SOP-NOD-0006 Treatment Groups

STANDARD OPERATING PROCEDURE
Pre-clinical Consortium on Combination Therapies for Type I Diabetes

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Applicable to: ITN Project 1	Category:

Title: Treatment Groups

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 is to describe the experimental agents, dosing and timing of administration of therapies in each treatment group.

DEFINITIONS

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.

IACUC: Institutional Animal Care and Use Committee.

Treatment Group	Category	n	Treatment
A	Negative Control	18	Insulin pellet (subcutaneous implant). One LinBit insulin implant to be implanted the same day as the second consecutive confirmatory hyperglycemic reading (d 1). Second insulin pellet to be implanted once the first is expended (day 10-18). Oral diluents (oral gavage, twice weekly for 6 weeks = 12 total administrations) begining the first Tuesday or Thursday following onset. Isotype control antibody (10 mg/kg IP injection on days 1, 2, 3, and 4).
B	Test	18	Insulin pellet (subcutaneous implant). One LinBit insulin implant to be implanted the same day as the second consecutive confirmatory hyperglycemic reading (d 1). Second insulin pellet to be implanted once the first is expended (day 10-18). Oral insulin (oral gavage, twice weekly for 6 weeks = 12 total administrations) begining the first Tuesday or Thursday following onset.

C	Test	18	Insulin pellet (subcutaneous implant). One LinBit insulin implant to be implanted the same day as the second consecutive confirmatory hyperglycemic reading (d 1). Second insulin pellet to be implanted once the first is expended (day 10-18). Anti-CD20 monoclonal antibody (10 mg/kg IP injection on days 1, 2, 3, and 4).
D	Test	18	Insulin pellet (subcutaneous implant). One LinBit insulin implant to be implanted the same day as the second consecutive confirmatory hyperglycemic reading (d 1). Second insulin pellet to be implanted once the first is expended (day 10-18). Oral insulin (oral gavage, twice weekly for 6 weeks = 12 total administrations) begining the first Tuesday or Thursday following onset. Anti-CD20 monoclonal antibody (10 mg/kg IP injection on days 1, 2, 3, and 4).
E	Test	18	Insulin pellet (subcutaneous implant). One LinBit insulin implant to be implanted the same day as the second consecutive confirmatory hyperglycemic reading (d 1). Second insulin pellet to be implanted once the first is expended (day 10-18). Anti-CD20 monoclonal antibody (10 mg/kg IP on day 1) Delayed oral insulin (oral gavage, twice weekly for 6 weeks beginning on day 21 = 12 total administrations running through day 63)
F	Positive Control for Diabetes Reversal	10	Anti-CD3 monoclonal antibody (5 micrograms on days 1-5 = 5 total administrations)
G	Test	18	Oral insulin (oral gavage, twice weekly for 6 weeks = 12 total administrations) begining the first Tuesday or Thursday following onset. Anti-CD20 monoclonal antibody (10 mg/kg IP injection on days 1, 2, 3, and 4).

MATERIALS

Insulin pellets: LinBit¨ Bio-erodible sustained release insulin implant (Linshin Canada, Inc., Toronto, Ontario, Canada). LinBits are composed of a mixture of insulin and microrecrystallized palmitic acid. Assay pack reference # 3315-J best by date 2012-07; reserve packs reference numbers 3315-H and 3315-C best by date 2010-07.

Oral Insulin: Human recombinant insulin (Humulin-R U-500, NDC 0002-8501-01, Eli Lilly & Co, Control # A810048A, Exp 10-2012)

Oral Diluent: The diluent consists of glycerin 16 mg/ml, metacresol 2.5 mg/ml and zinc oxide to supplement the endogenous zinc to obtain a total zinc content of 0.017 mg/100 units, and water for injection. The pH is 7.0 to 7.8. Sodium hydroxide and/or hydrochloric acid may have been added during manufacture to adjust the pH. (Sterile diluent, ND 800, Eli Lilly & Co, Control # A810048A, Exp 10-2012).

Anti-CD20 monoclonal antibody therapy: B-lymphocyte-depleting mouse CD20-specific 5D2 (IgG2a) monoclonal antibody (Genetech, San Francisco, CA). No lot number or other identifiers.

Isotype control antibody for anti-CD20: IgG2a non-B-lymphocyte-depleting C1.18 monoclonal antibody (Bio-X-Cell, Catalog # BE0085, 3842/0411).

