STANDARD OPERATING PROCEDURE

Pre-clinical Consortium on Combination Therapies for Type I Diabetes

Document #:	SOP-ITN3-001	**Version #:**	FINAL 02
Supersedes:	N/A	**Effective Date:**	September 6, 2013
Applicable to:	ITN Project 3	**Category:**	

Title: Pilot study to determine the efficacy of anti-CD3 plus IL-1 trap therapy in reversing new onset diabetes in the NOD mouse.

INTRODUCTION/PURPOSE

The goal of this pilot study is to ascertain whether the Regeneron IL-1 trap reagent, murine Rilonacept, is additive/synergistic with anti-CD3 therapy for reversing new onset disease in non-obese diabetic (NOD) mice. The outcome of this study will determine whether a larger study, adequately powered to determine the efficacy of this therapeutic combination, will be undertaken by the Consortium.

DEFINITIONS

Diabetes: Type 1 diabetes with blood glucose >= 250 mg/dl.

New onset diabetic: Blood glucose readings of >= 250 mg/dl on two consecutive days.

Reversal of diabetes: Blood glucose readings of < 250 mg/dl for 3 regularly scheduled readings following entry into the study. Following allocation to a treatment group, blood glucose will be monitored two-three times per week in the morning. Spacing between blood glucose measurements should be no more than four days. This definition may need to be revised if it does not permit us to clearly distinguish between spontaneous reversions in the untreated control group versus treated groups.

NOD mice: Female NOD mice between 10 and 26 weeks of age.

Day 1: The day of initiation of treatment. Study enrollment and treatment initiation will begin the same day as the second consecutive confirmatory hyperglycemic reading.

OVERVIEW OF STUDY DESIGN

Beginning at 10 weeks of age, the blood glucose of NOD mice will be monitored three times per week in the morning. Once a mouse registers a blood glucose reading greater than or equal to 250 mg/dL, diabetes onset will be confirmed by re-measurement of blood glucose levels the next day. Mice with blood glucose levels greater than or equal to 250 mg/dL for two consecutive days will be considered diabetic and will be entered into a treatment group the same day (day 1). Following allocation to a treatment group, blood glucose will be monitored three times per week in the morning. The study will run for 60 days. Mice should be sacrificed between 60-65 days for endpoint assessments.

Criteria for Removal from Study

Mice may be removed from the study for humane reasons. Animals will be removed from the study and euthanized according to IACUC protocol under the following conditions:

- After day 30 of study, if the animal has 3 regularly scheduled blood glucose readings > 250 mg/dl or significant weight loss as defined below.

- At any time, if a mouse's weight drops below that permitted by each site's IACUC (or 20% weight loss if IACUC does not have a weight loss restriction).
- Before day 30 of study, if there are 3 consecutive regularly scheduled maximum blood glucose measurements as permitted by each site's IACUC (or 3 consecutive blood glucose measurements exceeding the upper limit of detection for a meter if IACUC does not have a guideline for blood glucose measurements).
- The mouse exhibits signs of significant illness related distress (shaking, hunched, non-ambulatory, cool to the touch, etc.)

If possible, endpoint assessments should be performed at time of sacrifice.

PROCEDURES

Blood Glucose Monitoring

Female NOD mice between 10 and 26 weeks of age will be monitored for diabetes onset and the readings documented. Blood glucose monitoring will be performed three times per week in the morning. The spacing between monitoring days should be such that there is never more than two days that pass without blood glucose measurements. For example, monitoring on Friday and Monday is acceptable, but monitoring on Friday and Tuesday would result in too many days between blood glucose measurements.

Day 0 of treatment is defined as the day of the first of the two consecutive blood glucose readings.

Following allocation to a treatment group, blood glucose will be monitored two-three times per week in the morning. Spacing between blood glucose measurements should be no more than four days.

Procedure for Blood Glucose Monitoring: The tip of the tail is cut with scissors and a droplet of blood is placed into the slot on the end of a test strip inserted into a portable blood glucose monitor. The value is recorded.

Blood Glucose Monitoring Equipment: Each site should use the same model of blood glucose monitor during this study. The One Touch® Ultra 2® blood glucose meter and One Touch® Ultra® Blue test strips (LifeScan, Inc.) should be used for this study.

Body Weight Measurements

Body weights will be measured weekly and documented.

Identification of Animals Enrolled in Study

In order to prevent duplication in animal identifiers between sites, new onset diabetics should be assigned a unique identifier upon allocation to a study group. This unique identifier should have the following format: Site – ITN Project # - Treatment Group – Animal ID. The variables that may be used in each field are as follows:

- Site = F (Florida), Y (Yale), C (Colorado) or L (La Jolla)
- ITN Project # = 3P (Anti-CD3 + IL-1 trap pilot study)
- Treatment Group = 1, 2, 3, etc
- Animal ID = animal # (microchip #, ear tag, tattoo, etc)

For example, animal F-3P-1-1585 is from the Florida site during the project 3 pilot, received treatment 1, and its ID is 1585.

SOP-ITN3-001: Anti-CD3 plus IL-1 trap pilot study

Therapies

Anti-CD3 F(ab)2: Hamster anti-mouse CD3 F(ab)2 fragments of the 145-2C11 monoclonal antibody at a dose 5 mcg/mouse/day will be injected intraperitoneally (IP) for 4 days (days 1, 2, 3, and 4). Previous studies have demonstrated that this regimen is suboptimal at reversing T1D in NOD mice with only 50% of mice reverting to euglycemia. The F(ab)2 fragments are available from Bio-X-cell, West Lebanon, NH.

