INTRODUCTION/PURPOSE

The goal of this study is to determine the efficacy of anti-CD20 monoclonal antibody therapy (alone), oral insulin (alone), or the combination of anti-CD20 plus oral insulin to reverse hyperglycemia in NOD mice with recent onset autoimmune diabetes.

The purpose of this Standard Operating Procedure (SOP) is to describe the minimum analysis to be performed at study endpoint.

Definitions

None

Procedures

Terminal samples are to be collected as described in SOP-NOD-0011 Mouse Necropsy. Samples include blood (maximum attainable volume collected by cardiac puncture), spleen, pancreas, femur, and tibia.

Blood: serum frozen at -20°C. Serum will later be sent to UCDenver’s lab for insulin autoantibody analysis.

Spleen: cell isolation for flow cytometric analysis described below

Pancreas: Formalin fixed and scored for insulitis

Femur and Tibia: bone marrow cell isolation for flow cytometric analysis described below

Suggested flow cytometry analysis for both Spleen and Bone Marrow:

Tregs

surface stain: CD8, CD4, CD25
intracellular stain: FoxP3

B cells

surface stain: CD19, MHC II

Each lab may also choose to do additional studies.

REFERENCES TO OTHER APPLICABLE SOPS

SOP-NOD-0009 Blood Collection

SOP-NOD-0011 Mouse Necropsy