

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

761238Orig1s000

NON-CLINICAL REVIEW(S)

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research

Date: December 27, 2022

From: Lois M. Freed, Ph.D.
Director, Division of Pharmacology/Toxicology-Neuroscience
Office of Neuroscience

Subject: BLA 761238 (Briumvi, ublituximab)

BLA 761238 was submitted on October 5, 2021, by the sponsor (TG Therapeutics) for use of ublituximab as a treatment for relapsing forms of multiple sclerosis (MS), to include clinically isolated syndrome, relapsing-remitting disease, and active secondary progressive disease in adults. Clinical development for MS was conducted under IND 127265.

The nonclinical studies submitted in support of the BLA include the following:

- Pharmacology (in vitro, in vivo), tissue cross-reactivity (human, monkey)
- Toxicology (4-, 13-, and 26-week studies in monkey)
- Expanded pre- and postnatal development study (monkey)

This battery of nonclinical studies is consistent with the division's recommendations conveyed to the sponsor during clinical development. Carcinogenicity studies were not required because ublituximab is not active in rodent; therefore, standard carcinogenicity studies are not feasible.

The nonclinical studies have been reviewed by Dr. Wilcox (Pharmacology/Toxicology BLA Review and Evaluation, BLA 761238, Barbara J. Wilcox, Ph.D., December 5, 2022), who has concluded that "That the nonclinical data are adequate to support approval for the intended use."

Selected nonclinical data are briefly summarized below.

Pharmacology

Ublituximab is a recombinant chimeric IgG1 monoclonal antibody that binds to the CD20 antigen on the surface of B-lymphocytes, resulting in depletion of CD20+ B cells through one or more mechanisms, including antibody-dependent cellular cytotoxicity and complement-mediated cytotoxicity. The pharmacological activity of ublituximab was characterized in in vitro (using multiple cell lines and human whole blood) and in vivo (mouse models) assays. Tissue cross-reactivity studies in normal human and cynomolgus monkey tissue documented binding to CD20-expressing tissues.

The sponsor proposed conducting nonclinical studies of ublituximab only in monkey but did not conduct studies to characterize binding to CD20 in other animal species. Justification for this approach was submitted to the Division of Hematology and Oncology Toxicology (DHOT); DHOT found it acceptable.

Toxicology

The sponsor conducted 4-, 13-, and 26-week GLP intravenous toxicity studies in cynomolgus monkey. For these studies, ublituximab was administered intravenously, either by short infusion (3 or 15 min) or slow bolus.

In the 4-week study (0, 10, or 50 mg/kg QW), one high dose (HD) female was sacrificed moribund, with clinical signs, body weight loss, and histopathology findings considered to be consistent with immunosuppression. Similar clinical signs were observed in one HD male. Decreases in rbc parameters (count, hemoglobin) and increases liver enzymes (ALT, AST), bilirubin, LDH, and urea were observed in HD males. Histopathology findings consisted of lymphoid depletion (spleen, lymph nodes) and thymic atrophy at both doses (dose-related), and liver (single cell necrosis), lung (leukocytosis), bone marrow (atrophy), pancreas (acinar atrophy), and adrenal gland (reduced vacuolation) findings at the HD. Pharmacodynamic effects (peripheral B-cell depletion) was observed at both doses.

In the 13-week study (0, 3, 10, or 30 mg/kg QW), the only ublituximab-related findings were decreases in germinal center development and in size and number of follicles in lymph nodes and dose-dependent B-lymphocyte depletion, consistent with pharmacological activity. No histopathological changes in male or female reproductive organs were observed. TDAR (KLH antigen), assessed on Days 22 and 232, was not affected.

In the 26-week study (0 or 30 mg/kg QW), only one dose level was tested, based on the development of antidrug antibodies, resulting in loss of pharmacological activity, at the low and mid doses in the 13-week study. One ublituximab-dosed female was found dead on Day 50. This female was reported to have lost consciousness immediately post-dose on Day 50, with evidence of diffuse

alveolar hemorrhage in the lung at necropsy. Cause of death was considered to be immune-mediated hypersensitivity. There were no clearly ublituximab-related findings on other study parameters except for B-cell depletion (100%) and histopathological finding consistent with pharmacological activity (decreased germinal centers and size and number of follicles in the spleen, lymph nodes, and GALT) or immune-mediated effects resulting from antidrug antibody formation (mononuclear cell vascular/perivascular inflammation in various tissues, including the CNS and eye). In ublituximab-dosed animals exhibiting potential immune-mediated effects, the expected microscopic findings in lymphoid tissue were not detected and, in some cases, detection of antidrug antibodies. No effects on male or female reproductive organs were observed. Potential effects of ublituximab on immune function (TDAR) were not assessed.

The toxicokinetic (plasma AUC in $\mu\text{g}\cdot\text{hr}/\text{mL}$; $\text{AUC}_{(0-t)}$ for the 4-wk study; $\text{AUC}_{(0-144\text{h})}$ for the 13- and 26-week studies) data from the 4-, 13-, and 26-week studies (last sampling day) are summarized in the following table (doses are in mg/kg QW)

STUDY	DAY	MALES				FEMALES			
		3	10	30	50	3	10	30	50
4-wk	22		204 $\pm$ 4.55		6140 $\pm$ 1780		431 $\pm$ 329		8410 $\pm$ 2860
13-wk	78	6280	5800 $\pm$ 8760	26500 $\pm$ 25900		8.23	3360	20300 $\pm$ 20100	
26-wk	176*			56700 $\pm$ 32300				58700 $\pm$ 39000	
	176**			70800 $\pm$ 7190				78100 $\pm$ 3820	

*data for all animals; **data only for ADA-negative animals

For comparison, steady-state plasma AUC in humans at the maximum recommended human dose of 450 mg is $\sim 72000 \mu\text{g}\cdot\text{hr}/\text{mL}$. Maintenance dosing is every 24 weeks. Therefore, plasma exposures in monkeys at the high dose in the 26-week study are ~ 24 times that in humans, with exposures normalized to dosing interval (C_{ave}).

Genetic Toxicology

A battery of genetic toxicology assays was not conducted for ublituximab, a monoclonal antibody not expected to interact directly with DNA. An Ames assay to test for potential mutagenic impurities resulting from the manufacturing process was negative.

Carcinogenicity

Studies to assess the carcinogenic potential of ublituximab were not conducted, as previously agreed to by the division. Ublituximab is not pharmacologically active in rodent, making standard studies infeasible.

Reproductive and Developmental Toxicology

To assess the potential effects of ublituximab on development, the sponsor conducted an enhanced pre- and postnatal development study in pregnant cynomolgus monkey. Ublituximab was to be administered weekly by intravenous injection (slow bolus) at a single dose level (30 mg/kg) to three subsets of animals, as follows:

- Group 1 (control): GD 20-22 to parturition
- Group 2A (1st trimester): GD 20-22 to GD 63
- Group 2B (2nd trimester): GD 70 to GD 112
- Group 2C (3rd trimester): GD 119 to GD 161

However, 3 and 7 dams from Group 2A and Group 2B, respectively, were either found dead or sacrificed moribund after 3-6 doses, with severe clinical signs (including bleeding from gums, oral cavity, or nasal cavity, ataxia, or loss of consciousness). Postmortem findings included hemorrhage, edema, or necrosis in multiple organ (heart, liver, kidney, lungs); placental abnormalities (including infarction) were detected in all but one affected dam. Morbidity and death in all affected animals were considered secondary to hypersensitivity reaction.

Because of these deaths, ublituximab was administered only to two dams in Group 2C; each received 3 doses. The remainder (14) of Group 2C dams were “released from the study.”

Survival findings in Groups 2A and 2B are summarized in the following table:

GROUP	N (dams)	GESTATION LENGTH* (days)	MATERNAL DEATHS	FETAL DEATHS	LIVE INFANTS	INFANT DEATHS
1	16	161	0	2	14	3**
2A	16	162	3	6	7	0
2B	16	152	7	5	7	3
2C	2	136	0	1	1	1

*data from dams sacrificed premature or with fetal loss or sacrificed were excluded; **2 of the 3 Group 1 infant deaths attributed to maternal neglect

There were no clear ublituximab-related gross or histopathology findings identified in the aborted fetuses examined.

Upon examination of infants, gross and microscopic findings were observed in two infants in Group 2B, both of which were sacrificed prematurely due to multiple abnormalities. These findings were described in the study report as follows:

“External observations for Infant Nos. 2191 and 2321 included contractures and abnormal flexion of multiple limbs and tail resulting in fixed and limited range of motion of the joints...Other external findings included shortened mandible, elongated calvarium, and enlargement of ears for Infant No. 2191, and muscular atrophy in Infant No. 2321. On visceral examination, abnormalities were observed in the brain of Infant No. 2191 and included deepened sulci and narrowed gyri, and bilateral dilation of ventricles (microscopic correlate of moderate degeneration/necrosis). Taken together these findings were most consistent with a primary central nervous system effect resulting in secondary findings of limb contracture (arthrogryposis) and craniomandibular abnormalities due to decreased fetal movement precluding normal development...The incidence of such neuropathologic and secondary musculoskeletal changes...indicated that these changes were not spontaneous but rather associated with administration of [ublituximab].”

No ublituximab-related effects on CD20+ and CD19+ B lymphocytes were observed in infants. No effects on immune function (TDAR) were observed; however, TDAR was only evaluated in the 7 infants in Group 2A and 1 infant in Group 2B that survived to the scheduled sacrifice.

Toxicokinetic data (plasma AUC_(0-t) in µg*hr/mL) from dams in Groups 2A and 2B are summarized in the following table:

GESTATION DAY	GROUP 2A	GROUP 2B
20-22	55300	
42	56800	
63	70800	
70		52300
91		36800
112		42200

Conclusions and Recommendations

The nonclinical studies were conducted in (cynomolgus) monkey, the only relevant species. In the general toxicity studies, the primary findings were consistent with the pharmacological activity of ublituximab, i.e., binding to CD20+ B cells, resulting in depletion of B-lymphocytes through one or more mechanisms. However, the studies were complicated by immunogenicity, which resulted in reduced exposure and loss of pharmacological activity at lower doses.

The enhanced pre- and postnatal development study was conducted using subsets of animals, dosed during the first, second, or third trimester, to ensure, to the extent possible, adequate exposure throughout gestation. However,

ublituximab was substantially more immunogenic in pregnant animals, which resulted in maternal morbidity and death and embryofetal loss. Because of the deaths in dams dosed during the first and second trimesters, only 2 dams were dosed during the third trimester.

External, viscera, and skeletal abnormalities occurred in two infants from dams exposed to ublituximab during the second trimester of pregnancy. Findings in the two infants included contractures and abnormal flexion of multiple limbs and tail, shortened mandible, elongate calvarium, enlargement of ears, and/or craniomandibular abnormalities. Histopathology evaluation of these infants revealed minimal to moderate degeneration/necrosis in the brain. Abnormalities were absent in infants of dams exposed during the first trimester of pregnancy. A no-effect dose for adverse effects on embryofetal development in monkeys was not identified.

Potential adverse effects of ublituximab exposure during organogenesis on immune function could not be adequately assessed because of the immunogenic effects in dams.

Recommendations: The battery of nonclinical studies conducted on ublituximab are sufficient and adequate to support approval for the proposed indication, with appropriate labeling.

To support future clinical development of ublituximab in pediatric patients, it is recommended that the sponsor conduct, as a postmarketing requirement, a GLP-compliant study to assess the potential for ublituximab to have adverse effects on immune function when administered during the postnatal period to animals of a clinically relevant age. If such a study is not feasible (e.g., excessive immunogenicity), the sponsor should provide justification (with supportive data) for that conclusion and any other data that may be available to address this safety concern.

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/s/

LOIS M FREED
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**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

Application number: 761238
Supporting document: SDN1
Applicant's letter date: 9/24/2021
CDER stamp date: 10/5/2021
Product: Ublituximab (TG-1101)
Indication: Relapsing forms of multiple sclerosis
Applicant: TG Therapeutics
Clinical Division: DN2
Clinical Division Director: Nick Kozauer, MD
Review Division: DTP-N
Reviewer: Barbara J Wilcox, PhD
Supervisor: Lois M. Freed, PhD
Division Director: Lois M. Freed, PhD
Director, DPT-N
Project Manager: Rania Younes

All figures and tables are from the sponsor unless otherwise designated.

