CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125388Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Brentuximab vedotin (ADCETRIS)

Date: August 8, 2011
To: File for BLAs 125388 and 125399
From: John K. Leighton, PhD, DABT
Associate Director for Pharmacology/Toxicology
Office of Oncology Drug Products

John K. Leighton
8-8-2011

I have examined pharmacology/toxicology supporting review and labeling provided by Dr. Ouyang and the supervisory memorandum provided by Dr. Saber. I concur with their conclusions that Adcetris may be approved for the proposed indication and that no additional nonclinical studies are needed.

MEMORANDUM

Date: August 5, 2011
From: Haleh Saber, Ph.D.
Pharmacology/Toxicology Supervisor
Re: Approvability for Pharmacology and Toxicology
BLA: 125388 and 125399
Drug: Brentuximab vedotin (ADCETRIS)
Indication: Treatment of patients with Hodgkin lymphoma (BLA125388) and systemic anaplastic large cell lymphoma (BLA125399)

Brentuximab vedotin is an antibody-drug conjugate (ADC). The antibody is a chimeric IgG1 directed against CD30 antigen. The small molecule, monomethyl auristatin E (MMAE), is a microtubule disrupting agent. A protease-cleavable linker covalently attaches MMAE to the antibody. Brentuximab vedotin is indicated for treatment of patients with Hodgkin lymphoma (HL) who have relapsed after autologous stem cell transplant, and for treatment of relapsed or refractory systemic anaplastic large cell lymphoma (sALCL). CD30 is expressed on the surface of activated lymphocytes. It is known that HL and ALCL are associated with high levels of CD30 expression. In pharmacology studies, brentuximab vedotin caused cell death in CD30-positive cells. The free MMAE was toxic to cells regardless of CD30 expression status. Based on the specific targeting of the antibody moiety and the conjugation to a small molecule drug, the pharmacologic class assigned to this drug is "CD30-directed antibody-drug conjugate". The pharmacologic class of ADCs may be re-visited in future when more ADCs are approved. The assigned pharmacologic class is presently consistent with those assigned to antibodies targeting a specific CD antigen (e.g. Campath, Arzerra, and Rituxan).

Pharmacology, safety pharmacology, pharmacokinetic/ADME (absorption, distribution, metabolism, and excretion), and toxicology studies were conducted with either brentuximab vedotin or with MMAE, as appropriate. Cynomolgus monkey was considered an appropriate species for toxicology studies based on pharmacology and tissue cross-reactivity studies showing binding of the ADC to CD30-positive cells in monkeys and humans, with similar binding affinities. The main adverse finding in monkeys consisted of toxicity to the hematopoietic system, including bone marrow hypocellularity, lymphoid depletion, and neutropenia. Considering that the drug does not bind to the target cells in rats, toxicities observed in rats may be considered MMAE-related. In rats, toxicities were mainly to the hematopoietic, hepatobiliary, and male reproductive systems.

Safety pharmacology studies did not indicate brentuximab vedotin to be a cardiovascular toxicant.

Based on Section 6.1 of the label, the most common (≥20%) adverse reactions in patients include the following: neutropenia, anemia, thrombocytopenia, peripheral sensory neuropathy, nausea and vomiting, and upper respiratory infection. Toxicities to the

hematopoietic systems and GI tract were observed in animals. Neurotoxicity was not evident in animals; however, this effect is commonly observed with other microtubule disrupting agents.

MMAE was genotoxic in the rat bone marrow micronucleus study through an aneugenic mechanism. This effect is consistent with the pharmacologic activity of MMAE as a microtubule disrupting agent. The negative genotoxicity finding in the Ames (bacterial) assay was expected, due to the lack of eukaryotic microtubule structures in the bacterial system. The genotoxicity findings are consistent with those reported for other microtubule disrupting agents.

A designated fertility study was not conducted with brentuximab vedotin or MMAE. However, results of repeat-dose toxicity studies in rats indicate the potential for brentuximab vedotin to impair fertility in males. Weekly i.v. dosing in rats for 4 weeks resulted in seminiferous tubule degeneration, Sertoli cell vacuolation, reduced spermatogenesis, or aspermia. These effects appear to be related to the MMAE moiety. In general, systemic exposure (i.e. AUC) was a good predictor of toxicities in animals; therefore, animal-to-human comparisons based on AUC may be more appropriate. In the label; however, the animal-to-human ratios (Section 13.1) is reported based on the body weight as the AUC of brentuximab vedotin was not reported in the 4-week rat toxicity study.

In an embryo-fetal developmental study, pregnant rats were given 2 doses of brentuximab vedotin; doses were administered intravenously on gestation Days 6 and 13. Drug-related toxicities included pre-/post-implantation loss and embryo-fetal lethality or malformations. Effects were observed mainly at systemic exposures (AUCs) comparable to those reported for patients at the recommended dose of 1.8 mg/kg, when treated every 3 weeks.

Overall, toxicities associated with brentuximab vedotin are similar to what is generally observed with microtubule disrupting agents and are acceptable for the proposed indications based on the benefit: risk consideration. The nonclinical studies are reviewed in detail by Dr. Yanli Ouyang and findings are summarized in sections 1.2 (Brief Discussion of Nonclinical Findings) and 11 (Integrated Summary and Safety Evaluation) of the BLA review.

Recommendation: The non-clinical studies conducted with brentuximab vedotin and/or MMAE support the use of this drug for the proposed indications. There are no pharmacology/toxicology issues to preclude approval of brentuximab vedotin for the proposed patient populations at this time.

H. Salim
8/5/2011

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:	125388/125399
Supporting document/s:	0
Applicant's letter date:	28-Feb-2011
CDER stamp date:	28-Feb-2011
Product:	Brentuximab vedotin (ADCETRIS)
Indication:	Treatment of patients with Hodgkin lymphoma (BLA125388) and systemic anaplastic large cell lymphoma (BLA125399)
Applicant:	Seattle Genetics, Inc
Review Division:	Division of Hematology Products (DHP)
Reviewer:	Yanli Ouyang, MD, PhD, DABT
Supervisor/Team Leader:	Haleh Saber, PhD
Division Director:	Ann Farrell, MD
Project Manager:	Lara Akinsanya, M.S.

Yanli Ouyang
H. Saber
8/5/2011

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of BLAs125388 and 125399 are owned by Seattle Genetics, Inc or are data for which Seattle Genetics, Inc has obtained a written right of reference. Any information or data necessary for approval of BLAs125388 and 125399 that Seattle Genetics, Inc does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Seattle Genetics, Inc does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of BLAs125388 and 125399

TABLE OF CONTENTS

Table of Tables*

*All tables are excerpted from the submission unless otherwise stated.

Table of Figures*

*All figures are excerpted from the submission unless otherwise stated.

1 Executive Summary

Pharmacology and toxicology studies with brentuximab vedotin and/or MMAE were conducted according to ICHS9 and are considered adequate. Toxicities such as hematotoxicity, genotoxicity, and reproductive and developmental toxicities are consistent with those observed with microtubule disrupting cytotoxic agents.

There are no pharmacology/toxicology issues at this time that will preclude the approval of brentuximab vedotin for the proposed indications. An approval for this BLA is recommended from the pharmacology/toxicology perspective.

1.1 Recommendations

1.1.1 Approvability

Approval

1.1.2 Additional Non Clinical Recommendations

None

1.1.3 Labeling

The following pharmacologic class has been assigned to ADCETRIS: CD30-directed antibody-drug conjugate. This information is presented in HIGHLIGHTS section of the PLR label, under "INDICATIONS AND USAGE".

Applicant proposed labeling	FDA revised labeling
Warnings and Precautions • Use in Pregnancy (b) (4)	Warnings and Precautions • Use in pregnancy: Fetal harm can occur. Pregnant women should be advised of the potential hazard to the fetus (5.4).

USE IN SPECIFIC POPULATIONS----------	USE IN SPECIFIC POPULATIONS----------
[redacted] (b) (4)	[redacted] (b) (4)
[redacted] (b) (4)	**5.4 Use in Pregnancy** There are no adequate and well-controlled studies of ADCETRIS in pregnant women. However, based on its mechanism of action and findings in animals, ADCETRIS can cause fetal harm when administered to a pregnant woman. Brentuximab vedotin caused embryo-fetal toxicities, including [redacted] (b) (4) and malformations, in animals at maternal exposures that were similar to human exposures at the recommended doses for patients with HL and sALCL. If this drug is used during pregnancy, or if the patient becomes pregnant while receiving the drug, the patient should be apprised of the potential hazard to the fetus *[see Use in Specific Populations (8.1)]*.
8.1 Pregnancy Pregnancy Category D [*see Warnings and Precautions (5.6)*]. [redacted] (b) (4)	**8.1 Pregnancy** Pregnancy Category D *[See 'Warnings and Precautions (5.4)].* There are no adequate and well-controlled studies with ADCETRIS in pregnant women. However, based on its mechanism of action and findings in animals, ADCETRIS can cause fetal harm when administered to a pregnant woman. Brentuximab vedotin caused embryo-fetal toxicities in animals at maternal exposures that were similar to human exposures at the recommended doses for patients with HL and sALCL. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus.

(b) (4)	In an embryo-fetal developmental study, pregnant rats received 2 intravenous doses of 0.3, 1, 3, or 10 mg/kg brentuximab vedotin during the period of organogenesis (once each on Pregnancy Days 6 and 13). Drug-induced embryofetal toxicities were seen mainly in animals treated with 3 and 10 mg/kg of the drug and included increased early resorption (≥99%) (b) (4) post-implantation loss (≥99%), decreased numbers of live fetuses, and fetal malformations (e.g., umbilical hernia and malrotated hindlimbs). Systemic exposure in animals at the brentuximab vedotin dose of 3 mg/kg is approximately the same as exposure in patients with HL and sALCL who received the recommended dose of 1.8 mg/kg every three weeks.

12.1 **Mechanism of Action** (b) (4)	12.1 **Mechanism of Action** Brentuximab vedotin is an antibody-drug conjugate (ADC). The antibody is a chimeric IgG1 directed against CD-30. The small molecule, MMAE, is a microtubule disrupting agent. MMAE is covalently attached to the antibody via a linker. Nonclinical data suggest that the anticancer activity of ADCETRIS is due to the binding of the ADC to CD30-expressing cells, followed by internalization of the ADC-CD30 complex, and the release of MMAE via proteolytic cleavage. Binding of MMAE to tubulin disrupts the microtubule network within the cell, subsequently inducing cell cycle arrest and apoptotic death of the cells.
13.1 **Carcinogenesis, Mutagenesis, and Impairment of Fertility** (b) (4)	**13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility** Carcinogenicity studies with brentuximab vedotin or the small molecule (MMAE) have not been conducted. MMAE was genotoxic in the rat bone marrow micronucleus study through an aneugenic mechanism. This effect is consistent with the pharmacologic effect of MMAE as a microtubule disrupting agent. MMAE was not mutagenic in the bacterial reverse mutation assay (Ames test) or the L5178Y mouse lymphoma forward mutation assay. Fertility studies with brentuximab vedotin or MMAE have not been conducted. However, results of repeat-dose toxicity studies in rats indicate the potential for brentuximab vedotin to impair male reproductive function and fertility. In a 4 week repeat-dose toxicity study in rats with

(b) (4)	weekly dosing at 0.5, 5 or 10 mg/kg brentuximab vedotin, persistent seminiferous tubule degeneration, Sertoli cell vacuolation, reduced spermatogenesis or aspermia were observed. Effects in animals were seen mainly at 5 and 10 mg/kg of brentuximab vedotin. These doses are approximately 3 and 6-fold the human recommended dose of 1.8 mg/kg, respectively, based on body weight.

1.2 Discussion of Nonclinical Findings

Pharmacodynamics

Brentuximab vedotin (SGN-35) is an antibody-drug conjugate (ADC). The antibody is a chimeric IgG1 directed against CD30 and the small molecule, MMAE, is a microtubule disrupting agent. CD30 is a diagnostic marker for HL and is also highly expressed on subsets of NHL including ALCL. Binding studies demonstrated that SGN-35 bound to human and monkey CD30-positive cells but not murine CD30-expressing cells. Nonclinical studies demonstrated that binding of the SGN-35 to CD30-expressing cells initiated internalization of the SGN-35-CD30 complex, which was then trafficked to the lysosomal compartment, followed by MMAE release via proteolytic cleavage. MMAE inhibited microtubule polymerization with an activity comparable to that of vinblastine and disrupted the intracellular microtubule network. SGN-35 induced cell cycle arrest (G2/M phase cell cycle accumulation and sub-G0/G1 events), apoptosis, and cytotoxicity in CD30-positive cells but not in CD30-negative cells while MMAE produced the effects on both CD30-positive and CD30-negative cells, indicating CD30 targeting nature of SGN-35. SGN-35-mediated cytotoxicity was not observed in one CD30-positive cell line, which had lower intracellular MMAE concentration, suggesting the role of intracellular MMAE. SGN-35 treatment significantly delayed tumor growth in tumor xenograft models in a dose-dependent manner and in a tumor xenograft-related manner with the effect on ALCL Karpas 299 > HL L540cy > HL L428.

Toxicity

General toxicity

SGN-35 bound to human and monkey CD30-positive cells with similar affinity but did not bind to murine CD30-expressing cells. Therefore, monkey is considered a more appropriate animal species for the general toxicity studies. As expected for this class of drugs, main toxicities were dose-related hematological toxicity especially neutropenia, which led to premature deaths/sacrifices in the high dose group with white cell counts as low as 10/mcL. The decrease in white cell counts was more pronounced after the first dose and was at least partially recovery during the dosing phase and the recovery phase. Hematological changes correlated with histopathology findings of bone marrow hypocellularity and lymphoid depletion in thymus and spleen. A steep dose-response curve was evident as severe toxicities were observed in the 6 mg/kg group while toxicities were limited in the 3 mg/kg group.

Neurotoxicity consisting of peripheral sensory or motor neuropathy was the main adverse reaction observed in clinical trials. Similarly, neurotoxicity has been observed with other microtubule inhibitors. Only transient (on Days 10-14 only after the first dose, normal after this episode) lameness of hands was noted in one monkey (approximate 6%) given 6 mg/kg SGN-35.

Because SGN-35 did not bind to murine CD30-expressing cells, toxicities in rats may be considered MMAE-related. Drug-related hepatobiliary toxicities were noted in rats at the highest dose (10 mg/kg SGN-35) tested in a 4-week toxicity study. In these animals, significantly increased liver enzymes (≥ 3 folds) and total bilirubin (4-fold) were observed along with minimal coagulative necrosis. Considering that SGN-35 did not bind to murine CD30-expressing cells, hepatotoxicity in rats may be secondary to high levels of unbound ADC in the circulation and subsequent uptake by the liver.

Reproductive and developmental toxicity

Although fertility studies with SGN-35 or MMAE were not conducted, dose-related persistent seminiferous tubule degeneration, Sertoli cell vacuolation, reduced spermatogenesis, and aspermia were observed in a 4-week repeat-dose rat toxicity study with a weekly dosing regimen. In addition, dose-related decrease in testis weight and size and/or soft testis was noted. The adverse effects were seen at 5 and 10 mg/kg doses of SGN-35; approximately 3- or 6-fold the recommended human dose of 1.8 mg/kg, respectively, on the basis of body weight.

In an embryofetal toxicity study, SGN-35 was administered to rats twice during the period of organogenesis (once each on Pregnancy Days 6 and 13). Drug-related effects included increased early resorption, pre-implantation and post-implantation loss, decreased numbers of live fetuses, and fetal external malformations (e.g., umbilical hernia and malrotated hindlimbs). Embryofetal toxicities occurred at approximately the same level of brentuximab vedotin exposure (i.e. AUC) as in patients receiving the recommended dose of 1.8 mg/kg once every three weeks. At the dose level of 3 mg/kg, SGN-35 administration produced 99.4 % post-implantation loss and no viable fetuses in 92% (22/24) of dams. At 10 mg/kg, there was no viable fetus in all 25 dams.

Administration of MMAE produced similar toxicities but with a lower incidence. This appeared to be due to the differences in the pharmacokinetics of MMAE when it was free or conjugated, as conjugation resulted in continuous systemic exposure to MMAE.

Genetic toxicity

Standard genetic toxicity studies were conducted using MMAE. MMAE was not mutagenic in the bacterial reverse mutation assay and the L5178Y mouse lymphoma forward mutation assay. MMAE induced micronucleus formation via an aneugenic mechanism in rat bone marrow micronucleus study, which was consistent with the expected effect of MMAE as a microtubule disrupting agent.

