Antibody Mediated Killing of Mycobacteria by Macrophage Cells Correlates with *In Vivo* Blood Clearance of *Mycobacterium tuberculosis*

Clara J. Sei[1*], Nimisha Rikhi[1], Richard F. Schuman[3], Luke T. Daum[2], Kevin Muema[1], John D. Rodriguez[2], and Gerald W. Fischer[1]

[1]Longhorn Vaccines and Diagnostics, Gaithersburg, Maryland, USA
[2]Longhorn Vaccines and Diagnostics, San Antonio, Texas, USA
[3]Antibody and Immunoassay Consultants, Rockville, Maryland, USA

*Corresponding author: **Clara Jebet Sei**, Microbiology and Immunology Laboratory Director, Longhorn Vaccines and Diagnostics, LLC. Email: cs@lhnvd.com, Phone: +1.240.848.4293

Background: Macrophage mediated clearance of *Mycobacterium tuberculosis* (MTB) plays an important role in preventing progression of tuberculosis (TB). Monoclonal antibodies (MABs) directed against MTB not only promote macrophage opsonophagocytic killing activity (OPKA), but also enhance blood clearance of MTB and may improve treatment of multiple drug resistant and extensively drug resistant (MDR/XDR) TB. Additionally, immunoprophylactic therapies may benefit immunocompromised HIV patients co-infected with TB for prevention and treatment of MTB bacteremia and disseminated disease.

Materials/Methods: The functional capacities of two anti-MTB MABs to promote macrophage OPKA, *in vitro*, and enhance clearance of blood MTB, *in vivo*, was examined. The antibody-mediated killing was evaluated using MABs JG7 & GG9 with THP-1 & U-937 macrophage cells and live *Mycobacterium smegmatis* (MS). Female ICR mice were injected intravenously with 10^8 CFUs of gamma-irradiated MTB strain HN878 twenty-four hours after intraperitoneal injection of opsonic MABs. Blood specimens were collected post-challenge at 15 minutes, 4 and 24-hours and clearance of blood MTB was quantified by qPCR.

Results: Both MABs (JG7 and GG9) promoted macrophage opsonophagocytosis and killing of MS across a broad range of concentrations (0.05 – 250 µg/mL) using U-937 and THP-1 cells, with peak OPKA greater than 80%. Only MAB JG7 provided enhanced blood MTB clearance 15-minutes post-challenge at 1 and 5mg/kg doses (p values = 0.0060 and 0.0248, respectively) and at 4-hours with 10mg/kg (p value = 0.0017). However, both MABs enhanced clearance at 24-hours, albeit differentially; with JG7 at the high 10mg/kg dose (p value = 0.0018) and GG9 at the low 1mg/kg dose (p value = 0.0559), compared to the placebo. For MAB GG9, there was no significant clearance at 15-minutes. It was interesting to note that optimal clearance early and at 24-hours' post-challenge occurred at different dose levels between the MABs.

Conclusions: Two opsonic MABs were identified that enhanced clearance of MTB in murine blood. This data strongly suggests that opsonic MABs, directed against MTB, may provide useful adjunctive therapy to antibiotics for MDR/XDR TB patients. Furthermore, the use of MABs might contribute important strategies that could significantly impact prevention and treatment of MTB bacteremia and sepsis in patients.