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1.1 Introduction

Ublituximab is a chimeric monoclonal antibody directed against the CD20 antigen expressed on the cell surface of pre-B and mature B lymphocytes. The product consists of a murine variable region and human constant region (Fc) and shows the typical structure of an IgG1 immunoglobulin. Ublituximab is intended for the treatment of relapsing forms of multiple sclerosis.

Ublituximab binds with high affinity (low nM range) to CD20 on B cells from human and cynomolgus monkey. The product does not bind to rodent CD20. Therefore, cynomolgus monkey was determined to be the single relevant species for nonclinical evaluations. Binding of ublituximab results in destruction of the B lymphocyte through induction of antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC). The glycosylation profile of ublituximab provides increased affinity for the FcγRIIIa receptor and, thus, enhances ADCC activity.

1.2 Brief Discussion of Nonclinical Findings

A full battery of IV toxicity studies was conducted using cynomolgus monkeys. These consisted of general toxicology studies (single-dose, 4-weeks, 13-weeks, and 26-weeks) and an ePPND study to evaluate potential effects on embryofetal and postnatal development. Ublituximab was shown to be immunogenic in monkeys after repeated dosing. At lower doses, anti-drug antibody (ADA) development limited plasma exposure and resulted in loss of pharmacodynamic (PD) activity. However, in spite of ADA development, exposure was maintained at the HD of 30 mg/kg for 13 and 26 weeks in most animals, and pharmacodynamic activity (B lymphocyte depletion) was maintained.

In all the general toxicology studies, findings were observed that were considered to be the result of the known pharmacodynamic activity of ublituximab. These included near total depletion of B lymphocyte populations within 24 hours after the first dose, and depletion of B cell populations from lymphoid tissues (spleen, lymph nodes, and GALT). In the 4-week study, ublituximab-related toxicities were observed in lung, liver, and adrenal at the HD of 50 mg/kg (and at lower magnitude and incidence at the MD of 30 mg/kg). These toxicities were not observed in longer duration studies at the HD of 30 mg/kg.

The potential for adverse effects of ublituximab on embryofetal and postnatal development was evaluated in an ePPND study. Separate groups of 16 pregnant cynomolgus monkeys received weekly IV doses of 30 mg/kg of ublituximab during the first, second, or third trimester of pregnancy. One control group received vehicle alone weekly throughout pregnancy. Due to the development of significant immune-mediated toxicities, dosing was terminated early. Only the subgroup dosed during the first trimester received all 7 doses. Immune-mediated toxicity resulted in a high rate of unscheduled maternal, fetal, and infant deaths. Seven offspring from the females dosed during the first trimester and one infant from females dosed during the second trimester

survived the full 6-month postnatal period. The surviving offspring showed no adverse effects on embryofetal or postnatal development. However, 2 of the 4 infants born to females dosed during the second trimester showed significant abnormal findings: contractures and abnormal flexion of multiple limbs and tail, lack of grasp reflex, shortened mandible, elongated calvarium, enlarged ears, and muscle atrophy. Macroscopic findings noted were deepened sulci and narrow gyri in the brain, bilateral cerebral ventricular enlargement (correlated with degeneration/necrosis, graded moderate). These findings were considered related to ublituximab. (The 4th infant was euthanized due to premature delivery.) Because a single dose level was administered in this study, no NOAEL was identified.

No juvenile animal study has been conducted with ublituximab.

1.3 Recommendations

1.3.1 *Approvability*

The nonclinical data are adequate to support approval for the intended use.

If a clinical study in pediatric patients younger than 12 years old is conducted, a juvenile animal study will be needed, if feasible.

1.3.3 *Labeling*

8.1 Pregnancy

Data

Animal Data

(b) (4)

12.1 Mechanism of Action:

The precise mechanism by which ublituximab exerts its therapeutic effects in multiple sclerosis is unknown, but is presumed to involve binding to CD20, a cell surface antigen present on pre-B and mature B lymphocytes. Following cell surface binding to B lymphocytes, ublituximab results in

(b) (4)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No carcinogenicity studies have been conducted to assess the carcinogenic potential of BRIUMVI.

(b) (4)

2 Drug Information

2.1 Drug

USAN Name: Ublituximab

Code Name: TG-1101, TGTX1101, LFB-R603

Molecular Weight: 147 kDa

Structure or Biochemical Description: Chimeric antibody consisting of murine variable region fused to human constant region.

Pharmacologic Class: Chimeric monoclonal antibody (anti-CD20)

2.2 Relevant INDs, NDAs, BLAs, and DMFs

(b) (4)

BLA 761207 (OOD/DHM2) Withdrawn (TG Therapeutics)

2.3 Drug Formulation

Table 1: Composition of ublituximab

INGREDIENTS	FORMULA per mL	FUNCTION	REFERENCE TO STANDARDS
<u>Active substance:</u>			
Ublituximab (mg)	25.0	Active substance	USP <129>/Ph. Eur. (2031)
Sodium chloride (mg)	9.0	Tonicity agent	USP/Ph. Eur. (0193)
(b) (4)			USP/Ph. Eur. (0412)
Polysorbate 80 (mg)	0.7	Stabilizer	USP/Ph. Eur. (0428)
Water for injections (mL)	qs to 1	Solvent	USP/Ph. Eur. (0169)
<u>Excipient(s):</u>			
No additional excipient is added during manufacture of the finished product			

2.6 Proposed Clinical Population and Dosing Regimen

Patients: Adult patients with relapsing MS

Clinical dose and regimen: 150 mg for first infusion, followed 2 weeks later by a 450 mg infusion. Subsequent infusions of 450 mg administered every 6 months.

Route of administration: IV

2.7 Regulatory Background

Clinical development was conducted under IND 127265 (submitted on October 16, 2015). A pre-BLA meeting was held with the sponsor on April 6, 2021 (Meeting Minutes, April 29, 2021).

3.3 Previous Reviews Referenced

IND	Reviewer	Date Archived
127265	Barbara Wilcox	7/08/2020
(b) (4)		

4 Pharmacology

4.1 Primary Pharmacology

Ublituximab (TG-1101, LFB-R603) is a chimeric monoclonal antibody directed against the CD20 antigen that is widely expressed on the cell surface of B lymphocytes from the pre-B cell stage to mature B lymphocytes. The molecule consists of a murine variable region and human constant region (Fc) and shows the typical structure of an IgG1 immunoglobulin. Ublituximab is under development for treatment of relapsing forms of multiple sclerosis (RMS).

A series of studies (in vitro, ex vivo, and in vivo) were conducted to evaluate the pharmacodynamic activity, selectivity, and potential efficacy of ublituximab. Many of these studies were designed to include other products with anti-CD20 activity as comparators. The pharmacology studies were reviewed by Dr. Stacy Ricci. The data from these studies indicated that ublituximab binds with high affinity (low nM range) to CD20 on B cells from human and monkey. The Fc region of the antibody binds with high affinity to Fc gamma receptors on effector cells. Binding of ublituximab results in destruction of the B lymphocyte through induction of antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), or complement-dependent cytotoxicity (CDC). Studies using various human cell lines and primary cells from human blood demonstrated dose-dependent depletion of B lymphocytes by each process. The glycosylation profile of ublituximab provides increased affinity for the FCγRIIIa receptor and, thus, enhanced ADCC activity.

The potential for cytokine release by ublituximab was evaluated in monkey serum. Levels of four inflammatory cytokines (IL-6, IL-8, IFN-γ, and TNF-α) were evaluated after repeated dosing of ublituximab and no significant changes were observed.

Although at the time of IND submission, characterization of the binding specificity of ublituximab was minimal, in vivo studies demonstrated that dosing with ublituximab resulted in rapid and selective depletion of B lymphocytes (CD45+/CD3-/CD20+) from peripheral blood and lymphoid tissues in cynomolgus monkeys. Immunophenotyping conducted in the 13-week and 26-week IV toxicology studies demonstrated no significant effects of ublituximab on absolute levels of NK cells, total T-cells, cytotoxic T-cells, or helper T-cells.

Single species justification was provided under IND (b) (4). Published literature was provided to support the claim that NHP would be the single relevant species for the safety testing of ublituximab. The published data indicate that CD20 is highly conserved between humans and cynomolgus monkeys. Therefore, the cynomolgus monkey was selected as the single relevant species for nonclinical testing.

4.3 Safety Pharmacology

No stand-alone safety pharmacology studies were conducted with ublituximab. Safety pharmacology endpoints were evaluated in general toxicology studies.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Human PK:

Table 2: Pharmacokinetic results after the fourth infusion of 40 mg, 60 mg, 150 mg, 300 mg or 450 mg of ublituximab

PK Parameters ^a	40 mg Cohort A	60 mg Cohort B	150 mg Cohort C	300 mg Cohort D	450 mg Cohort E
n	6 ^b	3 ^c	3	3	6
C _{max} (mg/L)	7.7 ± 3.7	13.3 ± 8.1	61.4 ± 28.5	114.6 ± 68.4	142.9 ± 61.2
t _{max} (h)	4.3 (3.5-29.6)	3.5 (3.5-11.8)	3.7 (3.7-3.9)	5.2 (5.0-5.3)	3.6 (3.5-4.5)
AUC _{0-∞} (mg·h/L)	151.5 ± 127.4	384.5 ± 228.7	4109 ± 4463	12280 ± 8955	24510 ± 25310
t _{1/2term} (h)	10.6 ± 7.4	15.7 ± 4.1	41.8 ± 44.5	83.3 ± 54.3	171.4 ± 138.9
CL (mL/h)	680.8 ± 870.3	181.9 ± 73.4	114.7 ± 129.0	71.8 ± 72.1	56.1 ± 34.9
V _d (L)	1.5 ± 3.6	3.8 ± 1.1	3.3 ± 1.2	6.0 ± 1.0	6.4 ± 3.2

^a mean ± SD, t_{max}: median (range) , with respect to the start of infusion

^b n=5 for AUC_{0-∞}, t_{1/2term} and V_{dss}

^c n=2 for AUC_{0-∞}, t_{1/2term} and V_{dss}

Table 3: Pharmacokinetic results after the 1st (150 mg), the 4th (450 mg) and the 8th (450 mg) infusion of ublituximab

PK Parameters ^a	1 st Infusion 150 mg (Day 1)	4 th Infusion 450mg (Day 22)	8 th Infusion 450 mg (Day 50)
n	12	11	11
C _{max} (mg/L)	23.4 ± 11.2	168.6 ± 61.8	220.5 ± 141.9
t _{max} (h)	9.0 (5.0-30.3)	5.00 (3.1-52.0)	5.1 (3.1-23.5)
AUC _{0-∞} (mg·h/L)	732.1 ± 590	17890 ± 17730*	50760 ± 74460
t _{1/2term} (h)	13.43 ± 10.2	80.7 ± 58.5*	147.8 ± 133.8
CL (mL/h)	424.2 ± 389.3	57.69 ± 42.91	38.62 ± 26.63
V _d /V _{dss} (L)	4.8 ± 2.1	4.9 ± 2.3*	5.7 ± 3.3

^a mean ± SD, t_{max}: median (range) , with respect to the start of infusion

*Accurate determination not possible

5.2 Toxicokinetics

Toxicokinetic data were collected in the general toxicology studies.

6 General Toxicology

The initial toxicology (single-dose and 4-week) studies in cynomolgus monkey were reviewed by Dr. Stacey Ricci. In the single-dose study, cynomolgus monkeys received IV injections of ublituximab at dose levels of 0, 0.3, 10, or 100 mg/kg followed by a 14-day observation period. Near total depletion of B cell populations in plasma was observed at all dose levels by 24 hours post dosing. Near total B cell depletion was also observed at the MD and HD in cell suspensions from spleen, lymph node, and bone marrow. At the HD, an elevation of fibrinogen (graded moderate) was observed in both sexes. The sponsor did not consider this finding related to the test article, but because it occurred in only the HD group, a relationship cannot be ruled out. No other adverse findings were observed. Anti-drug antibodies (ADAs) were detected in all LD animals.

