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APPLICATION NUMBER:

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NON-CLINICAL REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

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Product:	Teplizumab (PRV-031)
Pharmacologic Class:	CD3 blocker immunosuppressant
Indication:	Evaluated for Delay/Prevention of T1DM
Applicant:	Provention Bio, Inc
Review Division:	Division of Diabetes, Lipid Disorders, and Obesity
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1 Executive Summary

1.1 Introduction

Provention Bio, Inc has submitted a BLA for teplizumab (hOKT3γ1 (Ala-Ala)) that is being developed for the delay/prevention of Type 1 Diabetes Mellitus (T1DM). Teplizumab is a humanized IgG1 monoclonal antibody (mAb) that binds the CD3ε epitope of the T cell receptor (TCR) complex on human T cells. Binding causes depletion/inactivation of CD3+ T cells resulting in transient, systemic immunosuppression.

1.2 Brief Discussion of Nonclinical Findings

The nonclinical support for teplizumab consists of a series of pharmacology, pharmacokinetic, and toxicologic studies. As teplizumab was shown to only cross-react with CD3+ T cells from humans, gorillas, chimpanzees, and gibbons, minimal nonclinical investigations were conducted with teplizumab itself. Instead, a (b) (4) surrogate monoclonal antibody (mAb) was developed and used to characterize the nonclinical profile of teplizumab. The surrogate antibody has an apparent affinity for the mouse CD3ε similar to that shown by teplizumab for human CD3ε.

In pharmacology studies in non-obese diabetic (NOD) mice, a model of spontaneous autoimmune development of diabetes, a single 5-day treatment cycle with the surrogate delayed the onset and reduced the incidence of diabetes by approximately 5 weeks and 30%, respectively. When a second treatment cycle was initiated 6 weeks later, but not after two weeks, anaphylactic reactions resulting in death occurred. Subsequent investigative work indicated that the IgG pathway played a role. Although this IgG-mediated anaphylaxis is well described in rodents, a similar phenomenon in humans is not well characterized.

A single dose study in chimpanzees was the only in vivo toxicology study conducted with (b) (4) hOKT3γ1 [Ala-Ala], similar to teplizumab but produced using a different cell line. Following single doses (up to 10 mg/kg) of teplizumab in chimps, both C_{max} and AUC increased with dose in a proportional to greater than proportional manner. The C_{max} was achieved within 2 hours and the half-life increased with dose. Dose-related reductions in T cell populations were observed across drug-treated groups. In this single dose chimp study, coughing and inappetence were observed in 3 high dose (HD) animals (and in one control (C)) and antibiotic treatment was initiated. All HD animals died or were euthanized while the C animal recovered. The cause of death was attributed to an infection that occurred as a result of T cell suppression that was related to the pharmacologic activity of the drug. The reductions in T cell populations persisted for approximately two weeks, although levels of some T cell populations remained reduced through study termination at the HD. Also observed were transient increases in the levels of cytokines, including TNFα, IL-6, IL-10, in all groups; IFN-$_\gamma$ levels were also increased at the HD. Cytokine levels generally peaked by 6 hours before declining. The NOAEL for the study was considered the mid dose (MD) of 1

mg/kg, approximately 4x the total human dose administered in a cycle of treatment based on body surface area (BSA).

Repeat dose toxicity studies were conducted in mice using the surrogate mAb. Because of intrinsic differences between the clinical candidate and the (b) (4) surrogate antibody, the clinical relevance of inter-species exposure comparisons is uncertain. In a 6-day study, reversible reductions in circulating T cells (e.g., CD3, CD4, CD8, cytotoxic, helper, total) were observed at all dosages. These effects were associated with a reduction in cellularity of the medulla in the thymus and hematopoiesis in spleen; these effects were also partially reversible. There were no off-target effects and the NOAEL was the HD of 20 mg/kg. In a second repeat dose toxicity study in mice in which the surrogate was administered every 3 days for 1 month, deaths occurred following the 4th dose administration that were preceded by labored breathing, hypoactivity, recumbency, ataxia, and swollen nose. These effects were consistent with an anaphylactic reaction, suggesting that intermittent dosing may have contributed to this finding in mice. The NOAEL was <0.3 mg/kg based on severe hypersensitivity reactions.

In a series of reproductive and developmental toxicity studies conducted with the surrogate in mice, treatment occurred approximately every 3 days. In contrast to the repeat dose study, no deaths occurred. The only notable effect in parental animals were reductions in T cell populations with corresponding increase in B cells that were observed at all dosages administered. There were no effects on fertility up to the HD of 20 mg/kg. In an embryofetal development study there was an increased incidence of post-implantation loss at the HD of 20 mg/kg that included the loss of the entire litter for multiple females leading to identifications of the MD (0.3 mg/kg) as the NOAEL. In a pre- and postnatal toxicity study, pharmacologic effects on T and B cells were the only observations in maternal animals. Reduced T cell populations and increased B cells were evident in the offspring of maternal animals administered the HD of the surrogate during gestation and lactation. Also observed in the HD offspring was a suppression of the primary IgM and IgG response and of the secondary IgG response, suggesting a potential risk to offspring immune system functioning when exposure occurs during gestation and/or lactation. Reduced sperm motility and a reduction in the number of resulting pregnancies were also evident at the HD in the F1 offspring. The effects in the offspring coincided with measurable drug levels in the serum of HD pups. The MD of 3 mg/kg was considered the NOAEL for effects on offspring development based on the altered immune system observations and effects on fertility in offspring at the HD.

In summary, pharmacologic investigations demonstrated a delay in the onset and reduction in the incidence of diabetes in the NOD mouse model. With the chimpanzee being the only relevant animal model for the clinical candidate, the safety profile was based on a single dose study in which reductions in T cell populations were evident. Increases in cytokines were also noted, indicating that cytokine release syndrome (CRS) may occur. Additional repeat-dose and reproductive toxicity studies were conducted with a surrogate mAb in mice. These studies revealed decreases in T cell populations and increased B cells that persisted for various periods of time. The effect

on these cell populations carried through to the F1 generation even though they were not directly dosed. Also observed in the F1 offspring was an impaired immune response and a possible reduction in fertility. These results suggest that precautionary statements about treatment during pregnancy/lactation should be considered in labeling.

1.3 Recommendations

1.3.1 Approvability

The nonclinical data support marketing approval of teplizumab to delay/prevent the onset of T1DM

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Revisions to the Established Pharmacologic Class (EPC) designation and nonclinical statements in sections 8 and 13 of the label will be necessary. EPC and labeling recommendations will continue beyond the finalization of this review and will be addressed separately.

2 Drug Information

OKT3 is a T cell-depleting mAb that binds to a CD3-ε epitope expressed on mature human T cells. OKT3 produces profound, transient T cell depletion in vivo, activates T cells, is strongly mitogenic, and its use in vivo is associated with severe cytokine release syndrome. T cell activation is strongly facilitated by the interaction of the Fc component of OKT3 with Fc receptors on lymphocytes (Fc/FcR engagement). To reduce its immunogenicity and side effects, OKT3 was humanized by grafting a human IgG1 Fc onto the OKT3 Fab and modified in the Fc region (Leu234 to Ala234 and Leu235 to Ala235) to limit TCR transactivation. The resultant product was hOKT3γ1(Ala-Ala), also called teplizumab. The modifications limit TCR transactivation leading to a reduction in cytokine release which mitigates the major adverse effect of mAb binding to CD3 (cytokine release syndrome).

CD3 is poorly conserved across species. Teplizumab cross-reacts only with human, gorilla, chimpanzee, and gibbon CD3+ T cells. As a result, a minimal number of nonclinical studies were conducted with teplizumab. To help in the characterization of teplizumab, a (b) (4) surrogate monoclonal antibody (mAb) that binds to CD3ε receptor on mouse T cells was developed (identified as 2C11-mG2a(Ala-Ala) or 2851055; 145-2C11 (b) (4)). This surrogate was used in the majority of nonclinical studies.

Teplizumab has been identified by numerous names during its development as summarized in the applicant table below. The term describing the test article in each review summarized is retained to be consistent with table/figure captions.

Table 1: Cross-Reference List of Test Article Names

Name	Alternate Name	Description
Teplizumab	hOKT3γ1(Ala-Ala)	Humanized, Fc-engineered IgG1 mAb that binds to CD3ε receptor on human and chimpanzee T-cells
	hOKT3aa	
	PRV-031	
	MGA031	
	(b) (4)311	
OKT®3	OKT®3	Murine IgG2 mAb that binds to CD3ε receptor on mouse T-cells; precursor to teplizumab
	Orthoclone	
Surrogate Antibody	2C11-mG2a(Ala-Ala)	Surrogate (b) (4) mAb that binds to CD3ε receptor on mouse T cells
	2851055	
hE16 mIgG2a(Ala-Ala)	-	Comparator IgG2 isotype control mAb
145-2C11	-	Surrogate (b) (4) mAb that binds to CD3ε receptor on mouse T cells but it has no Ala substitutions. It is used as a detection antibody in specific immunophenotyping studies

Applicant table

2.1 Drug

CAS Registry Number: 876387-05-2

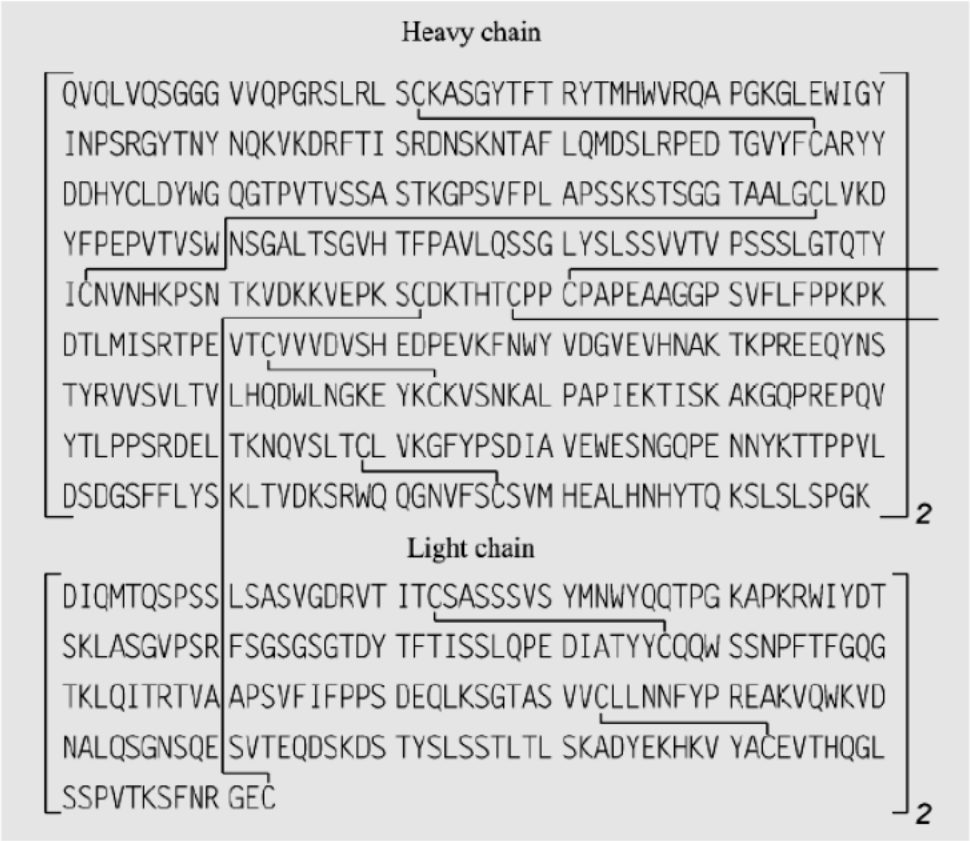
Generic Name: Teplizumab

Code Name: PRV-031 (refer to Table 1 also)

Chemical Name: hOKT3γ1 (Ala-Ala)

Molecular Formula/Molecular Weight: $C_{6462}H_{9938}N_{1738}O_{2022}S_{46}$ /~150 kDa

Structure or Biochemical Description: humanized monoclonal IgG1 (peptide sequence shown below [lines depict disulfide bonds])



Pharmacologic Class: CD3 blocker immunosuppressant

2.2 Relevant INDs, NDAs, BLAs and DMFs

INDs

Teplizumab (MacroGenics):

INDs: (b) (4) 100262 (DMEP, T1DM); (b) (4)

102629 (DMEP, T1DM) (b) (4)

(b) (4)

BLAs

(b) (4)

DMFs

DMFs: (b) (4)

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2.3 Drug Formulation

The drug product is provided as a sterile solution with a protein concentration of 1.0 mg/mL in a (b) (4) composed of (b) (4) sodium phosphate, (b) (4) sodium chloride, and 0.05 mg/mL polysorbate 80 at a pH of 6.1. The composition is summarized in the applicant table below:

Table 2 Teplizumab Drug Product Composition

Name of Ingredient	Concentration (mg/mL)	Nominal Amount per Vial (mg)[a]	Function	Grade
Teplizumab drug substance	1.0	2.0	Active ingredient	In-house
Sodium phosphate, dibasic, (b) (4)	0.26	0.52	(b) (4)	USP-NF, Ph. Eur., JP
Sodium phosphate monobasic, (b) (4)	(b) (4)	2.26		USP
Sodium chloride	8.78	17.56		USP-NF, Ph. Eur., JP
Polysorbate 80	0.05	0.10		USP-NF, Ph. Eur.
Water for Injection (WFI)	QS	QS		USP, Ph. Eur., JP

[a] Amount based on nominal 2 mL fill volume.
QS=quantity sufficient.

2.4 Comments on Novel Excipients

None of the excipients are considered novel.

2.5 Comments on Impurities/Degradants of Concern

Potential impurities in teplizumab are either process-related or product-related. The impurities that have been identified and the specification levels are considered acceptable to OBP. Refer to the CMC review for details.

2.6 Proposed Clinical Population and Dosing Regimen

Teplizumab is being developed to delay/prevent the onset of T1DM in individuals at risk for developing this disease. Treatment is comprised of a 14-day treatment cycle at a cumulative dose of 9 mg/m^2 (~0.24 mg/kg or 14.6 mg in a 60 kg adult). It is administered by intravenous infusion in a rising dose regimen (51 µg/m^2 on day 1, 103 µg/m^2 on day 2, 207 µg/m^2 on day 3, 413 µg/m^2 on day 4, and 826 µg/m^2 on days 5 through 14).

2.7 Regulatory Background

Summarized below are the major project milestones and interactions with the applicant in which nonclinical issues were a key component.

- IND 102629 filed 23 Dec 09
- Discussions at an EOP2 (13 Dec 18) meeting pertaining to nonclinical development for IND 100262, the same compound for treatment of the same underlying condition, are also considered pertinent. Key outcomes were:
  - The agency agreed that an in vivo carcinogenicity assessment was not needed
  - Agreement that the reproductive studies with the surrogate would be appropriate.
  - Agreement that the nonclinical package appeared adequate for filing the application
  - The agency also indicated that additional nonclinical studies may be needed to support a chronic recurring dosing regimen
- A meeting request was submitted 18 Mar 19 followed by receipt of the background package on 12 Apr 19 seeking confirmation that the nonclinical studies identified at the EOP2 meeting for IND 100262 would be adequate for support of this BLA. The agency agreed that the studies appear to be adequate, with responses sent to sponsor 31 May 19
- Meeting held 7 Nov 19 to discuss remaining development plans for IND 102629. Agency reiterated that nonclinical program was adequate for BLA submission as per written responses of 31 May 19
- Sponsor submitted a meeting request 3 Sep 19 followed by submission of a background package 4 Oct 19 and a meeting on 7 Nov 19. A nonclinical question pertained to the format of the nonclinical studies to which the agency agreed that pdf copies of all reports would be acceptable.
- Breakthrough Therapy designation granted 2 Aug 19
- Request for rolling submission for BLA submitted 31 Jan 20. Request granted 12 Feb 20
- Nonclinical component of BLA submitted 14 Apr 20
- An EOP2 meeting for IND 102629 was held 20 Aug 20. There were no nonclinical issues discussed

3 Studies Submitted

3.1 Studies Summarized

Although this BLA is supported by IND 102629, many of the nonclinical studies were conducted under IND 100262 which was filed by the same applicant for the treatment of recently diagnosed T1DM. (b) (4) [redacted]. All pertinent nonclinical studies supporting this marketing application have been fully reviewed under these INDs. For all nonclinical studies summarized, the test-article name in use at the time was retained to match information in tables.

3.2 Studies Not Reviewed

Analytical and methodological validation reports were not reviewed in detail.

3.3 Previous Reviews Referenced

The majority of the nonclinical studies supporting this NDA were originally submitted to IND 100262, 102629, (b) (4) where they were reviewed by either Dr. D. Carlson (review of IND 100262 dated 25 Aug 06), Dr. R. Wange (review of IND 102629 dated 14 Jan 10), or (b) (4). Pertinent studies have been briefly summarized within this review.

The following studies have not been previously reviewed under any of these INDs. As a result, they are summarized in more detail within this review.

Table 3 List of Studies Reviewed in Detail

Study Number	Study Title (abbreviated)
RND-MGA031-10-1001	Evaluation in Pre-diabetic NOD Mice
RND-MGA031-10-1002	Evaluation of Immunogenicity in Mice
(b) (4)-353209	Female Fertility
-353210	Male Fertility
-353232	Pre- and Postnatal Development
8233170	1-Month Tox with 3 Month Recovery

4 Pharmacology

(b) (4) surrogate monoclonal antibody (mAb), identified as 2C11-mG2a(Ala-Ala) or 2851055, was used in many of the pharmacology studies to characterize the pharmacologic activity in nonclinical species.

