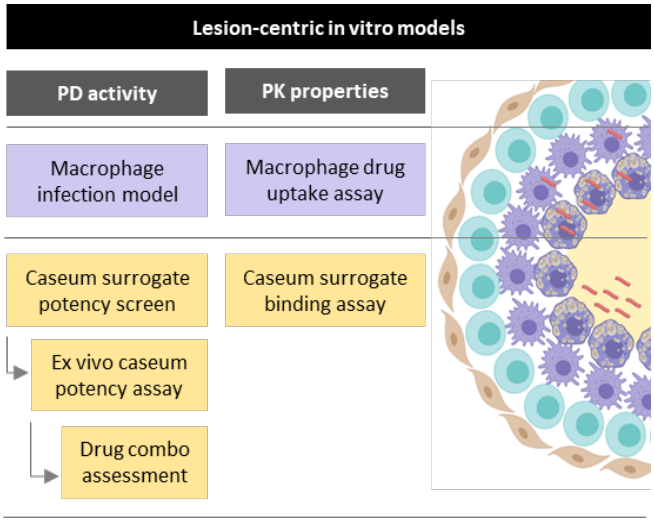
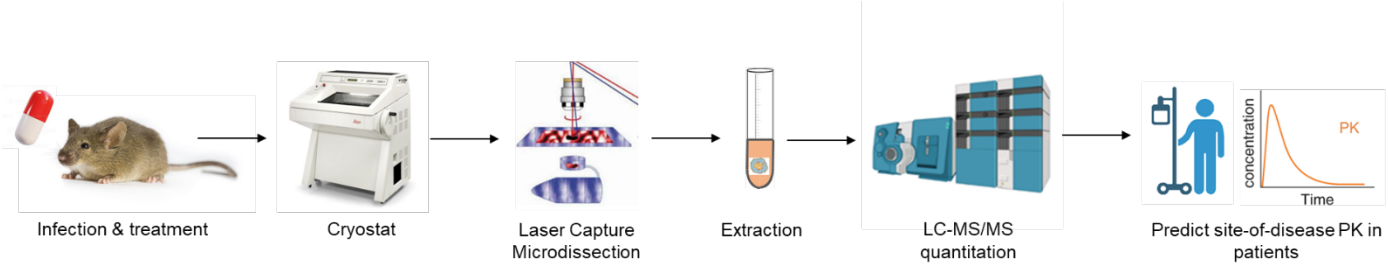
PReDiCTR-TB Consortium DMPK Preclinical Laboratory

Local environmental conditions in sub-lesion compartments drive metabolic and physiological changes in the pathogen. The ability of Mtb to enter a nonreplicating drug-tolerant state within the host is a major impediment to curing the disease. Hence, we offer a comprehensive platform for the measurement of drug potency against intracellular and extracellular Mtb in in vitro models that mimic conditions in these microniches. In addition, we have developed in vitro models that effectively predict drug partitioning in the cellular and caseous compartments of TB lesions. Overall, these in vitro tools guide medicinal chemistry and lead optimization efforts towards optimal PK-PD profiles. The **PReDiCTR-TB Consortium** further enables the integration of lesion-centric parameters of each drug candidate into systems pharmacology models to improve the translation of preclinical performance to clinical efficacy.



In vitro model systems at the DMPK PL. Several in vitro model systems were developed in-house to assess the PD and PK properties of drugs in macrophages (purple) and in caseum (yellow).

Traditional quantitative pharmacology approaches were previously focused on elucidating plasma PK and identifying human-equivalent doses that achieve efficacy in pre-clinical models. However, drug distribution at the site of action is now appreciated as a major determinant of treatment success. Clinical prediction platforms are being refined by incorporating differential drug exposure in distinct lesion compartments in the calculation of PK-PD models. Importantly, our lab has developed a novel protocol for the spatial resolution of drug distribution in granulomas and cavities by combining laser capture microdissection (LCM) technology with liquid chromatography -mass spectrometry (LC/MS) analysis. Using this workflow, we can quantify drug exposure in uninvolved lung, the outer cellular rim rich in lymphocytes, the inner cellular rim rich in foamy macrophages, the outer edge of the necrotic core and the center of caseum. Recent advances in our LCM technique have further allowed us to subdivide the caseous compartment into 'onion peels', with the sectioning of consecutive rings of necrotic tissue. This provides accurate drug distribution kinetics in the mostly avascular and acellular caseous core, the most problematic site of disease.



Workflow for the spatial resolution of drug distribution in lesion specimens. Plasma and lung lesion specimens are collected from *M. tuberculosis* -infected mice and rabbits that received drug treatment for predetermined durations. Frozen thin tissue sections (25µm) are visualized on a gravity-assisted laser capture microdissection (LCM) platform where specific lesion compartments are dissected and collected. Samples are extracted with organic solvents prior to analyte quantification by liquid chromatography -tandem mass spectrometry (LC-MS/MS).