2 Drug Information

2.1 Drug

2.1.1 CAS Registry Number (Optional)

2.1.2 Generic Name

brentuximab vedotin

2.1.3 Code Name

SGN-35 for brentuximab vedotin, the antibody-drug conjugate
cAC10-vc-MMAE for brentuximab vedotin
SGN-30 or cAC10 for the antibody
SGD-1010 for the small molecule, monomethyl auristatin E (MMAE)

2.1.4 Chemical Name

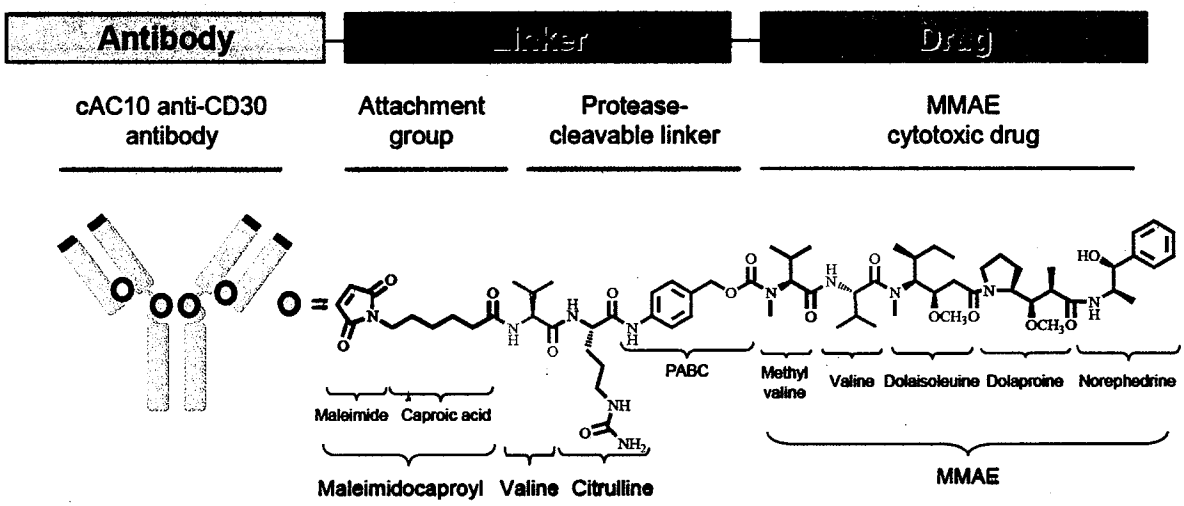
Not applicable.

2.1.5 Molecular Formula/Molecular Weight

Approximately 153 kDa

2.1.6 Structure

Figure 1. Structure of Brentuximab Vedotin



Brentuximab vedotin ADC is comprised of three parts: 1) Antibody - the cAC10 chimeric anti-human CD30 monoclonal antibody (IgG1); 2) Linker - a protease-cleavable linker comprised of a maleimidocaproyl attachment group, a valine-citrulline dipeptide, and a p-aminobenzylcarbamate (PABC) spacer; and 3) Drug – monomethyl auristatin E (MMAE), a penta-peptide consisting of methyl valine (MeVal), valine (Val), Dolaisoleuine (DIL), Dolaproine (DAP), and Norephedrine (E). MMAE was linked to the antibody via a thioether.

2.1.7 Pharmacologic class

CD30-directed antibody-drug conjugate

2.2 Relevant IND/s, NDA/s, and DMF/s

IND 71634

2.3 Clinical Formulation

2.3.1 Drug Formulation

ADCETRIS (brentuximab vedotin) will be supplied as a sterile, white or off-white, lyophilized cake or powder in single-use vials. Following reconstitution with 10.5 mL Sterile Water for Injection, USP, the solution will contain 5 mg/mL brentuximab vedotin, 70 mg/mL trehalose dihydrate, 5.6 mg/mL sodium citrate dihydrate, 0.21 mg/mL citric acid monohydrate, and (b) (4) polysorbate 80. The pH will be approximately 6.6. The quantitative composition of the DP is summarized in Table 1 .

Table 1 Quantitative composition of SGN-35 DP (per unit dose)

Component	Quantity per vial	Function	Quality Standard
Brentuximab vedotin	[redacted]	(b) (4)	NA
Citric acid monohydrate			USP, EP, JP
Sodium citrate dihydrate			USP, EP, JP
(b) (4) Trehalose dihydrate			USP/NF,EP
Polysorbate 80			USP/NF, EP, JP

2.3.2 Comments on Novel Excipients

None.

2.3.3 Comments on Impurities/Degradants of Concern

None.

2.4 Proposed Clinical Population and Dosing Regimen

Proposed Clinical Population:

- Patients with relapsed or refractory Hodgkin lymphoma (HL)
- Patients with relapsed or refractory systemic anaplastic large cell lymphoma (sALCL)

Dosing Regimen:

1.8 mg/kg administered as an intravenous infusion over 30 minutes once every 3 weeks until disease progression or unacceptable toxicity

2.5 Regulatory Background

Pre-BLA meetings on August 12, 2010, November 18, 2010 and December 7, 2010.

3 Studies Submitted

3.1 Studies Reviewed

Primary Pharmacology

1. Binding of SGN-30 and SGN-35 to HCD30 Presented Karpas 299 cells (Non-GLP, Study Number: TRN-0411-B)
2. Evaluation of the Binding of cAC10 and SGN-35 to activated human and cynomolgus lymphocytes (Non-GLP, Study Number: TRN-1435)

3. Evaluation of the Binding of cAC10 and SGN-35 to rat and mouse CD 30 by flow cytometry (Non-GLP, Study Number: TRN-1355)
4. Evaluation of Cross-Reactivity of SGN-30 with Human and Monkey (Cynomolgus) Tissues (GLP with exceptions, Study Number: 01-130)
5. Cross-Reactivity Study of Biotin-Conjugated SGN-35 with Normal Human Tissues and Normal Sprague-Dawley Rat Liver Tissues (GLP with exceptions, (b) (4) Study Number: IM1299)
6. Immunofluorescence imaging of SGN-35 internalization and lysosomal trafficking (Non-GLP, Study Number: TRN-1309)
7. MMAE is Released from SGN-35 by Cathepsin B and Lysosomal Proteases (non-GLP, TRN-1361)
8. MMAE is the Exclusive Product of SGN-35 Cellular Metabolism (Non-GLP, Study Number: TRN-0446-B)
9. Effects of MMAE and Vinblastine on Microtubule Polymerization Using Purified
10. Neuronal Tubulin (non-GLP, Study Number: TRN-1306)
11. Immunofluorescence Microscopy Imaging of MMAE and SGN-35 Medicated Effect on Microtubule Organization in CD30-positive Carcinoma Cells (non-GLP, Study Number: TRN-1357)
12. Cell Cycle Arrest and Apoptosis of CD30-positive Cells in Response to SGN-35 (Non-GLP, Study Number: TRN-1307)
13. Cytotoxic Activity of SGN-35 and MMAE against CD30-expressing Cell Lines (Non-GLP, Study Number: TRN-1363-A).
14. Effects of Three Metabolites of MMAE on the Viability of Normal Bone Marrow and CD30+ Tumor Cells (non-GLP, Study #: TRN-1201-A)
15. Evaluation of SGN-35 Mediated Antibody-Dependent Cellular Phagocytosis (ADCP), Antibody-Dependent Cellular Cytotoxicity (ADCC), and Complement-Dependent Cytotoxicity (CDC) (non-GLP, Study Number: TRN-1329)
16. Antitumor Activity of SGN-35 in the L428 Xenograft Model (non-GLP, Study Number: TRN-1304)
17. In Vivo Anti-tumor Activity of SGN-35 in SCID Mice Bearing Hodgkin Lymphoma
18. L540cy Tumor Xenografts (non-GLP, Study Number: TRN-1308)
19. Antitumor Activity of SGN-35 in Karpas 299 Xenograft Models (non-GLP, Study Number: TRN-1305)

Safety Pharmacology

1. Determine the dose-response relation of SGD-1010 block of hERG K+ channels heterologously expressed in Human Embryonic Kidney (HEK293) cells using the conventional whole-cell voltage clamp ((b) (4) Study 129-09-001)
2. A Safety Pharmacology Study (With Evaluation of Cardiovascular, Respiratory, and Central Nervous Systems) of SGN-35 Administered to Cynomolgus Monkeys by Intravenous Infusion (Study (b) (4) 00051)

PK/ADME

1. An 8-Week GLP Pharmacokinetic Study of SGN-35 Administered as an Intravenous Infusion to Cynomolgus Monkeys (Study 8201-470)

2. Single Dose IV Pharmacokinetic Study of SGN-35 in Sprague Dawley Rats (Study (b) (4)-08)
3. A Single Dose Pharmacokinetic Study of MMAE Following IV Administration of SGN-35 in Female Sprague Dawley Rats (Study (b) (4)-14)
4. Plasma Protein Binding Assay of MMAE by Ultracentrifugation (Study (b) (4)-0025)
5. In Vitro Evaluation of an NCE as an Inducer of Cytochrome P450 Expression in Cultured Human Hepatocytes (Study (b) (4)083016)
6. Reaction Phenotyping: Identification of Human CYP Enzymes Involved in the In Vitro Metabolism of [^{3}H]-MMAE (Study (b) (4)084006)
7. Metabolite Characterization of [^{3}H]-MMAE in Rat, Monkey, and Human Hepatocytes (Study (b) (4)084007)
8. In Vitro Evaluation of MMAE as an Inhibitor of Human Cytochrome P450 Enzymes (Study (b) (4)085021)
9. Cross-species comparison of MMAE metabolism and excretion (Study (b) (4)-SGN35-001)
10. Excretion, Mass Balance and Pharmacokinetics of Radioactivity in Spraque-Dawley Rats Following a Single Intravenous Bolus Dose of cAC10-vc-^{3}H-MMAE or ^{3}H MMAE (Study 420501)
11. Plasma Stability of cAC10-vcMMAE4 (SGN-35) (Study TRN-0512-A)

General Toxicology

6-Month Chronic Toxicity Study of SGN-35 in Cynomolgus Monkeys with a 6-Week Recovery Phase ((b) (4) Study Number 8216375)

Genetic Toxicology

1. Bacterial Reverse Mutation Assay: SGD-1010 (AA66EH.503.BTL)
2. L5178Y TK+/- Mouse Lymphoma Forward Mutation Assay with a Confirmatory Assay ((b) (4) Study Number 8204155)
3. *In Vivo* Rat Bone Marrow Micronucleus Assay ((b) (4) Study Number 8204151)

Reproductive and Developmental Toxicology

Intravenous Injection Study for Effects on Embryofetal Developmental and Toxicokinetics with SGN-35 and SGD-1010 in Rats ((b) (4) Study Number 8204397)

3.2 Studies Not Reviewed

1. *In vitro* Interaction Studies of MMAE with Human MDR1 (ABCB1/P-gp) ABC (efflux) Transporter (Study (b) (4)108004)
2. A Comparison Study of the Acute Effects of Monomethyl Auristatin E (MMAE) and MMAE-Based Antibody-Drug Conjugates Administered Intravenously in the Rat ((b) (4) Report 1019-001)
3. Single-Dose Intravenous Toxicity Study of Brentuximab Vedotin and MMAE in Sprague Dawley Rats (Seattle Genetics Report R-Tox-15)

4. Single-Dose Intravenous Kinetics of Bone Marrow Toxicity Study of Brentuximab Vedotin in Sprague Dawley Rats (Seattle Genetics Report R-Tox-17)
5. Single-Dose Intravenous Kinetics of Bone Marrow Toxicity Study of MMAE in Sprague Dawley Rats (Seattle Genetics Report R-Tox-33)
6. Single-Dose Intravenous Tolerability Study of Brentuximab Vedotin and MMAE in Cynomolgus Monkeys ((b) (4) Report 1644-167)
7. 4-Week (q1wkx4) Repeat-Dose Toxicity Study of Brentuximab vedotin, MMAE in Rats with a 2-Week Recovery (Seattle Genetics Report R-Tox-13)
8. 4-Week (q1wkx4) Repeat-Dose Intravenous Kinetics of Testicular Toxicity Study of Brentuximab Vedotin in Sprague Dawley Rats with up to a 16-Week Recovery (Seattle Genetics Report R-Tox-16)
9. Exploratory (q1wkx4, q2wkx4, and q3wkx4) Repeat-Dose Tolerability Study of Brentuximab Vedotin in Monkeys using Three Levels and Schedules ((b) (4) .163.13)
10. Exploratory (q3wkx4) Repeat-Dose Tolerability Study of Brentuximab Vedotin in Monkeys with a 4-Week Recovery (b) (4) .163.12)
11. Exploratory (q3wkx2) Repeat-Dose Tolerability Study of MMAE in Monkeys (b) (4) .163.19)
12. 4-Week (q1wkx4) Repeat-Dose Toxicity Study of Brentuximab Vedotin in Monkeys with a 2-Week Recovery ((b) (4) Report 8213-480)
13. 6-Week (q1wkx6) Repeat-Dose Toxicity Study of cAC10 in Monkeys with a 5-Week Recovery ((b) (4) Report 1151-167)
14. Exploratory Embryo-Fetal Development Study of Brentuximab Vedotin and MMAE in Rats (Seattle Genetics Report R-Tox-34)

3.3 Previous Reviews Referenced (reviewed by Dr. Haleh Saber under IND 71,634)

1. An 11-Week Repeat-Dose Intravenous Infusion Toxicity Study of SGN-35 and SGD-1010 (MMAE) in Cynomolgus Monkeys with a Five-Week Recovery Period (Study no.: (b) (4) .163.16)
2. A 4-Week Intravenous Injection Toxicity and Toxicokinetic Study of SGN-35, SGD-1010, and SGN-30 in Rats with a 4-Week Recovery ((b) (4) Number 7646-118)

4 Pharmacology

4.1 Primary Pharmacology

Brentuximab vedotin (SGN-35) is a CD30-directed antibody-drug conjugate (ADC) consisting of three components: 1) a chimeric antibody cAC10 (IgG1, SGN-30) targeting human CD30, 2) an antitubulin agent, monomethyl auristatin E (MMAE) and 3) a protease-cleavable linker that covalently attaches MMAE to cAC10.

CD30 is a member of the tumor necrosis factor receptor superfamily. Human CD30 is a type 1 glycoprotein with a molecular weight ranging from 105–120 kDa. Mature human CD30 is comprised of 577 amino acid residues, including a 365 amino acid residue extracellular domain, a 24 amino acid transmembrane segment, and a 188 amino acid cytoplasmic domain. The preprocessed form includes an additional 18 amino acid signal sequence. A soluble form of CD30 with a molecular weight of 85 kDa has been detected in the blood of patients with CD30-positive lymphomas and non-malignant autoimmune diseases.

CD30 was originally identified on Reed-Sternberg cells, the malignant cells in Hodgkin lymphoma (HL) and is recognized as a diagnostic marker for HL. CD30 is also highly expressed on subsets of non-Hodgkin lymphoma (NHL) including systemic anaplastic large cell lymphoma (sALCL) and cutaneous T cell lymphoma (CTCL), as well as on rare solid tumors such as embryonal carcinomas.

CD30 is also expressed on lymphoid cells in normal hematopoietic tissues. CD30 is mainly expressed on activated (but not on naïve or resting) lymphocytes (T, B, and natural killer cells) and weakly on activated monocytes.

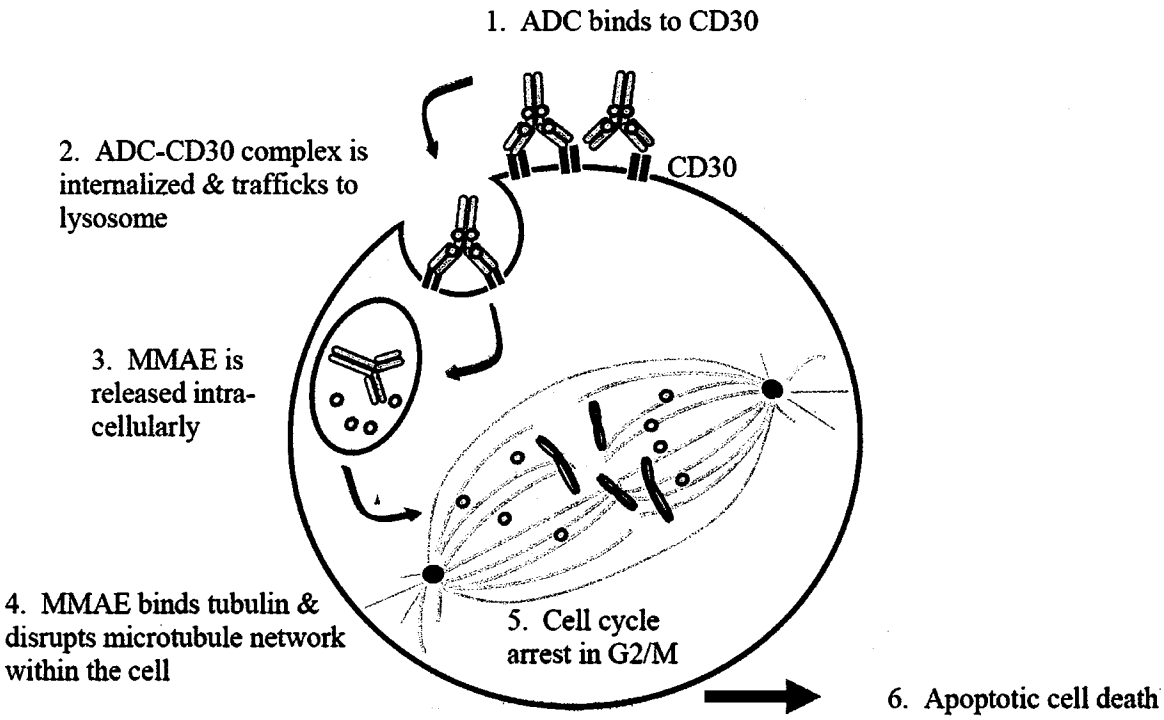
CD30 expression is absent on most normal, nonhematopoietic cells, but detected in cells of the pregnant uterus in the placental decidua and in decidual cells of the uterus in the secretory phase.

Pharmacodynamic studies were conducted in vitro using primary cells and cell lines and in vivo using xenograft models. The studies are reviewed below.

Mechanism of Action

The biological activity of brentuximab vedotin resulted from a multi-step process as proposed in Figure 2 and was confirmed in nonclinical studies (reviewed below), Binding of the ADC to CD30 on the cell surface initiated internalization of the ADC-CD30 complex, which then was trafficked to the lysosomal compartment. Within the cell, MMAE was released via proteolytic cleavage. Binding of MMAE to tubulin disrupted the microtubule network within the cell, inducing cell cycle arrest and apoptotic death of the CD30-expressing tumor cells.