Teplizumab (humanized hOKT3γ1 (Ala-Ala)) was designed from a (b) (4) mAb that binds mouse CD3+/TCR. Binding of the antibody causes a depletion in CD3+ T cells that results in transient, systemic immunosuppression. The Ala-Ala mutation in the Fc domain does not affect CD3 binding but does limit T cell receptor transactivation resulting in a reduction in cytokine release

In terms of the type 1 diabetes indication, the mechanism is not completely understood. According to the applicant:

> "…teplizumab appears to both decrease the activity of effector T cells and enhance the activity of regulatory T cells. The mechanisms of action of teplizumab appear to involve weak agonistic activity on signaling via the TCR-CD3 complex associated with the development of exhaustion, anergy, unresponsiveness, and/or apoptosis, particularly of unwanted activated T effector (Teff) cells (Long 2016; Long 2017). Exhausted T cells have regulatory functions, limiting T cell activation, and their expansion correlates with slow progression to T1D in subjects at risk in the absence of any

therapeutic agent (Wiedeman 2020). Thus, based on the observation that these exhausted T cells appear to be an inherent mechanism to modulate the course of T1D, teplizumab promotes a similar mechanism to delay autoimmunity in T1D. In addition, regulatory cytokines are released and regulatory T cells are expanded that may lead to the reestablishment of immune tolerance (Bisikirska 2005; Chatenoud 2007)."

(b) (4)

A surrogate form was also developed (see section 2) for use in safety assessments.

4.1 Primary Pharmacology

4.1.1 Binding of Teplizumab to Soluble Human CD3 and 211mG2a(ala,ala) to Soluble Mouse CD3

The binding of teplizumab and the surrogate to their respective CD3 target proteins was evaluated. The binding affinities were similar between teplizumab (K_d = 2.3 µM) and the surrogate (K_d = 3.0 µM), but the kinetic parameters did differ as the surrogate had a slower on-rate and off-rate, as shown in the applicant figure below:

Figure 1: SPR Analysis of hCD3 and mCD3 Binding to Teplizumab and Surrogate 2C11-mG2a(Ala-Ala)

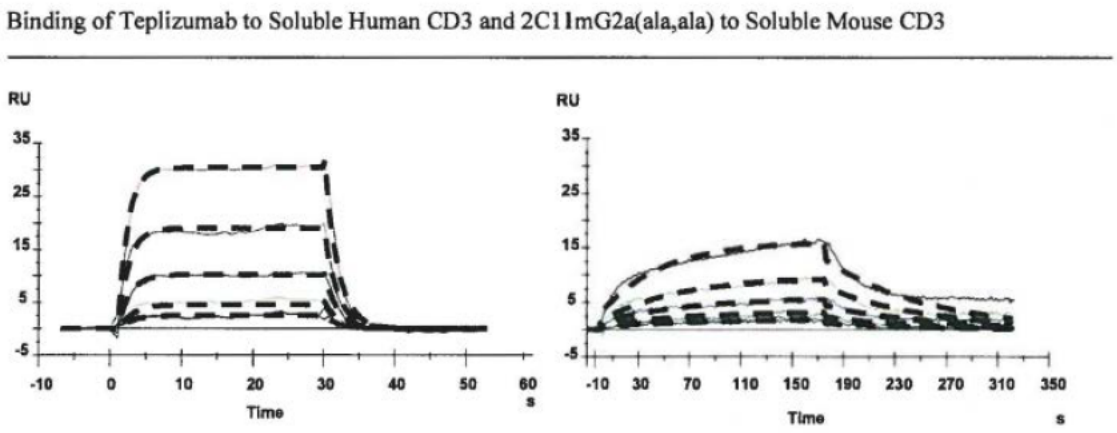


Figure 1 SPR analysis of hCD3 (left panel) and mCD3 (right panel) binding to immobilized teplizumab and 2C11mG2a(ala,ala) correspondingly. Dashed lines show 1:1 binding model fits of binding curves obtained at different concentrations of CD3 in solution.

Applicant figure from Pharmacology Written Summary

This pattern was seen when both the lgG was immobilized and antigen was passed over the surface or when antigen was immobilized and the respective Fab was passed over the immobilized sCD3. Although there were differences in the kinetics, the model was considered to be supportive of the use of the surrogate in nonclinical studies.

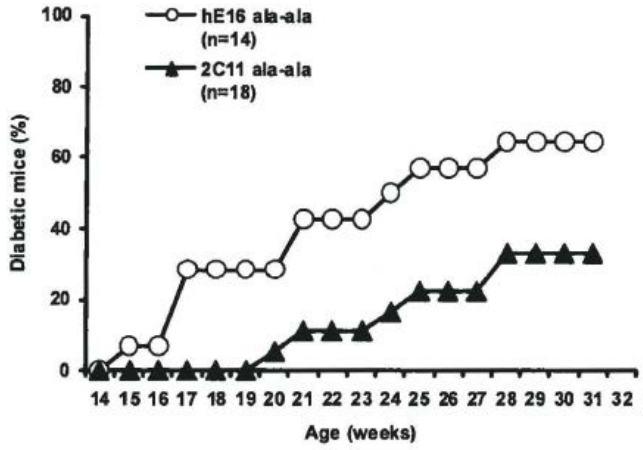
4.1.2 Evaluation of 2C11-mG2a(ala,ala) in Pre-diabetic NOD Mice

A study was performed in pre-diabetic (positive for insulin auto antibodies (IAA)) NOD mice to investigate the potential of the surrogate 2C11-mGa2(Ala-Ala) mAb to delay/prevent the onset of diabetic symptoms. Mice were administered 50 µg iv of the surrogate or control mAb (hE16-mlgG2a(ala-ala); same constant region but different Fv region that does not bind to CD3 or other proteins) for 5 days (1 treatment cycle) or 2 treatment cycles (planned for 6 weeks after the first cycle but a subset were treated after 2 weeks).

A single treatment cycle resulted in an approximate 5-week delay in the onset of diabetes and a 30% lower incidence of diabetes, as summarized in the applicant figure below:

Figure 2 Onset of Diabetes in NOD Mice Administered the Surrogate mAb

Figure 4-2. The diabetes incidence of mice treated with a single course of 2C11-mG2a(ala,ala)



Administration of 2C11-mG2a(ala,ala) mAb to NOD mice at 9–13weeks of age delays diabetes onset. Two groups of NOD female mice (9–13 weeks old) were given i.v. a single course of either 2C11-mG2a(ala,ala) mAb or control antibody (hE16-mIgG2a(ala,ala). Urinary glucose was monitored twice weekly. Diabetes was diagnosed after two consecutive blood glucose readings of ≥300 mg/dl in mice with positive urinary glucose. The graph shows cumulative survival of two independently treated cohorts. hE16 ala-ala=hE16-mIgG2a(ala,ala) mAb; 2C11 ala-ala=2C11-mG2a(ala,ala) mAb.

Applicant figure from study report

Following a second treatment cycle, no adverse effects occurred in mice re-dosed 2 weeks after the first cycle. However, all mice re-dosed 6 weeks after the first cycle died of apparent anaphylaxis within approximately 1 hour of dose administration, based on

the rapid onset of symptoms, the time interval between the end of the first cycle and initiation of the second treatment cycle that was consistent with the time required to generate an immune response, and the significant levels of ADAs observed in all mice 6 weeks following an initial treatment cycle, consistent with effects in NOD mice displaying a hyper-sensitivity reaction. Since deaths also occurred in the control mAb group, the deaths were not directly related to the mechanism of action of the surrogate. Furthermore, since ADA were seen with both the surrogate and control mAb, it is implied that the ADA were directed at least partially to the shared Fc regions.

4.1.3 Evaluation of 2C11-mG2a(ala,ala) Immunogenicity in Mice

To further characterize the immunogenic response, the surrogate 2C11-mGa2(Ala-Ala) was administered (50 µg iv for 5 days) to BALB/c, C57BL/6, CD-1, and NOD mice. Blood samples were obtained 5 weeks after treatment for the determination of ADA. NOD mice were subsequently challenged with a single dose of the surrogate mAb and assessed for anaphylaxis and determination of the mechanism (IgG dependent via PAF antagonism or IgE dependent via histamine antagonism).

Mean ADA titers were higher in the NOD mice (21.3 and 36.3 µg/mL in males and females, respectively) than in BALB/c, C57Bl/6, and CD-1 ♂ mice (ranging from 0.7 to 3.6 µg/mL), although the levels in female CD-1 mice (15.7 µg/mL) approached the level in NOD mice. The levels in females were higher than in males for each strain. In NOD mice re-challenged with the surrogate at 6-weeks after the first dosing cycle, anaphylaxis was seen. To determine the mechanism, NOD mice were treated with the anti-histamine H1 receptor antagonist triprolidone (IgE-based) and the PAF antagonist CV6209 (IgG- based) either together or alone prior to being administered the surrogate. Those treated with triprolidone alone exhibited clinical signs while those administered CV6209 or both antagonists demonstrated minimal to no symptoms, indicating that the IgG pathway plays a role in anaphylaxis.

The anaphylaxis response in the other mouse strains (2/sex) was also evaluated using mice with the highest or median ADA titers. In CD-1 mice, both females and 1 male exhibited anaphylaxis signs and were euthanized. In BALB/c and C57Bl/6 mice, anaphylaxis was only evident in a single BALB/c female, whereas all other mice showed no symptoms.

4.2 Secondary Pharmacology

4.2.1 Psoriatic Patient PBMC in vitro Cytokine Release Study: Induction of Cytokines from PBMC from Psoriasis Patients or Healthy Volunteers in Culture with the anti-CD3 mAb Teplizumab

The potential for teplizumab (MGA031) at concentrations of 1, 10, 100, 1000, or 10000 ng/mL to induce cytokines was evaluated in human peripheral blood mononuclear cells (PBMCs) obtained from patients with psoriasis to determine if teplizumab can be used in patients with diseases of the immune system. The levels of the cytokines IL-2, IL-4, IL-6, IL-10, TNF-α and IFN-γ where determined in patients and healthy volunteers.

Phytohemagglutinin (PHA) and OKT®3 were used as positive controls and Synagis (palivizumab, a human mAb used to treat RSV) was used as a negative control.

The levels of IFN-γ and TNF-α were increased over the levels in the media and negative controls at concentrations ≥ 100 ng/mL. The increases were minimal as compared to those induced by the positive control agents. There were no differences in cytokine levels between normal volunteers and patients with psoriasis. Data are summarized in the applicant table below (table split by reviewer for legibility):

Table 4 Cytokine Release from PBMC in Psoriasis Patients and Healthy Volunteers

	IFN-γ		TNF-α		IL-10	
Treatment (ng/ml)	Psoriasis	Normal	Psoriasis	Normal	Psoriasis	Normal
MGA031, 10,000	42 ± 52.5	134.5 ± 222.1	39.7 ± 75.6	40.4 ± 48.4	2.3 ± 2.1	2.9 ± 3
MGA031, 1,000	25.4 ± 33.3	65.4 ± 103.2	24.4 ± 35.8	26.1 ± 26.1	2 ± 1.7	2.2 ± 2.1
MGA031, 100	8.6 ± 8.2	25.2 ± 38.2	10 ± 8.6	20.6 ± 19.5	1.6 ± 0.4	2.1 ± 2.1
MGA031, 10	4.9 ± 4.1	15.5 ± 20.4	7.1 ± 5.3	11.7 ± 14	1.7 ± 0.4	2.2 ± 1.9
MGA031, 1	4.5 ± 4.5	9.5 ± 10.8	6.8 ± 4.4	12.6 ± 11.4	1.6 ± 0.4	1.8 ± 1.2
OKT3, 100	365.6 ± 828.6	561 ± 1037.6	236.1 ± 523.1	187.6 ± 248	19.2 ± 50.1	17.4 ± 24.2
OKT3, 0.1	242.9 ± 472	515.8 ± 977.9	210.6 ± 457	207.3 ± 223.5	10.2 ± 21.6	11.8 ± 17.2
OKT3, 0.0001	6.2 ± 8	13.9 ± 16.3	11.1 ± 9.3	17.9 ± 14.6	1.6 ± 1	2.4 ± 2.1
PHA, 5,000	470.3 ± 691.8	1090.8 ± 1395.9	529.2 ± 957.1	805.8 ± 746.5	16.1 ± 26.7	52.4 ± 72.9
Synagis, 10,000	4 ± 3.4	5.7 ± 3.3	5.1 ± 3.8	9.1 ± 9.2	1.5 ± 0.9	2.3 ± 1.3
Medium	4.5 ± 3.9	7.2 ± 5.4	7.3 ± 4.1	10.8 ± 9.6	1.9 ± 0.5	2.2 ± 1.8

	IL-6		IL-4		IL-2	
Treatment (ng/ml)	Psoriasis	Normal	Psoriasis	Normal	Psoriasis	Normal
MGA031, 10,000	369.2 ± 461	647.3 ± 1309.3	0.9 ± 0.9	1 ± 1.2	4 ± 3.1	3.8 ± 2.7
MGA031, 1,000	313.3 ± 346.6	540.7 ± 1080.1	1 ± 1.1	0.9 ± 0.9	3.2 ± 1.7	2.7 ± 1.2
MGA031, 100	253 ± 237.9	572.1 ± 1196.3	1.1 ± 1	0.9 ± 0.8	1.8 ± 1.1	1.9 ± 0.9
MGA031, 10	237.1 ± 216.6	521.4 ± 1030.8	1 ± 0.9	0.7 ± 0.9	1.1 ± 1	1.4 ± 1.3
MGA031, 1	247.6 ± 230	542.6 ± 1083.4	0.8 ± 0.8	0.5 ± 0.7	0.8 ± 0.9	0.9 ± 0.8
OKT3, 100	747.8 ± 1467.3	685.1 ± 1351.5	3.5 ± 3	6.9 ± 11.6	24.5 ± 39.6	25.1 ± 24.8
OKT3, 0.1	532.1 ± 983.4	547.4 ± 1028.8	3.2 ± 2.9	5.6 ± 7.4	27 ± 37.7	31.1 ± 33.8
OKT3, 0.0001	258.8 ± 243.7	631.9 ± 1351.7	0.8 ± 0.8	1 ± 1.1	1.8 ± 1.4	2 ± 1.3
PHA, 5,000	932 ± 1459.4	1383.4 ± 1741.6	16.3 ± 14.7	40 ± 53	317.9 ± 501.6	501.8 ± 320.7
Synagis, 10,000	218.1 ± 206.5	548.6 ± 1136.6	0.9 ± 0.9	1.3 ± 1.2	0.5 ± 0.8	1.5 ± 1.3
Medium	250.2 ± 245.9	542.4 ± 1109.5	1 ± 0.9	1.3 ± 1	0.8 ± 0.9	1 ± 1.1

Cytokine concentration in pg/mL; mean ± SD

4.3 Safety Pharmacology

No stand-alone safety pharmacology studies were performed. Cardiovascular and respiratory functioning was evaluated as part of the single dose study in chimps (see section 6.1.1). CNS functioning was indirectly evaluated in the various toxicology studies as a component of the clinical observations; no observations suggestive of an effect on the CNS were noted.

5 Pharmacokinetics/ADME/Toxicokinetics

Toxicokinetic data for teplizumab were collected as a component of a bioequivalence study in rats comparing teplizumab manufactured by two different methods.

Toxicokinetic analysis of teplizumab was also conducted in a single dose toxicity study in chimps while exposure data for the surrogate mAb were obtained in the single-dose, repeat-dose, and reproductive toxicology studies in mice. The bioequivalence study is summarized in section 5.1 while the exposure data from all other studies are discussed with each study in section 6 of this review.

5.1 PK/ADME

Pharmacokinetic parameters from different lots of teplizumab manufactured by the (b) (4) method and the MacroGenics method were compared. This mAb was initially (b) (4) MacroGenics acquired the technology from (b) (4) . In this study, pharmacokinetic profiles in rats were determined using test-article manufactured by the (b) (4) method produced using (b) (4) cells (lot# N11007F2 and 272-01-001) were compared to that produced by the MacroGenics method produced using CHO cells (lot# 141105001 and 060206001). For this study, (b) (4) Sprague Dawley rats were administered (iv) a 1 mg/kg dose of the mAb and followed for 14 days.

The AUC and C_{max} values were comparable between drug lots using the two manufacturing processes. Exposure in females was higher than in males, but the differences were not biologically meaningful. Based on the pre-determined comparison for bioequivalence (80% to 125% of each other), the different mAb products were considered bioequivalent to each other. The PK data are summarized in the applicant table below:

Table 5: Pharmacokinetic Parameters of hOKT3$_\gamma$1(Ala,Ala) Manufactured Using Different Cell Lines

Mean Pharmacokinetic Parameters (Mean ± Standard Deviation)

Gender	Half-Life (h)	C_{max} (µg/mL)	$AUC_{0-336\,h}$ (µg*h/mL)	$AUC_{0-\infty}$ (µg*h/mL)	Vz (mL/kg)	CL (mL/h/kg)	Vss (mL/kg)
Group 1: hOKT3γ1 (Ala-Ala) (Lot N11007F2)							
Male	255 ± 135	21 ± 3	1576 ± 219	2513 ± 532	143 ± 55	0.417 ± 0.103	137 ± 42
Female	284 ± 108	25 ± 4	1894 ± 165	3216 ± 670	125 ± 26	0.322 ± 0.059	118 ± 21
Combined	269 ± 120	23 ± 4	1735 ± 250	2864 ± 691	134 ± 43	0.369 ± 0.095	127 ± 34
Group 2: hOKT3γ1 (Ala-Ala) (Lot 272-01-001)							
Male	228 ± 52	23 ± 4	1656 ± 205	2550 ± 446	129 ± 25	0.403 ± 0.069	125 ± 21
Female	247 ± 53	27 ± 2	1856 ± 165	2922 ± 344	121 ± 20	0.347 ± 0.042	114 ± 15
Combined	237 ± 52	25 ± 4	1756 ± 208	2736 ± 432	125 ± 22	0.375 ± 0.063	120 ± 19
Group 3: MGA031 (Lot 141105001)							
Male	235 ± 62	25 ± 3	1852 ± 200	2897 ± 525	116 ± 21	0.355 ± 0.061	112 ± 16
Female	222 ± 74	28 ± 3	1986 ± 238	3041 ± 618	104 ± 17	0.339 ± 0.059	98 ± 15
Combined	228 ± 67	27 ± 4	1919 ± 225	2969 ± 563	110 ± 20	0.347 ± 0.059	105 ± 16
Group 4: MGA031 (Lot 060206001)							
Male	250 ± 120	24 ± 4	1783 ± 113	2821 ± 649	123 ± 29	0.370 ± 0.077	117 ± 21
Female	295 ± 77	28 ± 4	2061 ± 256	3546 ± 757	120 ± 18	0.293 ± 0.058	111 ± 17
Combined	273 ± 101	26 ± 4	1922 ± 240	3183 ± 781	121 ± 23	0.331 ± 0.077	114 ± 19

Applicant table

6 General Toxicology

A single dose study in chimpanzees was conducted with the (b) (4) hOKT3γ1 (Ala-Ala) mAb, considered analogous to teplizumab. No multiple dose studies were conducted in chimpanzees with the (b) (4) or current CHO cell-derived hOKT3γ1 (Ala-Ala) versions. To characterize the toxicologic profile of teplizumab, a (b) (4) surrogate monoclonal antibody (mAb) was developed (identified as 2C11-mG2a(Ala-Ala) or 2851055) and used in the majority of nonclinical studies.

