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

Jamal S. Lewis1,3, Matt Carstens1, Natalia Dolgova1, Greg Marshall3, Sufi Morshed1, Chang Qing Xia2, Michael Clare-Salzler2, Benjamin G. Kesselovsky1

1J. Crayton Pruitt Family Department of Biomedical Engineering, 2Dept. of Immunology/Pathology, University of Florida, Gainesville FL 32611. 3OneVax, LLC, Gainesville, FL 32601.

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 conventional particle degradation and drug detection methods. We confirmed loading and release kinetics of these drug-loaded using controlled-release materials (poly lactide-co-glycolide) (PLGA) was used to generate microparticles via a

Results: We fabricated two classes of MPs sized ~1 μm (phagocytosable) and 30 μm (un-phagocytosable). The phagocytosable MPs were loaded with D3 and insulin. The un-phagocytosable MPs were loaded with D3 and insulin. The un-

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.

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 translatable safety barriers.