Figure 2. Model of Mechanism of Action of Brentuximab Vedotin



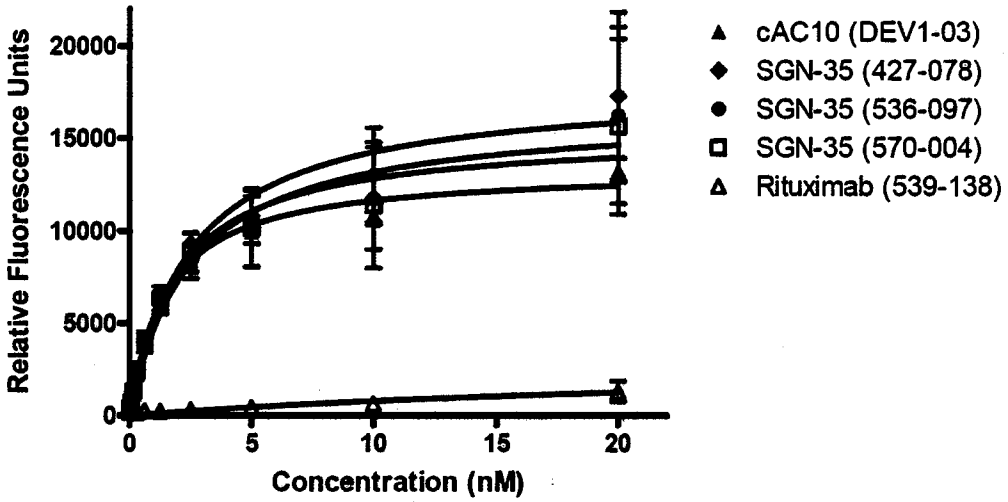
Binding studies

Binding of SGN-35 or cAC10 (SGN-30) to CD30 was assessed using the CD30-positive ALCL cell line Karpas 299 and human, monkey, and murine CD30-expressing cells. The results demonstrated that SGN-35 bound to human and monkey CD30-positive cells but not to murine CD30-expressing cells. The studies are briefly described below.

Binding of SGN-30 and SGN-35 to HCD30 Presenting Karpas 299 cells (Non-GLP, Study Number: TRN-0411-B)

Binding of SGN-35 or cAC10 (SGN-30) to human CD30 was assessed using the CD30-positive ALCL cell line Karpas 299 in this study (europium–labeling and time-resolved fluorescence spectrometry). Binding of cAC10 and SGN-35 to Karpas 299 cells appeared to be saturated at 5 nM (Figure 3). The apparent Kd for cAC10 was determined to be 1.38 ± 0.11 nM while the Kd for the 3 lots of SGN-35 ranged from 1.92 ± 0.77 to 2.64 ± 0.94 nM (Table 2).

Figure 3. Saturation Binding Curves of cAC10 and SGN-35*



* Each data point represented the mean ± standard error of the mean (SEM) of three independent experiments.

Table 2. Kd Values for SGN-35 and cAC10*

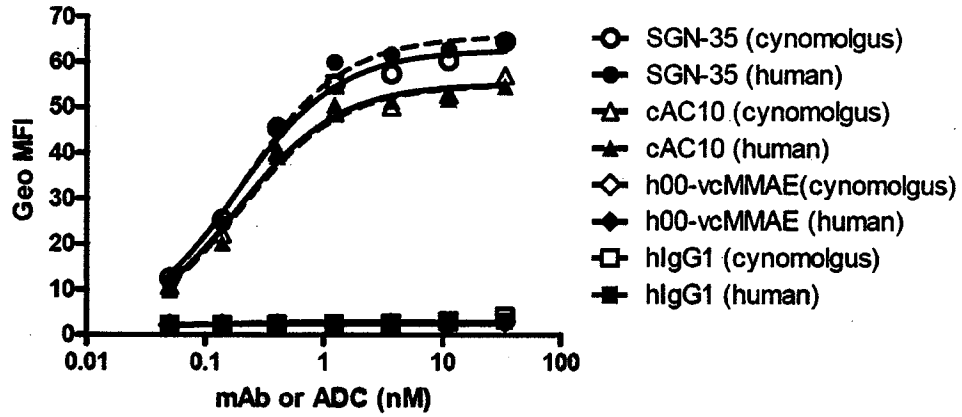
Molecule	Eu/mAb	A_{250}/A_{280} Ratio Pre-label	A_{250}/A_{280} Ratio Post Label	Mean ± SEM K_D (nM)
cAC10 (DEV1-03)	5.5	ND	ND	1.38 ± 0.11
SGN-35 (427-078)	5	0.76	0.79	2.64 ± 0.94
SGN-35 (536-097)	3.5	0.68	0.76	2.51 ± 1.01
SGN-35 (570-004)	4.8	0.68	0.79	1.92 ± 0.77
Rituxan	7.5	ND	ND	ND

* Kd values were determined on Karpas 299 cells for individual curves and reported as the mean ± SEM from the three independent experiments. ND = not determined. Eu = europium

Evaluation of the Binding of cAC10 and SGN-35 to activated human and cynomolgus lymphocytes (Non-GLP, Study Number: TRN-1435)

The bindings of cAC10 or SGN-35 to activated human and cynomolgus monkey lymphocytes were evaluated using a human peripheral blood mononuclear cell (PBMC)-derived T cell line, 40H7, and cynomolgus monkey PBMC (flow cytometry). The binding affinities of cAC10 or SGN-35 were determined to be the same with apparent Kd as 0.19 ± 0.01 nM and 0.20 ± 0.01 nM for activated cynomolgus monkey and human lymphocytes, respectively.

Figure 4. SGN-35 and cAC10 Binding Curves with Monkey and Human Lymphocytes*



*Points: the mean ± SD of triplicate values.

	Kd (nM)	
ADC, mAb	**Cynomolgus lymphocytes**	**Human lymphocytes**
SGN-35	0.19 ± 0.01	0.20 ± 0.01
cAC10	0.19 ± 0.01	0.20 ± 0.01

Evaluation of the Binding of cAC10 and SGN-35 to rat and mouse CD 30 by flow cytometry (Non-GLP, Study Number: TRN-1355)

The bindings of cAC10 and SGN-35 to rat and mouse CD 30 were evaluated using a murine CD30-potive cell line, EL4, and primary activated rat and mouse T cells by flow cytometry. The staining was not detected in these murine cells but detected in a human CD30-positive cell line, WIL2-S (used as a positive staining control).

Tissue cross-reactivity studies

Two tissue cross-reactivity (TCR) studies were performed using biotinylated cAC10 and biotinylated SGN-35 on tissue panels from human and cynomolgus monkey. The studies are reviewed below.

Evaluation of Cross-Reactivity of SGN-30 with Human and Monkey (Cynomolgus) Tissues (GLP with exceptions, Study Number: 01-130)

A TCR study using biotinylated SGN-30 (cAC10, 12.5 and 2.5 mcg/mL) was conducted using normal human and cynomolgus monkey tissue panel (Table 3). CD 30 positive (Karpas-299 lymphoma cell line) and negative (Ramos) cells were used as positive and negative controls, respectively.

Table 3. Tissue Panel (three separate individuals)*

- Adrenal
- Bladder
- Blood
- Bone Marrow
- Breast
- Cerebellum
- Cerebral Cortex
- Colon
- Endothelium (various tissues)
- Eye
- Fallopian Tube
- Gastrointestinal Tract
- Heart
- Kidney (glomerulus)
- Kidney (tubule)
- Liver
- Lung
- Ovary
- Pancreas
- Parathyroid
- Pituitary
- Placenta
- Prostate
- Skin
- Spinal Cord
- Spleen
- Striated Muscle
- Testes
- Thymus
- Thyroid
- Tonsils
- Ureter
- Uterus (cervix)
- Uterus (endometrium)
- Lymph Node

* Monkey placenta was not available for analysis.

Strong staining was observed on Karpas-299 cells while no staining was seen on Ramos cells.

Specific staining (low to moderate) was observed on thyroid epithelial cells (2/3 at 2.5 mcg/mL and 3/3 at 12.5 mcg/mL) in cynomolgus monkey only. No specific staining was observed on human tissues.

Cross-Reactivity Study of Biotin-Conjugated SGN-35 with Normal Human Tissues and Normal Sprague-Dawley Rat Liver Tissues (GLP with exceptions, (b) (4) Study Number: IM1299)

A TCR study using biotinylated SGN-35 (12.5 and 2.5 mcg/mL) was conducted using normal human tissue panel (Table 4). CD 30 positive (SR human lymphoma cell line) and negative (Ramos) cells were used as positive and negative controls, respectively. In addition, TCR study was conducted using two liver tissues from two separate Sprague-Dawley rats.

Table 4. Human Tissue Panel

Human Tissue (Normal) from Three Separate Donors[1]

• Adrenal	• Lung	• Spinal Cord
• Blood Cells[2]	• Lymph Node	• Spleen
• Blood vessels (endothelium)	• Ovary	• Striated (skeletal) Muscle
• Bone Marrow	• Fallopian Tube (oviduct)	• Testis
• Brain – cerebrum (cortex)	• Pancreas	• Thymus
• Brain – cerebellum	• Parathyroid	• Thyroid
• Breast (mammary gland)	• Peripheral Nerve	• Tonsil
• Eye	• Pituitary	• Ureter
• Gastrointestinal Tract[3]	• Placenta	• Urinary Bladder
• Heart	• Prostate	• Uterus- body (endometrium)
• Kidney (glomerulus, tubule)	• Salivary Gland	• Uterus- cervix
• Liver	• Skin	

[1] Only two donors of Parathyroid were evaluated, no other donor could be acquired in a timely fashion.
[2] Blood cells include granulocytes, lymphocytes, monocytes and platelets.
[3] Gastrointestinal Tract includes the colon (large intestine), esophagus, small intestine, and stomach.

There was specific staining in mononuclear cells, hematopoietic precursor cells, spindloid cells, oviduct and bronchial epithelium (cilia), and keratin squames/debris (Table 5). In addition, SGN-35-related staining was observed in kidney tubules, lens protein and thyroid colloid in 1-2 samples (3 total).

There was no SGN-35-related staining on rat liver samples.

Table 5. Cross-Reactivity of Biotin-Conjugated SGN-35 with Human Tissues

Tissue	Source	Biotin-Conjugated SGN-35		Biotin-Conjugated IgG1, human myeloma plasma in 1X PBS		Assay Control
		12.5 µg/mL	2.5 µg/mL	12.5 µg/mL	2.5 µg/mL	
Cryosections of SR cell line (Positive Control Material)	CP	2-4+ (freq)	2-4+ (freq)	Neg	Neg	Neg
Cryosections of Ramos cell line (Negative Control Material)	CP	Neg	Neg	Neg	Neg	Neg

Bone Marrow	HT107					
Hematopoietic precursor cells (cytoplasm, cytoplasmic granules)		1-2+ (freq)	1+ (occas)	Neg	Neg	Neg
Other elements		Neg	Neg	Neg	Neg	Neg
Bone Marrow	HT553	Neg	Neg	Neg	Neg	Neg
Bone Marrow	HT555					
Hematopoietic precursor cells (cytoplasm, cytoplasmic granules)		2-4+ (freq)	1-2+ (rare to occas)	Neg	Neg	Neg
Other elements		Neg	Neg	Neg	Neg	Neg
Spindloid cells (meninges, cytoplasmic granules)		2-3+ (very rare)	1+ (very rare)	Neg	Neg	Neg
Other elements		Neg	Neg	Neg	Neg	Neg
Eye	HT568-4					
Lens protein		2-3+ (occas)	Neg	Neg	Neg	Neg
Mononuclear cells (periocular muscle, interstitial, cytoplasm, cytoplasmic granules)		2-3+ (very rare)	Neg	Neg	Neg	Neg
Kidney (glomerulus, tubule)	HT241					
Contents of tubules, Bowman's space, and >>glomerular capillary loops		2-3+ (rare to occas)	1-3+ (very rare)	Neg	Neg	Neg
Lung	HT267					
Epithelium (bronchus, surface, cilia)		1+ (occas)	Neg	Neg	Neg	Neg
Amorphous material (extracellular, intra-airway)		3+ (rare)	Neg	Neg	Neg	Neg
Fallopian Tube (oviduct)	HT848					
Epithelium (surface, apical cilia)		1-3+ (occas)	1+ (rare to occas)	Neg	Neg	Neg
Thymus	HT873					
Mononuclear cells (interstitial, cytoplasm, cytoplasmic granules)		2-3+ (occas)	1+ (rare)	Neg	Neg	Neg
Other elements		Neg	Neg	Neg	Neg	Neg
Thyroid	HT478					
Colloid		1-3+ (occas)	Neg	Neg	Neg	Neg

± = equivocal, 1+ = weak, 2+ = moderate, 3+ = strong, 4+ = intense, Neg = Negative, M = Missing, freq = frequent, occas = occasional

SGN-35 Internalization and Trafficking to Lysosomes

Immunofluorescence imaging of SGN-35 internalization and lysosomal trafficking (Non-GLP, Study Number: TRN-1309)

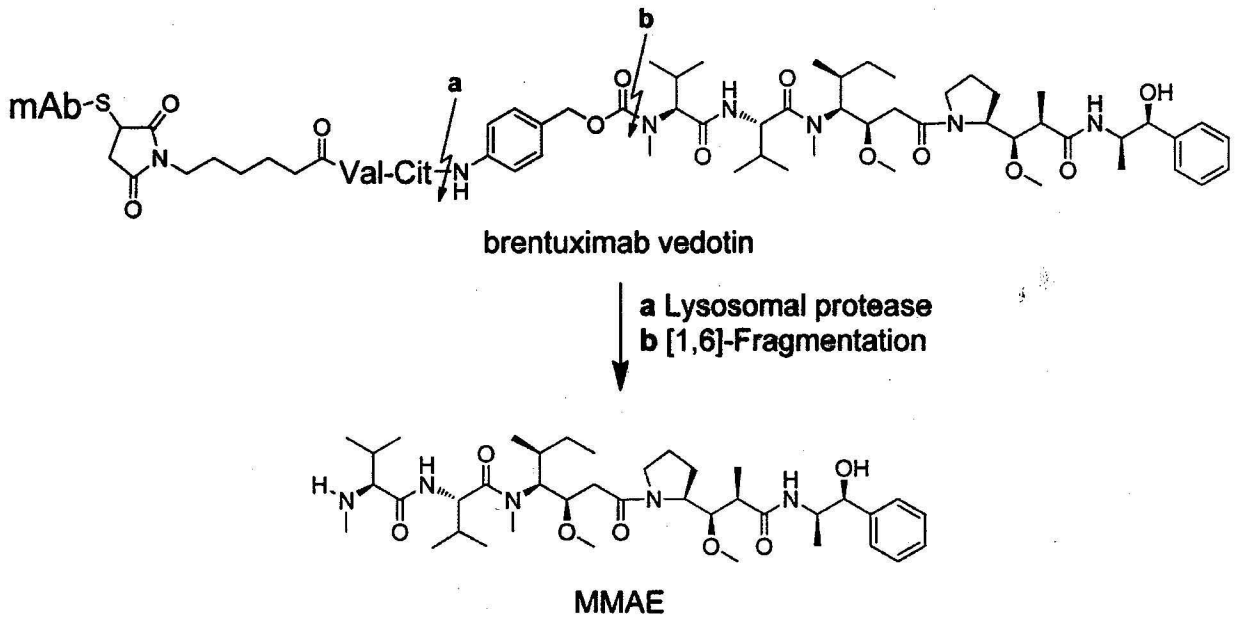
The internalization and trafficking of SGN-35 was visualized using fluorescence microscopy after binding to L540cy, a CD30-positive HL cell line. SGN-35 was detected inside L540cy cells and co-localized with Lamp-1 (a lysosomal marker) at 4 hours and increased at 16 and 24 hours while was not detected in Ramos, a CD30-negative Non-Hodgkin Lymphoma cell line.

MMAE Was Released by Cathepsin B and Lysosomal Proteases

MMAE is Released from SGN-35 by Cathepsin B and Lysosomal Proteases (non-GLP, TRN-1361)

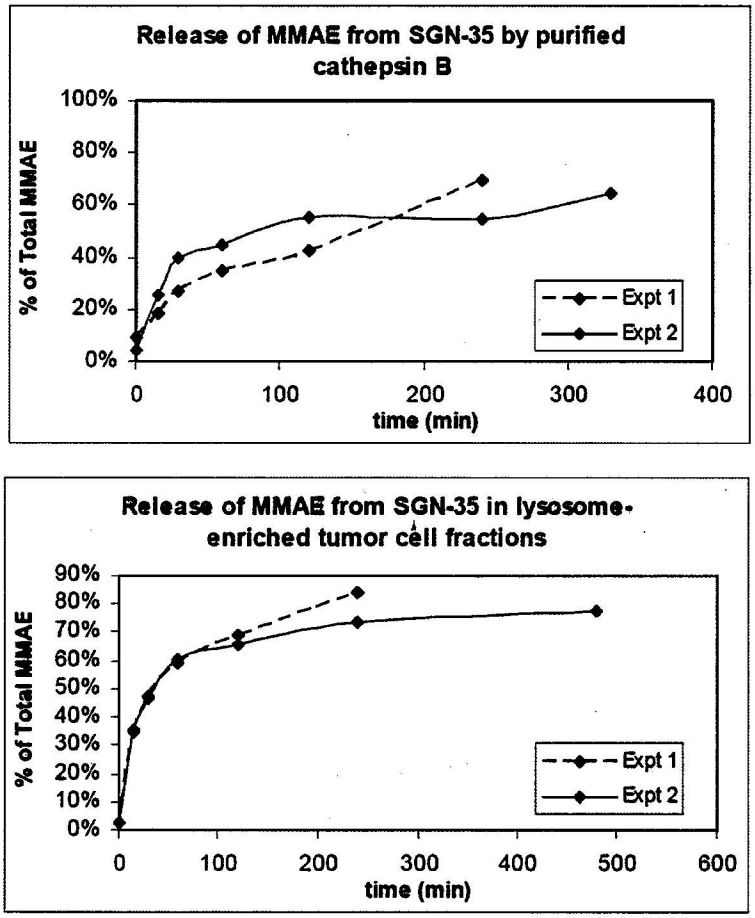
MMAE release from SGN-35 was evaluated using the purified lysosomal protease cathepsin B and lysosomal fractions purified from the human ALCL cell line Karpas 299 (Figure 5). As shown in Figure 6, MMAE was released by exposing SGN-35 to lysosomal protease. Addition of E64d, a cysteine protease inhibitor, blocked the release.