In the 4-week study, cynomolgus monkeys received ublituximab at weekly IV doses of 0, 10, or 50 mg/kg. The results showed dose-dependent depletion of circulating B lymphocytes and lymphoid depletion in the spleen, thymus, and lymph nodes. Significant body weight loss in males and stagnation of weight in females was observed at the HD. Small (59%) increases in urea and bilirubin (59%) were observed in HD males, fibrinogen levels were increased (graded moderate) in HD males (72%) and females (41%), and a small increase in aPTT was observed in HD males. One unscheduled death of a HD female was reported after reduced food consumption and loss of weight with marked dehydration, hypoactivity, and prostration were observed. At necropsy, findings were dark color of the adrenals, reduced thymus size, and red/purple area on the tongue. Microscopic findings were similar to those observed in other ublituximab-treated animals at the terminal sacrifice. This animal also showed tongue ulcer, leucocytosis of the kidneys, and salivary gland acinar atrophy. No clear cause of death was established but because the animal was in the HD group, the death was considered related to the test article. In the surviving animals at the terminal sacrifice, the following findings were reported:

- Spleen: Increased numbers of neutrophils and/or extramedullary hematopoiesis in red pulp at the HD, fibrinous thrombi at the HD, decreased follicles and germinal centers at the MD and HD.
- Lungs: Leucocytosis and fibrinous thrombi at the HD
- Bone marrow: Increased myeloid/erythroid ratio.
- Liver: single cell necrosis at the MD and HD, green pigment at the MD and HD, fibrinous thrombi at the HD, and extramedullary erythropoiesis at the HD
- Adrenals: Extramedullary hematopoiesis and decreased vacuolization of the zona fasciculata at the HD
- Pancreas: Acinar atrophy at the HD.
- Stomach: One HD animal showed acute erosions of the fundus.
- Anti-drug antibody (ADA) development was noted in the LD, MD, and HD groups. ADA levels increased with duration of dosing and, by SD22, significant reductions in AUC were observed in the LD and MD animals. Exposure was maintained through the study for the HD group.

In the 13-week study (reviewed by Dr. Barbara Wilcox), cynomolgus monkeys received IV injections (slow bolus) of ublituximab once weekly at doses of 0, 3, 10, or 30 mg/kg. Immunophenotyping evaluations demonstrated that B lymphocytes were reduced to undetectable levels at all doses by 24 hours post dosing. A strong immune-mediated response to the test article resulted dose-related reduction of pharmacodynamic (PD) effect at all doses, with trends toward reconstitution of B cell populations beginning SD22 in the LD, SD50 in the MD, and SD78 in the HD group. Only LD females showed nearly full repopulation of B cells by the end of the study. The reduction in PD effect in the LD and MD groups was considered due to the development of ADAs. Most animals in all dose groups tested positive for ADAs during the study. ADA levels correlated with reduction of in test article exposure in all dose groups, most significantly in the LD group. At the HD, although exposure was reduced, the PD effect was maintained through the dosing period (SD78). Microscopic examination of tissues showed decreased germinal center development in lymph nodes and spleen in males from all dose groups and in HD females. The TDAR test was conducted with KLH immunization on SD22 and SD232. No test article-related effects on functional immune activity were observed. At the end of the 6-month recovery period, no ublituximab-related findings were observed.

6.2 Repeat-Dose Toxicity

Study title: 26-week study of TG-1101 by intravenous slow bolus in cynomolgus monkeys

Study no.:	20264749
Testing Facility and location:	(b) (4)
Date of study initiation:	September 22, 2020
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TG-1101 (ublituximab), 2100205, 98.2%

Methods

Doses:	0 or 30 mg/kg
Frequency of dosing:	Once weekly
Route of administration:	IV
Dose volume:	1.67 mL/kg
Formulation/Vehicle:	(b) (4) sodium citrate (b) (4) NaCl, (b) (4) polysorbate 80, pH 6.5 (b) (4)
Species/Strain:	Cynomolgus monkey
Number/Sex/Group:	5
Age:	2.2 to 3.0 years old at initiation of dosing
Weight:	2.0 to 2.7 kg

Study design:

Text Table 2
Experimental Design

Group No.	Test Material	Dose Level (mg/kg) ^a	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	No. of Animals	
					Males	Females
1	Control	0	1.167	0	5	5
2	TG-1101	30	1.167	25.7	5	5

^a Based on the most recent body weight measurement.

Animals in the ublituximab group received IM diphenhydramine (5 mg/kg) within 30 minutes prior to dosing. No recovery period was included.

*Observations:*Text Table 8
General In-life Assessments

Parameter	Population(s) ^a	Frequency (Minimum Required)	Comments
Mortality	All animals	Once daily (afternoon) beginning by Week -1	The animals were observed within their cage unless necessary for identification or confirmation of possible findings.
Cage Side Observations	All animals	Once daily in the morning beginning by Week -1	The animals were observed within their cage unless necessary for identification or confirmation of possible findings. ^b
Individual Body Weights	All animals	Weekly; from at least Week -1	Fasted weight on the day of necropsy. Not collected from Group 2 Animal No. 2502 that was animals found dead.
Food Consumption	All animals	Once daily, from at least Week -1	Qualitatively evaluated
Veterinary Physical Examinations	All animals	Once pretreatment and during the last week of study in Week 26	A Testing Facility veterinarian I conducted the physical examination on a sedated animal.
Ophthalmic Examinations	All animals	Once pretreatment and during the last week of study in Week 26	See 4.7.1.
Electrocardiology (ECG) Exams	All animals	Once pretreatment and 1 to 4 hours postdose on Day 176	See 4.7.3.
Body Temperature	All animals	Once weekly, beginning Week -1.	Collected using a digital infrared thermometer (preferred) or a digital rectal thermometer.

^a Included alternate animals until released from study.^b For observations that could not be attributed to an individual animal due to social housing (e.g., watery feces), the observation was attributed to each animal in the socialized group.

Formulation Analysis: Not evaluated. Formulations were used as received from the sponsor.

Results:*Mortality:*

- One female was found dead on SD50 (#2502). On SDs 22 and 50, post dose drug levels were low, and ADAs were detected, accompanied by loss of PD. Cause of death was marked pulmonary hemorrhage “consistent with a fulminant immune-mediated hypersensitivity” to ublituximab.

Clinical Signs:

- One male showed head tremors and abnormal pupils on SD141 and increased salivation on SDs 141 to 178.
- Loss of consciousness was observed in one female on SD50.
- Liquid feces was observed in 60% of the dosed animals (3 males and 3 females) during the dosing period.

Body Weights:

- No test article-related effects were observed.

Food Consumption: Evaluated only qualitatively.

- No significant test article-related effects on food consumption were observed.

Ophthalmoscopy:

- No test article-related effects were observed.

ECG:

- No test article-related effects were observed.

Body temperature:

- No test article-related effects were noted.

Clinical Pathology:

Text Table 9
Clinical Pathology Sample Collection

Group Nos.	Time Point	Hematology	Coagulation	Clinical Chemistry
All animals	Week -1	X	X	X
Groups 1-2	Week 13	X	X	X
Groups 1-2	Week 26	X	X	X
Fasting		-	-	Yes
Method/Comments		Venipuncture	Venipuncture	Venipuncture
Target Volume (mL)		1	1.8	2
Anticoagulant		K ₃ EDTA	Sodium citrate	None
Special Requirements		-	-	-
Processing		None	Plasma	Serum

X = sample collected; - = not applicable.

Hematology: (Standard battery of parameters was evaluated.)

- No test article-related effects were noted.

- Elevation of reticulocytes (1.65X) was observed in males. No associated change in red cell mass was observed. Because only one sex was affected and the magnitude was small, the relationship to the test article is considered uncertain.

Clinical Chemistry:

The following results were observed in the dosed groups. The relationship to the test article is uncertain due to the small magnitude and the occurrence in only one sex.

- Decreased cholesterol, graded minimal to mild, in males was observed on SDs 86 and 182.
- Decreased albumin, graded minimal, was observed in males on SD182.
- Increased AST and ALT (less than 2X, graded minimal) was observed on SD86 and/or SD182 in females.

Coagulation:

- Increased fibrinogen (graded minimal) was observed in 2 males and 1 female (1.45 to 1.88X relative to baseline). Sponsor states that these results correlated with an acute phase response.

Immunogenicity: (Pre-dose samples were collected at Weeks 1, 4, 8, 12, 18, and 26.)

- One of 10 animals in each of the control and dosed groups tested positive for ADAs prior to the first dose. The control animal continued to test positive at Weeks 4 and 8. The reason for the apparent false positives is not provided.
- Anti-drug antibodies were detected in most animals in Group 2. Nine of 10 in Week 4, 6 of 10 in Week 8, and 2 of 9 animals in Weeks 12, 18 and 26. In 2 animals, the ADA levels correlated with reduced TG-1101 levels. Those animals were excluded from TK analyses.

Immunophenotyping: (Blood was collected from 5 animals/sex/group for immunophenotyping pre-dose during weeks 1, 4, 8, 12, 18, and 26.)

Text Table 4
Immunophenotyping Antigens and Cell Populations

Antigen Marker	Cell Population Identified ^a	Reported as Relative Percentages and Absolute Counts of the following Population:
CD45+/CD3+/CD16-	Total T-lymphocytes	Relative percentages and absolute counts
CD45+/CD3+/CD16-/CD4+/CD8-	T-helper lymphocytes	Relative percentages and absolute counts
CD45+/CD3+/CD16-/CD4-/CD8+	T-cytotoxic lymphocytes	Relative percentages and absolute counts
CD45+/CD3-/CD16-/CD20+	CD20+ B-lymphocytes	Relative percentages and absolute counts
CD45+/CD3-/CD16-/CD19+	CD19+ B-lymphocytes	Relative percentages and absolute counts
CD45+/CD3-/CD16-/CD19+/CD20+	CD20+ CD19+ B-lymphocytes	Relative percentages and absolute counts
CD45+/CD3-/CD16+	CD16+ Natural killer cells	Relative percentages and absolute counts

^a Absolute counts, relative percentages, and percent of baseline of absolute counts were calculated and reported.

- A significant reduction in B-cells (CD20+, CD19+, and CD20+/CD19+) was observed in all animals dosed with TG-1101. The maximum reduction in CD20+ cells was 100%, relative to baseline, in Week 4, and absolute counts generally remained low through the 26-week dosing period. CD19+ cell levels were similar, at 1% of baseline during Week 4.
- 2 animals showed recovery to baseline during Week 12. These animals were positive for ADAs.

B-Cell levels:

Summary of B lymphocyte levels is contained in the table below.

Study: STUDY 20264749
Compound: See Study Protocol
Sex: F
Analysis Framework: 1-Factor ANOVA
Primary Factor: Treatment Group Code
Parameter: CD45+/CD3-/CD16-/CD20+ (CD20+ B-lymphocytes) % baseline

		WEEK 4 PRE	WEEK 8 PRE	WEEK 12 PRE	WEEK 18 PRE	WEEK 26 PRE
GROUP 1	STATISTIC					
	Mean	104.40	113.00	125.00	121.20	105.40
	SD	23.84	26.82	33.64	32.14	38.00
	N	5	5	5	5	5
	Statistical Sig					
GROUP 2	Mean	0.20	0.00	0.00	0.00	0.00
	SD	0.45	0.00	0.00	0.00	0.00
	N	5	5	4	4	4
	Statistical Sig	*	*	*	*	*

* ANOVA with Dunnett's/Dunn's (p <= 0.05)
Groups with n<3 were excluded from statistical analysis

No significant test article-related effects on absolute levels NK cells, total T-cells, cytotoxic T-cells, or helper T-cells were observed.

Toxicokinetics:

Tables below show data excluding the 2 animals that had ADAs that resulted in reduced drug exposure.