Simultaneous treatment with IL-1 trap and anti-CD3 F(ab)2: IL-1 trap (murine Rilonacept; Regeneron) will be given at a dose of 60 mg/kg IP on days 1, 3, and 5. Anti-CD3 F(ab)2 dosing is the same as described above.

Anti-CD3 F(ab)2 treatment followed by IL-1 trap administration: IL-1 trap (Regeneron) will be given at a dose of 60 mg/kg IP on days 5, 7 and 9. Anti-CD3 F(ab)2 dosing is the same as described above.

The diluent for both the anti-CD3 fragments and IL-1 trap is Phosphate Buffered Saline (PBS).

Handling and Storage of Reagents

Anti-CD3 F(ab)2: Hamster anti-mouse CD3 F(ab)2 fragments of the 145-2C11 monoclonal antibody (lot 4425/1212) are supplied at a concentration of 2.36 mg/ml. Per Bio-X-Cell's recommendation, undiluted antibodies should be stored at 4°C in the dark. If the antibody is to be stored for more than 6 months, aliquot and freeze at -20°C in a freezer that is not frost-free. This lot of antibodies was originally received and aliquoted for distribution on May 21, 2013.

Mice are dosed with 5 mcg/mouse/day IP in a volume of 100 microliters. To prepare the reagent for administration, dilute the 2.36 mg/ml stock reagent to 0.05 mg/ml with sterile PBS without Mg2+/Ca2+ (such as PBS, pH 7.4 Invitrogen/Life Technologies Catalog #10010-02).

IL-1 trap (murine Rilonacept): the IL-1 trap reagent is supplied at a concentration of 58.1 mg/ml. Undiluted reagent should be stored at 4°C in the dark. If the reagent is to be stored for more than 6 months, aliquot and freeze at -20°C in a freezer that is not frost-free.

Mice are dosed with 60 mg/kg IP in a volume of 60-170 microliters. A dosing spreadsheet has been provided to make dose administration more convenient. To prepare the reagent for administration, dilute the 58.1 mg/ml stock to 15 mg/ml with sterile PBS without Mg2+/Ca2+ (such as PBS, pH 7.4 Invitrogen/Life Technologies Catalog #10010-02).

Treatment Groups

Following the second consecutive confirmatory hyperglycemic reading (day 1), mice will be serially allocated to the following groups:

Group	Group	n	Anti-CD3 Tx (5 mcg/dose)	IL-1 Trap (60 mg/kg)
1	Anti-CD3 alone	6	Days 1, 2, 3 & 4	None
2	Anti-CD3/IL-1 trap (simultaneous)	6	Days 1, 2, 3 & 4	Days 1, 3, & 5
3	Anti-CD3/IL-1 trap (sequential)	6	Days 1, 2, 3 & 4	Days 5, 7, & 9
4	Untreated control group	6	None	None

An allocation spreadsheet has been provided to assist in tracking allocation of mice to groups.

Sample Collection

All sites should collect at least 100 microliters of serum from each animal on study days 1 and 4 and at the end of study (one time in the day 60-65 window). Serum samples should be stored frozen, preferably at -80 degrees Celsius, but storage at -20 degrees Celsius is acceptable if -80 degree storage is unavailable.

The Yale site will perform a pilot study utilizing whole blood to determine whether the anti-CD3 reagent is functioning as expected. The design of and methods for this study will be determined by the Yale site. In the event that obtaining serum samples interferes with their ability to obtain whole blood samples, i.e., that much blood cannot be harvested from the animal without compromising its well-being, then the whole blood/anti-CD3 study will take precedence over obtaining serum samples for the Yale site.

DATA COLLECTION

Blood glucose measurements (3 times per week)
Body weights (once per week)
Administration of therapies

DATA ANALYSIS

Blood glucose data will be analyzed using GraphPad Prism (GraphPad Software Inc., San Diego, CA). 'Survival' curves will be generated from blood glucose data and compared using log rank tests. Test groups 1 through 3 will be compared to the untreated group. $P<0.05$ will be considered significant.

DOCUMENTATION TO BE MAINTAINED

Data sheets containing blood glucose values (3 times per week) and body weights (once per week).
Adverse effects of treatments, if any.

REFERENCES TO OTHER APPLICABLE SOPS

None

REFERENCES

Ablamunits V, et al. 2012. Synergistic reversal of Type 1 Diabetes in NOD mice with anti-CD3 and IL-1 blockade. *Diabetes* 61:145-154.

Chatenoud L, et al. 1994. Anti-CD3 antibody induces long-term remission of overt autoimmunity in nonobese diabetic mice. *PNAS* 91: 123-127.

Rudgren T, et al. 2013. Administration of IL-1 Trap prolongs survival of transplanted pancreatic islets to type 1 diabetic NOD mice. *Cytokine* 63: 123-129.

FORMS/ATTACHMENTS

None

REVISION HISTORY

Effective Date	Revision Version	Author	Description of Changes

6-Sept-2013	FINAL 02	TK	Blood glucose monitoring of subjects in treatment groups changed from 2-3 times per week to 3 times per week.