Figure 5. Proteolytic Release of MMAE from Brentuximab Vedotin*



* Lysosomal proteases cleaved at bond **a**. This cleavage was followed by spontaneous fragmentation of bond **b** to produce MMAE inside the cell.

Figure 6. MMAE Release by Lysosomal Protease*



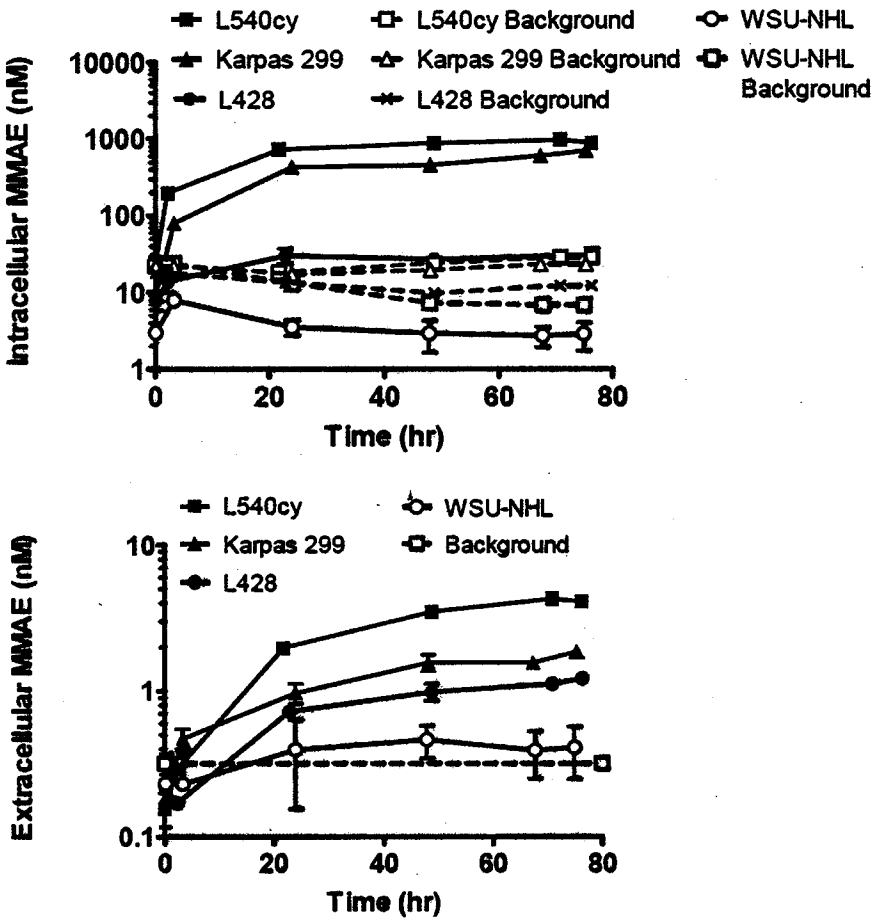
* The enzymatic release of MMAE from SGN-35 by cathepsin B and lysosome-enriched Karpas 299 cell fractions was detected over time (up to 8 hours) and quantified by LC-MS/MS.

MMAE is the Exclusive Product of SGN-35 Cellular Metabolism (Non-GLP, Study Number: TRN-0446-B)

The release of MMAE within CD30-positive cells (HL L540cy, L428 and ALCL Karpas 299 cells) after treatment with SGN-35 (containing radiolabeled [^{14}C]MMAE) was further evaluated in this study. As shown in Figure 7, increased intracellular free MMAE was detected in CD30-positive cells but not in CD30-negative WSU-NHL cells and the concentration of free MMAE was cell type-related with L540cy > Karpas 299 > L428.

In addition, the MMAE was released in a temperature- and pH-related manner. Reducing temperature from 37°C to 4 °C and increasing lysosomal pH by treating the cells with chloroquine reduced the MMAE release.

Figure 7. Distribution of [^{14}C]MMAE in Cell Culture*



* Intracellular and extracellular concentration of small molecule radioactivity detected in CD30-positive (L540cy, Karpas 299, and L428) and CD30-negative (WSU-NHL) cell cultures.

MMAE and SGN-35 Inhibited Microtubule Assembly and Disrupted the Microtubule Network in Cells

Effects of MMAE and Vinblastine on Microtubule Polymerization Using Purified Neuronal Tubulin (non-GLP, Study Number: TRN-1306)

Anti-microtubule activity of MMAE was evaluated using the CytoDYNAMIX in vitro tubulin assay. MMAE inhibited microtubule polymerization with an activity comparable to that of vinblastine (IC50: 1.00±0.50 micM for MMAE vs. 1.41±0.71 micM for vinblastine).

Immunofluorescence Microscopy Imaging of MMAE and SGN-35 Mediated Effect on Microtubule Organization in CD30-positive Carcinoma Cells (non-GLP, Study Number: TRN-1357)

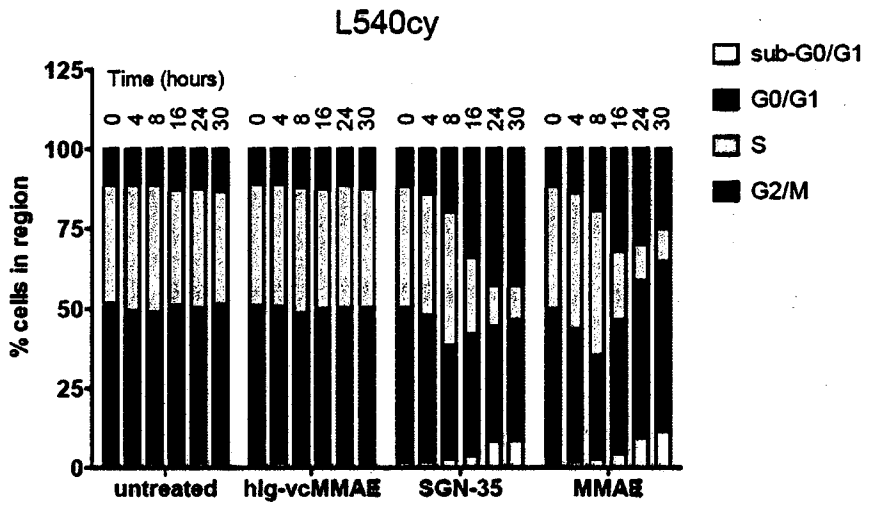
The effect of MMAE and SGN-35 on microtubules within cells was evaluated using Tera-2, a CD30-positive embryonal carcinoma cell line. Exposure of Tera-2 cells to MMAE or SGN-35 disrupted the intracellular microtubule network.

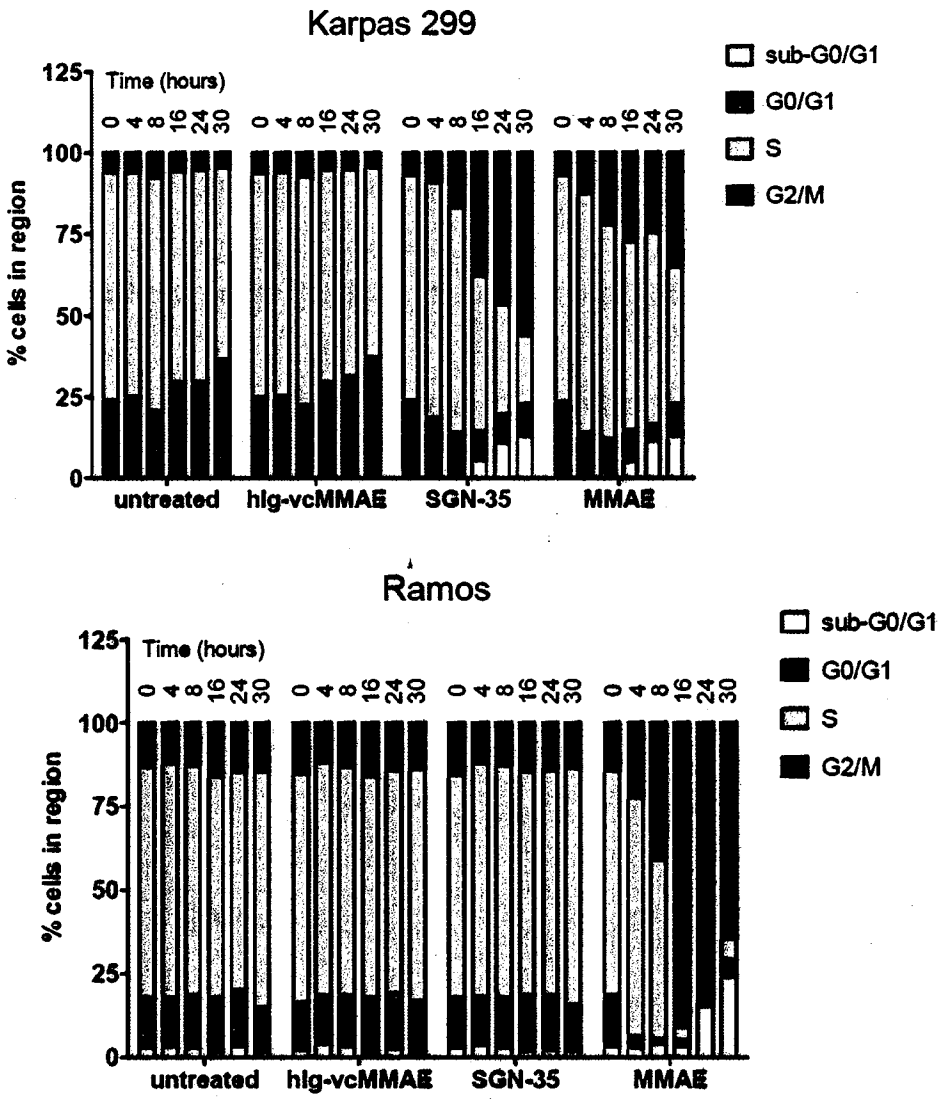
SGN-35 Induced Cell Cycle Arrest and Apoptosis

Cell Cycle Arrest and Apoptosis of CD30-positive Cells in Response to SGN-35 (Non-GLP, Study Number: TRN-1307)

Effects of SGN-35 (100 ng/mL, the approximate IC90) on the cell cycle were examined using CD30-positive HL L540cy and ALCL Karpas 299 cells and CD30-negative NHL Ramos cells. The equivalent molar concentration (2.7 nM) of MMAE was used as a comparator. SGN-35 treatment resulted in G2/M phase cell cycle accumulation and sub-G0/G1 events consistent with DNA fragmentation in CD30-positive cells (Figure 8). This effect was not observed in CD30-negative cells or in CD30-positive cells treated with a nonbinding antibody-drug conjugate control. MMAE exposure, however, produced the cell cycle arrest in G2/M, sub-G0/G1 events and apoptosis in both CD30-positive cells and CD30-negative cells, indicating CD30-targeting nature of SGN-35. These results suggest that G2/M phase cell cycle arrest is a mechanism by which SGN-35 inhibits proliferation of CD30-positive cells.

Figure 8. Effects of SGN-35 and MMAE on Cell Cycle





CD30-positive HL L540cy (top panel) and ALCL Karpas 299 (middle panel) and CD30-negative NHL Ramos (bottom panel) cells were untreated or treated with SGN-35, hIg-vcMMAE (a non-binding control ADC), or MMAE (an equimolar amount as SGN-35) and incubated for 0, 4, 8, 16, 24, or 30 hours. At each time point, the cells were labeled with EdU and Alexa Fluor647 azide to detect DNA synthesis and propidium iodide to detect total DNA content and the cell cycle position was measured by flow cytometry.

Cytotoxic Effect of SGN-35 on CD30-Positive Cells

Cytotoxic Activity of SGN-35 and MMAE against CD30-expressing Cell Lines (Non-GLP, Study Number: TRN-1363-A).

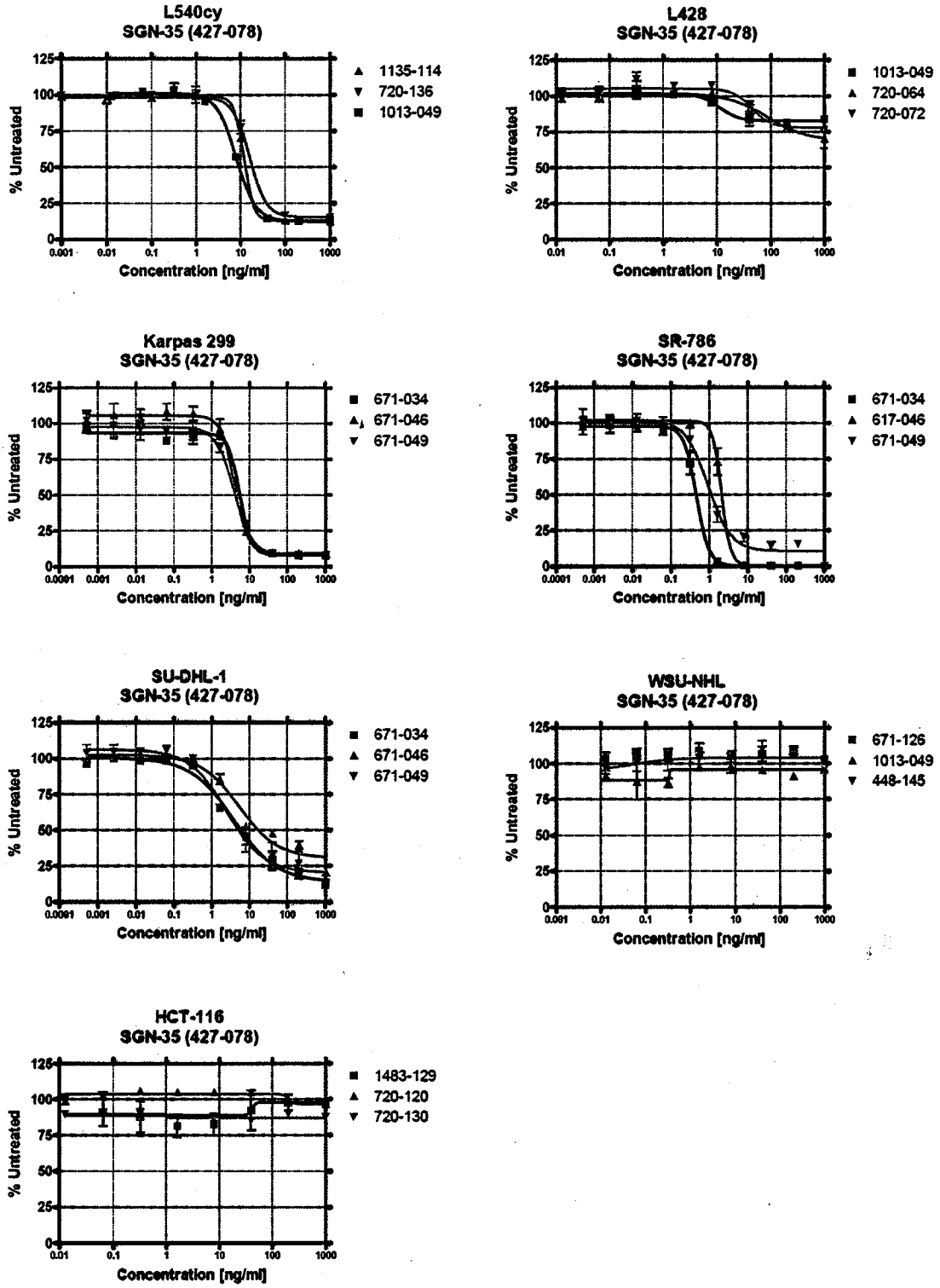
Cytotoxicity of SGN-35 was assessed by measuring the mitochondrial metabolism of the dye resazurin. Human CD30-positive cells (two HL cell lines: L540cy and L428, and three ALCL cell lines: Karpas 299, SR-786, and SU-DHL-1) and CD30-negative cells (WSU-NHL and HCT-116) were incubated with various concentrations of SGN-35 or MMAE for 96 hours.

SGN-35 mediated concentration-dependent cytotoxicity against 4 of the 5 CD30-positive cell lines tested in this study including L540cy, Karpas 299, SR-786, and SU-DHL-1 (Figure 9). The IC50 values are summarized in Table 6 and ranged from 0.4608 ng/mL (0.0031 nM) for SR-786 to 18.67 ng/mL (0.1245 nM) for L540cy. HL L428 is also a CD30-positive cell line but the cells were found to be relatively refractive to SGN-35 with cell viability about 75% at 1000 ng/mL (6.7 nM), which may be related to a lower intracellular MMAE concentration as described above.

In contrast to CD30-positive cells, SGN-35 did not affect the viability of the two CD30-negative cell lines tested in this study (Figure 9).

