Development of inhalable retinoic acid-loaded nanoparticles as targeted host-directed immunotherapy for tuberculosis

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Background: Tuberculosis (TB) is one of the top ten causes of death worldwide. The emergence of strains of multiple drug-resistant tuberculosis (MDR-TB) has pushed our available stock of anti-TB agents to the limit of effectiveness. An adjunctive, host-directed therapy (HDT) designed to act on the host, instead of the bacteria, by boosting the host immune response through activation of intracellular pathways could help address this issue. The integration of multidisciplinary approaches of repurposing currently FDA-approved drugs, with a targeted drug-delivery platform is a very promising option to accelerate new therapeutics reaching the clinic. Previous work conducted by our group showed the efficacy of All Trans Retinoic Acid (ATRA) as a HDT toward TB both *in vitro* and *in vivo.* The ultimate goal of this project is to develop Poly-Lactic-co-Glycolic Acid (PLGA) nanoparticles (NPs) to target ATRA to the lungs via inhalation and enhance uptake by alveolar macrophages (AM) which are the host cells for *Mycobacterium tuberculosis (Mtb)*.

Methods: ATRA was encapsulated into PLGA nanoparticles using a nanoprecipitation method. Efficacy studies were conducted *in vitro* using THP-1 derived macrophages infected with the avirulent *Mtb* strain (H37Ra) and determined by the BACT/ALERT® liquid culture system. MTS and propidium iodide (PI) exclusion assays were used to determine the cell viability in THP-1 derived macrophages and A549 alveolar epithelium. The formulation was integrated with a vibrating mesh nebulizer (Aerogen Solo®, Aerogen, Ireland); aerosol droplet size was characterized using laser diffraction (Spraytec, Malvern Instruments, UK) and apparatus 5/E impactor (Westech, UK). The inhaled dose percent in an adult breathing profile (500 mL tidal volume, 15 breaths per minute, Inhalation to exhalation ratio: 1:1) was generated using a breathing stimulator (BRS 2100, Copley Scientific Ltd., UK). A scalable nanomanufacturing approach was optimized using microfluidics mixing (Ignite Nanoassembler®,Precision nanosystems, Canada).

Results: We successfully developed a host-directed formulation for TB, using ATRA-loaded PLGA nanoparticles with a size of 261.6 nm and neutral zeta potential. Confocal laser scanning microscopy (CLSM) showed efficient cellular delivery of ATRA-loaded NPs into macrophages and extensive distribution in the cytoplasm. Cell viability studies indicated that the nanoparticles did not lead to significant cytotoxicity in macrophages or alveolar epithelial cells and efficacy studies conducted in an *in vitro* TB infection model have demonstrated a dose dependent reduction in *Mtb* growth (H37Ra). The generated aerosol had a volumetric median diameter (VMD) of 4.09 µm and mass median aerodynamic diameter (MMAD) of 2.13 µm. 65.1% of the dose was inhaled in an adult breathing simulation experiment.

Conclusions: : This type of targeted inhaled HDT offers an innovative approach for TB treatment with the potential to enhance current therapeutic regimens thereby providing better prognosis for patients, and reducing the incidence rate of MDR-TB.