Table 2.1: Individual and Summary TG-1101 Toxicokinetic Parameters in Cynomolgus Monkey Serum Following IV Bolus Injection of 30 mg/kg TG-1101 on Day 1

Day	Sex	Animal	C ₀ (µg/mL)	C ₀ /Dose (µg/mL /(mg/kg))	T _{max} (hr)	C _{max} (µg/mL)	C _{max} /Dose (µg/mL /(mg/kg))	T _{last} (hr)	AUC _{last} (hr*µg/mL)	AUC _{last} /Dose (hr*µg/mL /(mg/kg))	Cl (mL/hr/kg)	V _z (mL/kg)	T _{1/2} (hr)	AUC _(0 - inf) (hr*µg/mL)
1	Male	2002	874	29.1	0.083	872	29.1	144	52700	1760	0.467	36.8	54.7	64300
		2003	853	28.4	0.083	834	27.8	144	40800	1360	NRR	NRR	NRR	NRR
		2004	805	26.8	0.083	793	26.4	144	48000	1600	NRR	NRR	NRR	NRR
		2005	885	29.5	1	938	31.3	144	52700	1760	NRR	NRR	NRR	NRR
		2101	680	22.7	0.083	676	22.5	144	40400	1350	NRR	NRR	NRR	NRR
		Mean	819	27.3	-	823	27.4	-	46900	1560	0.467	36.8	54.7	64300
		N	5	5	5	5	5	5	5	5	1	1	1	1
		SD	83.8	2.79	-	97.6	3.25	-	6070	202	ID	ID	ID	ID
	Female	2501	833	27.8	0.083	817	27.2	144	50200	1670	0.479	42.0	60.7	62600
		2502	816	27.2	0.083	808	26.9	144	42900	1430	NRR	NRR	NRR	NRR
		2503	854	28.5	0.083	844	28.1	144	45900	1530	0.546	40.4	51.3	55000
		2504	728	24.3	0.083	719	24.0	144	42100	1400	0.578	46.7	56.1	51900
		2505	1010	33.6	0.083	974	32.5	144	45500	1520	NRR	NRR	NRR	NRR
		Mean	848	28.3	-	832	27.7	-	45300	1510	0.534	43.0	56.0	56500
		N	5	5	5	5	5	5	5	5	3	3	3	3
		SD	101	3.37	-	92.0	3.07	-	3180	106	0.0503	3.30	4.70	5510
	Combined	Mean	834	27.8	-	828	27.6	-	46100	1540	0.517	41.5	55.7	58400
		N	10	10	10	10	10	10	10	10	4	4	4	4
		SD	88.9	2.96	-	89.6	2.99	-	4640	155	0.0532	4.11	3.89	5940

NRR = No reportable result (Rs_q was less than 0.8 and/or the extrapolation of the AUC to infinity represented more than 20% of the total area).

ID = Insufficient Data.

Text Table 5

Summary (Mean $\pm$ SD) TG-1101 Toxicokinetic Parameters in Cynomolgus Monkey Serum Following IV Bolus Administration of TG-1101 on Days 1, 78 and 176 (TK Data from ADA-Negative Animals)

Day	Sex	T _{max} ^a (hr)	C _{max} (μ g/mL)	C _{max} /Dose (μ g/mL/(mg/kg))	T _{last} ^a (hr)	AUC _{tlast} (hr* μ g/mL)	AUC _{tlast} /Dose (hr* μ g/mL/ (mg/kg))	R _{AUC}
1	Male	0.083 (0.083 - 1)	823 $\pm$ 97.6	27.4 $\pm$ 3.25	144 (144 - 144)	46900 $\pm$ 6070	1560 $\pm$ 202	NA
	Female	0.083 (0.083 - 0.083)	832 $\pm$ 92.0	27.7 $\pm$ 3.07	144 (144 - 144)	45300 $\pm$ 3180	1510 $\pm$ 106	NA
	Combined	0.083 (0.083 - 1)	828 $\pm$ 89.6	27.6 $\pm$ 2.99	144 (144 - 144)	46100 $\pm$ 4640	1540 $\pm$ 155	NA
78	Male	1 (0.083 - 1)	906 $\pm$ 187	30.2 $\pm$ 6.25	144 (144 - 144)	70000 $\pm$ 11500	2330 $\pm$ 382	1.48 $\pm$ 0.423
	Female	0.083 (0.083 - 1)	975 $\pm$ 114	32.5 $\pm$ 3.80	144 (144 - 144)	72100 $\pm$ 7030	2400 $\pm$ 234	1.58 $\pm$ 0.249
	Combined	1 (0.083 - 1)	935 $\pm$ 153	31.2 $\pm$ 5.08	144 (144 - 144)	70900 $\pm$ 9140	2360 $\pm$ 305	1.52 $\pm$ 0.337
176	Male	0.083 (0.083 - 0.083)	1020 $\pm$ 94.2	34.0 $\pm$ 3.14	144 (144 - 144)	70800 $\pm$ 7190	2360 $\pm$ 240	1.49 $\pm$ 0.348
	Female	0.083 (0.083 - 1)	1120 $\pm$ 22.8	37.2 $\pm$ 0.759	144 (144 - 144)	78100 $\pm$ 3820	2600 $\pm$ 127	1.71 $\pm$ 0.198
	Combined	0.083 (0.083 - 1)	1060 $\pm$ 84.5	35.4 $\pm$ 2.82	144 (144 - 144)	74000 $\pm$ 6770	2470 $\pm$ 226	1.58 $\pm$ 0.296

^a Median (Min – Max).

NA = Not Applicable

R_{AUC} = Day 78 or 176 AUC_{tlast}/Day 1 AUC_{tlast}.

Terminal Procedures:

Gross Pathology

- No macroscopic findings were observed.

Organ Weights

- No test article-related effect on organ weights were observed.

Histopathology

Adequate Battery: Yes. (Signed and dated Pathology Report was provided.)

Peer Review: No

Histological Findings

- Reduced B cell populations were observed in lymphoid tissues (spleen, lymph nodes, GALT).
- Some animals showed vascular/perivascular inflammation in multiple tissues that was considered to be a result of immune response to TG-1101. The findings are summarized in the table below.

Text Table 27
Summary TG-1101-Related Microscopic Findings – Terminal Euthanasia (Day 183)

	Group Dose (mg/kg) No. animals examined	Males		Females	
		1	2	1	2
		0	30	0	30
GALT		5	5	5	4
Examined		5	5	5	4
Decreased size/number; follicle; germinal center		0	1	0	0
marked		0	1	0	0
LYMPH NODE, DRAINING					
Examined		5	5	5	4
Decreased size/number; follicle; germinal center		0	4	0	3
minimal		0	1	0	0
moderate		0	1	0	0
marked		0	0	0	2
severe		0	2	0	1
LYMPH NODE, MANDIBULAR					
Examined		5	5	5	4
Decreased size/number; follicle; germinal center		0	3	0	2
mild		0	1	0	0
moderate		0	0	0	1
marked		0	1	0	0
severe		0	1	0	1
LYMPH NODE, MESENTERIC					
Examined		5	5	5	4
Decreased size/number; follicle; germinal center		0	4	0	3
mild		0	1	0	0
moderate		0	0	0	2
marked		0	0	0	1
severe		0	3	0	0

	Group Dose (mg/kg) No. animals examined	Males		Females	
		1	2	1	2
		0	30	0	30
SPLEEN		5	5	5	4
Examined		5	5	5	4
Decreased size/number; follicle; germinal center		0	4	0	3
moderate		0	1	0	0
marked		0	1	0	2
severe		0	2	0	1

Severity grades for which there were no findings were omitted from the table.

Other findings (considered due to immune response to the drug):

Text Table 4
Summary Other Notable Microscopic Findings – Terminal Euthanasia (Day 183)

		Males		Females	
	Group	1	2	1	2
	Dose (mg/kg)	0	30	0	30
	No. animals examined	5	5	5	4
ARTERY, AORTA					
Examined		5	5	5	4
Inflammation, mononuclear cell; vascular/perivascular; adventitial		0	1	0	0
minimal			2101		
BRAIN					
Examined		5	5	5	4
Infiltration, mononuclear cell; parenchymal; perivascular		0	0	1	1
minimal				1503	2503
Infiltration, mononuclear cell; choroid plexus		3	1	2	1
minimal		1002	2101	1501	2503
		1003		1503	
		1005			
Infiltration, mononuclear cell; meninges		1	3	1	2
minimal		1002	2004	1503	2503
			2005		2505
mild			2003		
EPIDIDYMIS					
Examined		5	5	-	-
Inflammation, mononuclear cell; vascular/perivascular		0	1	0	0
minimal			2101		

	Males		Females	
Group	1	2	1	2
Dose (mg/kg)	0	30	0	30
No. animals examined	5	5	5	4
EYE				
Examined	5	5	5	4
Infiltration, mononuclear cell; choroid	0	2	0	0
minimal		2003		
		2005		
Infiltration, mononuclear cell; ciliary body	0	3	1	1
minimal		2002	1503	2503
		2003		
mild		2005		
GALLBLADDER				
Examined	5	5	5	4
Inflammation, mononuclear cell; vascular/perivascular	0	1	0	0
minimal		2101		
GLAND, SEMINAL VESICLE				
Examined	5	5	-	-
Inflammation, mononuclear cell; vascular/perivascular	0	1	0	0
mild		2101		
HEART				
Examined	5	5	5	4
Inflammation, mononuclear cell; vascular/perivascular	0	1	0	1
minimal		2101		2503
JOINT, FEMOROTIBIAL				
Examined	5	5	5	4
Inflammation, mononuclear cell; vascular/perivascular	0	1	0	0
minimal		2101		
KIDNEY				
Examined	5	5	5	4
Inflammation, mononuclear cell; vascular/perivascular	0	1	1	0
minimal		2101	1503	
MESENTERY				
Examined	0	1	0	0
Inflammation, mononuclear cell; vascular/perivascular	0	1	0	0
minimal		2101		
SMALL INTESTINE, ILEUM				
Examined	5	5	5	4
Inflammation, mononuclear cell; vascular/perivascular	0	1	0	0
mild		2004		
SMALL INTESTINE, JEJUNUM				
Examined	5	5	5	4
Inflammation, mononuclear cell; vascular/perivascular	0	1	0	0
mild		2004		

	Males		Females	
Group	1	2	1	2
Dose (mg/kg)	0	30	0	30
No. animals examined	5	5	5	4
SPINAL CORD				
Examined	5	5	5	4
Infiltration, mononuclear cell; meninges minimal	0	1 2003	0	0
Infiltration, mononuclear cell; nerve roots minimal	0	1 2003	0	0
SPLEEN				
Examined	5	5	5	4
Increased size/number; follicle; germinal center mild	0	1 2101	0	0
STOMACH				
Examined	5	5	5	4
Inflammation, mononuclear cell; vascular/perivascular minimal	0	1 2101	0	0
TESTIS				
Examined	5	5	-	-
Inflammation, mononuclear cell; vascular/perivascular minimal	0	1 2101	0	0

No NOAEL was determined for this study.

The dose of TG-1101 of 30 mg/kg was associated with an AUC value on SD1 of 46,900 hr*µg/mL for males and 45,600 hr*µg/mL for females.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Genotoxicity assays are not recommended for biological therapeutics. An Ames assay was conducted, and results were negative.

8 Carcinogenicity

Carcinogenicity studies were not considered feasible for ublituximab and were, therefore, not conducted.