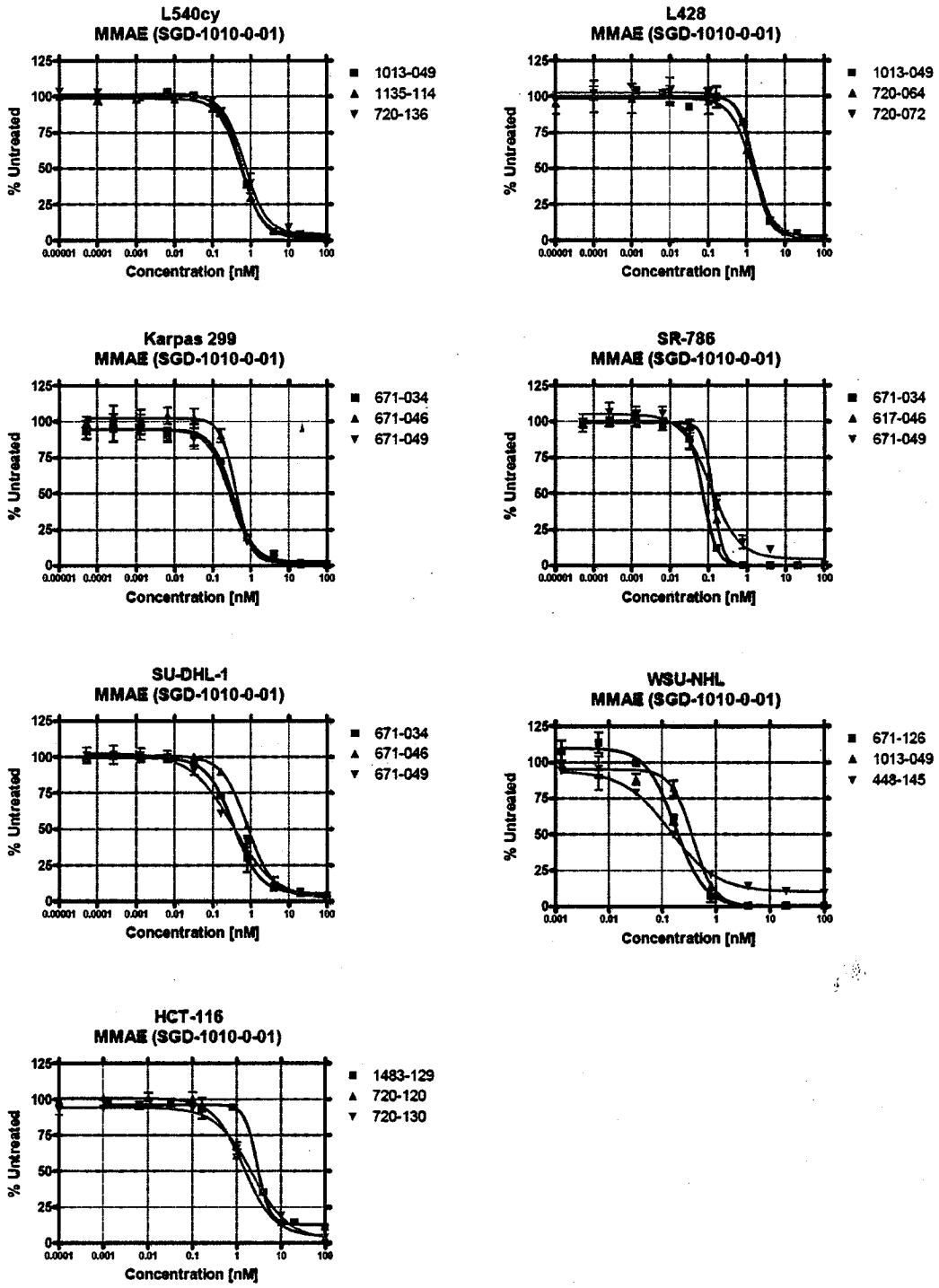
MMAE mediated concentration-dependent cytotoxicity in all cell lines regardless of CD30 expression (Figure 10). The IC50 values are summarized in Table 6 and ranged from 0.0716 nM for SR-786 to 3.111 nM for HCT-116.

Figure 9. Cytotoxicity of SGN-35



Cells were incubated with indicated concentrations of SGN-35 for 96 hours and cell viability was determined by resazurin dye conversion assay. Three independent experiments were conducted for each cell line.

Figure 10. Cytotoxicity of MMAE



Cells were incubated with indicated concentrations of MMAE for 96 hours and cell viability was determined by resazurin dye conversion assay. Three independent experiments were conducted for each cell line.

Table 6. Cytotoxicity of SGN-35 and MMAE

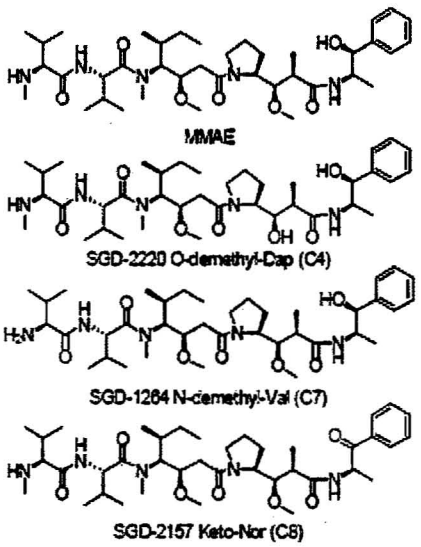
Cell Line	CD30 Expression	IC50 (nM*, Mean±SD, n=3)	
		SGN-35	MMAE
L540cy	+	0.091±0.031	0.6391±0.1091
L428	+	>6.7	1.5787±0.1511
Karpas 299	+	0.032±0.005	0.3339±0.0719
SR-786	+	0.008±0.005	0.1115±0.0348
SU-DHL-1	+	0.062±0.043	0.5367±0.2788
WSU-NHL	-	>6.7	0.2342±0.1010
HCT-116	-	>6.7	2.2553±0.4560

* Prepared by the reviewer based on the submission; IC50 in nM was calculated using 150,000 Daltons as an approximate molecular weight for SGN-35.

Effects of Three Metabolites of MMAE on the Viability of Normal Bone Marrow and CD30+ Tumor Cells (non-GLP, Study #: TRN-1201-A), reviewed by Dr. Miyun Tsai-Turton

Study Design: This study was to evaluate three MMAE (monomethyl auristatin E) metabolites (SGD-1264, SGD-2157, and SGD-2220) in *in vitro* cytotoxicity assays against a panel of $CD30^+$ tumor cell lines and normal human $CD34^+$ bone marrow cells. The cell lines used in this study included Karpas-299 ($CD30^+$ anaplastic large cell lymphoma cell line), L540cy ($CD30^+$ T-cell Hodgkin lymphoma cell line adapted for xenograft growth), L428 ($CD30^+$ B-cell Hodgkin lymphoma cell line), Donors 2121 and 1937 (primary normal human $CD34^+$ bond marrow cells from (b) (4). These cell lines were treated with MMAE and its metabolites in 96-well plates. The cell viability was measured by Alamar Blue (Invitrogen) or CelltiterGlo (Promega) and the IC_{50} was calculated.

Metabolites of MMAE



Cell Lines

Cell Line	Medium	Passage	Passage Conditions	Viability	Plating density / well for assay
L540cy	RPMI+ 20% FBS + 1% antibiotics	6 to 7	20,000 cells / ml in 50 mls	89 %	7000
Karpas	RPMI+ 10% FBS + 1% antibiotics	6 to 7	20,000 cells / ml in 50 mls	93 %	2000
L428	RPMI+ 10% FBS + 1% antibiotics	6 to 7	20,000 cells / ml in 50 mls	82 %	2000

Donor	Age	Sex	Height	Weight	Ethnicity	Cell Viability	Plating density / well for assay
1937	20	Male	68 inches	174 lbs	Caucasian	90 %	28,500
2121	24	Female	70 inches	147 lbs	Caucasian	95 %	23,300

Findings:

- Three MMAE metabolites were equal or less cytotoxic than the parent compound, MMAE. The SGD-2157 had similar cytotoxicity to MMAE whereas SGD-2220 and SGD-1264 were less cytotoxic than MMAE.
- Ranking of cytotoxic activities: SGD-1264 << SGD-2220 < SGD-2157 = MMAE

Table 7. IC_{50}. for MMAE and its Metabolites

Summary of Effects of MMAE and Metabolites on the Viability of Normal Human Bone Marrow Cells and CD30+ Tumor Cell Lines (IC50s expressed as nM)

Compounds	Notebook	Donor 2121*	Donor 1937*	Karpas 299	L540cy	L428
SGD-1264 (N-demethyl-Val (C7)-MMAE)	1625-107	> 100	> 100	31	549	1000
SGD-2157 (Keto-Nor (C8)-MMAE)	1554-073	0.39	0.75	0.01	0.2	0.6
SGD-2220 (O-demethyl-Dap (C4)-MMAE)	1554-139	4.4	8.6	0.9	6	30
MMAE	SGD-1010-0-01	0.31	0.92	0.01	0.2	0.2

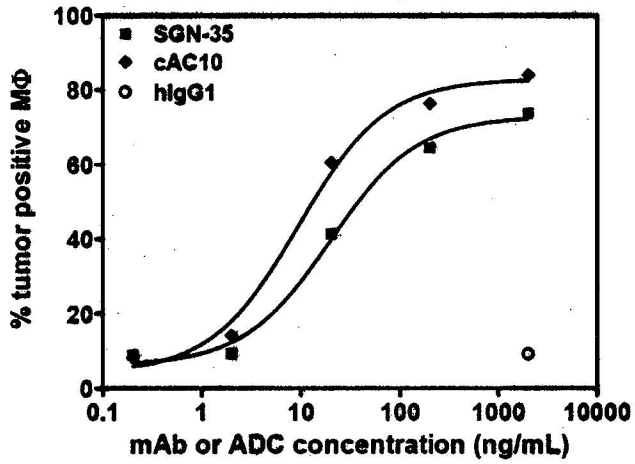
* Estimated IC50s from a 4-point dose curve.

Evaluation of SGN-35 Mediated Antibody-Dependent Cellular Phagocytosis (ADCP), Antibody-Dependent Cellular Cytotoxicity (ADCC), and Complement-Dependent Cytotoxicity (CDC) (non-GLP, Study Number: TRN-1329)

The cAC10 (SGN-30) has previously been shown to induce ADCP activity. Therefore, the ability of SGN-35 (three lots) to induce ADCP, ADCC, and CDC was evaluated in vitro. ADCP and CDC were evaluated using WIL2-S, a CD30-positive, CD20-positive EBV-transformed B lymphoblastoid cell line, and ADCC using L428 and L540cy, CD30-positive Hodgkin lymphoma cell lines. Ramos, a CD30-negative Burkitt's lymphoma cell line, was also used as CD30-negative control cells. hIgG1κ was used as a non-specific, non-binding control antibody.

Similar to cAC10, SGN-35 induced concentration-dependent ADCP of CD30-positive WIL2-S cells (Figure 11). SGN-35-coated tumor cell uptake was detected in >70% macrophages (MΦ, from cryopreserved PBMC) at the highest concentration tested. Non-specific uptake of WIL2-S cells (treated with hIgG1κ) was <20%. No SGN-35-mediated ADCP was observed in CD30-negative Ramos cells.

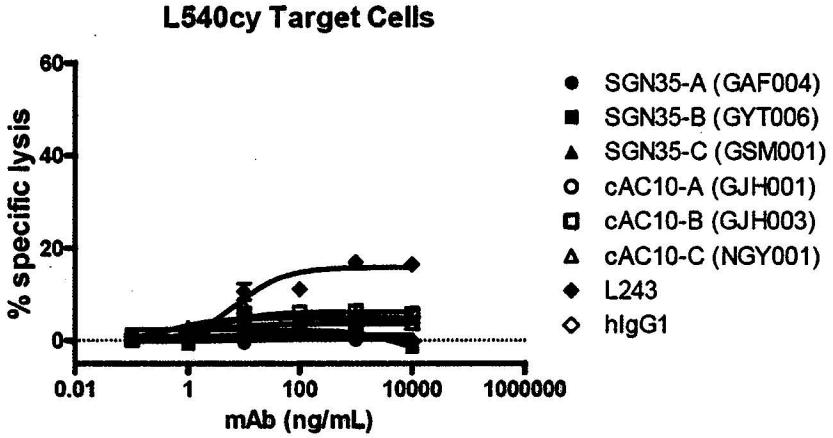
Figure 11. ADCP Assay Using WIL2-S Cells as Target Cells



ADCP assay using WIL2-S cells as target cells. PKH26 labeled target cells were treated with SGN-35, cAC10, or hIgG1κ followed by incubation with macrophages (MΦ) at a ratio of 1 MΦ to 4 target cells. Phagocytic activity was determined by calculating the percentage of total MΦ that engulfed tumor cells as measured by flow cytometry.

Neither SGN-35 nor cAC10 mediated ADCC against L428 cells. Minimal activity (≤ 11% specific lysis) was detected in assays using L540cy HL cells (Figure 12).

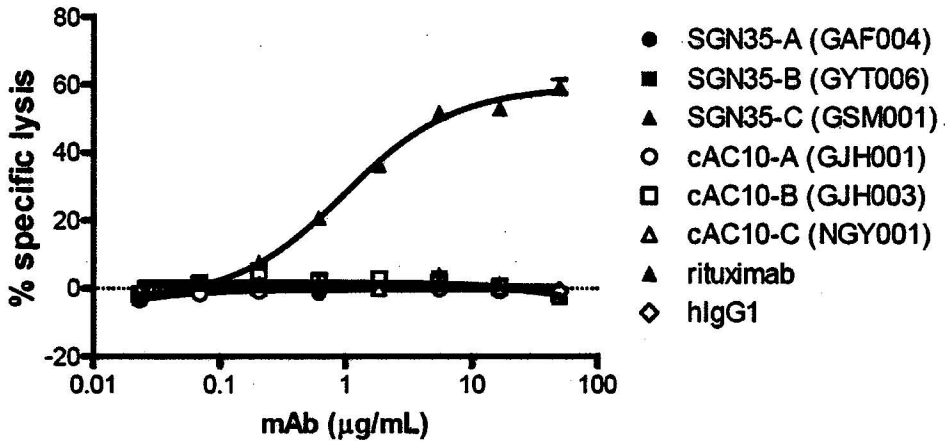
Figure 12. Effect of SGN-35 or cAC10 on ADCC



Cr-51 labeled target cells were treated with SGN-35 (three lots as SGN-35-A, B or C), cAC10, L243 (antihuman MHC class II mAb as a positive assay control) or hIgG1κ, then mixed with NK cells enriched from non-adherent PBMC at a ratio of 10 NK cells to 1 target cell. Counts per minute (CPM) were determined from released radionuclide in supernatants and used to calculate specific lysis of target cells. Data points: Mean ± SD.

SGN-35 did not induce lysis of CD30-positive WIL2-S cells in CDC assays (Figure 13).

Figure 13. Effect of SGN-35 or cAC10 on CDC Using WIL2-S target cells.



SYTOX® Green-labeled target cells were treated with SGN-35, cAC10, rituximab (as a positive control), or hIgG1κ in the presence of 10% human serum. Relative fluorescence units detected by a fluorescence microplate reader were used to calculate specific cell lysis. Data points: Mean ± SD.

In Vivo Pharmacodynamics

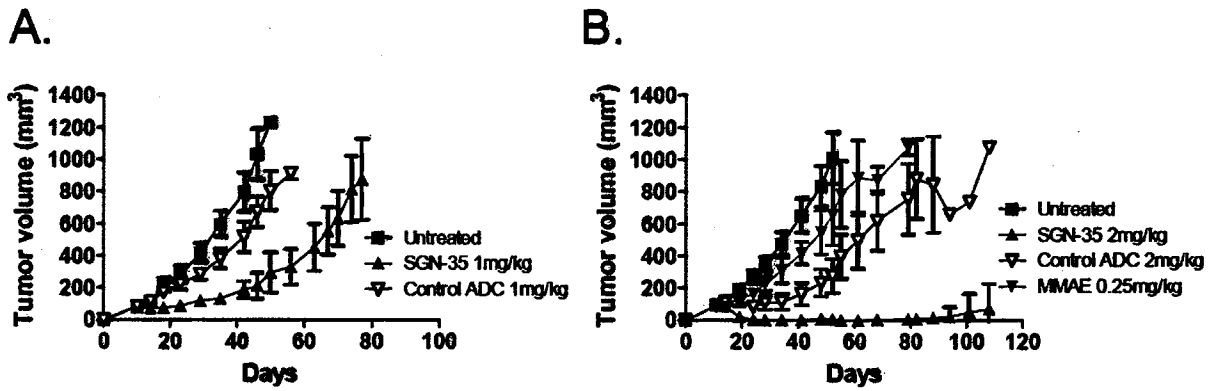
The antitumor activity of SGN-35 was evaluated using xenograft models because cAC10 did not bind to rodent CD30. The studies are reviewed below.

Antitumor Activity of SGN-35 in the L428 Xenograft Model (non-GLP, Study Number: (b) (4)-1304)

HL L428 cells (5 x 10^6) were injected subcutaneously in NSG female mice (10-13 wks). Mice (5 to 7/group) were administered SGN-35 (IP), the control ADC (IP) or MMAE (IV). The dosing was initiated when the average tumor volume of all mice was approximately 100 mm^3 (on Day 10). Mice were terminated when tumors reached approximately 1000 mm^3 or at the end of the study (Day 102).