6.1 Single-Dose Toxicity

6.1.1 Dose-Range Finding Study of (b) (4) 311 Following a Single Subcutaneous Administration to Chimpanzees

Single doses of (b) (4) 311 were administered by subcutaneous (sc) injection to chimps (11 to 23 years of age) at dosages of 0, 0.1, 1, or 10 mg/kg in a GLP-compliant ranging study (n=3/group). Dosages were administered using a staggered dose escalation design with each cohort of animals being evaluated for 21 days before escalating to the next higher dose. The vehicle (0.9% NaCl) was administered to control animals.

There were no effects on body temperature, ECGs, heart rates, blood pressure, or respiratory parameters. Inflammation or irritation at the injection site were not observed. Coughing and inappetence were noted in the HD cohort (1 C and 3 HD animals) beginning on approximately day 27. Antibiotic treatment was administered to all animals in this cohort, but only the control responded. One HD animal died (day 31) after being sedated for further evaluation and the other two were euthanized (day 31 or 33) based on deteriorating condition. The cause of death was determined to be necro-suppurative bacterial pneumonia with septicemia and lympho-proliferative disease, secondary to T cell suppression and suspected EBV-like lympho-cryptovirus infection.

Other notable effects were observed on hematologic/coagulation and clinical chemistry parameters in HD animals. In individual animals, marked increases in leukocytes (4x to 17x over baseline) and granulocytes (2x to 6x over baseline), increases in PT (up to 1.4x) and APTT times (up to 2.1x from baseline), and decreased fibrinogen (up to -79% from baseline) were observed that coincided in time with the animals becoming sick. Altered levels of multiple clinical chemistry parameters were observed at the HD. These effects are summarized in the applicant table below:

Table 6: Clinical Chemistry Alterations in Chimps Administered Teplizumab

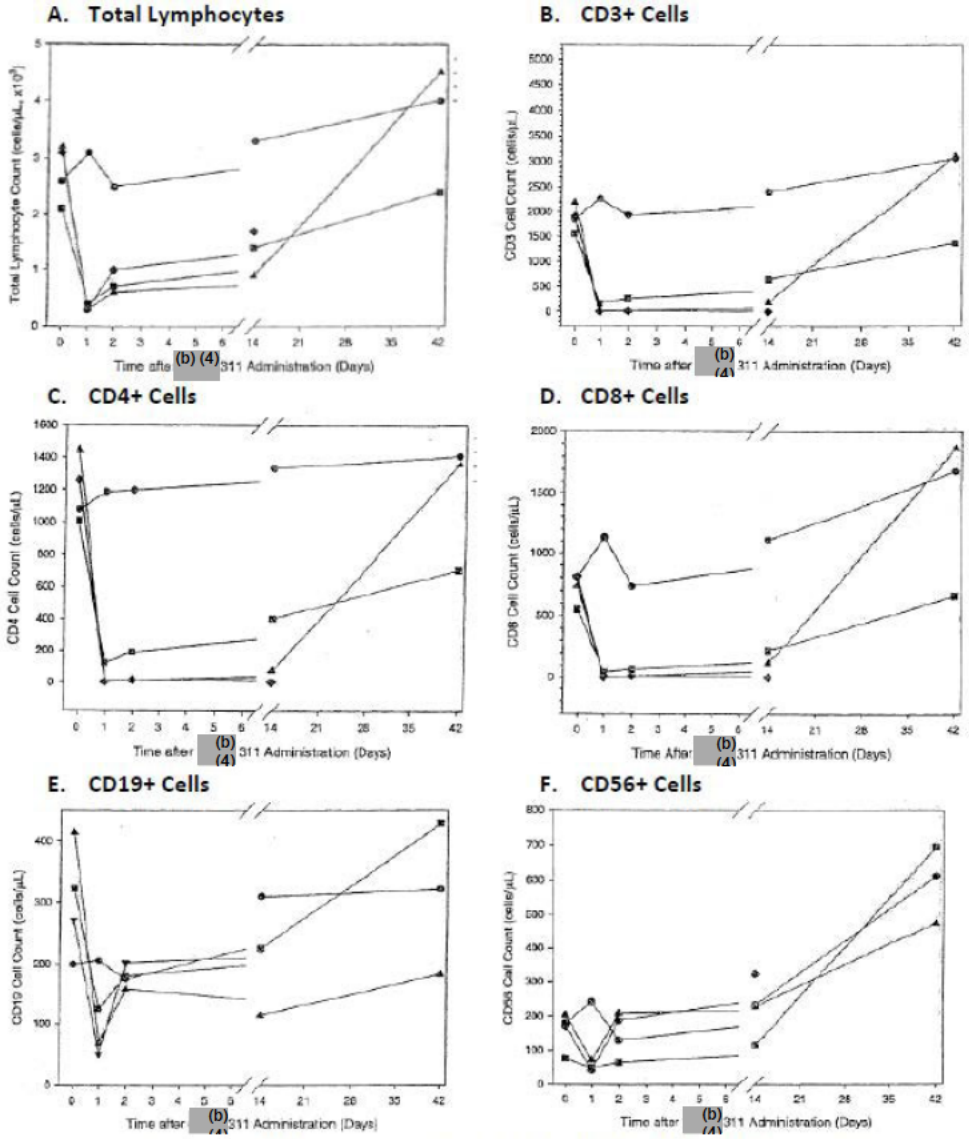
CLINICAL CHEMISTRY, 10 MG/KG (b) (4) 311		
Parameter	**# of animals (n=3)**	**Trend [a]**
Na^{2+}	2	↓ 10 – 11%
K^{+}	3	↓ 23 – 54%
Cl^{-}	3	↓ 18 – 22%
ALT	1	↑ 2-fold
AST	3	↑ 2 – 4-fold
ALP	2	↑ > 2-fold
GGT	2 [b]	↑ 3 – 6-fold
LDH	2	↑ > 3-fold
Cholesterol	3	↓ 2 – 3-fold

[a] Most consistent, large magnitude changes shown; various other findings seen in individuals.
[b] Not determined for one animal.

Necropsies were only performed on the 3 HD animals. Gross observations included enlargement of multiple tissues (lymph nodes, spleen, tonsils, liver, and thymus), necrosis (tonsils), discoloration (lungs), dehydration, and exudate (trachea and nasal passages). Microscopic observations in multiple tissues included atypical lymphocytes (kidney, thyroid, adrenal gland, submandibular salivary gland, mammary gland, tongue) and atypical proliferative lymphocytes (liver, lungs, trachea, spleen, lymph nodes (axillary, mesenteric, inguinal, submandibular, submaxillary), tongue, thymus, esophagus, gall bladder, bone marrow, and appendix). Pneumonia with abscess formation and pleuritis and the presence of bacteria were diagnosed in several tissues. In-situ hybridization studies of proliferative lymphoid cells identified the presence of Epstein-Barr virus while serum electrophoresis revealed increases in IgM and IgG.

Whole blood was analyzed by flow cytometric Fluorescence Activated Cell Sorting (FACS) for various lymphocyte types. Near complete depletion of total lymphocytes and of CD3+, CD4+, and CD8+ cells were noted the day after treatment at all doses. The reductions persisted at the LD/MD for approximately two weeks before returning to baseline values. However, at the HD the reductions were still evident at the time of death and/or euthanasia at days 31/33. In addition, CD19+ cells at all dosages and CD56+ cells at the MD/HD were depleted for one day before returning to baseline. Data are shown in the applicant figure below:

Figure 3 Lymphocyte Subset Populations Following Single Dose Administration in Chimpanzees



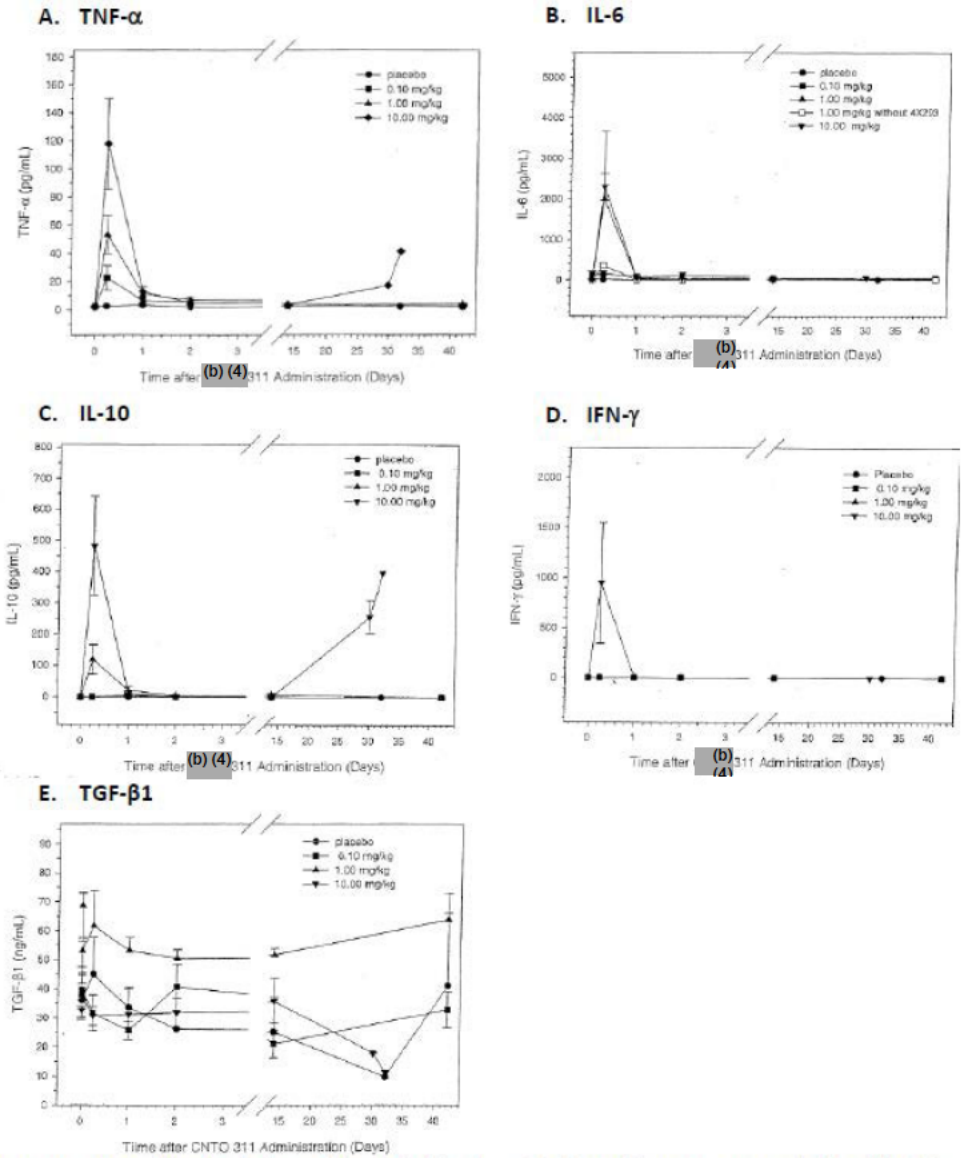
Group mean cell counts (cells/µL) are shown for total. CD3+, CD4+, CD8+, CD19+, or CD56+ lymphocytes in whole blood samples from chimpanzees treated with saline (●) or teplizumab at 0.1 mg/kg (■), 1.0 mg/kg (▲), or 10.0 mg/kg (♦ or ▼). CD3 is a marker for the total lymphocyte population. CD19 is a marker expressed on B lymphocytes, but not fully differentiated ones i.e., plasma cells. CD56 is a marker expressed on NK cells.
Source: (b) (4) Study Report T-2002-009 and Amendment.
Applicant figure from Pharmacology Written Summary

The mechanisms of lymphocyte reduction were investigated in peripheral blood mononuclear cells (PBMC) isolated from control and (b) (4) 311 treated chimpanzees at various time points after dosing. Apoptosis was estimated by Annexin-V expression on CD4+ and CD8+ cells. Increased Annexin-V was detected in LD animals that suggested induction of apoptosis on days 1 and 2, with cells returning to normal apoptotic status on day 3. Although CD4+ and CD8+ cells were depleted by all doses of (b) (4) 311, apoptosis could not be estimated at the MD/HD because of the lack of

adequate numbers of CD4+ and CD8+ cells. Regardless, these results suggest that the reduction in T cells by drug treatment may be partially due to induction of apoptosis.

Also noted were dose-dependent and transient increases in circulating levels of TNF-α, IL-6, and IL-10 at all doses, and INF-γ at the HD, peaking at 6 hours post-treatment. Increases in TNF-α and IL-10 levels were also evident in samples collected from HD animals when sampled at ~days 31-33 (unscheduled) prior to death/moribund sacrifice. There were no effects on TGF-□1 levels. Data are shown in the applicant figure below:

Figure 4 Cytokine Levels Following Single Dose Administration in Chimpanzees

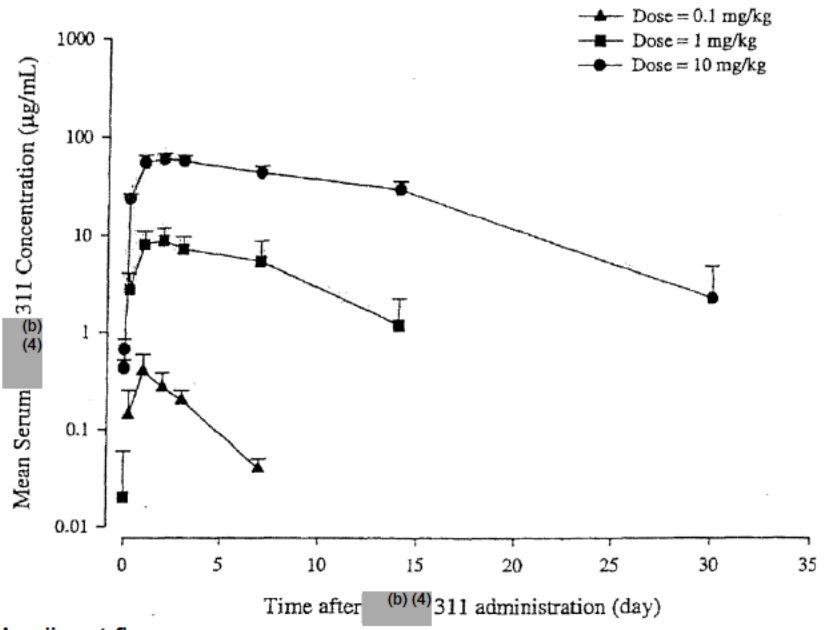


Group mean cytokine levels (±SEM) in whole blood samples from chimpanzees treated with saline (●) or teplizumab at 0.1 mg/kg (■), 1.0 mg/kg (▲), or 10.0 mg/kg (♦ or ▼). One animal (4X369) in the 0.1 mg/kg group had high levels of IL-6 at baseline and throughout the study.
Source: (b) (4) Study Report T-2002-009 and Amendment.
Applicant figure from Pharmacology Written Summary

Cytokine production was also evaluated as a measure of T cell function. PBMCs were isolated and re-stimulated by anti-CD3+ and anti-CD28 soluble antibodies. Proliferation indices were found to be similar for control and treated animals, independent of dose, after re-stimulation of isolated PBMCs, demonstrating that the limited population of T cells remaining after treatment were responsive to antibody stimulation. PBMCs also appeared to be able to produce cytokines upon re-stimulation, although the low levels of these cytokines made interpretation difficult.

Exposure to (b) (4)311 increased with dose. The increases were greater than proportional between the LD and MD and proportional between the MD and HD. The t_{max} was increased slightly at the MD/HD and the $t_{1/2}$ increased with dose. Exposure profiles are summarized in the applicant figure and table below:

Figure 5 Mean Serum Concentrations of Teplizumab in Chimps



Applicant figure

Table 7: Mean Serum Concentrations of Teplizumab Following a Single Dose in Chimps

Dose (mg/kg)	C_{max} (µg/mL)	T_{max}[1] (day)	$AUC_{0\text{-inf}}$ (µg*day/mL)	$AUC_{0\text{-7 days}}$ (µg*day/mL)	$t_{½}$[1] (day)
0.1	0.39 ± 0.18	1.00	1.29 ± 0.27	1.17 ± 0.30	1.94
1.0	8.84 ± 2.99	2.00	72.94 ± 41.00	45.65 ± 18.61	3.68
10	60.46 ± 7.14	2.00	788.53 ± 169.13	348.80 ± 40.24	5.20

Abbreviations: AUC=Area under the concentration versus time curve; C_{max}=maximum serum concentration; inf=infinity; SC=subcutaneous; SD=standard deviation; $t_{1/2}$=terminal phase half-life; T_{max}=time of C_{max}.
[1] T_{max} and $t_{½}$ are presented as median values.

Applicant table

In summary, all doses of (b) (4)311 caused a severe depletion of T cells leading to immunosuppression and mortality in HD animals and a dose-dependent increase in biomarkers for CRS. There was a limited response in CRS biomarkers in LD animals. The limited remaining T cell population was functional and responsive when stimulated in vitro. The response in chimpanzees was consistent with the expected pharmacodynamic response and similar to responses seen in clinical trials. The NOAEL was considered the MD of 1 mg/kg with a corresponding exposure of 72.9 µg·day/mL, based on the death/moribundity at the HD. However, it should be noted that increased cytokine levels (particularly TNFα, IL-6, and IL-10) were observed at all dosages that peaked at approximately 6 hours post-dose.