9 Reproductive and Developmental Toxicology

Study #:20083322

Title: Enhanced pre-postnatal toxicity of TG-1101 administered by intravenous injection in pregnant cynomolgus monkeys with 6-month postnatal evaluation

Testing Facility:

(b) (4)

Date of Study Initiation: 1/27/2018

GLP Compliance: Yes

Drug/lot#/Purity: TG-1101/AH4560/97.6%

Methods:

Animals: Cynomolgus monkey

Age/weight: 2.8 to 8.3 years old/ 2.9 to 5.1 kg

Number/group: 16 pregnant females/group

Doses: 0 or 30 mg/kg

Route: IV (Slow bolus)

Frequency: Once weekly, 7 doses were planned for each trimester

Study Design:

Text Table 1
Experimental Design

Group No.	Test Material	Day of Dose Administration (Gestation Day)	Dose Level (mg/kg/week)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Pregnant Females
1	Control	20-22, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 98, 105, 112, 119, 126, 133, 140, 147, 154, 161, and 168	0	3.0	0	16
2 (Set A)	TG-1101	20-22, 28, 35, 42, 49, 56, and 63	30	3.0	10.0	16
2 (Set B)	TG-1101	70, 77, 84, 91, 98, 105, and 112	30	3.0	10.0	16
2 (Set C)	TG-1101	119, 126, 133, 140, 147, 154, and 161 (and 168 if needed)	30	3.0	10.0	16

Pregnancy was confirmed by ultrasound between GD18-20.

Dosing for Set 2B was terminated early due to in-life findings (immune-mediated toxicity) before all females had received all 7 doses.

Dosing for Set 2C was also terminated on the same date as Set 2B. Only 2 of 16 animals from Set 2C were dosed (3 doses each). All remaining animals from Set 2C that had been confirmed pregnant but not yet dosed were released from the study.

Pregnancy outcomes are summarized in the following table:

Text Table 2
Pregnancy and Postpartum Outcomes

Group/Set No. (Dose Level)	No. of Pregnant Females	Mean Gestation Length ^a (Days)	Adult Female Losses Prior to Parturition	Total Fetal Losses (Trimester) ^b	No. of Infants Born M/F (Total Born)	No. of Infant Losses Excluding Maternal Neglect	Combined (Fetal and Infant) Losses as %
1 (0 mg/kg/week)	16	161 ± 4	0	2 (0/0/2)	8/6 (14)	1	3/14 (21.4%) ^c
2A (30 mg/kg/week 1st Trimester)	16	162 ± 5	3	6 (2/3/1)	3/4 (7)	0	6/13 (46.2%)
2B (30 mg/kg/week 2nd Trimester)	16	152 ± 14	7	5 (0/2/3)	4/0 (4)	3	8/9 (88.9%)
2C (30 mg/kg/week 3rd Trimester)	2	136	0	1 (0/0/1)	1/0 (1)	1	2/2 (100.0%)
Historical Control Data (n=22 ePPND studies, 2008-2017) (% , mean and range)							28.1% (20.0-42.9%)

^a Females with fetal losses: abortion or stillborn or females euthanized early on study were excluded from the calculations. Historical control gestation length: 159 ± 7 days.

^b First, 2nd, and 3rd trimesters are GD20-50, GD51-100, and > GD100, respectively, and the fetal losses per trimester are shown in parentheses.

^c Infant losses were calculated including maternal-induced trauma and/or inability to provide infant care due to maternal clinical condition, the combined fetal/infant loss rate for Group 1 was 31.3% (5/16).

No offspring were available for evaluation from Group 2C (3rd trimester dosing).
One offspring from Group 2B was available for evaluation (2nd trimester dosing).
Seven offspring were available for evaluation from Group 2A (1st trimester dosing).

Observations/Results:

Formulation analysis:

- Test article formulations were not evaluated.

Mortality (Maternal and fetal loss): Data were recorded for adults and offspring once daily.

- Ten adult females were euthanized early or found dead after 3-6 doses (3 from Set 2A and 7 from Set 2B). One control female was euthanized on PND1 due to moribund condition.

Maternal deaths are summarized in the table below:

Text Table 4
Early Deaths – Adult Females

Group No.	Dose Level (mg/kg/week)	Set	Adult Female No.	Gestation Day of Euthanasia or Death	Day(s) of Unscheduled Clinical Pathology Collection(s)	Comments
1	0	NA	1513	PPD1	PPD1	Euthanized due to clinical condition post delivery
2	30	A	2508	48	GD48	Euthanized due to clinical condition and non-viable fetus
			2511	45	NSC	Found dead
			2515	45	NSC	Euthanized due to clinical condition
		B	2521	88	GD88	Euthanized due to clinical condition and non-viable fetus
			2522	91	GD86	Euthanized due to clinical condition
			2527	87	GD86 and GD87	Euthanized due to clinical condition and non-viable fetus
			2528	102	GD94 and GD102	Euthanized due to clinical condition
			2529	88	GD88	Euthanized and non-viable fetus
			2530	91	NSC	Died during dose administration
			2531	109	GD101 and GD109	Euthanized due to clinical condition

No. = number; NA = Not applicable; NSC = no sample collected; PPD = Postpartum Days; GD = Gestation Days.

The death and/or and moribund euthanasias of adult females administered 30 mg/kg/week TG-1101 were not attributed to a direct effect of TG-1101, but rather to secondary effects resulting from an immunogenic response (hypersensitivity) to TG-1101, leading to formation of anti-therapeutic antibodies and subsequent adverse sequelae including adverse clinical signs (dose reactions), coagulopathy, and hemorrhage, edema and/or necrosis in multiple organs including liver, heart, lung and kidney ([Rojko et. al., 2014](#); [Mease and Kimzey 2019](#)).

- As stated above, due to the immune-mediated adverse effects, dosing was terminated early for Group 2B and 2C. The total number of doses received by each group were:
 - o Group 2A: 7 pregnant females that delivered a surviving infant received 7 doses
 - o Group 2B: 4 pregnant adults received 6-7 doses of the planned 7 doses
 - o Group 2C: 2 of 16 pregnant animals received 3 doses each. The remaining animals were released into the colony

Fetal loss for each group:

1. Control: 2 of 16 (12.5%)

2. Group 2A (First trimester dosing): 6 of 13 (46.2%) All living offspring from Set 2A survived to 6 months postpartum.
 3. Group 2B (second trimester dosing): 5 of 9 (55.6%)
 4. Group 2C (Third trimester dosing): 1 of 2 (50%)
- Seven adults aborted the fetuses during the first or second trimester (5 from set A, 2 from Set B). Four adults aborted during the 3rd trimester (1 control, 1 from Set A, 2 from Set B).
 - Three offspring were stillborn (1 from the control group, 1 from Set B, 1 from Set C).
 - Six infants died or were euthanized soon after birth due to moribund condition or deformities (2 Controls, 3 from Set B, 1 from Set C).

Summary of fetal losses:

Text Table 27
Fetal Losses (Abortions)

Group/Set No. (Dose Level)	No. Pregnant Enrolled	Adult Female Losses Prior to Parturition ^a	Gestation Day of Fetal Loss (Adult Female No. /Fetus No.)	Total Fetal Loss	1st TM GD20-50	2nd TM GD51-100	3rd TM > GD100 ^b
1 (0 mg/kg/week)	16	0	GD130 (1508/1086) GD159 (1511/1111)	2 of 16 (12.5%)	0 (0.0%)	0 (0.0%)	2 (12.5%)
2A (30 mg/kg/week 1st Trimester)	16	3	GD46 (2501/N/A) GD46 (2513/N/A) GD52 (2510/N/A) GD61 (2516/N/A) GD74 (2502/N/A) GD118 (2509/2096)	6 of 13 (46.2%)	2 (15.4%)	3 (23.1%)	1 (7.7%)
2B (30 mg/kg/week 2nd Trimester)	16	7	GD84 (2534/N/A) GD92 (2526/2261) GD102 (2523/2230) GD116 (2524/2240) GD141 (2520/2206)	5 of 9 (55.6%)	0 (0.0%)	2 (22.2%)	3 (33.3%)
2C (30 mg/kg/week 3rd Trimester)	2	0	GD137 (2517/2176)	1 of 2 (50.0%)	0 (0.0%)	0 (0.0%)	1 (50.0%)
Historical Control Data Mean (Range) ^c				20.2% 6.7 to 38.9%	8.6% 0 to 15.8%	1.6% 0 to 10.0%	12.% 0 to 28.6%

GD = gestation day; N/A = not applicable; TM = trimester.

^a All fetal losses were calculated excluding the adult female losses. Four of the adult females (Animal Nos. 2508, 2521, 2527 and 2529) had nonviable fetuses detected by ultrasound on the day of or day prior to unscheduled necropsy (UNEC). The severe moribund condition of the adults was such that it was considered likely causative in the fetal loss, therefore these situations were categorized as 'Adult Female Losses Prior to Parturition' rather than as 'Fetal Losses'. Fetal losses for all UNEC adult females still factored into the calculation of Total Fetal Loss (%) because the number of UNEC females was excluded from the denominator of that calculation.

^b Pregnancy losses after GD100 include abortions (up to GD139) and stillbirths ($\geq$ GD140), based on GD140 being the earliest age at which NHP infants are expected to survive ([Hendrie, 1996](#)).

^c Based on 22 ePPND studies conducted at the Testing Facility from 2008 to 2017.

The fetal losses were considered related to the test article because losses in each dose group exceeded the historical average range.

Clinical observations (adult females): Data were recorded for adults and offspring daily. Detailed clinical observations were conducted for infants once weekly.

- Bleeding from gums and nose was observed in Set A and B females.
- Sets A and B also showed increased time required to stop bleeding after blood draws.
- Other clinical observations for Set A and B were as follows: Salivation, shallow breathing, decreased activity, hunched posture, tremors, loss of consciousness, emesis, lying on side, pale skin, cold to touch, red urine, and sunken eyes.
- Set C animals (2 only) showed pale skin and red vaginal discharge (prior to abortion or preterm delivery).

Body weights: Data were recorded for adults prior to study enrollment, and weekly until parturition. After parturition, maternal and infant weight were recorded on PND2, 7 and weekly thereafter.

- Set A showed expected body weight gain for pregnant animals.
- Set B: Most animals lost weight between GDs 80 and 102. (~10% between day 81 and 102)
- Set C: Due to the small number of animals, body weight loss/gain could not be determined.

Food intake: (adult females): Data were recorded daily.

- Decreased food consumption was observed in Sets A and B females

Placental abnormalities:

Control group: 2 of 11 placentas were abnormal

Group 2: 11 of 24 placentas were abnormal

- Set 2A: 3
- Set 2B: 7
- Set 2C: 1

The sponsor reports that Group 2 placental abnormalities (except 1: green discoloration) were similar and “consistent with immune-mediated hemodynamic disturbances”: Nine abnormal placentas were from adults that were euthanized early due to moribund condition. All 9 were associated with fetal losses.

- Placental observations were described as: “disc infarction (wedge-shaped or regionally extensive coagulative necrosis of villi in the center of the disc, basal plate, and/or chorionic plate) retroplacental hematoma, and perivillous fibrin accumulation.”
- Also noted were mixed cell infiltrates, and hemorrhage graded minimal to abundant.
- One control placenta was reported to show increased macrophage numbers in the chorioamniotic membrane,
- One Group 2 Set A placenta showed green discoloration that was attributed to “intrauterine distress and fetal meconium release.”

- Placentas from successfully delivered offspring showed no consistent microscopic placental abnormalities.

Ultrasound evaluations: After pregnancy was confirmed, ultrasound evaluations were conducted Q2W during gestation. In cases where the fetus showed potential adverse effects, examinations were conducted more frequently.

- In surviving fetuses, fetal examinations showed no heart abnormalities or test article-related external or visceral abnormalities.

Cesarean Section: Four Cesarean sections were conducted because of poor maternal condition on animals from Group 2B (#2519, 2528, 2530, and 2531). During each of these surgeries, amniotic fluid, umbilical cord, placenta, and fetus were removed and examined (2B, infant with multiple deformities).

Birth observations (surviving adults and infants): Observations were recorded throughout the day of birth. Infants were not separated from the adult for detailed evaluation until PND2. On PND2, the following evaluations were conducted:

- Evidence of nursing behavior
- Body weight
- A physical examination (including inspection of the hard palate, spinal cord closure, digits, limbs, and joint angles for normal structural morphology, and lymph node palpation)
- Heart rate
- Respiration rate
- Adult females were checked for the availability of milk during the sedated separation from the infant.

Excluding infant and fetal losses described below, all surviving offspring were considered within normal limits for all parameters evaluated during the PND1/PND2 assessments.