SGN-35 treatment significantly delayed tumor growth in a dose-dependent manner (Figure 14, $p \leq 0.001$, T/C: 13% at 1 mg/kg and 0% at 2 mg/kg, % T/C=$\Delta T/\Delta C$ x 100, where ΔT and ΔC were changes in tumor volume). Tumor growth in mice treated with the control ADC (T/C: 62 % at 1 mg/kg and 20 % at 2 mg/kg) and MMAE (0.25 mg/kg, $p \leq 0.013$, T/C: 62%) was also delayed but to a lesser extent. Ultimately all mice in the control ADC and MMAE groups developed large tumors but four of five mice treated with 2 mg/kg SGN-35 had no detectable tumors at the end of the study (on Day 108).

Figure 14. Tumor Volume of NSG Mice Bearing L428 Xenografts



NSG mice were implanted subcutaneously with HL L428 cells in the right flank. The drug administration [every 4 days for 4 doses (A) or 3 doses (B)] was initiated when the tumor size for all mice averaged approximately 100 mm^3. Mice (5–7/group) were either untreated, or received SGN-35, a non-binding control ADC or free MMAE (B only). Data points represented the mean tumor volume of surviving mice. Bars represented standard deviation.

In Vivo Anti-tumor Activity of SGN-35 in SCID Mice Bearing Hodgkin Lymphoma L540cy Tumor Xenografts (non-GLP, Study Number: TRN-1308)

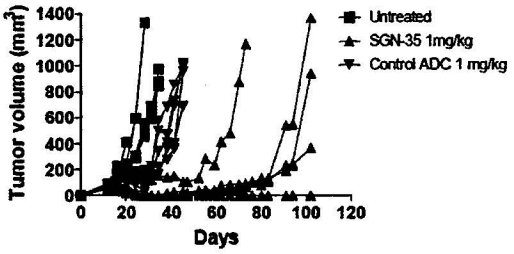
In this study, HL L540cy cells (5x10^6) were injected to female SCID mice (14 to 16 wks), the study design and conduct were the same as described above in study TRN-1304 unless indicated otherwise.

SGN-35 treatment significantly delayed tumor growth [Figure 15, T/C: 0 or 2 % at 1 mg/kg (two experiments) and 0% at 3 mg/kg]. Tumor growth in mice treated with the control ADC was also delayed but to a lesser extent [T/C: 34 or 68 % at 1 mg/kg (two experiments) and 0 % at 3 mg/kg]. Ultimately all mice in the control ADC group developed large tumors while SGN-35 induced complete and durable tumor regressions

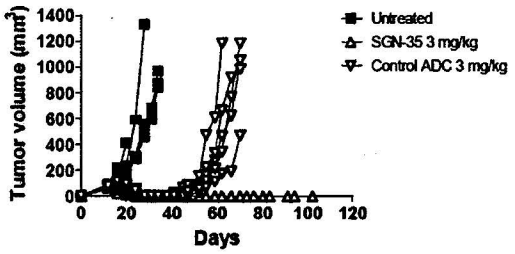
in all five mice in the 3 mg/kg group but in fewer mice in the 1 mg/kg group (1/5 mice or 3/7 mice).

Figure 15. Tumor Volumes of SCID Mice Bearing L540cy Xenografts

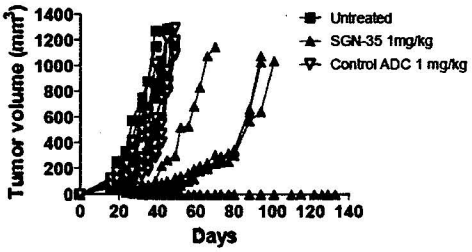
A.



B.



C.



SCID mice were implanted subcutaneously with HL L540cy cells in the right flank. The drug administration [every 4 days for 4 doses] was initiated when the tumor size for all mice averaged approximately 100 mm^3. Mice (5–7/group) were either untreated, or treated with SGN-35, or a non-binding control ADC. Points represent the tumor volume of individual mice. Each line represent an individual animal.

Antitumor Activity of SGN-35 in Karpas 299 Xenograft Models (non-GLP, Study Number: TRN-1305)

In this study, ALCL Karpas 299 cells ($5x10^6$) were injected either SC (experiment A) or IV (experiment B) to female SCID mice (7 to 11 wks old), the study design and conduct were the same as described above in study TRN-1304 unless indicated otherwise.

SGN-35 at 0.5 mg/kg significantly delayed tumor growth (Figure 16 A, T/C: 0 %) and induced a complete and durable response in 7 of 8 mice. Tumor growth in other groups was not significantly delayed (Table 8).

SGN-35 increased the median survival (Figure 16 B) in mice with the disseminated disease in a dose-related manner [36 days, 43 days ($p \leq 0.009$), > 89 days ($p \leq 0.001$), or >100 days ($p \leq 0.001$) in the untreated, 0.125, 1.0, and 3.0 mg /kg SGN-35 groups, respectively]. Ten of 10 mice treated with 3.0 mg /kg SGN-35 were still alive at the end of the study on Day 106. cAC10 (3.0 mg/kg) plus MMAE (0.06 mg/kg) also significantly

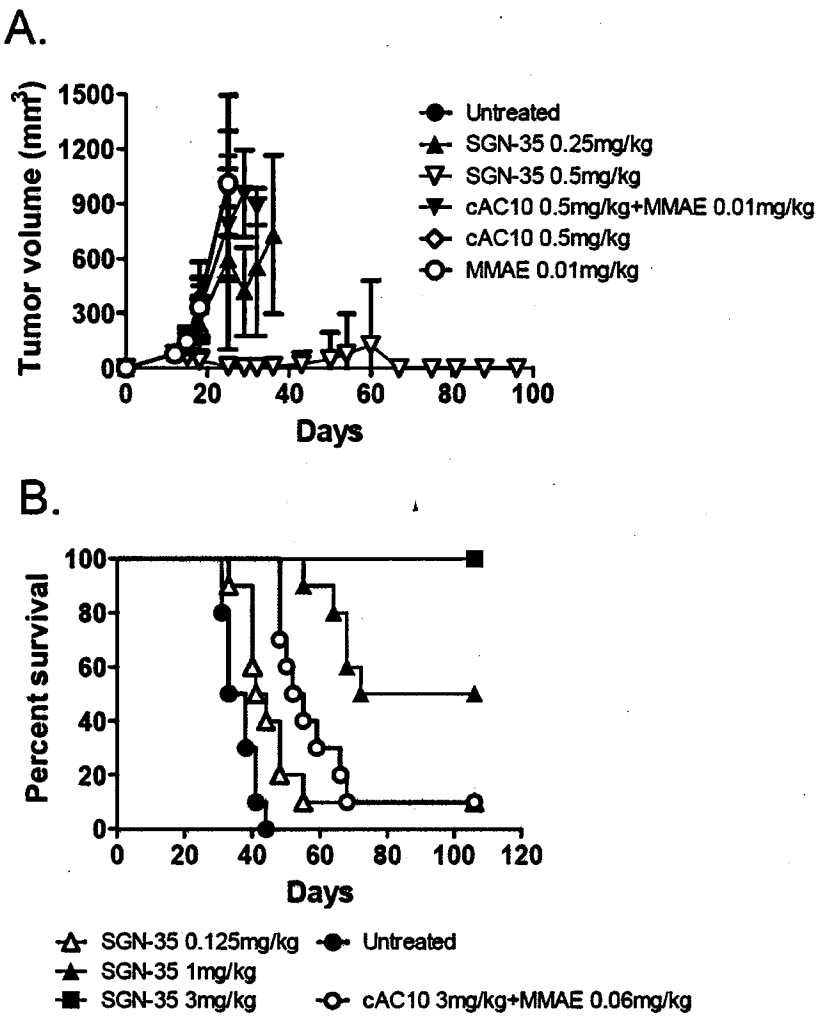
increased the median survival (Day 54, p≤0.001) but was less effective than the equivalent dose of SGN-35 (3 mg/kg, p<0.001).

Table 8. Study Design and Findings for Experiment A

Treatment Group	Dose (mg/kg)	Route	Schedule	Sex/ Number Per Group	Species/Strain	Mean Tumor Volume (cubic mm) ± SD[a]	%T/C[b]	P Value ΔT vs. ΔC[c]	CR[d]	DR[e]
Untreated	N/A	N/A	N/A	F/8	*Mus musculus*/ C.B-17/IcrHsd-PrkdcSCID	995 ± 498	N/A	N/A	0/8	0/8
SGN-35	0.25	IV	q4d x 4	F/8	*Mus musculus*/ C.B-17/IcrHsd-PrkdcSCID	596 ± 493	56	p = 0.1	0/8	0/8
SGN-35	0.5	IV	q4d x4	F/8	*Mus musculus*/ C.B-17/IcrHsd-PrkdcSCID	11 ± 13	0	p <0.001	7/8	7/8
cAC10 + MMAE	0.5 /0.01	IV	q4d x 4	F/8	*Mus musculus*/ C.B-17/IcrHsd-PrkdcSCID	694 ± 379	67	p = 0.2	0/8	0/8
cAC10	0.5	IV	q4d x 4	F/8	*Mus musculus*/ C.B-17/IcrHsd-PrkdcSCID	1022 ± 402	103	p = 0.9	0/8	0/8
MMAE	0.01	IV	q4d x 4	F/8	*Mus musculus*/ C.B-17/IcrHsd-PrkdcSCID	1012 ± 287	102	p = 0.9	0/8	0/8

N/A=not applicable
[a] Average tumor volume on Day 25.
[b] %T/C=ΔT/ΔC x 100, where ΔT and ΔC were changes in tumor volume for each treated and untreated control groups, respectively, as determined on Day 25 [tumor volume on Day 25 – tumor volume on Day 12 (the first day of treatment)].
[c] ΔT compared to ΔC using t-test (differences were considered significant at p≤0.01).
[d] CR=complete response defined by no discernable tumor mass at some point during the study.
[e] DR=durable response. No measureable tumor mass at the end of the study (Day 96).

Figure 16. Effects of SGN-35 on ALCL Karpas 299 Tumor Xenograft Model



A. The mice bearing tumor were intravenously administered SGN-35 at 0.25 or 0.5 mg/kg, cAC10 at 0.5 mg/kg, MMAE at 0.01 mg/kg, or cAC10 (0.5 mg/kg) plus MMAE (0.01 mg/kg) once every 4 days for a total of 4 doses. The treatment was initiated on Day 12 post tumor inoculation when the mean tumor volume of all groups was approximately 100 mm^3. Data represented mean ± standard deviation (SD) (n=8).

B. Survival curves of mice bearing disseminated Karpas 299. The mice were intravenously administered SGN-35 at 0.125, 1.0, or 3.0 mg/kg, cAC10 at 0.5 mg/kg, MMAE at 0.01 mg/kg, or cAC10 (0.5 mg/kg) plus MMAE (0.01 mg/kg) once every 4 days for a total of 4 doses. The treatment was initiated on Day 9 post tumor inoculation.

4.2 Secondary Pharmacology

No secondary pharmacodynamic analyses were conducted.

4.3 Safety Pharmacology

The safety pharmacology studies were reviewed by Dr. Miyun Tsai-Turton.

Determine the dose-response relation of SGD-1010 block of hERG K^+ channels heterologously expressed in Human Embryonic Kidney (HEK293) cells using the conventional whole-cell voltage clamp ((b) (4) Study 129-09-001, by CRO: (b) (4))

Study Design: This GLP study was to evaluate the effect of SGD-1010 on hERG K^+ channels, heterologously expressed in HEK293 cells using whole cell voltage clamp technique. The cells were treated with 1) 10 micM SGD-1010, 2) 100 μM SGD-1010, 3) saline as negative control, or 4) 25 nM cisapride hydrate as positive control. The effects on hERG K^+ were determined by measuring peak hERG tail current before and during test at 35±1 °C. The IC_{50} can be derived if it can produce an effect of > 0.5 mean fractional block (hERG current).

Study groups

Group	Study Group	Target concentration	Number of cells
	Negative Control	0	4
A	SGD-1010	10 μM	4
B	SGD-1010	100 μM	4
	Positive Control	25 nM	2

Note: Batch (Lot) Number for SGD-1010: 2002E.

Findings:

- Dose solution analysis confirmed that test article (SGD-1010) dose solution was homogenous and prepared at the target concentration.
- SGD-1010 at 10 micM produced a mean fractional block of 0.103±0.03 (not significantly different from negative control) whereas SGD-1010 at 100 micM produced a mean fractional block of 0.237±0.056 (significantly different from negative control).
- The IC_{50} is estimated to be > 100 micM since 100 micM SGD-1010 did not produce > 0.5 fractional block of peak hERG tail current.

Table 9 Study No 129-19-001: IC_{50}. for hERG K^+ Block

Mean fractional block of peak hERG tail current and summary statistics.

Dose Perfusate	Mean	SD	SEM	Number of Cells
Negative Control	0.063	0.046	0.023	4
10 μM	0.103	0.059	0.030	4
100 μM*	0.237	0.112	0.056	4
Positive Control†	0.743	0.007		2

* Indicates statistically significant difference from the negative control ($p < 0.05$)
† Not tested for difference from the negative control

A Safety Pharmacology Study (With Evaluation of Cardiovascular, Respiratory, and Central Nervous Systems) of SGN-35 Administered to Cynomolgus Monkeys by Intravenous Infusion (Study (b) (4) 00051 by CRO: (b) (4))

Study Design: This GLP study was to identify any potential effect of SGN-35 on the CV, respiratory, and CNS following 1 hr IV infusion (0.3, 1, 3 mg/kg) to unrestrained and radiotelemetry-implanted Cynomolgus monkeys (age 2.8-5 yrs, wt 2.9-4.6 kg). All animals were dosed with control article via IV infusion on Day 1, followed by the test article (SGN-35) on Day 6.

Group Assignments and Dose Levels

Group No.	Number of animals	Dose Day	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)
1,2,3	9	1	0 (control)	10	0
1	3	6	0.3		0.03
2	3	6	1		0.10
3	3	6	3		0.30

Note: Batch (Lot) Numbers for SGD-35: SSB003 and for control article (formulation buffer: 20 mM sodium citrate, 70 mg/ml trehalose, 0.16 mg/ml Tween® 80, pH 6.6): 1327001.

Findings:

- Clinical signs (mortality and cage side observations once daily on Days 7-10)

SGN-35 had no effects on clinical observation. Few animals had reddening/abrasion at the incision site from the telemetry implantation (most seen on Day 5). There were also bruising, periorbital swelling, and watery feces prior to the administration of SGN-35 on Day 6. These observations were not related to SGN-35.

- Food consumption (once daily on Days 7-10)

SGN-35 had no effects on food consumption.

- Body weight (Days 1, 5, and 10)

SGN-35 had no effects on body weight.

- Respiratory system (respiration rate prior to each dose, 2, 24, and 72 hrs after the end of infusion and blood gasses)

There were no SGN-35 related effects on the respiratory rates and blood gas.

- CV system (ECG, heart rate, and blood pressure prior to, during, and following dosing on Days 1 and 6)

The ECG showed no abnormal findings attributable to SGN-35. The heart rates and mean arterial pressure were similar among groups.

- Neurological exams (pre-study, at 24 and 72 hrs after the end of infusion Days 2, 4, 7, and 9)

There were no SGN-35 related effects on neurological endpoints (behavior, visual evaluations, reflex evaluation, motor/sensory, facial movement, pupils, visual field,

vestibulocochlear, prehension and swallowing, and proprioception). In addition, there was no SGN-35 related effect on body temperature. The body temperatures showed the expected diurnal cycles during each recording period.

- Blood samples were collected for clinical pathology analysis, including plasma chemistry, hematology, and coagulation (pre-study, Days 4, and 9), blood gas (prior to each dose, 2, 24, and 72 hrs following the end of infusion).

Plasma Chemistry Parameters	
Alanine aminotransferase (ALT)	Total protein
Aspartate aminotransferase (AST)	Albumin
Alkaline phosphatase (ALP)	Globulin
Gamma-glutamyltransferase (GGT)	Albumin/globulin ratio
Lactate dehydrogenase (LD)	Glucose
Total bilirubin	Cholesterol
Urea nitrogen (BUN)	Triglycerides
Creatinine	Sodium
Calcium	Potassium
Phosphorus	Chloride
	Carbon dioxide

Hematology Parameters	
Red blood cell (RBC) count	Mean corpuscular hemoglobin (MCH)
Hemoglobin concentration	Reticulocyte count
Hematocrit	Platelet count
Mean corpuscular volume (MCV)	White blood cell (WBC) count*
Mean corpuscular hemoglobin concentration (MCHC)	Blood cell morphology**

* Included total white blood cell, polysegmented neutrophil, band neutrophil, lymphocyte, monocyte, eosinophil, basophil, and other cell counts as appropriate.

** The blood smear from all animals was examined at each time point (including prestudy).

Coagulation Parameters
Prothrombin time (PT)
Activated partial thromboplastin time (APTT)
Fibrinogen

There were no SGN-35 related effects on plasma chemistry, hematology, and coagulation parameters.

Plasma chemistry: There were increased ALT, AST, and LD on Day 4 with two animals after vehicle administration (lower by Day 9). This might be secondary to procedure-related activities. The albumin level was higher on Day 4 with all animals (normal by Day 9). This change might be due to inadequate water intake, secondary to procedure related stress.

Hematology: There were lower platelet counts for all animals on Days 4 and 9 without progressive decline compared to pre-study. There were also higher reticulocyte counts at the Day 6 compared to values on Days 4 and 9. The reasons for such differences were not evident.

Coagulation: There were increased PT and APTT for few animals on Day 4 compared to pre-study. This change was attributed to sample quality (inappropriate anticoagulant: blood volume ratio).

- Dosing solution analysis is provided by (b) (4).

The SGN-35 dosing solution for Group 1 (0.3 mg/kg) had 83.3% of target concentration (below acceptance criteria) on Day 6. For Groups 2 (1 mg/kg) and 3 (3 mg/kg), dosing solutions were within ± 10% of the target concentration.