6.1.2 Single Dose Toxicity Study of Teplizumab in C57Bl/6 Mice

hOKT3$_\gamma$1(Ala,Ala) was administered intraperitoneally (ip) as single doses at 0, 5, or 10 mg/kg followed by a 14-day observation period.

There were no effects on survival, clinical signs, or body weight and the LD_{50} was considered > 10 mg/kg. The absence of effects was not surprising given that hOKT3$_\gamma$1(Ala,Ala) does not cross-react with mouse CD3+ cells.

6.1.3 A Dose-Ranging Study in CD-1 Mice Given a Single Subcutaneous Dose of Compound 2851055 (b) (4) Monoclonal Antibody 2C11-mG2(ala,ala)]

The surrogate 2851055 was administered to CD-1 mice as a single sc injection at dosages of 0, 0.03, 0.1, 0.3, 1, 3, 10, 20, or 60 mg/kg or as a single iv injection at 10 or 30 mg/kg. All mice survived the 3-day observation period. Terminal blood collections were performed at 72-hour post administration for immunophenotyping and tk. Total T cell counts at 72 hr post-dose were reduced in male mice given 0.1-10 mg/kg and in females given 0.1-1 mg/kg, with the largest decreases at 0.3 and 1.0 mg/kg (~73% in males and 76% in females). T cell counts were slightly reduced (-23%) at 60 mg/kg, but decreases were not seen in male mice given 0.03 mg/kg sc, 10 mg/kg iv, 20 mg/kg sc, or 30 mg/kg iv, or in female mice given 0.03, 3, or 10 mg/kg sc. The reductions in T-cell levels were accompanied by concurrent increases of up to 40% (♂) and 66% (♀) in percentage of B cells at doses $\geq$ 1 mg/kg. Following iv administration, there were either no or minimal reductions in relative T-cell proportions. Data are summarized in the applicant tables below:

Table 8: Immunophenotype Assessment in Male Mice

M00255 Male Mouse Peripheral Blood Immphen - Compound 2851055(Hamster Mab 2C11mG2)

Dose (mpk)[1]		CD3%[3]	CD3 ABS	CD4 %	CD4 ABS	CD8 %	CD8 ABS	Total T% (calc)	Total T ABS (calc)	CD4:CD8 (calc)	B220%	B220 ABS
Naïve Males[2]	MEAN	33.7	866.4	19.8	507.4	10.4	269.0	30.2	776.4	1.9	51.9	1624.0
	SD	12.72	607.98	9.36	400.64	3.89	188.28	12.73	583.13	0.55	14.51	1404.69
0.03	MEAN	35.0	666.3	24.0	457.7	8.8	166.7	32.8	624.3	2.8	43.1	713.3
	SD	13.44	437.89	12.11	332.03	2.21	93.36	12.90	412.66	1.54	11.18	387.77
0.1	MEAN	23.7	1264.0	13.7	739.3	7.1	370.0	20.8	1109.3	1.7	65.7	3189.3
	SD	20.09	1202.99	14.46	852.08	5.45	327.07	19.88	1178.98	0.54	18.97	1209.18
0.3	MEAN	10.4	341.3	6.4	213.7	2.0	63.7	8.4	277.3	3.51	69.8	2667.7
	SD	3.83	29.87	2.31	9.29	1.07	14.57	3.36	13.05	1.02	12.84	1553.06
1	MEAN	NA	NA	5.4	144.3	2.8	77.7	8.2	222.0	2.02	72.9	2070.7
	SD	NA	NA	1.02	9.02	0.45	29.67	0.85	38.59	0.65	3.17	986.18
3	MEAN	NA	NA	6.7	168.0	2.8	69.5	9.5	237.5	2.48	70.5	1821.5
	SD	NA	NA	1.01	72.12	0.65	36.06	1.66	108.19	0.25	4.32	673.87
10	MEAN	NA	NA	12.6	416.3	6.0	198.0	18.6	614.3	2.13	64.9	2555.0
	SD	NA	NA	3.28	262.21	1.03	123.96	3.65	383.30	0.54	10.09	2577.30
10, IV	MEAN	NA	NA	17.1	703.3	13.6	593.0	30.6	1296.3	1.22	62.0	2478.0
	SD	NA	NA	9.05	240.43	3.66	161.16	12.47	331.80	0.37	14.88	1417.97
20, SC	MEAN	NA	NA	17.6	803.3	14.0	643.3	31.6	1446.7	1.24	60.2	2978.7
	SD	NA	NA	4.80	201.33	1.35	79.29	6.12	263.75	0.24	7.98	870.57
30, IV	MEAN	NA	NA	19.7	832.0	14.0	587.7	33.6	1419.7	1.44	55.7	2789.7
	SD	NA	NA	1.28	436.22	2.56	320.06	3.11	739.93	0.25	6.84	662.73
60, SC	MEAN	NA	NA	13.3	674.3	10.1	464.0	23.4	1138.3	1.64	64.4	2894.3
	SD	NA	NA	6.45	582.58	6.90	323.02	11.84	865.04	0.75	10.14	1114.82

[1] All samples collected approximately 72 hours post-dose except for 10mg/kg IV and 20mg/kg SC groups, which were collected at approximately 48 hours post-dose.

[2] Naïve male data from lab method development study, Notebook reference D3858

[3] CD3 data NA at doses over 0.3 mg/kg since the dosed antibody 2851055 bound to the same site as the CD3ε detection antibody used for immunophenotyping

Applicant table

Table 9: Immunophenotype Assessment in Female Mice

M00255 Female Mouse Peripheral Blood Immphen - Compound 2851055(Hamster Mab 2C11mG2)

Dose (mpk)[1]		CD3%[3]	CD3 ABS	CD4 %	CD4 ABS	CD8 %	CD8 ABS	Total T% (calc)	Total T ABS (calc)	CD4:CD8 (calc)	B220%	B220 ABS
Naïve Females[2]	MEAN	45.3	2011.0	27.9	1243.8	14.3	619.7	45.4	2548.7	1.9	45.0	2026.2
	SD	11.64	1582.43	8.85	1089.30	2.97	412.51	7.17	1964.55	0.39	14.58	1287.68
0.03	MEAN	66.0	1561.0	45.6	1082.3	18.0	422.3	63.6	1504.7	2.54	20.4	593.3
	SD	3.64	604.49	3.31	441.53	0.87	143.63	2.67	582.49	0.28	3.84	405.38
0.1	MEAN	26.6	1040.3	15.4	602.7	8.2	319.7	23.6	922.3	1.87	61.7	1960.3
	SD	6.99	286.82	5.12	207.47	2.12	87.52	7.16	291.88	0.20	9.24	331.48
0.3	MEAN	13.7	240.0	8.6	153.7	2.9	44.7	11.5	198.3	4.35	65.8	1303.0
	SD	1.99	99.98	2.23	85.17	1.75	25.48	2.01	77.24	3.28	12.80	857.64
1	MEAN	NA	NA	6.8	316.7	4.2	192.0	11.0	508.7	1.71	74.8	3618.0
	SD	NA	NA	3.85	106.64	2.20	58.92	5.87	147.76	0.57	7.92	1102.17
3	MEAN	NA	NA	39.4	1737.0	15.1	631.5	54.6	2368.5	2.74	30.5	1119.7
	SD	NA	NA	6.89	236.17	1.19	48.79	8.00	284.96	0.16	4.33	187.47
10	MEAN	NA	NA	22.4	533.7	21.8	572.7	44.1	1106.3	1.03	39.3	1315.3
	SD	NA	NA	7.91	112.25	2.36	287.95	9.10	379.83	0.32	12.10	1107.01
10, IV	MEAN	NA	NA	16.9	551.0	10.0	312.0	26.9	863.0	1.75	62.7	1983.0
	SD	NA	NA	2.96	229.41	2.37	74.18	3.58	290.77	0.47	1.42	592.99
20, SC	MEAN	NA	NA	24.1	819.7	15.0	507.0	39.1	1326.7	1.61	48.9	1683.3
	SD	NA	NA	4.63	223.00	2.90	132.14	7.47	353.51	0.09	4.48	120.97
30, IV	MEAN	NA	NA	19.5	712.3	11.9	414.0	31.4	1126.3	1.75	58.9	2067.7
	SD	NA	NA	2.09	207.32	4.25	107.35	6.31	267.04	0.49	14.69	902.25
60, SC	MEAN	NA	NA	21.1	812.0	14.3	579.3	35.4	1391.3	1.54	52.3	2358.3
	SD	NA	NA	7.90	205.36	2.50	209.36	6.85	229.21	0.70	10.24	784.06

[1] All samples collected approximately 72 hours post-dose except for 10mg/kg IV and 20mg/kg SC groups, which were collected at approximately 48 hours post-dose.

[2] Naïve male data from lab method development study, Notebook reference D3858

[3] CD3 data NA at doses over 0.3 mg/kg since the dosed antibody 2851055 bound to the same site as the CD3ε detection antibody used for immunophenotyping

Applicant table

Plasma concentrations were indicated as being collected from animals at all dosages, but data were only reported for those administered sc dosages of 0.03 to 10 mg/kg Mean plasma concentrations are shown in the applicant table below:

Table 10: Mean Plasm Concentration (µg/mL) in CD-1 Mice Following a Single SC Administration

Study Day	Time (hrs)	0.03 mg/kg	0.1 mg/kg	0.3 mg/kg	10 mg/kg
1	72	<0.015	<0.015	0.0451 ± 0.0271	5.96 ± 1.30

Abbreviations: N=number of animals; SC=subcutaneous; SD=standard deviation.

Applicant table

Based on the absence of off-target effects, the NOAELs were 60 and 30 mg/kg by sc and iv administration, respectively.

6.2 Repeat-Dose Toxicity

6.2.1 A Repeat-Dose Toxicity and Toxicokinetic and Immunotoxicology Study in Mice Given Compound 2851055 by Subcutaneous or Intravenous Injection for 6 Days with a 6-Week Recovery

Date of study initiation:	30 Sep 08
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	2851055, lot# AE7-A11633-69 (purity not determined) and lot# P265.026 (purity >95%)

Key Study Findings

2851055 was administered for 6 consecutive days at dosages of 0, 0.03, 0.3, or 20 mg/kg by sc administration or 0.3 mg/kg by iv administration followed by a 6-week recovery period.

Following treatment with 2851055, reductions in lymphocyte counts were observed in all drug-treated groups, regardless of the route of administration. The most pronounced effects occurred at the LD. Counts remained lower than controls following recovery, but none of the effects were statistically significant. Immunophenotyping revealed decreases in absolute and relative lymphocyte counts (CD3+, CD4+ and CD8+, total lymphocytes, and various T-cell subsets) in peripheral blood, spleen, and/or thymus. Reductions in absolute B cell and natural killer cell counts were also observed. Microscopic observations at the end of the dosing phase in all test article treated animals included reduction in cellularity of thymus medulla, hematopoiesis in spleen, neutrophil infiltration in mandibular lymph nodes, and myeloid hyperplasia comprised primarily of mature neutrophils in bone marrow. The effects observed in this study were generally in the process of reversing at the end of the recovery period. The CD3+ T cell reduction and histology findings were consistent with the pharmacological effects of the

test article. There was no off-target toxicity evident. For this short-term study, the NOAEL could be considered the HD of 20 mg/kg. However, the observed effects may become adverse with longer treatment periods.

Methods

Doses:	0, 0.03, 0.3, 20 mg/kg sc + 0.3 mg/kg iv
Frequency of dosing:	daily
Route of administration:	sc and iv
Dose volume:	0.5 mL/kg sc, 2 mL/kg sc
Formulation/Vehicle:	(b) (4) NaPO4, (b) (4) NaCl, and (b) (4) polysorbate 80 prepared in sterile water for injection, pH 6.1.
Species/Strain:	Mouse / Crl:CD1(ICR)
Number/Sex/Group:	15, the last 5/group used for recovery
Age:	6 – 7 weeks
Weight:	Males: 35.6-24.7 g, Females: 32.2-19.6 g
Satellite groups:	16 control or 34 drug treated sex/group for tk, 25 sex/group for immunophenotyping
Unique study design:	Appropriate for study purposes
Deviation from study protocol:	Minor – none affecting study interpretability

Observations and Results

Mortality: No treatment-related effects.

Clinical Signs: No treatment-related effects.

Body Weights: No treatment-related effects.

Feed Consumption: A slight dose-dependent decrease in food consumption was noted during the dosing phase. Food consumption was 101, 97, 94, and 94% for males and 100, 92, 87, and 95% for females given 0.03, 0.3, 20 mg/kg SC or 0.3 mg/kg IV, respectively, compared with control.

Ophthalmoscopy: No treatment-related effects.

ECG: NA

Hematology: In males, statistically significant reductions in lymphocyte counts were evident on Day 7 at the LD (-51%) and MD (-40%) compared to control. Counts were still lower following recovery but were not statistically different from controls. There were no significant effects at the HD or following iv administration. In females, lymphocyte counts were statistically decreased on Day 7 at the LD (-53%) compared to control. The lymphocyte counts of females at the MD and HD by sc administration and the iv treated group were decreased by 46%, 22%, and 45%, respectively, although these decreases did not achieve statistical significance. Counts were still lower

following recovery, particularly at the LD and HD, but none of the effects were statistically significant. Lymphocyte count data are summarized below:

Table 11 Mean Lymphocyte Data in Mice

Mean Hematology Data

Test Article	Control	2851055			
Group	1	2	3	4	5
Level (mg/kg)	0.0	0.03	0.3	20	0.3
Dosing Route	SC	SC	SC	SC	IV

Group/ Sex		LYM E3/uL DSNG 7	LYM E3/uL RECO 43	Group/ Sex		LYM E3/uL DSNG 7	LYM E3/uL RECO 43
1M	Mean	2.63	2.67	1F	Mean	2.62	2.09E
	SD	1.025	0.909		SD	1.516	1.017
	N	9	5		N	8	3
2M	Mean	1.29*	1.91	2F	Mean	1.22*	1.15E
	SD	0.628	0.893		SD	0.407	0.438
	N	8	4		N	8	2
3M	Mean	1.57*	2.19E	3F	Mean	1.42	1.92
	SD	0.622	0.589		SD	0.505	0.868
	N	9	3		N	9	5
4M	Mean	2.51	1.68	4F	Mean	2.05	1.67
	SD	0.759	0.844		SD	0.870	0.618
	N	10	4		N	9	5
5M	Mean	2.24	2.41	5F	Mean	1.45	1.98
	SD	1.086	0.937		SD	0.753	0.890
	N	10	5		N	6	5
Statistics		P	P	Statistics		PK	X

* P< or =0.05

E = group excluded from analysis
P = ANOVA (and Dunnett's, if applicable)

Applicant table

Clinical Chemistry: No treatment-related effects.

Urinalysis: NA

Immunophenotyping: Pharmacodynamic activity was evaluated by quantifying lymphocyte and T-cell levels in peripheral blood, spleen, and thymus. In general, T-cell populations were decreased in all compartments at all dose levels. Although recovery was seen in some cases, levels still tended to be lower than controls. The strongest effect in the blood and spleen appeared to be at the MD and was often more pronounce in females than in males. In the thymus, the strongest effect was at the HD and appeared to occur sooner in males than in females. More specifically:

Peripheral Blood
Mild to marked reductions (not necessarily dose-related) in lymphocytes (LYM) and absolute total T lymphocytes (ATLY) were observed in all treated groups for all intervals when compared with controls, with many of the effects reaching statistical significance. Absolute counts were nearly undetectable at the MD (both routes) and HD by day 2 and levels stayed extremely low in the MD (sc) and HD groups. Also evident were lower absolute and/or relative T cell sub-populations and/or ratios. Mean levels of B cells and natural killer (NK) cells were slightly lower than controls on day 2

but were comparable to controls thereafter. Effects on peripheral blood immunophenotyping persisted through recovery, although there was evidence of partial reversal for some of the populations. Lymphocyte data are summarized in the applicant tables below while T cell data are summarized at the end of the section.

Table 12 Lymphocyte Data - Males

Test Article	Control		2851055		
Group	11	12	13	14	15
Level (mg/kg)	0.0	0.03	0.3	20	0.3
Dosing Route	SC	SC	SC	SC	IV

Group/ Sex		LYM E3/uL DSNG 2	DSNG 4	DSNG 7	RECO 37	ATLY E3/uL DSNG 2	DSNG 4	DSNG 7	RECO 37
11M	Mean	2.90	2.49	3.29	2.42	1.50	1.02	1.63	1.22
	SD	0.705	1.108	0.851	1.463	0.536	0.407	0.351	1.002
	N	5	4	5	8	5	4	5	8
12M	Mean	1.41*	1.46	2.04	1.91	0.43*	0.09	0.06*	0.48
	SD	0.623	0.709	1.336	0.871	0.369	0.083	0.047	0.350
	N	6	5	6	6	6	5	6	6
13M	Mean	0.62*	0.95*	1.44	2.23	0.00*	0.01*	0.13*	0.02*
	SD	0.452	0.384	0.627	1.060	0.002	0.004	0.204	0.023
	N	6	6	6	6	6	6	6	6
14M	Mean	1.44*	2.00	2.14	1.22	0.00*	0.02*	0.01*	0.01*
	SD	0.630	0.837	0.755	0.421	0.001	0.010	0.005	0.002
	N	6	6	5	5	6	6	5	5
15M	Mean	0.55*	1.79	2.31	1.51	0.00*	0.03*	0.01*	0.13*
	SD	0.206	0.741	0.952	0.507	0.001	0.021	0.005	0.109
	N	6	6	6	6	6	6	6	6
Statistics		P	P	P	P	PK	PK	PK	PK

* P< or =0.05

K = rank-transformed data
P = ANOVA (and Dunnett's, if applicable)

Applicant table

Table 13 Lymphocyte Data – Females

Test Article	Control		2851055		
Group	11	12	13	14	15
Level (mg/kg)	0.0	0.03	0.3	20	0.3
Dosing Route	SC	SC	SC	SC	IV

Group/ Sex		LYM E3/uL DSNG 2	DSNG 4	DSNG 7	RECO 37	ATLY E3/uL DSNG 2	DSNG 4	DSNG 7	RECO 37
11F	Mean	1.81	2.17	3.15	1.69	0.90	0.93	2.13	1.07
	SD	0.757	0.734	0.750	0.647	0.231	0.657	0.514	0.464
	N	4	4	5	8	4	4	5	8
12F	Mean	1.91	1.57	0.98*	1.14	0.95	0.75	0.04	0.48
	SD	1.059	1.829	0.205	0.212	0.636	1.416	0.037	0.241
	N	6	6	4	5	6	6	4	5
13F	Mean	0.91	1.34	1.79*	1.01	0.04*	0.02*	0.00*	0.20*
	SD	0.737	0.556	0.791	0.166	0.045	0.010	0.001	0.296
	N	6	4	6	5	6	4	6	5
14F	Mean	0.90	2.08	1.85*	1.38	0.00*	0.02*	0.01*	0.01*
	SD	0.407	0.669	0.945	0.228	0.001	0.005	0.007	0.006
	N	6	5	5	4	6	5	5	4
15F	Mean	0.42*	0.92	1.32*	1.68	0.00*	0.01*	0.00*	0.03*
	SD	0.327	0.393	0.365	0.975	0.003	0.006	0.003	0.027
	N	6	6	5	5	6	6	5	5
Statistics		PK	P	P	PK	PK	PK	PK	PK

* P< or =0.05

K = rank-transformed data
P = ANOVA (and Dunnett's, if applicable)

Applicant table

Spleen

Slightly higher lymphocyte counts (1.5x) in HD males and spleen cellularity values (up to 1.6x) were seen at the MD (both routes) relative to controls. Absolute and mean CD3+ cells in all groups and helper, cytotoxic, and total T cell values at the LD and MD (both routes) were lower during the dosing interval in all groups relative to control. At the HD, mean absolute and relative T cell (helper, cytotoxic, and total T cell) values were mildly higher than controls through day 4 but thereafter declined to below control levels where they remained through recovery. B and NK cell counts were slightly higher than controls throughout the study. T cell data are shown at the end of this section.