The following table summarizes the infant loss data:

Text Table 28
Infant Losses

Group/Set No. (Dose Level)	Total No. of Infants Born	Infant No.	Adult Female No.	Birth Day of Infant Loss	GD at Delivery ^a	Comments	Total Infant Loss	Total Infant Loss Adjusted
1 (0 mg/kg/ week)	14	1036	1503	BD88	GD162	Infant euthanized due to overall poor condition	3/14 (21.4%)	1/12 ^b (8.3%)
		1041	1504	BD1	GD155	Infant euthanized due to maternal-induced injuries		
		1136	1513	BD2	GD166	Adult female euthanized with a retained placenta with no cross-foster available (infant euthanized due to lack of available maternal animal)		
2A (30 mg/kg/ week 1st Trimester)	7	N/A	N/A	N/A	N/A	N/A	0/7 (0.0%)	0/7 (0.0%)
2B (30 mg/kg/ week 2nd Trimester)	4	2191	2519	BD1	GD173	Infant euthanized due to abnormal appearance (external abnormalities)	3/4 (75.0%)	3/4 (75.0%)
		2321	2532	BD1	GD164	Infant euthanized due to abnormal appearance (external abnormalities) and inability to grasp		
		2331	2533	BD7	GD137	Infant delivered prematurely; euthanized due to overall poor condition		

Group/Set No. (Dose Level)	Total No. of Infants Born	Infant No.	Adult Female No.	Birth Day of Infant Loss	GD at Delivery ^a	Comments	Total Infant Loss	Total Infant Loss Adjusted
2C (30 mg/kg/ week 3 rd Trimester)	1	2181	2518	BD1	GD136	Infant delivered prematurely; euthanized due to overall poor condition.	1/1 (100.0%)	1/1 (100.0%)
Historical Control Data mean and range (n=22 ePPND studies, 2008-2017)							9.5% (0 to 20%)	4.4% (0 to 16.7%)

BD = birth day; GD = gestation day; N/A = not applicable.

- ^a Deliveries that occurred between GD152 and GD141 were considered pre-term (based on historical control data for average gestation length of 159 ± 7 days for 22 ePPND studies conducted at the Testing Facility from 2008 to 2017). Deliveries that occurred prior to GD140 were considered premature based on this being the earliest age at which NHP infants are expected to survive ([Hendrie et al., 1996](#)).
- ^b The 2 infant losses for Group 1 related to maternal neglect or inability to provide infant care due to maternal condition were excluded in this calculation; therefore, the total number of infants was decreased by 2.

Detailed description of the infant losses provided by the sponsor:

Controls

- 1503/1036 (BD88): delivery GD162, infant euthanized on BD88 due to poor overall clinical condition including lethargy, dehydration, and watery feces.
- 1504/1041 (BD1): delivery GD155, infant euthanized on BD1 due to maternal-induced injuries on the day of delivery.
- 1513/1136 (BD2): delivery GD166, infant euthanized on BD2. Adult female had a retained placenta and had to be euthanized due to poor clinical condition. There was no cross-foster female available; therefore, the infant was euthanized.

Set 2B

- 2519/2191 (BD1): C-section delivery on GD173, infant euthanized due to external abnormalities considered TG-1101-related (contractures and abnormal flexion of multiple limbs and tail resulting in fixed and limited range of motion of the joints). See results Section 8.12.
- 2532/2321 (BD1): delivery GD164, infant euthanized on BD1 due to external abnormalities (contractures and abnormal flexion of multiple limbs and tail resulting in fixed and limited range of motion of the joints) and a lack of grasp reflex, both of which were considered TG-1101-related. See results Section 8.12.
- 2533/2331 (BD7): delivery GD137, infant euthanized on BD7 due to poor overall clinical condition (weakness, dehydration and body weight loss). Due to the delivery date of this infant being before GD140, it was unlikely to survive and considered a premature delivery. The early delivery was considered TG-1101-related.

Set 2C

- 2518/2181 (BD1): delivery GD136, infant euthanized on BD1 due to poor overall clinical condition and maternal neglect. Due to the delivery date of this infant being before GD140, it was unlikely to survive and considered a premature delivery. The early delivery was considered TG-1101-related. Maternal neglect was involved, common for premature births.

Clinical observations (surviving infants):

- No test article-related observations were reported in surviving infants.

Body weight (surviving infants):

- Body weight and body weight of surviving infants were similar to control infants.

Infant external assessments: External assessments were conducted on infants on the day of birth, on PND28, PND91, and at necropsy.

- For surviving infants, no test article-related effects were observed.
- Infants from Group 2 Set 2B showed test article-related external findings as described above.

Morphometric measurements: Data were recorded on PNDs 28 and 91, and at necropsy.

- Crown-rump length
 - Femur length (bilateral)
 - Foot length (bilateral)
 - Horizontal head circumference
 - Biparietal diameter
 - Occipitofrontal diameter
 - Chest circumference
 - Anogenital distance
- No test article-related effects were observed in fetal measurements in surviving fetuses.
 - Infants that died or were euthanized early showed no test article-related morphometric abnormalities.

Neurobehavioral Assessments: Assessments were conducted on alert infants on PNDs 7 and 154.

- Righting
- Palmar grasp
- Clasp-grasp
- Visual following
- Prone progression
- Lipsmack orient
- Oral reflexes (rooting, sucking, and snout reflex)
- Eye reflexes (pupil constriction, nystagmus, and glabellar tap)
- Moro reflex
- Negative geotaxis
- Buildup (increasing arousal levels in response to manipulation)

In surviving infants, no test article-related effects were observed. One infant (2331, Group 2B) was too weak to grasp and was euthanized moribund on PND7.

Skeletal evaluations (Infants): Infant skeletal evaluation was conducted by X-Ray on PND28.

- Radiographic evaluations for one infant (2191, Set 2B) showed shortened mandible, elongated cranium, and sternal concavity. This infant was euthanized due to external abnormalities.

Laboratory evaluations:

Adults: Blood samples were collected according to the schedule below:

Text Table 5
Samples for Clinical Pathology Evaluation – Adult Females

Group Nos.	Time Point	Hematology	Clinical Chemistry	Coagulation	PFA-100
1-2 (Set A)	GD20-22	X	X	-	-
2 (Set A)	GD63 Pre	X	X	-	-
1-2 (Set B)	GD70 Pre	X	X	X ^a	X ^b
2 (Set B)	GD93 (48 hr postdose GD91)	X	-	X	X ^b
2 (Set B)	GD112 Pre	X	X	-	-
1-2 (Set C) ^d	GD119 Pre	X	X	X ^a	X ^c
1-2	PPD35	X	X	-	-
1-2	PPD91	X	X	-	-
1-2	PPD180 ± 2	X	X	-	-
Unscheduled euthanasia (when possible)	Before euthanasia	X	X	X	-
At the time of abortion confirmation	At confirmation or the time of abortion	X	X	X	-

GD = gestation; PPD = postpartum day; Pre = predose; hr = hour; X = sample collected; - = not applicable.

^a Coagulation samples were collected from Group 2 only.

^b Samples for PFA-100 were collected as close to GD70 as possible with flexibility to allow for multiple animals to be collected on the same day. The GD93 samples were collected in the range of GD91: 36-72-hour postdose. Samples were collected from approximately 3 control animals at roughly the same GD for comparison.

^c PFA-100 samples were collected for Group 2 Set C Animals 2517 and 2518 only. This included GD119 predose and GD119.

^d Samples for this time point were only collected for animals that received a dose on GD119.

Hematology:

- For Set 2A adults, no test article-related effects on hematology were observed.
- For Set 2B adults, decreased neutrophils (0.28X), lymphocytes, monocytes, and eosinophils (0.43X, 0.28X, 0.28X, and 0.42X, respectively), as well as decreased platelets and decreased RBC mass (~0.85X) with associated increased reticulocytes were observed on GD93 and/or GD112.
- One adult from Set 2B showed no effects on hematology evaluations.

Clinical Chemistry:

- Adults in Set 2A showed increased albumin (graded mild, 0.66X) and elevated globulin (1.09X). The sponsor considers these results consistent with an acute phase response. The albumin and globulin levels returned to baseline during the post-partum period.
- Set 2B adults showed elevated globulin (graded mild) that returned to baseline post-partum.

Coagulation (Adults):

- No samples were collected from adult Set A
- Set B adults showed increased fibrinogen (graded moderate, 2.22X) with prolonged aPTT (graded minimal) for 2 adults (+4.7 and 6.9 seconds) on GD93.

Infants: Samples were collected from surviving infants according to the schedule below.

Text Table 6
Samples for Clinical Pathology Evaluation – Infants

Group Nos.	Time Point	Hematology	Clinical Chemistry
1-2	BD42	X	X
1-2	BD91	X	X
1-2	BD180 ± 2	X	X
Unscheduled euthanasia (when possible)	Before euthanasia	X	X

X = sample collected.

Hematology: A standard battery of parameters was evaluated.

- No test article-related findings were noted in hematology evaluations for surviving infants.

Clinical Chemistry: A standard battery of parameters was evaluated.

Text Table 9
Clinical Chemistry Parameters

Alanine aminotransferase	Total protein
Aspartate aminotransferase	Albumin
Alkaline phosphatase	Globulin
Gamma-glutamyltransferase	Albumin/globulin ratio
Lactate dehydrogenase	Glucose
Total bilirubin ^a	Cholesterol
Urea nitrogen	Triglycerides
Creatinine	Sodium
Calcium	Potassium
Phosphorus	Chloride
	Sample quality

^a When total bilirubin was >1.0 mg/dL, indirect and direct bilirubin were also measured.

- 6 infants were euthanized on PND1, 2, 7, or 88. These infants showed no Tg-1101-related effects on clinical chemistry.
- No TG-1101-related effects on clinical chemistry were observed for surviving infants.

PFA-100 (Evaluation of platelet function): Samples were available from 3 control animals and 2 group 2 Set B animals. The values from the Group 2 animals appeared generally higher than controls, but insufficient samples were available to draw conclusions. The sponsor was silent on these results.

Toxicokinetics (adults and infants):

Text Table 10
Toxicokinetic (TK) Sample Collection Schedule – Adult Females

Adult Female Sample Collection Time Points		
Group Nos.	Study Day	Time Points (Relative to Dosing)
1-2 (Set A)	GD20-22	Pre
2 (Set A)	GD20-22	15 min, 1, 6, 48, 96, and 144 hr Post
1	GD42	Pre
2 (Set A)	GD42	Pre, 15 min, 1, 6, 48, 96, and 144 hr Post
1	GD63	Pre
2 (Set A)	GD63	Pre, 15 min, 1, 6, 48, 96, and 144 hr Post
1	GD70	Pre
2 (Set B)	GD70	Pre, 15 min, 1, 6, 48, 96, and 144 hr Post
1 ^c	GD91	Pre
2 (Set B)	GD91	Pre, 15 min, 1, 6, 48, 9,6 and 144 hr Post
1 ^c	GD112	Pre
2 (Set B) ^b	GD112	Pre, 15 min, 1, 6, 48, 96, and 144 hr Post
1 ^c	GD119	Pre
2 (Set C) ^d	GD119	Pre, 15 min, 1, 6, 48, 96, and 144 hr Post
1 ^c	GD140	Pre
1 and 2 (all sets)	PPD2 ^a	N/A
1 and 2 (all sets)	PPD14	NA
1 and 2 (all sets)	PPD28	NA
1 and 2 (all sets)	PPD91	NA
1 and 2 (all sets)	PPD180 ± 2	NA
Day of abortion/pregnancy loss confirmation (including stillbirth and any C-sections) and infant losses	As applicable	NA
Unscheduled necropsy of adult female	As applicable	NA

GD = gestation day; PPD = postpartum day; Pre = predose; post = postdose; min = minute; hr = hour;
NA = not applicable.

^a When the need arose for separation of the maternal/infant pair on PPD1, this sample was collected at that time.

^b For Set B animals not receiving a dose on GD112, only the predose sample was collected.

