Decoding Drug Efficacy and Host–Pathogen Interactions in Tuberculosis Using a Standardized *In Vitro* Granuloma-Like Structures Model

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Tuberculosis remains a major global health challenge, largely due to the ability of *Mycobacterium tuberculosis* (*Mtb*) to evade immune responses and persist within granulomatous lesions. Replicating these complex host–pathogen interactions *in vitro* remain difficult, as widely used models fail to capture the multicellular organization and dynamic microenvironment of granulomas, ultimately limiting drug development.

In this study, we developed and refined a human PBMC-derived Granuloma-Like Structure (GLS) model and directly compared it with conventional macrophage monolayers to evaluate drug activity in a more representative setting. A panel of 11 antitubercular compounds was tested across a range of concentrations and experimental replicates. We also investigated drug combinations mimicking the clinically used BPaL regimen within the GLS system.

To improve robustness, we integrated CFU-based bacterial quantification with imaging-driven analysis of GLS formation and evolution, combining brightfield and time-lapse microscopy. This approach, supported by a hybrid human- and AI-assisted pipeline, enabled quantitative assessment of GLS size and number, highlighting how similar CFU values may arise from structurally distinct granuloma populations. Consistently, drug efficacy varied with GLS size distribution, a relationship further supported by a simple mathematical framework and *in silico* modeling.

Comparison with monolayer cultures revealed distinct drug response profiles, with GLS better capturing immune-related effects. Interestingly, early GLS conditions appeared to favor bacterial expansion. Altogether, this work supports GLS as a reproducible and scalable platform for studying tuberculosis and improving preclinical drug evaluation.