Current translation efforts for a microparticle-based vaccine against Type 1 Diabetes

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Introduction: Current paradigms for diabetes treatment are inadequate at responding accurately to short term homeostatic imbalances and cannot prevent chronic diabetes-related complications. Predictably, novel approaches to re-establish homeostatic conditions in patients afflicted by T1D. Notably, the ex vivo generation and injection of tolerance-promoting dendritic cells (DCs) is being pursued in clinical trials for applications in T1D. While instructive, exogenously-conditioned cellular-based vaccines for T1D treatment have numerous limitations. Dissemination of exogenously delivered DCs is inefficient, and treatment involves a personalized medicine approach involving the generation of cultured DCs, which amounts to high production and treatment costs that prohibit widespread application. To circumvent these limitations, we are developing a multifunctional, synthetic microparticle-encapsulating vaccine that can be easily administered with simultaneous and continuous delivery using controlled-release materials (poly lactide-co-glycolide) for the in vivo conditioning of DCs and amelioration of T1D. Moreover, these microparticle-based vaccines are engineered to target DCs, and provide both intracellular and extracellular delivery of immunomodulatory agents (Vitamin D3 [VitD3], Transforming growth factor- beta 1[TGF-β1], and Granulocyte macrophage colony stimulating factor [GM-CSF]) as well as antigen. Our ultimate goal is to develop a microparticle-based (MP) vaccine capable of reversal of T1D in humans. To date, we have demonstrated (i) the ability of targeted MPs to improve in vivo DC uptake and translocation, (ii) the effect of our non-targeted MP vaccine on bone marrow-derived DC phenotype and downstream effects on allogenic T cells, and (iii) the efficacy of the non-targeted MP vaccine to prevent diabetes onset in NOD mice. Current investigative work is focused on espousing the cellular mechanisms behind the observed prevention in NOD mice, reversal of type 1 diabetes and, evaluating the safety of this biomaterial formulation in rodent models (at OneVax, LLC), with an eye on full translation of this technology.

Methods: A 50:50 polymer composition of poly (d lactide-co-glycolide) (PLGA) was used to generate microparticles via a standard oil-water solvent evaporation technique and sized using standard DLS equipment. Loading efficiencies and release kinetics of encapsulated factors were determined by various spectrophotometric methods. Purified splenocyte populations from NOD mice treated with the particle vaccine were adoptively co-transferred with diabetic splenocytes in NOD.SCID mice to determine the cellular elements involved in the observed protection. Endotoxin contamination and pyrogenicity of the MP vaccine formulation were assessed using the in vitro LAL assay and the in vivo rabbit pyrogen test (RPT) respectively. Evaluation of MP distribution and systemic levels of particle-dissociated agents over time were also performed. MP distribution over 72 h after MP inoculation was determined using a Xenogen IVIS and flow cytometry, whilst ELISAs and HPLC were used to determine systemic levels of different factors.

Results: We fabricated two classes of MPs sized ~ 1μm (phagocytosable) and 30 μm (un-phagocytosable). The phagocytosable MPs were loaded with D3 and insulin. The un-phagocytosable MPs encapsulated TGF-B1 and GM-CSF. We confirmed loading and release kinetics of these drug-loaded using conventional particle degradation and drug detection methods.

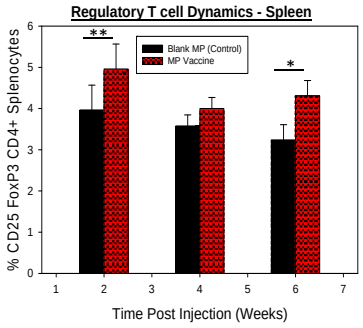

Figure 1. **Regulatory T cells (Tregs) are boosted following MP vaccination**. Briefly, a cohort of 8 weekold female NOD mice were injected with the MP vaccine (or control) 3 times between weeks 8 – 9 weeks and given a booster at 12 weeks old. At 2, 4 and 6 weeks after initial injection, mice were sacrificed (n=5/group/timepoint) and their spleen and pancreatic draining lymph nodes analyzed for Tregs using flow cytometry.

The observed 60% effeicacy of prevention of T1D in NOD mice conferred by MP vaccine inoculation seems to be linked to an increase in the number of splenic Tregs (**Figure 1**). To investigate the MP vaccine efficacy at diabetes reversal, MPs were injected into a cohort of diabetic female NOD mice, two weeks after they became normoglycemic (via an implanted insulin pellet). Diabetes was reversed in 20% mice injected with GM-CSF+D3+TGF-B1+insulin MP formulation (**Figure 2, Left Panel**). Rabbit Pyrogen Testing of the MP vaccine does not induce any significant rise in body temperatures, following administration (Not shown).

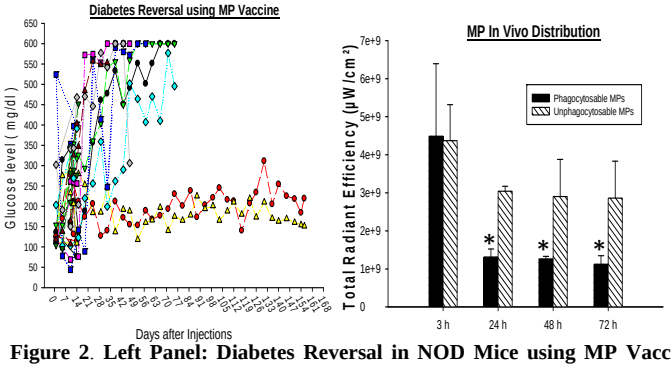

Figure 2. **Left Panel: Diabetes Reversal in NOD Mice using MP Vaccine**. **Right Panel: MP Accumulation at the site of innoculum for 72 h period.**

Using a Xenogen IVIS, the movement of MPs containing the previously described vaccine agents as well as infrared, fluorescent dyes (IR Dye 800RS – Phagocytosable MPs and IR Dye 700DX – Unphagocytosable MPs) were tracked throughout the live mice at 3 h, 24 h, 48 h and 72 h after MP injection. The mean radiance per area was quantified for each MP type around the site of inoculum for a 72 h monitoring period (n = 3/ time point). . After 24 h, there is a reduction of approximately 70% in the fluorescence intensity of this MP type at the injection site, which remains at the same level for the next 48 h (**Figure 2, Right Panel**).

Conclusions: These studies demonstrate that our engineered microparticle vaccine formulation is effective at not only prevention, but also reversal of T1D in NOD mice. Additionally, these results help to highlight the importance of MP size in the mechanics of our vaccine and demonstrate compliance with initial translational safety barriers.