^c Samples for noted time points were only collected for control animals that received a dose on the corresponding day listed prior to discontinuation of dose administration. Samples for these days were not collected from any control animal beginning on 20 Jun 2018. Animal Nos. 2532, 2533, 1511, and 1512 had GD112: predose sample collected although they were not dosed on GD112.

^d Samples for noted time points were only collected for Set C Female Nos. 2517 and 2518 only. All other Set C females were released from the study prior to GD119.

Toxicokinetic results in adults (Set A-C):

Table 2.8: Summary (Mean $\pm$ SD) TG-1101 Toxicokinetic Parameters in Maternal Cynomolgus Monkey Serum Following Once Weekly IV Bolus Injection of 30 mg/kg TG-1101 from GD20-22 Through GD140 (Set A)

GD	T _{max} ^a (hr)	C _{max} (ng/mL)	T _{last} ^a (hr)	AUC _(0-t) (hr*ng/mL)	T _{1/2} (hr)	CL (mL/hr/kg)	Vd (mL/kg)	R _{AUC} (RATIO)
20-22	1 (0.25 - 1)	1020000 $\pm$ 151000	144 (144 - 144)	55300000 $\pm$ 9040000	NC	NC	NC	NC
42	0.63 (0.25 - 1)	1080000 $\pm$ 238000	144 (48 - 144)	56800000 $\pm$ 27700000	22.5 $\pm$ 17.7	1.60 $\pm$ 1.45	28.8 $\pm$ 11.9	1.03 $\pm$ 0.509
63	0.25 (0.25 - 6)	1110000 $\pm$ 295000	144 (96 - 144)	70800000 $\pm$ 26500000	35.0 $\pm$ ID	1.17 $\pm$ ID	40.2 $\pm$ ID	1.31 $\pm$ 0.450

^a Median (Min – Max); ID = Insufficient data; NC = Not calculated; R_{AUC} = GD42 or GD63 AUC_(0-t)/GD20-22 AUC_(0-t).

Table 2.9: Summary (Mean $\pm$ SD) TG-1101 Toxicokinetic Parameters in Maternal Cynomolgus Monkey Serum Following Once Weekly IV Bolus Injection of 30 mg/kg TG-1101 from GD70 Through GD112 (Set B)

GD	T _{max} ^a (hr)	C _{max} (ng/mL)	T _{last} ^a (hr)	AUC _(0-t) (hr*ng/mL)	T _{1/2} (hr)	CL (mL/hr/kg)	Vd (mL/kg)	R _{AUC} (RATIO)
70	0.25 (0.25 - 1)	1020000 $\pm$ 162000	144 (144 - 144)	52300000 $\pm$ 6700000	57.6 $\pm$ ID	0.440 $\pm$ ID	36.6 $\pm$ ID	NC
91	0.25 (0.25 - 1)	835000 $\pm$ 266000	144 (48 - 144)	36800000 $\pm$ 22200000	22.8 $\pm$ 17.4	1.96 $\pm$ 2.46	43.4 $\pm$ 34.4	0.741 $\pm$ 0.532
112	0.63 (0.25 - 1)	1070000 $\pm$ 249000	144 (48 - 144)	42200000 $\pm$ 32000000	12.6 $\pm$ ID	2.62 $\pm$ ID	36.4 $\pm$ ID	0.771 $\pm$ 0.568

^a Median (Min – Max); ID = Insufficient data; NC = Not calculated; R_{AUC} = GD91 or GD112 AUC_(0-t)/GD70 AUC_(0-t).

Table 2.10: Summary (Mean $\pm$ SD) TG-1101 Toxicokinetic Parameters in Maternal Cynomolgus Monkey Serum Following IV Bolus Injection of 30 mg/kg TG-1101 on GD119 (Set C)

GD	T _{max} ^a (hr)	C _{max} (ng/mL)	T _{last} ^a (hr)	AUC _(0-t) (hr*ng/mL)	T _{1/2} (hr)	CL (mL/hr/kg)	Vd (mL/kg)	R _{AUC} (RATIO)
119	3.13 (0.25 - 6)	941000 $\pm$ ID	144 (144 - 144)	58200000 $\pm$ ID	NC	NC	NC	NC

^a Median (Min – Max); ID = Insufficient data; NC = Not calculated.

Text Table 11
Toxicokinetic (TK) Sample Collection Schedule – Infants

Infant Sample Collection Time Points	
Group Nos.	Study Day
1-2 (all sets)	BD14
	BD28
	BD91
	BD180 $\pm$ 2
Unscheduled necropsy of infant (including rejection or health reasons)	As applicable

BD = birth day.

Table 3.1: Infant-to-Maternal Serum Concentration Ratio of TG-1101

	Mother								
	Set A							Set B	
	2505	2507	2512	2503	2504	2506	2514	2525	2533
Day	Concentration (ng/mL)								
PPD7	-	-	-	-	-	-	-	-	11200 ^a
PPD14	BQL ⁺	57.9 ⁺	43.9	46.8 ⁺	BQL ⁺	BQL ⁺	BQL ⁺	145 ⁺	-
PPD28	BQL ⁺	BQL ⁺	BQL ⁺	BQL ⁺	BQL ⁺	BQL ⁺	BQL ⁺	BQL ⁺	-
PPD91	BQL ⁺	BQL ⁺	BQL ⁺	BQL ⁺	BQL ⁺	BQL ⁺	BQL ⁺	BQL ⁺	-
PPD180	BQL ⁺	BQL ⁺	BQL ⁺	BQL ⁺	BQL ⁺	BQL ⁺	BQL ⁺	BQL ⁺	-
	Infant								
	2051	2071	2121	2036	2046	2066	2146	2251	2331
	Concentration (ng/mL)								
BD7	-	-	-	-	-	-	-	-	BQL
BD14	BQL	211	310	165	BQL	BQL	BQL	1510	-
BD28	BQL	109	123	66.8	BQL	BQL	BQL	306	-
BD91	BQL	BQL	BQL	BQL	BQL ⁺	BQL	BQL	BQL	-
BD180	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	-
Day	Infant to Mother Ratio								
PPD7	NA	NA	NA	NA	NA	NA	NA	NA	NA
PPD14	NA	3.64	7.06	3.53	NA	NA	NA	10.4	NA
PPD28	NA	NA	NA	NA	NA	NA	NA	NA	NA
PPD91	NA	NA	NA	NA	NA	NA	NA	NA	NA
PPD180	NA	NA	NA	NA	NA	NA	NA	NA	NA

BD = Birth day; BQL = Below quantitation limit (LLOQ = 39.3 or 78.5 ng/mL); NA = Not applicable.
PPD = Postpartum Day.

Immunogenicity:

Text Table 14
Immunogenicity Sample Collection Schedule – Adult Females

Group Nos.	Study Day	Time Points (Relative to Dosing)
1-2 (all sets) ^a	GD20-22	Pre
1-2 (Set A)	GD42	Pre
1-2 (Set A)	GD63	Pre
1-2 (Set B) ^b	GD91	Pre
1-2 (Set B) ^b	GD112	Pre
1 ^b	GD140	Pre
1-2 (all sets)	PPD14, PPD28, PPD91, and PPD180 ± 2	NA
Day of abortion/pregnancy loss confirmation (including stillbirth and any C-sections) and infant losses	As applicable	NA
Unscheduled necropsy of adult female	As applicable	NA

GD = gestation day; PPD = postpartum day; Pre = predose; NA = not applicable.

^a For any late enrollments placed in Sets B or C, the first sample was collected on the day of enrollment.

^b Samples for noted time points were only collected for control animals that had reached the GD140 time point prior to discontinuation of dose administration. Samples for GD91, GD112, and GD140 were not collected from any control animal beginning on 20 Jun 2018. Animal Nos. 2532, 2533, 1511, and 1512 had GD112: predose sample collected although they were not dosed on GD112.

Text Table 15
Immunogenicity Sample Collection Schedule – Infants

Infant Sample Collection Time Points	
Group Nos.	Study Day
1-2 (all sets)	BD35, BD91, and BD180 $\pm$ 2
Unscheduled necropsy of infant (including rejection or health reasons)	As applicable

BD = birth day.

Immunophenotyping:

Text Table 16
Immunophenotyping Sample Collection Schedule – Adult Females

Group Nos.	Study Day	Time Points (Relative to Dosing)
1-2 (all sets) ^a	GD20-22	Pre
1-2 (Set A)	GD42	Pre
1-2 (Set A)	GD63	Pre
1-2 (Set B) ^b	GD91	Pre
1-2 (Set B) ^b	GD112	Pre
1 ^b	GD140	Pre
1-2 (all sets)	PPD14, PPD28, PPD91, and PPD180 $\pm$ 10 ^c	NA
Day of abortion/pregnancy loss confirmation (including stillbirth and any C-sections) and infant losses.	As applicable	NA

GD = gestation day; PPD = postpartum day; Pre = predose; NA = not applicable.

^a For any late enrollments placed in Sets B or C, the first sample was collected on the day of enrollment.

^b Samples for noted time points were only collected for control animals that had reached the GD140 time point prior to discontinuation of dose administration. Samples for GD91, GD112, and GD140 were not collected from any control animal beginning on 20 Jun 2018. Animal Nos. 2532, 2533, 1511, and 1512 had GD112: predose sample collected, although they were not dosed on GD112.

^c Collections for adult females for the PPD180 time point were collected between PPD172 and PPD182 in order to combine days that samples were sent to the flow cytometry laboratory as detailed in [Text Table 17](#).

Text Table 19
Cellular Antigens and Cell Populations

Antigen Marker	Cell Population Identified ^a
CD45+/CD3-/CD19+	CD19+ B-lymphocytes
CD45+/CD3-/CD20+	CD20+ B-lymphocytes
CD45+/CD3+	T-lymphocytes
CD45+/CD3-/CD16+	Natural-killer cells

^a Relative percent, absolute count and absolute count percent of baseline were calculated and reported.

- All adults that received TG-1101 showed reductions in B lymphocytes (CD20+, CD19+)
- No changes in NK or T lymphocyte populations were observed in adults.
- B cell populations showed a trend toward recovery on PND91.

Text Table 18
Immunophenotyping Sample Collection Schedule – Infants

Infant Sample Collection Time Points	
Group Nos.	Study Day
1-2 (all sets)	BD14, BD28, BD91, and BD180 $\pm$ 10 ^a
Unscheduled necropsy of infant (including rejection or health reasons)	As applicable

BD = birth day.

^a Collections for infants mirrored the schedule for their dam for the BD180 time point and were collected between BD172 and BD182 in order to combine days that samples were sent to the flow cytometry laboratory.

- No test article-related effects on the various cell subpopulations were observed in surviving infants.

TDAR (F1 offspring): Immunizations with KLH were conducted on PND140 and 161. Samples were collected as described below:

Samples were collected at BD140 (pre-KLH dose), BD147, BD150, BD154, BD161 (pre-KLH dose), BD168, BD171, BD175, and BD182.

- All immunized infants demonstrated primary, secondary, and memory responses. No test article-related effect was observed in surviving infants.

Terminal Procedures:

The table below contains a summary of terminal procedures at scheduled euthanasia.

Text Table 20
Terminal Procedures Infant (and Fetal) and Adult Female Procedures

Group No./ Animals	No. of Animals	Scheduled Euthanasia Day	Procedures				Histology ^b	Histopathology ^a
			External Eval.	Visceral Eval. and Necropsy	Tissue Coll. ^a	Organ Wts ^a		
Aborted Fetuses (prior to GD100)			X	-	-	-	-	-
Aborted Fetuses (GD100 or later) and Stillbirths (GD140 or later)			X	X	X	-	X	X
1 Infants (control)	11	BD180 ± 2	X	X	X	X	X	X
2 Infants (30 mg/kg/week) 1 st Trimester	7		X	X	X	X	X	X
2 Infants (30 mg/kg/week) 2 nd Trimester	1		X	X	X	X	X	X
2 Infants (30 mg/kg/week) 3 rd Trimester	0		X	X	X	X	X	X
Unscheduled Infant Necropsy			X	X	X	-	X	X
Adult Female Procedures								
Unscheduled Adult Female Necropsy			Necropsy	Tissue Coll. ^a		Organ Wts ^c	Histology ^b	Histopathology ^a
			X	X		X	X	X

X = procedure conducted; - = not applicable.