Thymus

Mean absolute lymphocyte and cellularity values were lower than controls in the majority of drug-treated groups throughout the study with the reductions persisting through recovery, but only a few statistically significant effects were noted. CD3+, helper, cytotoxic, CD4+CD8+, and CD4-CD8- levels were generally lower than controls in most drug-treated groups during the study, with some parameters demonstrating recovery, particularly at the LD and MD. However, at some timepoints for some of the groups the levels were higher than controls (e.g., absolute helper counts in LD females and MD both routes on day 4, CD4+CD8+ counts in MD and HD males on day 4 and/or 7 and females on day 7, relative CD4-CD8- levels in HD males and all female groups on day 2, MD sc and HD animals on day 4, MD either route and HD at recovery).

Data for absolute T cell populations in blood, spleen, and thymus are summarized in the applicant tables below:

Table 14 Percent Change from Control in T cell Populations - Males

Loading Dose (mg/kg)	Males Percent Change from Control											
	0 SC				0.03 SC				0.3 SC			
Interval	DSNG 2	DSNG 4	DSNG 7	REC 37	DSNG 2	DSNG 4	DSNG 7	REC 37	DSNG 2	DSNG 4	DSNG 7	REC 37
Blood Absolute CD3+ T cells	1.50	1.02	1.63	1.22	-71.3%*	-91.2%	-96.3%*	-60.7%	-100%*	-99.0%*	-92.0%*	-98.4%*
Blood Absolute Sum CD4+ and CD8+ T cells	1.52	1.08	1.67	1.26	-70.4%*	-82.4%*	-88.0%*	-42.9%	-95.4%*	-91.7%*	-85.6%*	-41.3%
Spleen Absolute CD3+ T cells	6.14	2.41	2.35	2.74	-6.80%	-56.4%*	-78.3%*	-28.9%	-43.2%*	-98.8%*	-98.3%*	-35.7%*
Spleen Absolute Sum CD4+ and CD8+ T cells	6.21	2.60	2.37	2.75	-5.00%	-47.3%*	-71.3%*	-29.5%	-16.90%	-58.5%*	-60.3%	-35.9%*
Thymus Absolute CD3+ T cells	4.88	2.76	1.85	2.40	-5.94%	-25.4%	-32.4%	-23.8%	-21.3%	-17.8%	-36.8%	-17.8%

* = Denotes mean values which are statistically significant when compared with controls; P< or =0.05. Absolute values are E3/µL for blood and E6/µL for spleen and thymus.
+ or - Indicate when a value is increased or decreased vs. controls, respectively.

Loading Dose (mg/kg)	Males Percent Change from Control							
	20 SC				0.3 IV			
Interval	DSNG 2	DSNG 4	DSNG 7	REC 37	DSNG 2	DSNG 4	DSNG 7	REC 37
Blood Absolute CD3+ T cells	-100%*	-98.0%*	-99.4%*	-99.2%*	-100%*	-97.1%*	-99.4%*	-89.3%*
Blood Absolute Sum CD4+ and CD8+ T cells	-81.6%*	-32.4%	-67.7%*	-78.6%*	-98.0%*	-87.0%*	-84.4%*	-48.1%*
Spleen Absolute CD3+ T cells	-98.0%*	-99.6%*	-98.3%*	-97.9%*	-60.1%*	-99.2%*	-98.3%*	-37.9%*
Spleen Absolute Sum CD4+ and CD8+ T cells	+16.7%	+46.2%	-32.5%	-68.4%*	-38.3%	-51.9%*	-65.4%*	-39.7%*
Thymus Absolute CD3+ T cells	-66.6%*	-100%*	-99.5%*	-98.3%*	-56.1%*	-39.9%	-47.0%*	+9.43%

* = Denotes mean values which are statistically significant when compared with controls; P< or =0.05. Absolute values are E3/µL for blood and E6/µL for spleen and thymus.
+ or - Indicate when a value is increased or decreased vs. controls, respectively.

Applicant table

Table 15 Percent Change from Control in T Cell Populations - Female

Loading Dose (mg/kg)	Females Percent Change from Control											
	0 SC				0.03 SC				0.3 SC			
Interval	DSNG 2	DSNG 4	DSNG 7	REC 37	DSNG 2	DSNG 4	DSNG 7	REC 37	DSNG 2	DSNG 4	DSNG 7	REC 37
Blood Absolute CD3+ T cells	0.90	0.93	2.13	1.07	+5.60%	-19.4%	-98.1%	-55.1%	-95.6%*	-97.8%*	-100%*	-81.3%*
Blood Absolute Sum CD4+ and CD8+ T cells	0.95	1.13	2.16	1.08	+6.00%	-27.4%	-93.1%*	-38.9%	-83.2%*	-88.5%*	-87.5%*	-52.8%*
Spleen Absolute CD3+ T cells	5.05	2.21	2.55	2.83	+30.5%	+2.26%	-70.2%*	-23.3%	-19.8%	-99.1%*	-98.4%*	-24.0%
Spleen Absolute Sum CD4+ and CD8+ T cells	5.05	2.35	2.54	3.28	38.4%	+6.38%	-65%*	-33.8%	+23.6%	-63.4%*	-68.1%*	-35.1%
Thymus Absolute CD3+ T cells	5.67	2.92	3.37	3.67	+11.56%	+18.2%	-18.4%	+12.4%	-3.35%	-6.85%	-42.4%*	+15.8%

* = Denotes mean values which are statistically significant when compared with controls; P< or =0.05. Absolute values are E3/µL for blood and E6/µL for spleen and thymus.
+ or - Indicate when a value is increased or decreased vs. controls, respectively.

Loading Dose (mg/kg)	Females Percent Change from Control							
	20 SC				0.3 IV			
Interval	DSNG 2	DSNG 4	DSNG 7	REC 37	DSNG 2	DSNG 4	DSNG 7	REC 37
Blood Absolute CD3+ T cells	-100%*	-97.8%*	-99.5%*	-99.1%*	-100%*	-98.9%*	-100%*	-97.2%*
Blood Absolute Sum CD4+ and CD8+ T cells	-85.3%*	-24.8%	-79.6%*	-81.5%*	-93.7%*	-91.2%*	-89.8%*	-31.5%
Spleen Absolute CD3+ T cells	-98.4%*	-99.5%*	-98.8%*	-97.5%*	-37.8%	-99.5%*	-95.3%*	-31.1%*
Spleen Absolute Sum CD4+ and CD8+ T cells	+6.34%	+23.8%	-53.1%	-82.3%*	-10.9%	-59.6%*	-48.4%*	-40.9%*
Thymus Absolute CD3+ T cells	-79.5%*	-100%*	-100%*	-98.6%*	-56.1%*	-10.7%	-8.61%	-21.5%

* = Denotes mean values which are statistically significant when compared with controls; P< or =0.05. Absolute values are E3/µL for blood and E6/µL for spleen and thymus.
+ or - Indicate when a value is increased or decreased vs. controls, respectively.

Applicant table

Gross Pathology: No treatment-related effects.

Organ Weights: No treatment-related effects.

Histopathology
Adequate Battery: Yes
Peer Review: Yes

Microscopic findings in males and females at the end of dosing occurred in the thymus, spleen, lymph node, and bone marrow. In all test article treated groups of males and/or females, minimal to moderate reduction in cellularity of the medulla in the thymus, minimal to moderate hematopoiesis in spleen, minimal to slight neutrophil infiltration in mandibular lymph nodes, and minimal to marked myeloid hyperplasia in bone marrow comprised primarily of mature neutrophils were noted. These changes were most severe at the HD for both sexes, except for the thymus in females which was similarly affected at all dose levels. The microscopic findings at the MD were similar with both routes of administration.

The effects in the thymus and spleen were still noted at the end of recovery, although occurring at a lower incidences/severity. Neutrophil infiltrates were evident in single MD iv and HD males, but not in any other group. There were no effects on bone marrow in any drug-treated groups.

Toxicokinetics: Following SC administration, t_{max} was reached between 4 and 24 hours post-dosing on days 1 and 6. Exposure increased with dose and the increases in C_{max} and AUC_{0-t} were greater than dose proportional. The systemic exposure to 2851055 was generally higher in males than in females on both Days 1 and 6. The C_{max} values were higher on Day 6 than on Day 1, indicating accumulation of 2851055 following multiple SC dosing. The $t_{1/2}$ could only be determined at the HD (465 and 353 hours for

male and female, respectively). The absolute bioavailability (Day 6) comparing the SC to IV 0.3 mg/kg doses, based on AUC_{0-t}, was 132% and 87.5% for male and females, respectively. The half-life was determined at the HD on day 6 (465 and 353 hours in males and females, respectively). Exposure following iv administration was comparable to that following sc administration. Data are summarized in the applicant table below:

Table 16 Exposure Summary in Mice

Mean Serum Toxicokinetic Data Summary

		Subcutaneous Injection Dose (mg/kg)						Intravenous Injection Dose (mg/kg)	
Parameter		0.03		0.3		20		0.3	
	Sex	M	F	M	F	M	F	M	F
2851055									
Day 1									
C_{max} (ng/mL)		NC	12.9	1367	1077	221333	230667	921	2673
Day 6									
C_{max} (ng/mL)		59.3	44.6	7783	7130	1826667	1031000	22533	10953
AUC_{0-t} (ng·hr/mL)		1246	909	1410295	657211	503786000	475431500	1068137	751072

Applicant table

Dosing Solution Analysis: Test article formulations ranged from 98% - 117% of claim on day 1 and 103% - 133% of claim on day 4. For both preparations, the highest values were at the lowest dose levels.

6.2.2 A Repeat-Dose Toxicity and Toxicokinetic and Immunotoxicology Study in Mice Given Compound 2851055 by Intravenous Injection Every 3 Days for 1 Month with a 3 Month Recovery

Key Study Findings

2851055 was administered to 15 CRI:CDI(ICR) mice/sex/group at dosages of 0, 0.3, 3, or 20 mg/kg by iv injection (2 mL/kg) every 3 days for 1 month (10 doses administered). [redacted] (b) (4)

The most significant finding was mortality. Death occurred in 4 LD (3♂,1♀) and 15 MD (2♂,13♀) animals. The deaths were observed at the LD beginning after the 4th dose and at the MD beginning after the 5th dose. All deaths occurred within minutes to hours after dose administration, with the exception of 1 LD animal found dead the day after drug administration. Swollen nose, irregular respiration, hypoactivity, ataxia, and/or recumbency were observed in animals subsequently found dead. These clinical signs were also observed in surviving LD and MD animals. The clinical signs appeared within minutes of dose administration and were consistent with an anaphylactic reaction.

These findings are summarized as this is the only toxicology study with the surrogate antibody in which anaphylactic reactions were observed. No other data from this study have been summarized or evaluated.

7 Genetic Toxicology

No genetic toxicology studies have been conducted with teplizumab or the surrogate.

8 Carcinogenicity

Carcinogenicity studies were not performed with teplizumab or the surrogate. The short duration of treatment, the absence of pharmacologic activity in the rodent (for teplizumab itself), and the observations of anaphylaxis and hypersensitivity following various dosing regimens in the rodent limit the utility of carcinogenic assessments in rodents with either teplizumab or the (b) (4) surrogate. For these reasons, carcinogenicity studies to support the safety of teplizumab were not considered appropriate. As a result, the long-term effects of treatment were not ascertained. However, suppression of the immune system is associated with an increased risk of cancers.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

9.1.1 A Male Fertility, Toxicokinetic and Immunophenotyping Study in Mice of 2851055 Administered via Subcutaneous Injection

Date of study initiation:	20 May 09
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	2851055, lot P265.059, 97.2%

Key Study Findings

The surrogate antibody was administered by sc injection to male mice once every 3 days at doses of 0, 0.3, 1, 3, or 20 mg/kg. A group was also administered the comparator IgG2 isotope control at a dose of 20 mg/kg. Drug-related reductions in T cell populations were seen at all doses relative to the comparator group that were associated with corresponding increases in B cell populations. No drug-related mortality, adverse clinical signs, or effects on body weight/food consumption were observed in males. There were no effects on the number of animals mating or resulting pregnancies. Sperm counts, motility, and morphology were unaffected as were necropsy observations. The NOAEL for effects on fertility with the surrogate was the HD of 20 mg/kg.

Methods

Doses:	0, 0.3, 1, 3, or 20 mg/kg of 2851055 or 20 mg/kg of the IgG2a(ala,ala) isotope control
Frequency of dosing:	Every 3 days
Dose volume:	2 mL/kg
Route of administration:	SC injection
Formulation/Vehicle:	(b) (4) NaPO4, (b) (4) NaCl, and (b) (4) Tween®80 prepared in sterile water for injection, pH of 6.1
Species/Strain:	Mouse / Crl:CD1(ICR)
Number/Sex/Group:	30 drug-treated males and 30 untreated females per group
Satellite groups:	28 males/group for tk and 20 males/group for immunophenotyping
Study design:	Males treated for 4 weeks prior to cohabitation and continuing until 1 to 3 days prior to termination. 15 doses were administered

Observations and Results

Mortality: There were two deaths on study, neither of which were considered drug-related.

A single male administered 0.3 mg/kg was euthanized on day 10 due to poor clinical condition. Clinical signs observed in days preceding termination included unkempt appearance, hunched posture, decreased defecation, body cool to touch, pale bod, and prolapsed penis. Postmortem examination was suggestive of malignant lymphoma.

A male administered 20 mg/kg was euthanized in extremis on day 6 due to impaired use of forelimbs and hindlimbs. There were no other signs in this animal.

Clinical Signs: No observations considered drug-related. There also were no reactions at the injection site.

Body Weight: A transient loss of weight was observed following the first dose administration at the HD, but there were no effects on absolute weights. There were no effects on weight gain or body weights thereafter.

Feed Consumption: No effects observed considered drug-related.

Toxicokinetics: Blood samples were taken after the first dose and at the end of the cohabitation period to assess exposure. Exposures increased with dose, with the increases being approximately dose proportional at doses $\geq$ 1 mg/kg. Following treatment, drug levels at each dose remained comparable through at least 72 hours. At doses $\geq$3 mg/kg, exposure on day 27 was higher than on day 0. The minimal exposure on day 27 at doses $\leq$1 mg/kg suggests the production of anti-surrogate antibodies or changes in clearance. Data are summarized in the applicant table below:

Table 17 Mean Serum Concentration of 2851055 in Male Rats

Dose (mg/kg):	0.3	1.0	3.0	20
Study Day 0 (ng/mL)				
24 hour	1152.6[a]	8332.1	26534.1	186371.3
48 hour	273.6	5016.1	24427.8	208985.9
72 hour	83.7	1828.7	29377.3	175247.3
Study Day 27 (ng/mL)				
0 hour	<15.0	NR[b]	149998.0	1166102.5
24 hour	<15.0	<15.0	180066.8	1132530.0
48 hour	<15.0	NR[c]	146324.4	1242916.3
72 hour	<15.0	<15.0	NR[d]	1072939.7

[a] = N = 3

[b] = below the quantifiable limit of <15.0 mg/mL for 3 animals with the fourth at 359332.0 ng/mL

[c] = below the quantifiable limit of <15.0 mg/mL for 3 animals with the fourth at 51228.9.0 ng/mL

[d] = 2 values below the quantifiable limit of <15.0 ng/mL and third and fourth values at 178631.3 and 166565.3 ng/mL, respectively

Applicant table

Immunophenotyping: Blood samples were collected after the first dose and following the pre-cohabitation period on day 27. At both time points, decreases in T cell populations (total, T helper, and T cytotoxic T cells) were seen in all 2851055-treated groups relative to comparator IgG2 controls. These decreases were associated with corresponding increases in B cell populations. The effects on T and B cell populations were more pronounced after a single dose than following multiple dose administrations. The reduction in the magnitude of response is potentially a reflection of the formation of anti-surrogate antibodies. Data are summarized in the applicant table below:

Table 18 Mean Percent Change for Lymphocyte Populations Relative to Comparator IgG2 in Male Mice Following Treatment with 2851055

Lymphocyte Subset	Mean % Change from Comparator IgG2 Isotype Control Values				
	Vehicle Control	0.3 mg/kg	1.0 mg/kg	3.0 mg/kg	20 mg/kg
24 Hours Post-dose on Study Day 0					
Total T cells (CD5+)	-5.2%	-91%**	-91%**	-88%**	-75%**
T-helper cells (CD5+CD4+)	-5.4%	-93%**	-93%**	-90%**	-72%**
T-cytotoxic cells (CD5+CD8+)	-9.7%	-89%**	-90%**	-90%**	-83%**
Total B cells (CD45R+/B220+)	-26%	+41%	+85%*	+178%**	+142%**
24 Hours Post-dose on Study Day 27					
Total T cells (CD5+)	-15%	-48%**	-43%**	-40%**	-53%**
T-helper cells (CD5+CD4+)	-15%	-46%**	-44%**	-54%**	-64%**
T-cytotoxic cells (CD5+CD8+)	-16%	-60%**	-48%**	-19%	-47%**
Total B cells (CD45R+/B220+)	+17%	+73%**	+57%*	+63%*	+43%

* Significantly different from comparator IgG2 isotype control at $p \leq 0.05$.