Overall Findings: The cynomolgus monkeys showed no adverse effects on CV, respiratory, and CNS systems after 4 days following a single dose of SGN-35 (0.1, 1, or 3 mg/kg) via 1 hr IV infusion.

Overall Safety Pharmacology Conclusions: One *in vitro* safety pharmacology study was conducted to see the effect of MMAE (at 10 and 100 mcM) on hERG K^+ channels expressed in HEK cell, using whole cell voltage clamp. The study showed that IC_{50} was > 100 micM. When compared to human clinical dose of 1.8 mg/kg brentuximab vedotin (with the mean C_{max} of MMAE as 6.92 nM), the 10 micM MMAE (no biologic effect on hERG channel) was approximately 1000x higher than MMAE C_{max} in patients. One *in vivo* safety pharmacology study was conducted to see the effects of brentuximab vedotin on CV, respiratory, CNS systems in monkeys given a single dose (0.3, 1, 3 mg/kg) via 1 hr infusion. This study showed that there were no effects of brentuximab vedotin within 4 days after following a single 1 hr IV infusion at doses up to 3 mg/kg. These studies suggested that it is unlikely that brentuximab vedotin would block hERG K^+ channels or have adverse effects on QTc interval.

Reviewer's comments (by Yanli Ouyang)

According to QT-IRT review, no large changes (i.e., greater than 20 ms) in QTc interval were detected following brentuximab vedotin 1.8 mg/kg i.v. infusion in patients with CD30-positive malignancies, which is consistent with the findings in nonclinical studies described above. The largest upper bound of the 2-sided 90% CI for the mean change from baseline was 2.9 ms, observed at one hour post-dose on Day 1 of Cycle 1. In addition, within the range of concentrations observed in this clinical study, no apparent concentration-QT relationship was identified.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The PK/ADME studies were reviewed by Dr. Miyun Tsai-Turton.

Absorption

An 8-Week GLP Pharmacokinetic Study of SGN-35 Administered as an Intravenous Infusion to Cynomolgus Monkeys (Study 8201-470 by CRO: (b) (4))

Study Design: 12 female monkeys (age 2.9-4.7 yrs) were given a single dose of 0.3 or 1 mg/kg (1 hr IV infusion via a saphenous vein). These monkeys were observed twice daily and their body weights were recorded prior to dosing and weekly throughout the test period. Food consumption was also measured daily through the end of the test period. To determine immunogenicity background, the blood samples (approximately 1 mL) were collected via a femoral vein on Day 4 for Group 1 and Day 11 for Group 2. For clinical chemistry and hematology analysis, the blood samples (approximately 3.5 mL) were collected predose and up to Day 57. Several PK parameters including total antibody, ADC, free MMAE, Cmax, Tmax, t½, AUC, CL, and V_z, were calculated.

STUDY DESIGN

Group	Number of Females	Dose Route	Target Dose Level (mg/kg)	Target Dose Volume (mL/kg)	Target Dose Concentration (mg/mL)
1	6	IV	0.3	10	0.03
2	6	IV	1	10	0.1

Note: The dose was administered as an approximately 60-minute intravenous (IV) infusion.

Note: The purity of SGN-35 (Batch Lot No SSB004) was 93.1% (monomer).

The dose concentrations were approx 94% (Group 1) and 97% (Group 2) of the target concentrations.

Sample Collection Schedule		
Study Day	**Time points – relative to dosing**	**Sample(s) Collected**
-4 (Group 1) and -11 (Group 2)	Predose	Immunogenicity screening

1	Predose (just before infusion)	PK / immunogenicity
1	Post infusion: 10 min (±1 min), 1 hr (±4 min), 6 hr (±10 min), and 12 hr (±20 min)	PK
2	24 hr (±30 min)	PK
3	48 hr (± 1hr)	PK
4	72 hr (± 2hr)	PK
6	120 hr (± 2hr)	PK
8	168 hr (± 2hr)	PK / immunogenicity
11	240 hr (± 2hr)	PK
15	2 weeks post dose	PK / immunogenicity
22	3 weeks post dose	PK
29	4 weeks post dose	PK / immunogenicity
36	5 weeks post dose	PK
43	6 weeks post dose	PK / immunogenicity
50	7 weeks post dose	PK
57	8 weeks post dose	PK / immunogenicity

Findings:

- SGN-35 was well-tolerated in female monkeys given 0.3 and 1 mg/kg via 1 hr IV infusion.
- The clinical chemistry and hematology parameters were within acceptable ranges.
- No significant changes were seen in post-dose body weight or food consumptions over the 8 week study.
- Antibodies to SGN-35 were seen in all animals.
- Serum total antibody and ADC exposures increased with increasing doses (from 0.3 to 1 mg/kg). The majority of free MMAE at 0.3 mg/kg SGN-35 was <0.0100 ng/ml (lower limit of quantitation). However, at 1 mg/kg SGN-35, MMAE level peaked at approximately 1 day postdose and slowly declined thereafter.
- Serum Cmax and AUC values were slightly greater than dose proportional.

Table 10. PK Parameters for Female Monkeys (0.3 and 1 mg/kg)

Summary of the Mean Pharmacokinetic Parameters for ADC in Female Monkey Serum

Group	SGN-35 Dose Level (mg/kg)		C_{max} (µg/mL)	DN C_{max} [(µg/mL)/(mg/kg)]	T_{max} (day)	C_{last} (µg/mL)	T_{last} (day)	AUC_{0-t} (µg·day/mL)	$AUC_{0-\infty}$ (µg·day/mL)	DN $AUC_{0-\infty}$ [(µg·day/mL)/(mg/kg)]	$t_{1/2}$ (day)	CL (mL/day/kg)	V_{ss} (mL/kg)
1	0.3	Mean[a]	6.95	23.2	0.0486	0.109	10.0417	10.7	11.0	36.8	1.82	27.7	68.0
		SD[b]	1.25	NA	(0.0486, 0.0486)	0.099	(7.0417, 10.0417)	1.8	1.7	NA	0.37	4.3	11.8
		N	6	NA	6	6	6	6	6	NA	6	6	6
2	1[c]	Mean[a]	29.2	29.2	0.0486	0.476	14.0417	53.3	55.3	55.3	2.69	18.5	67.4
		SD[b]	5.5	NA	(0.0486, 0.0486)	0.337	(10.0417, 21.0417)	8.3	9.2	NA	0.56	3.0	19.8
		N	5	NA	5	5	5	5	5	NA	5	5	5

NA Not applicable.
DN Dose normalized.
Note: T_{max} values presented to 4 decimal places for clarity.
a Median presented for T_{max} and T_{last}.
b Range (min, max) presented for T_{max} and T_{last}.
c Animal 109620 was excluded from descriptive statistics for all parameters because C_{max} and AUC_{0-t} are outliers per Dixon Test.

Summary of the Mean Pharmacokinetic Parameters for TAb in Female Monkey Serum

Group	SGN-35 Dose Level (mg/kg)		C_{max} (µg/mL)	DN C_{max} [(µg/mL)/(mg/kg)]	T_{max} (day)	C_{last} (µg/mL)	T_{last} (day)	AUC_{0-t} (µg·day/mL)	DN AUC_{0-t} [(µg·day/mL)/(mg/kg)]
1	0.3	Mean[a]	8.30	27.7	0.0486	0.261	10.0417	17.4	58.0
		SD[b]	1.77	NA	(0.0486, 0.0833)	0.183	(7.0417, 10.0417)	3.0	NA
		N	6	NA	6	6	6	6	NA
2	1	Mean[a]	24.8	24.8	0.0486	0.876	14.0417	68.6	68.6
		SD[b]	2.8	NA	(0.0486, 0.0833)	0.914	(10.0417, 21.0417)	7.5	NA
		N	6	NA	6	6	6	6	NA

NA Not applicable.
DN Dose normalized.
Note: T_{max} values presented to 4 decimal places for clarity.
a Median presented for T_{max} and T_{last}.
b Range (min, max) presented for T_{max} and T_{last}.

Summary of the Mean Pharmacokinetic Parameters for free MMAE in Female Monkey Serum

Group	SGN-35 Dose Level (mg/kg)		C_{max} (ng/mL)	T_{max} (day)	C_{last} (ng/mL)	T_{last} (day)	AUC_{0-t} (ng·day/mL)	Apparent $t_{1/2}$[d] (day)
1[a]	0.3	Mean[b]	NC	NC	NC	NC	NC	NC
		SD[c]	NC	NC	NC	NC	NC	NC
		N	NA	NA	NA	NA	NA	NA
2	1	Mean[b]	0.0268	1.0417	0.0135	5.0417	0.111	NC
		SD[c]	0.0059	(1.0417, 2.0417)	0.0016	(5.0417, 7.0417)	0.034	NC
		N	6	5	6	6	6	NA

NA Not applicable.
NC Not calculated when the number of measurable values is less that 50% of the total number of animals.
Note: T_{max} and T_{last} values presented to 4 decimal places for clarity.
a Only one animal had measurable concentrations above the lower limit of quantitation.
b Median presented for T_{max} and T_{last}.
c Range (min, max) presented for T_{max} and T_{last}.
d Apparent $t_{1/2}$ was not calculated for most animals due to insufficient data in the terminal phase.

Single Dose IV Pharmacokinetic Study of SGN-35 in Sprague Dawley Rats (Study R-PK-08 by Seattle Genetics)

Study Design: This non-GLP study was to characterize the PK profile of SGN-35 following single IV dose (0.5 or 5 mg/kg) to 24 female rats (age 8-12 weeks and weight 208-230 g). Blood samples were collected for PK analysis (see table below for time points). Samples were also analyzed for SGN-35 antibody drug conjugate (ADC) and total antibody (TAb).

Study design for R-PK-08

Group	Test Article	Dose Level (mg/kg)	N	Rat ID in Sub-group A (collection timepoints of 1, 6, 24, 30, 48, and 96 hours)	Rat ID in Sub-group B (collection timepoints of 7, 14, 21, 35, 49, 63, and 77 days)
1	SGN-35	0.5	12	1, 2, 3, 4, 5, 6	7, 8, 9, 10, 11, 12
2		5.0	12	13, 14, 15, 16, 17, 18	19, 20, 21, 22, 23, 24

Note: Drug Lot No: SGD-1006-0-07

The dose concentrations were within 15% of the target concentrations for all doses.

Findings:

- SGN-35 was well-tolerated in female rats given 0.5 and 5 mg/kg via a single IV bolus dose.
- The overall ATA (anti therapeutic antibodies, i.e. anti-SGN-35 antibodies) was seen in 3/12 (25%) animals given SGN-35. No ATA were detected in rats given 0.5 mg/kg SGN-35. However, ATA was detected in 3/6 (50%) animals given 5 mg/kg SGN-35.
- Serum ADC concentrations declined in a multi-exponential manner. Serum TAb concentrations were consistently higher than those for SGN-35 ADC.
- Serum total antibody and ADC exposures (C_{max} and AUC) increased with increasing doses (from 0.5 to 5 mg/kg, approximately dose proportional). The C_{max} for TAb was higher than it was for ADC. The AUC for TAb was approximately 2X higher than it was for ADC. The T_{max} for TAb was similar to that of ADC. The t ½ was estimated 9-15 days for ADC.

Table 11. PK Parameters of (TAb and ADC) SGN-35 for Female rats (0.5 and 5 mg/kg)

PK parameters for SGN-35 ADC and TAb following administration of a single IV bolus dose of SGN-35 to female Sprague Dawley rats

Analyte	SGN-35 Dose (mg/kg)	N	ATA	C_{max} (µg/mL)	T_{max} (day)	AUC_{last} (day-µg/mL)	$AUC_{0-\infty}$ (day-µg/mL)	$t_{1/2}$[c] (day)	CL (mL/day/kg)	V_{ss} (mL/kg)
SGN-35 ADC	0.5	6	neg	13	0.042	20	20	14.6	25	183
	5.0	3	neg	171	0.042	253	253	8.5	20	135
	5.0	3	pos[a]	171	0.042	245	246	10.1	20	152
TAb[b]	0.5	6	neg	15	0.042	40	NA	NA	NA	NA
	5.0	3	neg	240	0.042	557	NA	NA	NA	NA
	5.0	3	pos[a]	240	0.042	475	NA	NA	NA	NA

NA = not applicable

a. PK parameters were calculated separately for animals with ATA (n=3) in the 5 mg/kg group.

b. For TAb $t_{1/2}$ (and consequently, $AUC_{0-\infty}$, V_{ss}, and CL) was not estimated as TAb is a measure of at least 2 distinct entities, cAC10 and ADC each with unique elimination rates.

c. In an exploratory analysis, a truncated ADC $t_{1/2}$ was estimated based on the concentration-time data up through 7 days postdose; this truncated ADC $t_{1/2}$ was approximately 2.5 and 2.0 days at dose levels of 0.5 and 5 mg/kg SGN-35, respectively.

A Single Dose Pharmacokinetic Study of MMAE Following IV Administration of SGN-35 in Female Sprague Dawley Rats (Study R-PK-14 by Seattle Genetics)

Study Design: This non-GLP study was to characterize the PK profile of free MMAE following single IV dose (3 or 10 mg/kg) to 18 female rats (age 8-12 weeks, weight 200-250 g). Blood samples were collected for PK analysis at following time points: 1, 10, 30 min, 1, 2, 4, 6, 24 hr, 2, 3, 4, 5, 6, 7, 10, 14, 21, and 28 days postdose.

R-PK-14 study design

Group	No. of Animals (female)	Test Article	Dose Level (mg/kg)	Rat ID Numbers
1	9	SGN-35	3	1-9
2	9		10	10-18

Note: Drug Lot No: NBZ5084/42 with purity of 93.2%

The dose concentrations were within 10% of the target concentrations for all doses.

Findings:

- SGN-35 was well-tolerated in female rats given 3 and 10 mg/kg via a single IV bolus dose.
- Exposures increased approximately proportionally with dose from 3 to 10 mg/kg.
- T_{max} was approximately 1 day postdose at 3 and 10 mg/kg.
- The t½ ranged from 2.2 days (for 3 mg/kg) to 2.5 days (for 10 mg/kg).

Table 12. PK Parameters of (free) MMAE for Female Rats (3 and 10 mg/kg)

Mean MMAE PK following administration of a single IV bolus dose of SGN-35 to female Sprague Dawley rats

Parameter	3 mg/kg	10 mg/kg
AUC_{last} (d·ng/mL)	0.94	3.6
$AUC_{0-\infty}$ (d·ng/mL)	1.2	3.9
$C_{max} \pm SE$ (ng/mL)	0.34 ± 0.013	0.93 ± 0.014
T_{max} (d)	1.0	1.0
Apparent $t_{1/2}$ (d)	2.2	2.5

Distribution

Plasma Protein Binding Assay of MMAE by Ultracentrifugation (Study XS-0025 by CRO: (b) (4).)

Study Design: This non-GLP study was to evaluate the plasma protein binding of MMAE in ICR mouse, Wistar rat, Cynomolgus monkey, and human by calculating in vitro plasma protein binding ratios. Three concentrations (1, 10, and 100 nmol/L) of [^{3}H]-MMAE (Lot No 646-116-0242) was added to plasma and radioactivity (in plasma and supernatant) was measured by (b) (4). Note: Unlabeled test article (reference standard – MMAE, SGD-1010) Lot No: 2002E with 92.8 % purity

Experimental Conditions

Species	[^{3}H]-MMAE concentrations (nmol/L)	Sample replicates
Mouse	1, 10 and 100	3/concentration
Rat		
Monkey		
Human		

Plasma Collection

Mouse

Species	Strain	Sex	Weeks of age at blood collection	Number of animals
Mouse	Crlj:CD1 (ICR)	Male	9	33
Supplier and address of head office	(b) (4)			

Rat

Species	Strain	Sex	Weeks of age at blood collection	Number of animals
Rat	Crlj:WI (Wistar)	Male	9	11
Supplier and address of head office	(b) (4)			

Monkey

Species	Sex	Years of age at blood collection	Number of animals
Cynomolgus	Male	2-3	3
Facility engaged and address of head office			(b) (4)

Plasma was purchased from Hamri Co., Ltd.

Human

Species	Sex	Years of age at blood collection	Number of humans for blood collection
Human (Volunteer)	Male	29 to 36	3
Facility engaged and address			(b) (4)

The exclusion criteria for volunteers were as follows:
1) Systolic blood pressure below 90 mmHg
2) Body weight below 45 kg
3) Subjects who have a disease that is or might be aggravated by the blood collection
4) Subjects with fever
5) Subjects receiving medication
6) Any others who were not in good physical condition

Findings:

- The [^{3}H]-MMAE plasma protein binding ratio was 19-29% in mouse, 72-74% in rat, 17-19% in monkey, and 68-82% in human.
- The plasma protein binding of [^{3}H]-MMAE was species-dependent.
- There was no change in binding from 1 to 100 nM with rat and monkey plasma, but small differences were observed with mouse and human plasma.

Table 13. Protein Binding Ratios in Mouse, Rat, Monkey, and Human

Species	1 nmol/L	10 nmol/L	100 nmol/L
Mouse	18.8%	19.6%	28.5%
Rat	72.9%	73.5%	72.0%
Monkey	17.1%	17.8%	18.9%
Human	67.9%	77.5%	82.2%

Metabolism

***In Vitro* Evaluation of an NCE as an Inducer of Cytochrome P450 Expression in Cultured Human Hepatocytes** (Study (b) (4) 083016 by CRO: (b) (4))

Study Design: This non-GLP study was to see the effects of MMAE (Batch Lot No: 2002E with 93.7% purity) on cytochrome P450 enzymes in primary human hepatocyte cultures. Primary hepatocytes from three separate livers (see table below for donor

information) were treated once daily with DMSO, MMAE (0.1, 1, or 10 μM), or one of the three human CYP inducers (100 μM omeprazole, 750 μM phenobarbital, and 10 μM rifampin) for three days. After treatment, the cells were harvested and microsomes were prepared. The microsomal CYP450 enzyme activities (see table below for a specific maker with each CYP450 enzyme) were measured by LC-MS.