^a See Text Table 23 for adults and Text Table 24 for infants.

^b Histology = process to paraffin blocks and slides.

^c For adult females delivered to necropsy alive, organ weights were collected. For adult females previously euthanized or delivered dead, no organ weights were collected.

Infant external and visceral evaluation and gross pathology:

Teratology evaluation (internal and external) was conducted on all surviving offspring at necropsy.

- For infants 2191 and 2321 (from Set 2B) observation consisted of contractures, abnormal flexion of multiple limbs and tail, shortened mandible, elongated calvarium, enlargement of ears, and muscular atrophy.
- Internal findings for infants 2191 and 2321: deepened sulci and narrow gyri, bilateral ventricular enlargement, which correlated with microscopic findings of degeneration/necrosis (graded moderate)
- The sponsor suggested that the findings were consistent with primary CNS effects that resulted in the limb contractures and craniomandibular abnormalities. The sponsor considers these findings due to the test article.

Surviving infants showed no internal or external abnormalities.

Organ weights:

- No test article-related effects on organ weights were observed in infants that survived to the terminal necropsy.

Text Table 21
Organs Weighed at Necropsy – Adult Females

Brain	Liver
Gland, adrenal ^a	Ovary ^a
Gland, pituitary	Placenta
Gland, thyroid ^a	Spleen
Kidney ^a	Thymus
	Uterus

^a Paired organ weight.

Text Table 22
Organs Weighed at Necropsy – Infants

Brain	Liver
Epididymis ^a	Ovary ^a
Gland, adrenal ^a	Placenta
Gland, pituitary	Spleen
Gland, prostate	Testis ^a
Gland, thyroid ^a	Thymus
Kidney ^a	Uterus

^a Paired organ weight.

Histology:

Text Table 23
Tissue Collection and Preservation – Adult Females

Animal identification	Liver
Bone marrow smear ^a	Lung
Brain	Lymph node, mandibular
Eye ^b	Lymph node, mesenteric
Gland, adrenal	Muscle, skeletal
Gland, mammary	Ovary
Gland, parathyroid	Oviduct
Gland, pituitary	Pancreas
Gland, salivary	Placenta
Gland, thyroid	Small intestine, duodenum
Gross lesions/masses	Small intestine, ileum
Gut-associated lymphoid tissue	Small intestine, jejunum
Heart	Spleen
Kidney	Stomach
Large intestine, cecum	Thymus
Large intestine, colon	Urinary bladder
Large intestine, rectum	Uterus
	Vagina

^a Bone marrow smears were collected from the 7th rib at scheduled and unscheduled necropsies (for possible examination). Smears were not collected from animals that were found dead. Bone marrow smears were allowed to air dry and were not fixed in formalin.

^b Preserved in Davidson's fixative.

Text Table 24
Tissue Collection and Preservation – Infants

Animal identification	Large intestine, colon
Artery, aorta	Large intestine, rectum
Bone marrow smear ^a	Liver
Bone marrow	Lung
Bone, femur	Lymph node, mandibular
Bone, sternum	Lymph node, mesenteric
Brain	Muscle, skeletal
Cervix	Nerve, optic ^b
Epididymis	Nerve, sciatic
Esophagus	Ovary
Eye ^b	Oviduct
Gallbladder	Pancreas
Gland, adrenal	Placenta
Gland, mammary	Skin
Gland, parathyroid	Small intestine, duodenum
Gland, pituitary	Small intestine, ileum
Gland, prostate	Small intestine, jejunum
Gland, salivary	Spinal cord
Gland, seminal vesicle	Spleen
Gland, thyroid	Stomach
Gross lesions/masses	Testis ^c
Gut-associated lymphoid tissue	Thymus
Heart	Tongue
Kidney	Trachea
Large intestine, cecum	Urinary bladder
	Uterus
	Vagina

^a Bone marrow smears were collected from the 7th rib (or femur if the infant necropsied was less than 1 month in age or if unobtainable from the 7th rib) at scheduled and unscheduled necropsies (for possible examination). Smears were not collected from animals that were found dead. Bone marrow smears were allowed to air dry and were not fixed in formalin.

^b Preserved in Davidson's fixative.

^c Preserved in Modified Davidson's fixative.

- No test article-related microscopic findings were observed in surviving offspring at terminal necropsy.

Histopathology for infant early deaths:

- Both infants # 2191 and 2321 that were euthanized early showed similar effects: degeneration/ necrosis of the brain and spinal cord (graded minimal to moderate), adrenocortical hypertrophy, optic nerve atrophy, and atrophy of the tongue (2191).
- The brain degeneration was characterized by decreased number of neurons, calcified and/or necrotic neurons, loss of/or vacuolation of the white matter, infiltration of foamy macrophages, areas of rarefaction, gliosis, thinning of the cerebral cortex, dilation of ventricles and central canal of the spinal cord.
- Other infants that died or were euthanized early showed no test article microscopic effects.

Integrated Summary

Background

Ublituximab is a recombinant chimeric monoclonal antibody directed against the CD20 antigen widely expressed on the cell surface of pre-B and mature B lymphocytes. The molecule consists of a murine variable region and human constant region (Fc) and shows the typical structure of an IgG1 immunoglobulin.

Pharmacology

Pharmacology studies demonstrated that ublituximab binds with high affinity (Kd in the low nM range) to CD20 on B cells from human and cynomolgus monkey. The Fc region of the antibody binds with high affinity to Fc gamma receptors on effector cells. Binding of ublituximab results in destruction of B lymphocytes through induction of antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC). The glycosylation profile of ublituximab was selected to increase affinity for the FcγRIIIa receptor and, thus, enhance ADCC activity.

Although the characterization of binding specificity was initially inadequate, specificity for CD20 was demonstrated by immunophenotyping in the general toxicity studies after 13 and 26 weeks of exposure in cynomolgus monkeys. In both studies, B lymphocytes were depleted rapidly from lymphoid organs and plasma after the first dose, with no significant effect on NK cells, total T lymphocytes, cytotoxic T cells, or helper T cells. In addition, tissue cross reactivity studies in human and cynomolgus monkey tissue showed specific binding in lymphoid tissues and areas known to be positive for CD20+ cells, and no off-target or unexpected binding was observed.

Justification for testing in a single species was provided in the original submission of IND (b) (4). Published reports were provided indicating that CD20 is highly conserved between humans and cynomolgus monkeys. Based on these data, cynomolgus monkey was selected as the single relevant species for nonclinical testing of ublituximab.

General Toxicology

General toxicology studies of up to 26 weeks' duration were conducted in cynomolgus monkeys. In the single-dose study, ublituximab was administered IV at dose levels up to 100 mg/kg. In the 4-week study, doses were administered IV once weekly at doses up to 50 mg/kg; and in the 13 and 26-week studies, ublituximab was administered IV at weekly doses of up to 30 mg/kg. In all studies, near total depletion of plasma B-cell populations was observed at all doses by 24 hours after the first dose.

In the 4-week study, one unscheduled death occurred in the HD group. Pre-mortem observations were reduced food consumption associated with body weight loss and dehydration, hypoactivity, and prostration. At necropsy, this animal had a tongue ulcer (red/purple area on the tongue), and reduced thymus size. Microscopic findings

were leucocytosis of the kidneys and salivary gland acinar atrophy. No cause of death was identified, but, because it was a HD animal, the death was considered related to the test article.

In surviving animals, toxicities were observed in spleen (fibrinous thrombi at the HD and decreased follicles and germinal centers at the MD and HD), lungs (leucocytosis and fibrinous thrombi at the HD), liver (single cell necrosis at the MD and HD, green pigment at the MD and HD, fibrinous thrombi at the HD, and extramedullary erythropoiesis at the HD), and adrenal gland (extramedullary hematopoiesis and decreased vacuolization of the zona fasciculata at the HD). The reduced follicles and germinal centers in spleen were considered to be a result of the pharmacological activity of ublituximab. No recovery period was included.

In the 13-week study, the HD was reduced to 30 mg/kg and all findings at the end of the dosing period were considered due to the pharmacological activity of ublituximab; B cell populations were to undetectable levels by 24 hours post dose in all dose groups, decreased germinal centers in lymphoid organs in males from all dose groups and females in the HD group. No test article effects on functional immune activity were observed. No ublituximab-related findings were observed at the end of the 6-month recovery period.

ADA development and reduction of PD activity were observed in all dose groups, with the greatest magnitude in the LD group. In the HD group, exposure was reduced in some animals, but pharmacodynamic activity was maintained through the study.

The 26-week toxicity study was designed using only a single IV dose level of 30 mg/kg because of the ADA development and loss of pharmacodynamic activity observed at the LD and MD in the 13-week study, cynomolgus monkeys received weekly doses of 0 or 30 mg/kg ublituximab. No recovery period was included in the study design. One female from the dosed group was found dead on SD50. The death was considered immune-mediated, and this animal was positive for ADAs; low levels of test article and loss of PD activity were also noted. No other unexpected test article-related effects were observed in surviving animals. ADA development was observed in most animals that received Ublituximab, but exposure and PD activity were maintained. As expected, significant depletion of B lymphocytes (100% relative to baseline) was observed, with no significant effects on levels of total T lymphocytes, NK cells, T-cytotoxic lymphocytes, or T-helper lymphocytes. Histopathology showed reductions of B cell populations in lymphoid tissues. Also noted were signs of immune-mediated response, including immune cell infiltration in multiple organs and signs of perivascular inflammation. These findings were the result of an immunogenic response to the test article and were expected based the same finding observed in other toxicology studies. Since only one dose level of ublituximab was administered, no NOAEL was identified.

Reproductive toxicology

Because ublituximab is not pharmacologically active in rodents, an ePPND study was conducted in cynomolgus monkey to evaluate the potential effects of ublituximab on embryofetal and postnatal development. Pregnant monkeys received weekly IV injections of ublituximab at doses of 0 or 30 mg/kg. Due to the expected immunogenicity of this drug in monkeys, a unique design was applied to allow dosing of confirmed pregnant females through each trimester of gestation. Separate groups of 16 pregnant monkeys per group were dosed during the first (DG20 to GD 63, Set 2A), second (GD70 to GD112, Set 2B), and third (GD119 to 161, Set 2C) trimester. Each group was expected to receive 7 total doses. Due to very high immunogenic response, dosing of Sets 2B and 2C was terminated early. Only Set 2A received the full 7 doses. Four adults from Set 2B received 6-7 doses, and two animals from Set 2C received 3 doses each. (The remaining 13 animals from Set 2C were returned to the colony.)

An unusually high rate of early death in pregnant females was observed. The deaths were considered to be due to immune-related toxicity and not directly due to ublituximab. Ten were euthanized early or found dead (3 from Set 2A and 7 from Set 2B), all due to immune-mediated toxicity. (One control was euthanized on PND1 due to moribund condition. The cause of death was not identified.) Due to the high rate of early loss of pregnant females, a high rate of fetal abortion and stillborn infants, very few offspring were available for evaluation: 14 from the control group, 7 from Set 2A, 3 from Set 2B, and 0 from Set 2C.

Seven infants from Set 2A and 1 from Set 2B survived the 6-month postnatal period and showed no adverse effects related to ublituximab.

Of the 4 infants born to Set 2B adults, 3 were euthanized (one due to premature delivery and 2 due to abnormal appearance). The single infant from Set 2C was delivered prematurely and euthanized due to poor condition. Abnormal findings consisted of contractures and abnormal flexion of multiple limbs and tail, lack of grasp reflex, shortened mandible, elongated calvarium, enlarged ears, and muscle atrophy. Macroscopic internal findings noted were deepened sulci and narrow gyri, bilateral ventricular enlargement, which were correlated with microscopic findings of degeneration/necrosis, (graded moderate). These findings were considered to be related to ublituximab.

No juvenile animal study has been conducted. Due to the known immunogenicity of ublituximab in monkeys, such a study may not be feasible.

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/s/

BARBARA J WILCOX
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