** Significantly different from comparator IgG2 isotype control at $p \leq 0.01$.

Applicant table. Note: The 24 hour data on day 27 at 20 mg/kg was revised by reviewer to reflect directionality of change for total T, T helper, and T cytotoxic cells. Correction was based on data in study report.

Fertility Parameters: There were no effects on the number of animals mating or the ensuing number of pregnancies. No effects were seen on sperm motility or morphology in any group. Sperm counts were also similar between groups (only control, HD, and comparator groups evaluated).

Cesarean sectioning of untreated females revealed no effects on corpora lutea, implantation sites, pre- or post-implantation loss, or the number of viable embryos per group.

Necropsy: Macroscopic examination revealed no drug-related effects, including in the two animals terminated early. Reproductive organ weights were similar between groups, and microscopic evaluation of reproductive organs from HD males revealed no effects.

Dosing Solution Analysis: Dosing formulations ranged from 104% to 107% of claim.

9.1.2 A Female Fertility and Early Embryonic Development, Toxicokinetic and Immunophenotyping Study in Mice of 2851055 Administered via Subcutaneous Injection

Date of study initiation:	20 May 09
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	2851055, lot P265.059, 97.2%

Key Study Findings

Female mice were administered the surrogate antibody once every 3 days at doses of 0, 0.3, 1, 3, or 20 mg/kg from prior to cohabitation through gestation day (GD) 6. A group was also administered the comparator IgG2 isotope control at a dose of 20 mg/kg. The only treatment-related effects observed were reductions in T cell populations with corresponding increases in B cell populations, occurring in all groups administered the surrogate relative to the comparator IgG2. There was no drug-related mortality, adverse clinical signs, or effects on body weight/food consumption. There were no effects on the time to mate, the number of animals mating, or on the ability of maternal animals to maintain a pregnancy or on intrauterine survival. The HD of 20 mg/kg was considered the NOAEL for effects on female fertility.

Methods

Doses:	0, 0.3, 1, 3, or 20 mg/kg of 2851055 or 20 mg/kg of the IgG2a(ala,ala) isotope control
Frequency of dosing:	Every 3 days
Dose volume:	2 mL/kg
Route of administration:	SC injection
Formulation/Vehicle:	(b) (4) NaPO4, (b) (4) NaCl, and (b) (4) Tween®80 prepared in sterile water for injection, pH of 6.1
Species/Strain:	Mouse / Crl:CD1(ICR)
Number/Sex/Group:	30 drug-treated females and 30 untreated males per group
Satellite groups:	32 females/group for tk and 20 females/group for immunophenotyping
Study design:	Females were treated for 2 weeks prior to cohabitation and continuing through gestation day (GD) 6. Animals were terminated on GD 15. 9 - 12 doses were administered.

Observations and Results

Mortality: There was no surrogate-related mortality.

A single female administered 1 mg/kg was euthanized on study day 10 due to an inability to use of the right forelimb and hindlimb. There were no effects noted during the postmortem exam.

A single female administered 3 mg/kg was euthanized on GD 10 due to poor clinical condition (cool to touch, pale body, decreased defecation, red discoloration or urogenital region). The postmortem exam was unremarkable.

Clinical Signs: No treatment-related effects observed. Injection site observations were similar between groups.

Body Weight: There were no effects on weight gain or body weights considered treatment-related during the pre-cohabitation period. Increases in weight gain for various intervals at the higher doses administered or in the group administered the IgG2 comparator were considered incidental as there was no corresponding effect on body weight. Body weights and weight gains were also similar between groups during gestation.

Feed Consumption: There were no effects on food consumption considered treatment-related.

Toxicokinetics: Blood samples were taken at various timepoints after the first dose, at the end of the cohabitation period, and after the last dose on GD 6 to assess exposure. Surrogate drug levels were detected only at 24 and 72 hours after the initial dose at

doses ≤1 mg/kg. At doses ≥3 mg/kg, surrogate levels were detected at all timepoints. Exposures increased with dose and were higher after multiple doses than that reported following a single dose. The minimal exposure following multiple dosing at the lower doses could be a result of the formation of anti-surrogate antibodies, assay interference, or altered clearance. Exposure data are summarized in the applicant table below:

Table 19 Mean Serum Concentrations of 2851055 in Female Mice

Dose (mg/kg):	0.3	1.0	3.0	20
Study Day 0 (ng/mL)				
24 hour	691.9	5650.0	17720.0	112000.6
48 hour	NR[a]	NR[b]	14245.6	143815.5
72 hour	1341.8	1288.3	21688.8	123826.3
Study Day 15 (ng/mL)				
0 hour	NR[c]	NR[d]	72146.6	401196.9
24 hour	NR[e]	NR[f]	108903.0	670406.9
48 hour	<15.0	NR[g]	106222.3	807346.3
72 hour	NR[h]	<15.0	48657.5	638606.3
Gestation Day 6 (ng/mL)				
24 hour	NR[i]	<15.0	84233.5	852030.6

Abbreviations: NR = no result, and BQL = below quantifiable limit of 15.0 mg/mL
a = BQL for 2 animals and 109.3 and 105.6 ng/mL.
b = BQL for 2 animals and 3127.4 and 4480.6 ng/mL.
c = BQL for 3 animals with 4th at 286.3 ng/mL.
d = BQL for 2 animals and 20.5 and 127.8 ng/mL.
e = BQL for 2 animals and 2305.8 and 880.1 ng/mL.
f = BQL for 2 animals and 27077.8 and 1070151.0 ng/mL.
g = BQL for 3 animals with 4th at 27491.6 ng/mL.
h = BQL for 3 animals with 4th at 464.5 ng/mL.
i= BQL for 3 animals with 4th at 84.5 mg/mL.

Applicant table

Immunophenotyping: Blood samples were collected after the first dose and at the end of the pre-cohabitation period. Surrogate-related decreases in T cell populations (not dose-related) were seen at both timepoints in all groups, with the exception of cytotoxic T cells at the HD which were no different from levels seen with the IgG2 control. These changes were associated with increased B cells in all groups, although not all increases were statistically significant.

Table 20 Mean Percent Change for Lymphocyte Populations Relative to Comparator IgG2 in Female Mice Following Treatment with 2851055

Lymphocyte Subset	Mean % Change from Comparator IgG2 Isotype Control Values				
	Vehicle Control	0.3 mg/kg	1.0 mg/kg	3.0 mg/kg	20 mg/kg
24 Hours Post-dose on Study Day 0					
Total T cells (CD5+)	+38%*	-50%*	-81%**	-60%*	-23%
T-helper cells (CD5+CD4+)	+35%	-53%*	-82%**	-58%*	-14%
T-cytotoxic cells (CD5+CD8+)	+39%	-67%**	-87%**	-51%*	+2.8%
Total B cells (CD45R+/B220+)	-65%	+11%	+64%	+16%	+82%
24 Hours Post-dose on Study Day 15					
Total T cells (CD5+)	-1.9%	-51%**	-53%**	-42%**	-35%**
T-helper cells (CD5+CD4+)	-0.8%	-43%**	-45%**	-48%**	-49%**
T-cytotoxic cells (CD5+CD8+)	-7.3%	-74%**	-72%**	-40%**	-18%
Total B cells (CD45R+/B220+)	-2.8%	+69%**	+70%**	+84%**	+64%*

* Significantly different from comparator IgG2 isotype control at $p \leq 0.05$.
** Significantly different from comparator IgG2 isotype control at $p \leq 0.01$.

Applicant table

Fertility Parameters: There were no effects on estrous cyclicity, the number of females mating, the time to mate, or the number of pregnancies. The number of corpora lutea, implantations, pre- and post-implantation loss rates, and viable number of embryos were comparable between groups.

Necropsy: Postmortem examination revealed no treatment-related effects.

Dosing Solution Analysis: Dosing formulations ranged from 100% to 107% of claim.

9.2 Embryonic and Fetal Development

9.2.1 An Embryo-Fetal Development, Toxicokinetic and Immunophenotyping Study in Mice of 2851055 Administered Subcutaneously on Gestation Days 6, 10 and 14

Date of study initiation:	29 Sep 08
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	2851055, lot# AE7-A11633-69 (purity not determined) and lot# P265.026 (purity >95%)

Key Study Findings

The surrogate 2851055 was administered to presumed gravid mice at dosages of 0, 0.03, 0.3, or 20 mg/kg on GD 6, 10, and 14. Treatment-related reductions in T cell populations with associated increases in B cell populations were evident at all dosages. Transient reductions (in excess of 20%) in body weight gain and food consumption were observed during the initial treatment period (GD 6-10) at the HD. However, there were no corresponding effects on absolute body weights. At the HD, 6 animals showed early resorption of the entire litter; it is not clear if this is a result of reduced maternal body weights or if the litter loss induced the effect on maternal animals. The total litter loss in these dams resulted in a surrogate-related increase in the incidence of post-implantation loss with a corresponding reduction in the numbers of viable fetuses/litter at the HD. The effects at the HD were considered adverse, leading to identification of the MD (0.3 mg/kg) as the NOAEL for the study.

Methods

Doses:	0, 0.03, 0.3, 20 mg/kg
Frequency of dosing:	GD 6, 10, 14
Dose volume:	0.5 mL/kg for LD, 5 mL/kg for other groups
Route of administration:	SC injection
Formulation/Vehicle:	(b) (4) NaPO4, (b) (4) NaCl and (b) (4) Polysorbate 80 in sterile water for injection, pH 6.1
Species/Strain:	Mouse / Crl:CD1(ICR)
Number/Sex/Group:	30 presumed pregnant females
Satellite groups:	20 presumed pregnant females for tk and immunophenotyping
Study design:	Mice euthanized on GD 18

Observations and Results

Mortality: A LD animal was euthanized on GD7 based on impaired use of the hindlimbs. Postmortem examination revealed no surrogate-related effects. This animals had a normal number of implants. A MD animal died on day 10 prior to dosing after becoming opisthotonic. No effects were noted during the postmortem exam; this female had normal appearing implants. Neither of these were considered treatment-related based on the absence of death at the HD or of other surrogate-related signs of toxicity.

Clinical Signs: There were no effects observed considered treatment-related. There were no effects at the injection sites.

Body Weight: Weight gain (GD 6-10) following the first dose was significantly reduced (-26.5%) at the HD but the difference in absolute weights was minimal (0.3%). No effects on weight gain or absolute weights were seen after subsequent doses or for the entire treatment period. Carcass weight change (absolute terminal weight minus gravid uterine weight compared to GD 0 weight) was 30% lower at the HD relative to controls (5.1 g at HD as compared to 7.3 g in control). This effect appeared to be due primarily

to the 6 animals that had 100% post-implantation loss (early resorption of entire litter). These animals (and those found to be not pregnant) showed weight loss or minimal weight gain during the GD 6 – 10 period which contributed to either loss of weight or minimal weight gain over the entire dosing period as compared to other animals in the group. There were no effects at the LD or MD.

Feed Consumption: HD animals consumed 1 – 2 g less food (g/animal/day data) than controls each day from GD 6 – 10 and approximately 1g less per day from GD 11 - 14. Although none of these effects were significant by themselves, they contributed to statistically lower food consumption for the GD 6-10 (-21%) and 10-14 (-11%) intervals. These reductions also were the basis for reduced food consumption (-12.7%) at the HD during the treatment period (GD 6 – 18). The HD dams that had entire litter resorptions also tended to have lower food consumption during the GD 6-10 and 6-18 intervals than the dams that successfully carried their litters to term.

Toxicokinetics: Blood samples were collected for the measurement of surrogate levels at various timepoints after the first and last dose administration. With the exception of 72 hours after the initial dose, surrogate was not detected at the LD or MD. The applicant considered this to likely reflect rapid receptor-mediated clearance. At the HD, exposure was higher after multiple doses than seen following a single dose. Data are summarized in the applicant table below:

Table 21 Mean Serum Concentrations in Gravid Mice

Dose (mg/kg):	0.03	0.3	20.0
Day 6 Mean Serum Concentration (ng/mL), (standard error)			
Predose (0 hr)	<15.0 (NC)	<15.0 (NC)	<15.0 (NC)
Postdose (72hr)	<15.0 (NC)	20.5 (11.5)	98633 (7819)
Day 14 Mean Serum Concentration (ng/mL), (standard error)			
Predose (0 hr)	<15.0 (NC)	<15.0 (NC)	21075 (7856)
Postdose (72hr)	<15.0 (NC)	NC (NC)	14367 (1802)

NC = not calculated

Applicant table

Immunophenotyping: Lymphocyte levels were determined 72 hours after dosing on GD 14. Reductions in T cell populations, with most attaining statistical significance, were seen in all groups relative to comparator with the exception of cytotoxic T cells at the HD which were increased. Corresponding statistically significant increases in B cells were seen at the LD and MD; increases were also seen at the HD but they were not statistically significant. Data are summarized in the applicant table below:

Table 22 Mean Percent Change in Lymphocyte Populations in Pregnant Mice

Lymphocyte Subset	Mean % Change from Vehicle Control Values		
	0.03 mg/kg	0.3 mg/kg	20 mg/kg
72 Hours Post-dose on GD14			
Total T cells (CD5+)	-41%**	-50%**	-19%
T-helper cells (CD5+CD4+)	-40%**	-48%**	-40%**
T-cytotoxic cells (CD5+CD8+)	-40%*	-61%**	+33%
Total B cells (CD45R+/B220+)	+63%**	+69%**	+19%

Abbreviations: GD=gestation day; SC=subcutaneous.
* Significantly different from the vehicle control at $p \leq 0.05$.
** Significantly different from the vehicle control at $p \leq 0.01$.

Applicant table

Necropsy: No treatment-related effects were observed grossly. Single LD and HD animals delivered on GD18. For the LD female, 4 live pups, 2 dead pups, and 2 cannibalized pups were found; there were no other pups in utero. For the HD female, 4 dead pups and 3 cannibalized pups were delivered while an additional 7 live and 2 dead pups were found in utero.

Cesarean Section Data: The number of pregnant females at term was similar between groups. There were no effects on the numbers of corpora lutea/dam, implants/dam, or gravid uterine weights. An increase in mean post-implantation loss was evident at the HD. Although not statistically significant, the incidence did exceed the historical range. The increased post-implantation loss reflected the early resorption of all implantations in 6 of the 26 pregnant females in this group (no control group females had complete litter resorption). This increase in post-implantation loss resulted in a reduction in the mean number of viable fetuses/dam (statistically significant and outside the historical range) and in the mean percentage of viable fetuses/litter (outside of historical range). There were no effects on fetal weights or on any parameters at the LD/MD. Data are summarized in the applicant table below:

Table 23 GD 20 Cesarean Sectioning Data in Mice

Dose (mg/kg):	0[a]	0.03	0.3	20	HC mean (range)
Pregnant females at laparohysterectomy	26	25	27	26	NA
Mean corpora lutea/dam	13.7	14.4	13.8	13.0	14.6 (11.7-17.3)
Mean implantations/dam	12.6	13.0	13.0	11.4	13.1 (10.8-14.8)
Mean pre-implantation loss (% per litter)	9.5	10.1	7.0	12.4	9.6 (2.1-21.2)
Mean postimplantation loss (% per litter)	4.9	9.6	7.0	26.8	7.7 (2.2-16.5)
Mean viable fetuses/dam	11.9	11.9	12.1	9.1*	12.1 (9.9-13.8)
Mean viable fetuses (% per litter)	95.1	90.4	93.0	73.2	92.3 (83.5-97.8)
Mean fetal weights (grams)	1.38	1.38	1.37	1.37	1.33 (1.21-1.41)

All data expressed as mean values for the group, except for the number of pregnant females.

NA = not applicable

HC = WIL Historical Control data

[a] Vehicle

*p<0.05

Applicant table

Offspring Examinations: Treatment with the surrogate did not affect fetal gross, visceral, or skeletal development. An incidental observation was a statistically significant reduction in the incidences of a 14th rib in the LD group.

Dosing Solution Analysis: Dosing formulations ranged from 97.5% to 100% of claim.

9.3 Prenatal and Postnatal Development

9.3.1 A Prenatal and Postnatal Development, Including Maternal Function, Toxicokinetic, Immunophenotyping and Antibody Assessment Study of 2851055 Administered via Subcutaneous Injection in Mice

Date of study initiation:	1 Mar 10
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	2851055, lot P265.059, 97.2%

Key Study Findings

The surrogate was administered at doses of 0, 0.3, 3, or 20 mg/kg once every 3 days to mice from GD 6 through LD 19 (with a break in dosing at the time of expected parturition). A group was also administered the comparator IgG2 isotope control at a dose of 20 mg/kg. There were no effects on the ability of maternal animals to maintain a pregnancy, deliver a litter, and rear the litter. The most notable effects in maternal animals were on the immune system. Consistent with the pharmacologic activity of the compound, reductions in T cell populations with corresponding increases in B cell populations were evident at all dosages. As a result, the F0 maternal NOAEL was considered the HD of 20 mg/kg.