Anti-CD3 monoclonal antibody therapy: F(ab)2 fragments from the T cell-depleting anti-CD3 monoclonal antibody 145-2C11 (hamster IgG). No lot number or other identifiers

DOSING AND ROUTE OF ADMINISTRATION

Insulin pellets: diabetic mice in groups A, B, C, D, and E will receive one LinBit sustained release insulin implant the same day as the second consecutive confirmatory hyperglycemic reading (d 1). A second insulin pellet will be implanted following the expenditure of the first pellet (defined as two consecutive regularly scheduled blood glucose readings >= 250 mg/dl or 14 mmol/L). This is expected 10-18 days following the first implantation.

Oral insulin: Twenty-seven units (1 mg) of human recombinant insulin (Humulin R U-500) in a 0.4-ml bolus (or, if a site's IACUC will not allow this large of a volume, in the largest volume approved by IACUC) will be given by oral gavage to Groups B, D, E and G twice weekly for a period of six weeks (12 total administrations). Groups B, D and G will begin oral insulin therapy the the first Tuesday or Thursday following onset. Group E will receive delayed therapy beginning on day 21 post-onset. Subsequent gavages will occur on Tuesdays and Thursdays.

Oral diluent: A 0.4-ml bolus (or, if a site's IACUC will not allow this large of a volume, in the largest volume approved by IACUC) of the diluent used in Humulin R U-500 will be administered via oral gavage to Control Group A twice weekly for a period of six weeks (12 total administrations) beginning the first Tuesday or Thursday following onset.

Anti-CD20 monoclonal antibody therapy: 10 mg/kg of body weight in a volume of approximately 200 microliters of the B-lymphocyte-depleting mouse CD20-specific 5D2 monoclonal antibody (IgG2a) will be injected intraperitoneally. Groups C, D and G will receive four doses of anti-CD20 on days 1, 2, 3, and 4. Group E will receive one dose of anti-CD20 on day 1. Recommended dilution procedure: To minimize potential dosing error, the anti-CD20 antibody stock should be diluted to a working solution of 1 mg/ml saline with sterile, GMP-grade Phosphate Buffered Saline. A dilution of 1 mg/ml will result in a deliverable dose of 200 microliters for a 20 g mouse.

Isotype control antibody therapy: 10 mg/kg of body weight in a volume of approximately 200 microliters of the non-B cell-depleting mouse C1.18 monoclonal antibody (IgG2a) will be injected intraperitoneally. Group A will receive four doses of control antibody on days 1, 2, 3, and 4. Recommended dilution procedure: To minimize potential dosing error, the isotype control antibody stock should be diluted to a working solution of 1 mg/ml with sterile, GMP-grade Phosphate Buffered Saline. A dilution of 1 mg/ml will result in a deliverable dose of 200 microliters for a 20 g mouse.

Anti-CD3 monoclonal antibody therapy: 5 mgs of F(ab)2 fragments from the T cell-depleting anti-CD3 monoclonal antibody 145-2C11 will be injected intraperitoneally on days 1-5 post-onset (5 consecutive doses) into Control Group F. To minimize potential dosing error, the anti-CD3 stock should be diluted to a working solution of 25 micrograms/ml. A dilution of 25 micrograms/ml will result in a deliverable dose of 200 microliters for all recipients.

DOCUMENTATION TO BE MAINTAINED

Animal Data Sheets should capture the following information: animal ID, sex, age, source (vendor or bred in-house), proper strain name, length of time in colony, age at onset, details of treatments, initials of individual performing treatment.

REFERENCES TO OTHER APPLICABLE SOPS

None

REFERENCES

L Chatenoud, E Thervet, J Primo, and J F Bach. Anti-CD3 antibody induces long-term remission of overt autoimmunity in nonobese diabetic mice. Proceedings of the National Academy of Sciences. 1994 91 (1) 123-127

FORMS/ATTACHMENTS

REVISION HISTORY

Effective Date	Revision	Author	Description of Changes
8/17/11	.01	T Kupfer	Added the definition of day 1, added additional details of timing of dosing, added details of control antibody administration, added missing lot numbers and expiration dates, added data fields to be captured in documentation.
8/21/11		Amanda Posgai	changed day of anti-CD20 administration for group E
8/24/11	.02	TKupfer	Modified control isotype antibody and anti-CD20 injections to days 1, 2, 3, and 4. Added second insulin pellet implantation at 10-18 days post-implantation. Added recommended dilutions and deliverable volumes of therapies.