatures), survival, bacterial load measurements by CFU determination, viral load by plaque assays or $TCID_{50}$, bioanalysis, pathology (gross observations, histopathology), and immunohistochemistry.

Galleria Mellonella Model

The *Galleria mellonella* (wax moth larvae) model enables early screening of antimicrobials to provide information on compound toxicity and efficacy, and help design optimal dosing regimens, including the assessment of combination therapies, prior to use in a murine model of infectious disease. The main readout is survival (Kaplan-Meier); the additional use of bioluminescent bacteria enables the infection to be tracked using GloMAX® and IVIS technology.

Neutropenic Thigh Model

The thigh infection model provides a sensitive experimental system for the study of antimicrobial efficacy. The model allows the evaluation of antimicrobial-microbial interactions combined with antimicrobial pharmacokinetics assessed in serum and tissues samples. Typically, mice are rendered neutropenic by cyclophosphamide treatment prior to infection. A wide variety of bacterial species of clinical interest may be employed.

Bacterial Peritonitis Models

Mice are infected with a bacterial suspension via intraperitoneal injection. A wide variety of clinically relevant strains can be utilized. Bacteria spread to multiple organs including kidney, spleen, liver, leading to sepsis. Spread of bacteria following assessment of CFU in different tissues can be compared following treatment with potential antimicrobials, vehicle, and control compounds following a variety of treatment regimen. Improved survival may also be demonstrated by Kaplan-Meier survival curves.

Intravenous Infection Model of Sepsis

Intravenous infection as a model of sepsis results in bacteria colonizing different organs leading to organ failure and death. Our murine sepsis model can be performed with neutropenic and non-neutropenic animals, and can be modelled using single or combination treatments. Bacterial load in different organs and survival can be assessed to determine efficacy of client treatments.

Bacterial Lung Infection Models

Bacterial infections in the lung leading to pneumonia are a major cause of death in the young, elderly, immunocompromised, and cystic fibrosis patients. The development of multi-drug resistant strains makes effective treatment difficult. Potential novel treatments may be assessed in several models of murine lung infection, including *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, and *Staphylococcus aureus*. The duration and severity is dependent on the bacterial strain. Efficacy of client treatments can be determined by reduction in clinical disease, improved survival, and bacterial load in the lung. The lung can also be assessed for gross pathology, cellular infiltrate and cytokines in the bronchial lavage fluid, and histopathology.

Deep Wound and Topical Skin Infection Models

Deep wound infections as a result of injury or surgery often lead to persistent chronic infections which are difficult to treat due to multi-drug resistance and formation of biofilms. Charles River has established several models of wound infection using *Staphylococcus aureus* and *Pseudomonas aeruginosa*, including the use of bioluminescent strains to enable in-life analysis of bacterial infection at the wound site. Wounds may be excised for determination of bacterial load by enumeration of CFU in homogenized tissue, and biopsies removed for histopathology.

Urinary Tract Infection Model

Urinary tract infections (UTIs) are amongst the most common infections in humans and represent the most frequent urological disease affecting bladder and kidneys. Those suffering from UTI often develop bacteriuria, cystitis, kidney infections, acute pyelonephritis, and fever, representing a significant burden on the healthcare system. In the murine model, there are a range of readouts including CFU in urine, kidney and bladder, pathology, bioanalysis, and bioluminescent imaging (IVIS) to monitor in-life infection.

Influenza Models

The pandemic of swine flu which arose from Mexico and the constant threat of new strains such as H5N1, has highlighted the potential risk to human health that influenza poses globally, and the importance of developing novel anti-influenza drugs and improved vaccines. Charles River models influenza virus infection in both the mouse and the ferret. The mouse model uses murine-adapted virus strains, and due to the highly characterized nature of the murine immune system is particularly suited for lead identification and for analysis of multiple immunological parameters in vaccine design. Ferrets are uniquely sensitive to human influenza strains, and display all of the key symptoms of infection, allowing the effects of novel drugs and vaccines to be tested on a wide range of human influenza viruses in a setting where the effects on classical influenza symptoms can be monitored.

Herpes Simplex Virus Models

Herpes simplex virus type-1 is a major cause of skin infection and blindness in the developed world. The ability of the virus to become latent in nerve ganglia results in recurrent infection. Following viral replication, subsequent damage to tissue is driven by immunopathology. A range of antiviral compounds or potential vaccines aimed at viral antigens and/or immunomodulation can be tested in our cutaneous or ocular HSV-1 models of infection. Clinical observations and viral load in tissues, as well as bioanalysis and histopathology, are useful endpoints for assessment of efficacy.

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