In the F1 offspring, surrogate levels were measurable following treatment of maternal animals during the lactational period. It is uncertain if the levels reflect in utero and/or lactational exposure. Other notable effects include reductions in male sperm count and motility and a reduction in the number of resulting pregnancies at the HD. It is not known if the effect on fertility was male or female mediated as mating was between treated animals. Regardless, there were no effects on the ability of F1 animals to maintain a pregnancy and deliver a viable litter. Also observed in the F1 at the HD were reductions in T cell populations and increased B cells, although only a few of the values attained statistical significance at the various timepoints. Decreases in the primary IgM/IgG response and secondary IgG response in the TDAR assay using KLH were also evident at the HD. Postmortem examination of F1 generation animals revealed increased thymus weights that were associated with an increased incidence of animals with increased cellularity of the thymic medulla at the HD. As a result of the effects on reproductive-related endpoints and the immune system functional effects observed at the HD, the MD of 3 mg/kg was considered the NOAEL for effects on the F1 generation

Methods

Doses:	0, 0.3, 3, or 20 mg/kg of 2851055 or 20 mg/kg of the IgG2a(ala,ala) isotype control
Frequency of dosing:	Once every 3 days during gestation (GD 6-18) and lactation (LD 1-19)
Dose volume:	2 mL/kg
Route of administration:	SC injection
Formulation/Vehicle:	(b) (4) NaPO4, (b) (4) NaCl, and (b) (4) Tween 80, pH 6.1
Species/Strain:	Mouse / Crl:CD1(ICR)
Number/Sex/Group:	30 females
Satellite groups:	An additional 30 females/group were used for tk and 20 females/group for immunophenotyping
Deviation from study protocol:	Multiple, but none affecting interpretability of study

Observations and Results

F0 Dams

Mortality: No deaths occurred on study.

Clinical Signs: No treatment-related clinical signs or effects at the injection sites were observed.

Body Weight: No effects on body weight parameters were observed with the surrogate or the comparator IgG2 during either gestation or lactation. Increases and decreases relative to controls were noted for various intervals in the IgG2 comparator group, but the effects were of small magnitude and had no effect on overall weight change for the study. As a result, they were considered incidental and unrelated to treatment.

Food Consumption: There were not considered to be any surrogate-related effects on food consumption. Slight increases relative to the IgG2 comparator were seen during mid-gestation, but the effects were small in magnitude, transient, and not considered related to treatment.

There were no surrogate-related effects during the lactational period. Slightly higher (~1g/animal) food consumption occurred during the second week of lactation period in the IgG2 comparator group relative to controls but was considered incidental based on the small magnitude of effect.

Gestation Length/Parturition: No effects were observed in maternal animals. There were no effects on the number of pups delivered, live litter size, or sex ratio.

Necropsy: There were 2, 0, 3, and 4 females in the Control, LD, MD, and HD groups, respectively, that did not deliver. Of these animals, 0, 0, 1, and 2 in the Control, LD, MD, and HD groups, respectively, were pregnant while the others were not. Of those that were pregnant, 1 MD and 1 HD animal had total litter resorptions (uterine contents for 1 HD female not described). For those animals terminated at the end of lactation, there were no effects on the number of implantation sites or maternal tissues.

Toxicokinetics: Following the initial dose, surrogate levels were measurable in all groups for various periods of time. Exposure increased with dose in a less than proportional manner from the LD to MD and in an approximately proportional manner between the MD and HD. At the middle of the lactational period, surrogate levels were consistently measurable only at the HD. Comparing the exposures during gestation to those during lactation, exposures are lower with increased treatment duration. Following each dose administration, surrogate levels decreased over the subsequent 72 hour period. Data are summarized in the applicant table below (modified by reviewer to only present maternal data; pup data are in Table 27):

Table 24 Mean Serum Concentration of Surrogate in Maternal Animals

Time of TK Sample Draw	Mouse Type	Dose (mg/kg)								
		0.3			3.0			20.0		
		Mean (ng/mL)	SEM (ng/mL)	n	Mean (ng/mL)	SEM (ng/mL)	n	Mean (ng/mL)	SEM (ng/mL)	n
GD 7 (GD 6, 24 hr post-dose)	Dams	510.8	112.6	5	19860.0	1825.5	5	151013.6	37870.5	5
GD 8 (GD 6, 48 hr post-dose)	Dams	108.3	43.7	5	15180.0	2302.0	5	139140.0	11946.9	5
GD 9 (GD 6, 72 hr post-dose)	Dams	NR[a]	NR[a]	4	9390.0	799.1	5	105540.0	4676.7	5
LD 11 (LD 10, 24 hr post-dose)	Dams	NR[b]	NR[b]	4	11265.6	4495.8	5	202250.0	19830.0	4
LD 12 (LD 10, 48 hr post-dose)	Dams	< 15.0	NC	4	NR[c]	NR[c]	4	41950.0	14547.9	4
LD 13 (LD 10, 72 hr post-dose)	Dams	< 15.0	NC	5	NR[d]	NR[d]	4	26675.0	2913.0	4

Abbreviations: SEM = standard error of the mean, n = number of dam serum samples or number of pooled pup serum samples, GD = Gestation Day, NR = not reported, LD = Lactation Day, NC = Not calculated, PND = Post-Natal Day, M = Male, F = Female.

a 3 values below the quantifiable limit of < 15.0 ng/mL, and one value of 58.8 ng/mL.

b 3 values below the quantifiable limit of < 15.0 ng/mL, and one value of 847.0 ng/mL.

c 2 values below the quantifiable limit of < 15.0 ng/mL, and two values of 9630.0 and 9570.0 ng/mL.

d 2 values below the quantifiable limit of < 15.0 ng/mL, and two values of 2080.0 and 846.0 ng/mL.

Applicant table

Immunophenotyping: Lymphocyte population levels were determined (24 hours after treatment) in maternal animals on GD 6 and lactation day (LD) 10. Reductions in T cell populations and increases in B cell populations relative to those induced by the comparator IgG2 were evident at all surrogate dosages. These effects were seen on both GD 6 and LD 10, with the effects after multiple dose administrations being of lower magnitude. At both time points, the reductions followed an inverse dose-response and the majority of effects reached statistical significance. The levels in the vehicle control group were not different from the levels in the IgG2 group. Data are summarized in the applicant table below:

Table 25 Mean Percent Change for Lymphocyte Populations in Maternal Animals

Lymphocyte Subset	Mean % Change from Comparator IgG2 Isotype Control Values			
	Vehicle Control	0.3 mg/kg	3.0 mg/kg	20 mg/kg
24 Hours Post-dose on GD6				
Total T cells (CD5+)	+6.0%	-84%**	-79%**	-57%**
T-helper cells (CD5+CD4+)	+6.9%	-84%**	-77%**	-50%**
T-cytotoxic cells (CD5+CD8+)	+2.4%	-92%**	-92%**	-80%**
Total B cells (CD45R+/B220+)	-18%	+36%	+79%**	+76%**
24 Hours Post-dose on LD10				
Total T cells (CD5+)	+5.6%	-50%**	-38%**	-26%
T-helper cells (CD5+CD4+)	+12%	-38%*	-29%	-38%*
T-cytotoxic cells (CD5+CD8+)	-11%	-78%**	-61%**	-7.6%
Total B cells (CD45R+/B220+)	-19%	+36%	+14%	+23%

Abbreviations: GD=gestation day; LD=lactation day; SC=subcutaneous.
* Significantly different from comparator IgG2 isotype control at $p \leq 0.05$.
** Significantly different from comparator IgG2 isotype control at $p \leq 0.01$.
Applicant table

F1 Generation

Survival: There were no effects on pup survival during the pre-weaning period. Following weaning, elective euthanasia and deaths were observed in all study groups (1, 5, 5, 2, 2 in the control, LD, MD, HD, and IgG groups, restively), with most occurring in the first two weeks after weaning. Associated clinical signs included hypoactivity, unkempt appearance, cool body, and/or yellow material around urogenital area. No effects were noted during macroscopic or microscopic examination and the causes of death were not determined.

Clinical Signs: No surrogate-related effects were observed during the pre-weaning or post-weaning periods.

Body Weight: No effects were observed that were considered treatment-related during the pre- or post-weaning periods. For the F1 animals that were mated, no effects were seen on body weight during gestation and post-delivery lactation day 1 body weights.

Food Consumption: Food consumption of F1 females that were mated was not considered affected by treatment with the surrogate or IgG2 comparator.

Reflex and Morphological Development: There were no treatment-related effects on negative geotaxis or the age of balano-preputial fold separation (or on the weights at the time criteria met). The age at which vaginal patency was observed in the IgG2 comparator group was approximately 1.4 days later than controls. As a result, the body weights were also slightly (<2g) higher. This effect is considered to reflect the early age at which criteria were met in controls which was below the historical age range.

Necropsy Observations: No treatment-related effects observed in pups necropsied on post-natal day (PND) 21.

Grip Strength: Mean forelimb grip strength was increased (<12%; statistically significant) in HD females relative to control and IgG2 groups on PND 60. This observation was not considered toxicologically meaningful.

Motor Activity: No effects were seen that were considered treatment-related.

Startle Response: No effects occurred that were considered treatment-related. The t_{max} for a response occurred more quickly for specific blocks of time in HD males (-10% from 41-50 msec on PND 62) and females (-5% from 11-20 msec on PND 22) than in controls during the same blocks of time. An increase (+56%) in the maximum response was seen in HD females administered the IgG2 comparator from 41-50 msec on PND 62. Based on the transient appearance and lack of effect overall across all blocks of time, these observations were considered incidental and unrelated to treatment. There were no effects at lower dose levels and no effects on the habituation patterns.

Learning and Memory: In the initial straight-channel swim trial used to acclimate animals to being in the water and familiarize them with how to complete the task (Biel water maze), all surrogate and IgG2-treated groups of males performed this task more quickly than controls. In the actual learning and memory component of the test, there were no effects on the time to complete the trials or the number of errors made during the trials that were considered treatment-related as any effects observed were only evident during a single trial and there was no overall effect on the parameter when evaluated across all trials.

Reproductive Performance: Female estrous cyclicity, the time to mate, and the numbers of male and females mating were similar between groups. However, the number of pregnancies that ensued (83%) was reduced at the HD of the surrogate compared to controls and the IgG2 comparator. Although the reductions were not statistically significant, they were below the historical range (84% to 100%) and attributed to treatment with the surrogate. A similar effect was not seen with the comparator IgG2. Gestation lengths were not considered to be affected by treatment and there were no signs of dystocia.

Sperm Evaluation: Sperm motility was lowest (80%) in the HD group compared to percent motile in controls (86%, not statistically significant) and the IgG2 comparator (86%, statistically significant). This reduction was considered surrogate-related even though it was within the historical range (79.4% to 90.0%). Sperm counts were also lower (-18%) relative to control and to the IgG2 comparator (-14%), but the effects did not reach statistical significance. There was no effect on sperm morphology in any group or on any parameter at the LD and MD.

Necropsy: Treatment of the F0 generation did not result in any treatment-related macroscopic observations in F1 animals. However, F1 generation organ weight and microscopic alterations were evident.

The weights of the thymus were increased in both sexes of the F1 generation at the HD as compared to both the control and comparator IgG2 group. The increases are summarized in the applicant table below:

Table 26 Thymus Weights of F1 Animals at the High Dose

Parameter	Direction and magnitude of the difference when compared with the vehicle control group	Direction and magnitude of the difference when compared with the IgG2a(ala,ala) isotype group
Thymus		
Absolute (males)	↑32.4%**	↑18.5%**
Absolute (females)	↑28.2%*	↑29.0%
Relative to Body (males)	↑34.8%**	↑20.3%**
Relative to Body (females)	↑20.9% *	↑23.8%
Relative to Brain (males)	↑28.3%**	↑17.6%*
Relative to Brain (females)	↑29.3% *	↑29.5%*

* $p<0.05$.

** $p<0.01$.

Applicant table

Statistically significant alterations in organ weights were also noted for some of the reproductive organs when compared to the comparator IgG2. In males, reductions in the weights of the testis (left -7% and right -9%) and epididymis (left -6%) were observed following adjustment for brain weight, but absolute and body weight adjusted differences were not statistically significant. In females, absolute and brain weight adjusted ovarian weights were increase up to 12%. However, there were no differences compared to controls. Based on the small magnitude of change and absence of microscopic correlates, none of the effects on the reproductive organs were considered adverse.

Microscopic examination of tissues from F1 animals revealed effects in the medulla of the thymus. Increased cellularity was seen that was attributed to expanding populations of lymphocytes. The percentage of F1 animals with increased cellularity of the thymic medulla was 44% and 35% in MD and HD females, respectively, and 25% in HD males.

Toxicokinetics: Surrogate levels were measurable in pooled serum samples collected from MD and HD pups at various timepoints following treatment of maternal animals on LD 10. There did not appear to be any sex effect for the majority of samples collected, although the high variability in data from pups at the MD was confounding. Serum levels of the surrogate appeared to slowly decline over time, at least at the HD. Mean serum concentrations in HD pups (based on combined sex data) were $\leq$ 1.6% of the level in maternal animals at the corresponding timepoint (ranging from 0.1% to 1.8% for individual pups compared to their dam levels). At the MD, pup levels averaged 27% of the maternal level 24 hours after dosing on LD 10. For the individual maternal/offspring

pairs at the MD for which both maternal and pup data exist, pup levels ranged from 2.6% to 7.5-fold maternal levels at the timepoints where full data exist for individual dam/pups. At the LD, pup levels ranged from 16% to 37% of maternal levels at the two timepoints for the single dam/pup for which data were measurable. Since pup levels were not obtained at birth, it was not possible to determine if the levels in pups reflected placental transfer, transfer from milk, or a combination of the two. Mean exposure data in pups are summarized in the applicant table below (table modified by reviewer to only present pup data; maternal data are in Table 24):

Table 27 Mean Concentration of Surrogate in F1 Animals

Time of TK Sample Draw	Mouse Type	Dose (mg/kg)								
		0.3			3.0			20.0		
		Mean (ng/mL)	SEM (ng/mL)	n	Mean (ng/mL)	SEM (ng/mL)	n	Mean (ng/mL)	SEM (ng/mL)	n
PND 11 (PND 10, 24 hr post-dose)	Pups, M	NR[e]	NR[e]	4	3283.6	2630.7	5	613.0	163.2	4
PND 12 (PND 10, 48 hr post-dose)	Pups, M	< 15.0	NC	4	520.7	297.7	4	399.5	43.1	4
PND 13 (PND 10, 72 hr post-dose)	Pups, M	NR[f]	NR[f]	5	1729.8	1138.6	4	375.8	45.0	4
PND 11 (PND 10, 24 hr post-dose)	Pups, F	NR[g]	NR[g]	4	2862.6	2138.2	5	603.8	75.0	4
PND 12 (PND 10, 48 hr post-dose)	Pups, F	< 15.0	NC	4	7215.8	6996.3	4	551.3	99.5	4
PND 13 (PND 10, 72 hr post-dose)	Pups, F	NR[h]	NR[h]	5	1621.8	969.6	4	454.5	103.5	4
PND 11 (PND 10, 24 hr post-dose)	Pups, M+F	NR[e,g]	NR[e,g]	8	3073.1	1599.6	10	608.4	83.1	8
PND 12 (PND 10, 48 hr post-dose)	Pups, M+F	< 15.0	NC	8	3868.3	3479.8	8	475.4	57.8	8
PND 13 (PND 10, 72 hr post-dose)	Pups, M+F	NR[f,h]	NR[f,h]	10	1675.8	692.6	8	415.1	54.3	8

Abbreviations: SEM = standard error of the mean, n = number of dam serum samples or number of pooled pup serum samples, GD = Gestation Day, NR = not reported, LD = Lactation Day, NC = Not calculated, PND = Post-Natal Day, M = Male, F = Female.

e 2 values below the quantifiable limit of < 15.0 ng/mL, and two values of 316.0 and 38.4 ng/mL
f 4 values below the quantifiable limit of < 15.0 ng/mL, and one value of 15.7 ng/mL.
g 2 values below the quantifiable limit of < 15.0 ng/mL, and two values of 316.0 and 31.4 ng/mL.
h 4 values below the quantifiable limit of < 15.0 ng/mL, and one value of 17.3 ng/mL.

Applicant table

Immunophenotyping: Reductions in T cell populations and increases in B cell populations were observed in samples collected from F1 pups prior to weaning on PND 10. In general, the effects were analogous to those seen in maternal animals, but the majority of effects did not reach statistical significance in the pups. There were no statistical differences between the control and IgG2 comparator groups. Data are summarized in the applicant table below:

Table 28 Mean Percent Change in Lymphocyte Population in F1 Animals on PND 10

Lymphocyte Subset	Mean % Change from Comparator IgG2 Isotype Control Values			
	Vehicle Control	0.3 mg/kg	3.0 mg/kg	20 mg/kg
Blood collected on PND10 in Males				
Total T cells (CD5+)	-38%	-50%*	-24%	-55%
T-helper cells (CD5+CD4+)	-42%	-52%	-31%	-78%*
T-cytotoxic cells (CD5+CD8+)	-21%	-66%*	-27%	-40%
Total B cells (CD45R+/B220+)	+18%	+51%	+37%	+101%
Blood collected on PND10 in Females				
Total T cells (CD5+)	+8.8%	-16%	-20%	-38%
T-helper cells (CD5+CD4+)	+32%	-10%	-26%	-44%
T-cytotoxic cells (CD5+CD8+)	+34%	+20%	+23%	+8.8%
Total B cells (CD45R+/B220+)	+15%	+27%	+21%	+18%

Abbreviations: PND=postnatal day; SC=subcutaneous.
* Significantly different from comparator IgG2 isotype control at p≤ 0.05.

