Project Title: Diabetes Prevention with proinsulin mRNA Vaccines in the NOD Mouse Model

Project Summary:
We hypothesized that a multi-lamellar liposome vaccine with proinsulin mRNA will prevent diabetes in an at-risk diabetes model by conferring immune tolerance. Our specific aims were to measure the effect of different doses of proinsulin mRNA multi-lamellar liposome vaccine on the time to diabetes development and overall rate of diabetes in the mouse model.

In our in vivo study, we used non-obese diabetic mouse model (NOD/ShiLtJ strain) wherein 90% of female mice are diabetic by 30 weeks of age. We utilized four groups of 25 female mice each: IV injections of proinsulin II mouse mRNA nanoparticle vaccine starting at 8 weeks of life (3 vaccines within one week) with a booster (group 1), proinsulin II mRNA weekly for 6 weeks starting at 8 weeks of life (group 2); irrelevant GFP mRNA at 8 weeks with a booster (group 3); and an untreated group (group 4). We are performing weekly glucose checks starting at 8 weeks of age and obtaining insulin autoantibody levels at 8 weeks and endpoint (diabetes or 32 weeks of age). We are also obtaining pancreatic tissue and splenocytes.

Progress to date:
Thus far, we have completed the treatment of the NOD mice with mRNA vaccines. They received four IV mRNA liposome vaccines, but additional treatment was stopped due to anaphylactic-like reactions immediately after the fourth vaccine in the weekly group (group 2), at vaccine #3 in group 1 and during the booster vaccine for group 3 (GFP). This highlights the inflammatory reaction of the vaccine independent of type of RNA. Overall, proinsulin II mRNA vaccine given at 8 weeks with a booster was the best to significantly delay diabetes compared to untreated mice (p=0.03) with a median age of diabetes at 21 weeks, compared to 18 weeks in the GFP group, and 16 weeks in untreated mice. However, as noted below GFP and weekly proinsulin II vaccines demonstrated similar total number of surviving mice without diabetes (n=5 in each vaccine group) at the end of the study (32 weeks) compared to untreated NOD mice (N=1).
Plan for the remaining project time:
We plan to analyze this data over the next 3 months and score insulitis in pancreata collected for histopathology. We also will use collected serum to evaluate insulin antibody levels overtime and analyze for significance. Cross-sectional evaluation of autoimmunity and insulitis of preselected mice at 14 weeks of age will also be conducted.

Future Direction
We will try different formulations in the future including 1-methylpseudouridine RNA to silence the innate immune system. This may abrogate the potentially lethal inflammatory reaction upfront. We will also create an anionic vaccine (opposed to cationic in traditional vaccine), which will specifically target the spleen and assess this effect on vaccine reactions and diabetes prevention.