Applicant table

In samples collected from pups at the end of the study on PND 84, minimal differences from the IgG2 comparator were seen in lymphocyte populations at the LD and MD. Effects of a larger magnitude were seen at the HD, but only a few of the differences achieved statistical significance. The only effects attaining statistical significance in males were for cytotoxic T cells (-44%). In females, decreases in total T cells at the HD (-20%) and cytotoxic T cells at the MD (-38%) and HD (-49%) were noted as were increases in B cell populations at the HD (148%). Data are summarized in the applicant table below:

Table 29 Mean Percent Change in Lymphocyte Populations in F1 Animals on PND 84

Lymphocyte Subset	Mean % Change from Comparator IgG2 Isotype Control Values			
	Vehicle Control	0.3 mg/kg	3.0 mg/kg	20 mg/kg
Blood collected on PND84 in Males				
Total T cells (CD5+)	-13%	+0.4%	-1.2%	-23%
T-helper cells (CD5+CD4+)	-12%	-2.6%	+0.8%	-22%
T-cytotoxic cells (CD5+CD8+)	-21%	+7.9%	-13%	-44%*
Total B cells (CD45R+/B220+)	+38%	-8.3%	+1.7%	+37%
Blood collected on PND84 in Females				
Total T cells (CD5+)	-7.5%	-3.8%	-10%	-20%**
T-helper cells (CD5+CD4+)	-7.0%	-1.5%	-1.8%	-21%
T-cytotoxic cells (CD5+CD8+)	-4.7%	-10%	-38%*	-49%**
Total B cells (CD45R+/B220+)	+16%	0.0%	+37%	+148%**

Abbreviations: PND=postnatal day; SC=subcutaneous.
* Significantly different from comparator IgG2 isotype control at p≤ 0.05.
** Significantly different from comparator IgG2 isotype control at p≤ 0.01.

Applicant table

KLH Assay: Immune system functioning of the offspring was also evaluated using a KLH assay. Treatment of maternal animals with the surrogate resulted in a reduction of the primary IgM and IgG antibody responses to KLH (administered day 28) when evaluated on PND 35 in the F_1 animals. The effects at the HD were statistically significant for both sexes. To determine if treatment had the ability to affect the memory response to KLH, a second KLH immunization was administered (PND 70) and the IgG response was evaluated on PND 84. The secondary IgG response was decreased significantly in HD males and females. No differences in the primary or secondary responses were seen between the IgG2 comparator and controls. The IgM and IgG responses are summarized in the applicant table below:

Table 30 IgM and IgG Response to KLH in F1 Animals

Antibody Response	Vehicle Control	0.3 mg/kg	3.0 mg/kg	20 mg/kg
Blood collected on PND35 in Males				
Primary IgM	+0.9%	+47%	+2.3%	-57%**
Primary IgG	-47%	-22%	-53%	-93%**
Blood collected on PND35 in Females				
Primary IgM	-3.9%	+13%	+7.0%	-63%**
Primary IgG	-4.7%	-25%	-18%	-100%**
Blood collected on PND84 in Males				
Secondary IgG	+0.4%	-1.6%	-26%	-90%**
Blood collected on PND84 in Females				
Secondary IgG	+5.6%	+12%	-28%	-85%**

Abbreviations: KLH=keyhole limpet hemocyanin; PND=postnatal day; SC=subcutaneous.
** Significantly different from comparator IgG2 isotype control at p≤ 0.01.

Applicant table

F2 Observations

Birth Examinations: Live litter size, sex ratios, survival through PND 1, and pup weights were not affected by treatment with the surrogate or comparator IgG2. A comparable number of pups were found dead in all groups, including controls. No treatment-related gross macroscopic effects were observed.

10 Special Toxicology Studies

10.1 Cross-Reactivity

10.1.1 Cross Reactivity of Humanized Monoclonal Antibody hOKT3r1 (Ala-Ala) with Human Tissues

Teplizumab at 0.3 and 2.4 µg/mL was evaluated for its' potential to bind to a panel of human tissues from 3 donors. Immunohistochemistry was used to identify targeted tissues.

Strong binding of teplizumab to perivascular and parafollicular splenic T cell regions of the spleen and the paracortical regions of the lymph nodes was seen in all donors. Specific staining was also seen for the T cell regions in the mucosa-associated lymphoid tissues (tonsils, lung, intestine), T lymphoid cells in the broncho-alveolar mucosal compartments of the bronchiolar and alveolar epithelium, and the medullary and cortical thymocytes of the thymus. Staining was seen infrequently in interstitial lymphoid cells in the mammary gland, esophagus, kidney, prostate, salivary gland, and ureter. Lymphocytes in the bone marrow were also stained. No binding to lymphoid cells in B regions or non-T regions of lymphoid tissues was observed.

It was concluded that teplizumab binds to T lymphocytes in multiple tissues but not to B or non-T regions.

10.2 Cytokine Release

10.1.2 A Single-Dose Cytokine Release Study in Mice

Because OKT3, the precursor to teplizumab, is associated with the induction of cytokine release syndrome, the potential for cytokine release to occur was investigated using the murine surrogate 2851055. CD-1 mice were administered a single sc dose of 0, 1, or 30 mg/kg (actual doses were 0.65 and 19.5 mg/kg based on concentration analysis). There were no clinical signs observed in the animals and the doses were well-tolerated.

At both doses there were slight increases in IL-6 (~1- to 3-fold) and IL-12 levels (~1- to 3.5-fold) across timepoints with recovery seen by 24 hr post-dose.

At the LD, levels of IL-4, IL-5, IL-10, IL-17, and TNFα were increased over control levels in a few animals at various time points, but there were no consistent patterns of effects seen or a clear relationship to treatment. Since no pre-treatment samples were obtained, it is not known if the increases reflect inter animal variability or represent a treatment-related increase. There were no effects noted on the levels of IL-1□, IL-2, INF-γ, and GM-CSF. The dose of 0.65 mg/kg corresponded to a mean (sexes combined) C_{max} of 4505 ng/mL at 24 hr post-dose.

At the HD there were sporadic increases (e.g., seen at one of the timepoints and/or in a single sex) in the levels of IL-2, IL-4, IL-5, IL-17, INF-γ, and TNFα relative to the level in controls at 1 hour post-dosing, but no clear pattern was evident that could be considered drug-related in the absence of pre-dose data. There were no effects on the levels of IL-1□, IL-10, and GM-CSF. The dose of 19.5 mg/kg corresponded to a mean (sexes combined) C_{max} of 178600 ng/mL at 24 hr post-dose.

In contrast, with the reference control 145-2C11 ((b) (4) that binds CD3Ɛ receptor but doesn't have alanine substitutions to prevent Fc binding), the level of all cytokines measured (except IL-1β) were substantially increased over multiple timepoints following a single IV dose of 1 mg/kg.

11 Integrated Summary and Safety Evaluation

Teplizumab is being developed to delay/prevent the onset of T1DM. The OKT®3 mAb has been modified by substituting alanine into two sites of the IgG1 Fc region. This modification retains the immunomodulatory properties of the mAb and at the same time reduces the side-effects associated with T cell activation and cytokine release.

In a tissue cross-reactivity study using human tissues, staining of perivascular and parafollicular splenic T cell regions of the spleen and the paracortical regions of the lymph nodes occurred as did staining of the T cell regions in the mucosa-associated lymphoid tissues (tonsils, lung, intestine), T lymphoid cells in the broncho-alveolar mucosal compartments of the bronchiolar and alveolar epithelium, and the medullary and cortical thymocytes of the thymus. Staining of lymphoid cells in B regions or non-T regions of lymphoid tissues was not observed.

Teplizumab cross-reacts with CD3+ T cells from humans, gorillas, chimpanzees, and gibbons. Because of the impracticality of conducting studies in these species, minimal nonclinical investigations were conducted with teplizumab itself. Instead, a (b) (4) surrogate monoclonal antibody (mAb) was developed (identified as 2C11-mG2a(Ala-Ala) or 2851055) and used to characterize the nonclinical profile of teplizumab. The Fc portion of this mouse IgG2a has the same alanine substitutions as in teplizumab and the affinity of this mAb for mouse CD3 (3 µM) was similar to that of teplizumab for human CD3 (2.3 µM).

Efficacy was demonstrated in a study in pre-diabetic NOD mice administered the surrogate (50 µg iv) where a single 5-day treatment cycle delayed the onset of diabetes by ~5 weeks. No adverse effects were seen when a second treatment cycle was initiated two weeks after the first cycle. However, when the second cycle was initiated 6 weeks later, death occurred at all dosages within an hour of dose administration as a result of apparent anaphylaxis; death was also seen with the control mAb that has the same constant region but a different variable region that does not bind CD3 or other mouse proteins. ADA titers were found to be elevated with both the surrogate and control mAb, implying that they were directed at least partially to the shared Fc regions. The multi-week time interval between treatment regimens is consistent with the time required to elicit an immune response.

NOD mice were subsequently challenged with a single dose of the surrogate mAb to determine the mechanism for the induction of anaphylaxis (IgG dependent via platelet activating factor antagonism or IgE dependent via histamine antagonism). NOD mice were treated with the anti-histamine H1 receptor antagonist triprolidone and the PAF antagonist CV6209 either together or alone prior to being administered the surrogate

mAb. Those treated with triprolidone alone exhibited anaphylactic signs while those administered CV6209 or both antagonists demonstrated minimal to no symptoms, indicating that the IgG pathway plays a role in anaphylaxis. Although anaphylaxis mediated through IgG is well described in rodents, mediation through IgG in humans is not as well characterized.

The most relevant in vivo study with the (b) (4) version of teplizumab was conducted in the chimp in which a single sc dose of 0, 0.1, 1, or 10 mg/kg was administered. At the HD, coughing and inappetence were observed. Antibiotic treatment was initiated but all HD animals died or were euthanized. The cause of death was attributed to a suspected EBV-like lympho-cryptovirus infection. The T cell suppression occurring as a result of the pharmacologic activity of the drug was a contributing factor. Pharmacologic effects observed at all doses included dose-related reductions in CD3+, CD4+, and CD8+ counts that persisted for approximately two weeks and transient (single day) reductions in CD19+ cells at all dosages and CD56+ cells at the MD/HD. Also observed were transient increases in the cytokines TNFα, IL-6, IL-10 in all groups and increased IFN-γ at the HD that peaked at approximately 6 hours after treatment. The decreases in T cells and increases in cytokines are consistent with effects seen clinically. Necropsies were only performed on HD animals. Gross and microscopic observations were consistent with polymorphic lymphoproliferative disease with secondary sequelae of pneumonia and sepsis while in-situ hybridization studies identified the presence of an Epstein-Barr-like virus. Based on the death/moribundity at the HD resulting from a pharmacologic depletion of T cells (transient) leading to immunosuppression and the inability to respond to an infection, and increase in biomarkers for cytokine release syndrome, the NOAEL for the study was considered the MD of 1 mg/kg, approximately 4x the total human dose administered in a cycle of treatment based on BSA. The response in chimpanzees was consistent with the expected pharmacologic activity and similar to effects observed in previous clinical trials. No multiple dose studies were conducted in the chimp and no studies with the current CHO-derived hOKT3γ1 (Ala-Ala) test article have been conducted in the chimp.

The surrogate mAb was considered to be an appropriate 'stand-in' for teplizumab based on the similar affinity for the CD3 target for each compound in their respective species. In a 6-day repeat dose toxicology study in CD1 mice, surrogate dosages of 0, 0.03, 0.3, and 20 mg/kg were administered sc or a dose of 0.3 mg/kg iv, and pharmacodynamic endpoints were evaluated. Reductions in circulating T cells (e.g., CD3, CD4, CD8, cytotoxic, helper, total) were seen in peripheral blood, spleen, and thymus that showed a trend to partial recovery following at least a 2-week recovery period. The reduction in T cells was associated with a reduction in cellularity of the medulla in the thymus and hematopoiesis in spleen; these effects were also partially reversible. As there were no off-target effects, the NOAEL is considered the HD of 20 mg/kg.

A second repeat dose toxicity study was performed in CD1 mice in which surrogate dosages of 0, 0.3, 3, or 20 mg/kg were to be administered every 3 days for 1 month. Deaths began to occur at all dosages following the 4th dose administration that were preceded by labored breathing, hypoactivity, recumbency, ataxia, and swollen nose.

These effects were consistent with an anaphylactic reaction, suggesting that intermittent dosing may be a concern.

The potential for the surrogate to induce a release of cytokines was also evaluated in mice. At both doses evaluated, slight increases (<3.5-fold) in IL-6 and IL-12 levels were evident, consistent with the release of cytokines seen following treatment of chimps with teplizumab. There were no clear treatment related effects on the levels of the other cytokines evaluated.

In a series of reproductive and developmental toxicity studies conducted with the surrogate in mice, reductions in T cell populations with corresponding increase in B cells were noted at all doses. In the male and female fertility studies at dosages of 0, 0.3, 1, 3, or 20 mg/kg, no effects were noted on reproductive endpoints and the HD of 20 mg/kg was the NOAEL. Similar effects on T and B cell populations were evident in a mouse embryofetal development study in which dosages of 0, 0.03, 0.3, or 20 mg/kg were administered on GD 6, 10, and 14. In addition, there was an increased incidence of post-implantation loss at the HD that included the loss of the entire litter for multiple females. As a result, the numbers of viable fetuses/litter were reduced. The effects at the HD were considered adverse and the MD (0.3 mg/kg) was the NOAEL.

In a pre- and postnatal toxicity study in which surrogate dosages of 0, 0.3, 3, or 20 mg/kg once every 3 days to mice from GD 6 through LD 19, reductions in T cell populations with corresponding increases in B cell populations were evident at all dosages relative to the comparator (no differences between comparator and control). As these are expected pharmacologic effects, the NOAEL was the HD of 20 mg/kg. Reduced T cell and increased B cells were evident (statistically significant in only a few cases) in the offspring of maternal animals administered the HD of the surrogate during gestation and lactation. Suppression of the primary IgM and IgG response and of the secondary IgG response to KLH relative to the response seen with the comparator was also evident in F1 animals exposed to the HD of the surrogate (no differences between controls and comparator). Increased thymus weights associated with increased cellularity were also evident in the HD F1 animals. These effects coincided with measurable drug levels in the offspring exposed to the HD. Also observed at the HD in the F1 was lower sperm motility and a reduction in the number of resulting pregnancies. These data indicate that there is a potential risk to offspring immune system functioning when exposed during gestation and/or lactation that will need to be addressed in the label. Based on these observations, the MD of 3 mg/kg was considered the NOAEL for effects on offspring development.

In summary, treatment with teplizumab leads to a reduction in T cell populations with a corresponding increase in B cells. Increases in the levels of multiple cytokines were evident that persisted for various periods of time indicating that there may be the potential for the induction of cytokine release. Treatment of mice with a surrogate induced analogous effects on T-cell populations, B-cells, and cytokines (where evaluated) which supports the use of the surrogate as a model for teplizumab. The potential for an anaphylactoid response exists based on the results of a pharmacology

study in diabetic mice and a repeat dose toxicity study. In both studies, clinical signs and death occurred following intermittent dosing. A potential risk also exists for effects on the offspring of maternal animals administered high doses of the mAb as an increased incidence of embryofetal loss was observed in an embryofetal development study. Furthermore, treatment of maternal animals through pregnancy and lactation was associated with pharmacologic effects on T and B cell populations, primary and secondary immune responses, and fertility of the F1 animals. These effects will be summarized in the proposed label. Key observations and the safety margins (Note: the relevance of safety margins based on the surrogate are uncertain) are summarized in the table below:

Table 31 Summary of Observations and Safety Margins

Study Duration / Study #	Dosages	NOAEL and Safety Margin (Based on BSA)[a]	Notable Observations
Chimpanzee – teplizumab			
Single dose T-2002-2009	0, 0.1, 1, or 10 mg/kg	1 mg/kg (34 mg/m^2) 3.8x	20 mg/kg: Infection leading to death resulting from T cell suppression, ↑ INF-γ levels All dosages: ↓ T cell subpopulations, ↑ B cells, transient ↑ in circulating levels of TNF-α, IL-6, and IL-10
Mouse – surrogate			
6-day + recovery 7608-797	0, 0.03, 0.3, or 20 mg/kg sc or 0.3 mg/kg iv	20 mg/kg (60 mg/m^2) 6.7x	All dosages: ↓ T cell subpopulations and ↑ B cells that were partially reversible, ↓ cellularity of the medulla in the thymus, ↑ hematopoiesis in spleen; both effects partially reversible. No off target effects
1-month + recovery 8233170	0, 0.3, 3, or 20 mg/kg administered every 3 days	ND	All dosages: difficulty breathing, hypoactivity, recumbency, ataxia, swollen nose, and death occurring after 4 [h] dose administration.
Male fertility (b) (4)-353210	0, 0.3, 1, 3, or 20 mg/kg A comparator group was administered 20 mg/kg of an IgG2a (ala,ala)	20 mg/kg (60 mg/m^2) 6.7x	All dosages: ↓ T cell subpopulations and ↑ B cells relative to comparator. No differences between controls and comparator.
Female fertility (b) (4)-353209	0, 0.3, 1, 3, or 20 mg/kg A comparator group was administered 20 mg/kg of an IgG2a (ala,ala)	20 mg/kg (60 mg/m^2) 6.7x	All dosages: ↓ T cell subpopulations and ↑ B cells relative to comparator. No differences between controls and comparator.

Embryofetal development (b) (4)-353187	0, 0.03, 0.3, or 20 mg/kg on GD 6, 10, and 14	0.3 mg/kg (0.9 mg/m^2) 0.1x	20 mg/kg: Transient ↓ (in excess of 20%) in body weight gain and food consumption but no corresponding effects on body weights, ↑ post-implantation loss (including loss of entire litter in multiple dams), ↓ numbers of viable fetuses/litter All dosages: ↓ T cell subpopulations, ↑ B cells,
Pre/postnatal toxicity (b) (4)-353232	of 0, 0.3, 3, or 20 mg/kg once every 3 days to mice from GD 6 through LD 19. A comparator group was administered 20 mg/kg of an IgG2a (ala,ala)	F0 20 mg/kg (60 mg/m^2) 6.7x F1 3 mg/kg (9 mg/m^2) 1x	F0 animals All dosages: ↓ T cell subpopulations and ↑ B cells relative to comparator. F1 animals 20 mg/kg: ↓ T cell subpopulations and ↑ B cells relative to comparator, ↓ IgM and IgG primary response and IgG secondary response in TDAR assay using KLH, ↓ sperm motility, ↓ number of pregnancies, ↑ thymus weights, ↑ cellularity of the thymic medulla ≤ 3 mg/kg: ↓ in some T cell subpopulations

a) Based on a cumulative clinical dose of 9 mg/m^2 administered over a 14-day treatment cycle

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