Human hepatocyte preparation: Organ donor information and hepatocyte viability

(b) (4) liver number	Gender	Age (years)	Ethnicity	Blood type	Tobacco [a]	Drug use [a]	Alcohol use [a]	Cell viability [b]
H877	Female	48	Caucasian	A	Yes [c]	Yes [d]	Yes [e]	86.15%
H879	Male	41	Caucasian	A	Yes [f]	No	Yes [g]	77.4%
H882	Male	46	Caucasian	O+	Yes [h]	Yes [i]	Yes [j]	85.0%

Summary of assay conditions to measure microsomal CYP enzyme activity

Enzyme	Reaction monitored	SOP followed	Substrate concentration (μM)	Quantity of protein (mg/incubation)	Incubation volume (mL)	Incubation time (min)
CYP1A2	Phenacetin *O*-dealkylation	L3260.02	80	0.008	0.2	30
CYP2B6	Bupropion hydroxylation	L3260.02	500	0.008	0.2	30
CYP2C8	Amodiaquine *N*-dealkylation	L3260.02	20	0.004	0.2	10
CYP2C9	Diclofenac 4′-hydroxylation	L3260.02	100	0.008	0.2	10
CYP2C19	*S*-Mephenytoin 4′-hydroxylation	L3260.02	400	0.02	0.2	30
CYP3A4/5	Testosterone 6β-hydroxylation	L3260.02	250	0.008	0.2	10

Findings:

- Treatment of cultured human hepatocytes once daily for 3 days with < 10 μM MMAE caused significant decrease in the CYP450 enzyme activities tested. Treatment with MMAE caused a decrease in CYP1A2 (49-55%), CYP2B6 (62-70%), CYP2C8 (55-63%), CYP2C9 (34-47%), CYP2C19 (35-39%), and CYP3A4/5 (32-53%).
- However, in general, such decrease in CYP enzyme activity among MMAE-treated groups was not concentration-dependent. The mechanism underlying such decrease in CYP activity was unclear.

Table 14. The effect of MMAE on P450 enzymatic activities

The effects of treating cultured human hepatocytes with MMAE or prototypical inducers on microsomal cytochrome P450 (CYP) enzyme activity: Fold increase (treated/vehicle control)

Treatment	Concentration	Fold increase [a]					
		Phenacetin *O*-dealkylation (CYP1A2)	Bupropion hydroxylation (CYP2B6)	Amodiaquine *N*-dealkylation (CYP2C8)	Diclofenac 4′-hydroxylation (CYP2C9)	*S*-Mephenytoin 4′-hydroxylation (CYP2C19)	Testosterone 6β-hydroxylation (CYP3A4/5)
Dimethyl sulfoxide	0.1% (v/v)	1.00 ± 0.50	1.00 ± 0.49	1.00 ± 0.11	1.00 ± 0.18	1.00 ± 0.72	1.00 ± 0.18
MMAE	0.1 μM	0.510 ± 0.095 †	0.380 ± 0.112 †	0.447 ± 0.147 * †	0.655 ± 0.173	0.613 ± 0.084 †	0.560 ± 0.133
MMAE	1 μM	0.449 ± 0.149 †	0.299 ± 0.088 †	0.374 ± 0.134 * †	0.529 ± 0.189 *†	0.610 ± 0.183 †	0.470 ± 0.173 †
MMAE	10 μM	0.475 ± 0.137 †	0.344 ± 0.062 †	0.436 ± 0.086 * †	0.605 ± 0.166 †	0.647 ± 0.165 †	0.680 ± 0.309
Omeprazole	100 μM	33.1 ± 16.1	4.57 ± 2.12	2.67 ± 0.46 *	1.19 ± 0.18	0.830 ± 0.194	1.32 ± 0.19
Phenobarbital	750 μM	2.29 ± 0.60	6.66 ± 1.38	4.28 ± 0.24 *	1.36 ± 0.18	2.35 ± 0.93	3.36 ± 0.60 *
Rifampin	10 μM	1.71 ± 0.49	3.73 ± 0.99	3.79 ± 0.15 *	1.64 ± 0.34 *	5.23 ± 1.45	3.35 ± 0.51 *

a Values are the mean ± standard deviation of three determinations (human hepatocyte preparations H877, H879 and H882).

† Significantly different from the vehicle control (dimethyl sulfoxide) as a result of One-way Analysis of Variance ($p < 0.05$) with the positive control groups (omeprazole, phenobarbital and rifampin) excluded from the statistical analysis.

* Significantly different from the vehicle control (dimethyl sulfoxide) as a result of One-way Analysis of Variance ($p < 0.05$) with all treatment groups included in the statistical analysis.

Reaction Phenotyping: Identification of Human CYP Enzymes Involved in the In Vitro Metabolism of [^{3}H]-MMAE (Study (b) (4) 084006 by CRO: (b) (4))

Study Design: This non-GLP study was to evaluate the role of CYP450 in the metabolism of [^{3}H]-MMAE using NADPH-fortified human liver microsomes and HPLC as analytical method. Human liver microsomes were from a pool of 50 individuals (Cat No (b) (4), Note: Batch Lot No for [^{3}H]-MMAE: 646-062-0258 and 646-116-0242 and MMAE (unlabeled): 2002E). When incubated with human liver microsomes, 0,9 μM [^{3}H]-MMAE was converted to 8 radioactive compounds (which were numbered based on HPLC retention time).

Findings:

- [^{3}H]-MMAE was converted to 8 radioactive compounds by NADPH-fortified human liver microsomes.
- The major components (metabolites) were 7.0 (by O-demethylation), 8.6 (by N-demethylation), and 11.1 (by dehydrogenation).
- The formation of these major components was primarily mediated by CYP3A4. Additional CYP450 enzymes (i.e. CYP2D6) might be minor contributors to the formation of these components.

Table 15. The Role of CYP450 in MMAE Metabolism

Characterization of MMAE and proposed metabolites in incubations with recombinant CYP enzymes and proposed routes of biotransformation

Original retention time (min)	Characterization retention time (min)	Relative retention time to MMAE (min)	Observed *m/z*	Change in mass (amu) from MMAE	Proposed biotransformation of MMAE	Test system
7.0	6.5	0.8	704	−14	*O*-demethylation	rCYP 3A4
8.6	7.5	0.9	704	−14	*N*-demethylation	rCYP3A4 and 2D6
9.2 (MMAE)	8.3 (MMAE)	1	718	0	NA	NA
11.1	10.3	1.2	716	−2	Oxidation of secondary alcohol to a ketone	rCYP3A4

NA Not applicable

Metabolite Characterization of [^{3}H]-MMAE in Rat, Monkey, and Human Hepatocytes (Study (b) (4) 084007 (by CRO: (b) (4))

Study Design: This non-GLP study was to determine in vitro metabolic profile of [^{3}H]-MMAE (Batch Lot No 646-063-0258) following incubation with cryopreserved Spraque Dawley rat, Cynomolgus monkey and human hepatocytes by using LC/MS/MS as analytical method. The hepatocytes, prepared from rat, monkey, and human, were incubated with 10 μM [^{3}H]-MMAE for 0, 60, 120, and 240 min. The supernatant fractions of samples were analyzed by LS-MS/MS using electrospray ionization and incorporating inline radiometric detection.

Findings:

- The substrate loss data over 240 min time course showed that the 240- min incubation with hepatocytes resulted in 32% (in rat), 18% (in monkey), and 31% (in human) loss of [^{3}H]-MMAE. The rank order (greatest to least) of observed substrate loss over the 240 min incubations was rat ≈ human > monkey.
- 12 components were detected in incubation of 10 μM [^{3}H]-MMAE with hepatocytes. Mass shifts observed from the majority of metabolites were consistent with formation of primary metabolites by hydroxylation, demethylation, dehydrogenation or hydrolysis.
- All 12 metabolites were detected from monkey hepatocytes, 9 of which were seen in rat hepatocytes and a different set of 9 were detected in human hepatocytes. Strong signals were seen with C4 and C6, indicating C4 (O-demethylation) and C6 (hydroxylation) could be major metabolites in all three species. No human specific components were detected.

Table 16. MMAE metabolism in rat, monkey, and human hepatocytes

Substrate loss of 10 μM [^{3}H]-MMAE in rat, monkey and human hepatocytes (1 million cells/mL)

Species	Incubation time (min)	Cell concentration (million/mL)	Peak area counts	Percent loss of substrate	Percent substrate remaining
Rat	0	1	1780	NA	100%
	60		1450	18.3%	81.7%
	120		1280	27.9%	72.1%
	240		1200	32.4%	67.6%
	0	1 (boiled)	1530	NA	100%
	240		1350	11.5%	88.5%
Monkey	0	1	1580	NA	100%
	60		1310	17.1%	82.9%
	120		1320	16.8%	83.2%
	240		1300	18.0%	82.0%
	0	1 (boiled)	1500	NA	100%
	240		1420	5.7%	94.3%
Human	0	1	1520	NA	100%
	60		1320	13.2%	86.8%
	120		1330	12.5%	87.5%
	240		1040	31.5%	68.6%
	0	1 (boiled)	1530	NA	100%
	240		1410	8.2%	91.8%

Peak area counts of the parent compound are the mean of duplicate determinations and are rounded to three significant figures.

Percent values are rounded to one decimal place.

Data are shown graphically in Figure 2.

Metabolite profile and characterization

(b) (4) metabolite assignment	*m/z*	Retention time (min)	Change in mass (amu) from MMAE	Proposed transformation from MMAE	Rat	Monkey	Human
C1	734	10.7	+16	Hydroxylation	+	+	ND
C2	734	11.0	+16	Hydroxylation	ND	+	ND
C3	734	11.2	+16	Hydroxylation	+	+	+
C4	704	11.3	−14	*O*-Demethylation	+	+	+
C5	605	11.7	−113	Amide hydrolysis	+	+	+
C6	734	12.0	+16	Hydroxylation	+	+	+
C7	704	12.3	−14	*N*-Demethylation	+	+	+
C8	716	14.2	−2	Oxidation of alcohol to form a ketone	+	+	+
C9	734	14.8	+16	Hydroxylation	+	+	+
C10	718	17.9	+0	*N*-demethylation + hydroxylation to form a nitroso compound	+	+	+
C11	734	18.9	+16	Hydroxylation	ND	+	ND
C12	716	19.4	−2	Oxidation of alcohol to form a carbonyl (following formation of the nitroso compound)	ND	+	+
Parent	718	12.6	+0	-	+	+	+

\+ Peak detected
ND Not detected

In Vitro Evaluation of MMAE as an Inhibitor of Human Cytochrome P450 Enzymes

(Study (b) (4) 085021 by CRO: (b) (4))

Study Design: This non-GLP study was to evaluate the ability of MMAE to inhibit major CYP enzymes (i.e. CYP1A2, CYP2B6, CYP2C8, CYP2C9, aCYP2C19, CYP2D6, and CYP3A4/5) in human liver microsomes (a pool of 16 individuals). Human liver microsomes were incubated with marker substrates in the presence or absence of MMAE (ranged from 0.1 to 100 μM) to evaluate MMAE as a direct inhibitor of CYP activity. MMAE was also evaluated for its ability to function as a time-dependent inhibitor at the same concentrations. MMAE was pre-incubated with human liver microsomes and an NADPH-generating system for 30 min to allow for the generation of metabolites that might inhibit CYP activity

Summary of experimental conditions for enzyme assays: Direct and time-dependent inhibition of CYP enzymes by MMAE (IC_{50} determinations)

Enzyme	CYP Reaction	Substrate concentration (μM)	Incubation volume (μL)	Protein [a] (μg/mL)	Incubation time (min)	Pre-incubation time (min)	MMAE Target concentrations (μM)	MMAE Solvent volume [b] (μL)
CYP1A2	Phenacetin *O*-deethylation	40	400	100	5	30	0, 0.1, 0.3, 1, 3, 10, 30, 100	4
CYP2B6	Bupropion hydroxylation	50	400	100	5	30	0, 0.1, 0.3, 1, 3, 10, 30, 100	4
CYP2C8	Amodiaquine *N*-dealkylation	7	400	100	5	30	0, 0.1, 0.3, 1, 3, 10, 30, 100	4
CYP2C9	Diclofenac 4′-hydroxylation	6	400	100	5	30	0, 0.1, 0.3, 1, 3, 10, 30, 100	4
CYP2C19	S-Mephenytoin 4′-hydroxylation	40	400	100	5	30	0, 0.1, 0.3, 1, 3, 10, 30, 100	4
CYP2D6	Dextromethorphan *O*-demethylation	7.5	400	100	5	30	0, 0.1, 0.3, 1, 3, 10, 30, 100	4
CYP3A4/5	Testosterone 6β-hydroxylation	100	400	100	5	30	0, 0.1, 0.3, 1, 3, 10, 30, 100	4
CYP3A4/5	Midazolam 1′-hydroxylation	4	400	50	5	30	0, 0.1, 0.3, 1, 3, 10, 30, 100	4

a The human liver microsomal sample used for these experiments was a pool of sixteen individuals (samples 286, 290, 312, 313, 315, 333, 334, 335, 336, 339, 348, 359, 364, 383, 389 and 390).

b Methanol (1% final incubation concentration) was the vehicle used to dissolve the test article.

Experiments were conducted to further characterize the possible time-dependent inhibition of CYP3A4/5 measured by testosterone 6β-hydroxylation: 1) to determine if the inhibition observed upon pre-incubation with MMAE was dependent upon NADPH, 2) to determine to what extent (K_I value) and how quickly (K_{inact} value) MMAE inactivates CYP3A4/5, and 3) to investigate the ability of MMAE to form a metabolite inhibitory complex (MIC) with CYP3A4/5.

Summary of experimental conditions: Metabolism-dependent inhibition of CYP3A4/5 by MMAE

(b) (4)

Enzyme	CYP activity	Substrate concentrations (μM)	Incubation volume (μL)	Protein [a] (μg/mL)	Incubation time (min)	Preincubation time (min)	MMAE: Target concentrations (μM) [b]	MMAE: Solvent volume [c] (μL)
CYP3A4/5	Testosterone 6β-hydroxylation	200 [d]	200	100	5	0 and 30	0 and 10	4

a The human liver microsomal sample used for these experiments was a pool of sixteen individuals (samples 286, 290, 312, 313, 315, 333, 334, 335, 336, 339, 348, 359, 364, 383, 389 and 390).

b Represents the concentration in the preincubation.

c Methanol (1% final incubation concentration) was the vehicle used to dissolve the test article.

d Represents the concentration of substrate at $2K_m$.

Best Available Copy

Summary of experimental conditions: Direct inhibition of CYP3A4/5 by MMAE (K_I/k_{inact} determinations)

Enzyme	CYP activity	Substrate concentrations (μM)	Incubation volume (μL)	Protein [a] (μg/mL)	Incubation time (min)	Preincubation time (min)	MMAE: Target concentrations (μM) [b]	MMAE: Solvent volume [c] (μL)
CYP3A4/5	Testosterone 6β-hydroxylation	100 and 200 [d]	400	100	5	0, 1, 2.5, 5, 10, 15 and 20	0, 0.5, 1, 3, 10 and 20	4

a The human liver microsomal sample used for these experiments was a pool of sixteen individuals (samples 286, 290, 312, 313, 315, 333, 334, 335, 336, 339, 348, 359, 364, 383, 389 and 390).

b Represents the concentration in the preincubation.

c Methanol (1% final incubation concentration) was the vehicle used to dissolve the test article.

d Represents the concentration of substrate at $2K_m$.

Summary of experimental conditions: Metabolism-dependent inhibition of CYP3A4/5 by MMAE (MIC formation)

Enzyme	Incubation volume (μL)	Protein [a] (μg/mL)	Time lapse between scans (min)	Total scan time (min)	Wavelengths monitored (nm)	MMAE: Target concentrations (μM)	MMAE: Solvent volume [b] (μL)
CYP3A4/5	1000	1000	1	15	380 – 520	0 and 10	10

a The human liver microsomal sample used for these experiments was a pool of sixteen individuals (samples 286, 290, 312, 313, 315, 333, 334, 335, 336, 339, 348, 359, 364, 383, 389 and 390).

b Methanol (1% final incubation concentration) was the vehicle used to dissolve the test article.

Findings:

- MMAE caused direct inhibition of CYP3A4/5 by midazolam 1'-hydroxylation with an IC_{50} of 10 μM.
- There was little or no direct inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 or CYP3A4/5 by testosterone 6β-hydroxylation, with IC50s of > 100 μM.
- MMAE is a time-dependent inhibitor of YCP3A4/5 (measured by testosterone 6β-hydroxylation and midazolam 1'-hydroxylation) as an increase inhibition was seen with pre-incubation period.

- The increased inhibition upon pre-incubation by MMAE was dependent upon NADPH and was partially reversed with microsomal re-isolation, indicating that metabolism-dependent inhibition was likely quasi-irreversible.
- MMAE had a K_{inact} value of 0.1 (min^{-1}), indicating that at saturating concentrations of MMAE, approximately 10% CYP3A4/5 activity would be inactivated per min. The K_I value was 1.12 μM, indicating the concentration of MMAE that gave half the maximum rate of inactivation.
- MMAE formed MIC with CYP3A4/5 (of human liver microsomal sample), suggesting that the metabolism inhibition being seen here was quasi-irreversible.

Table 17. MMAE as an inhibitor of human CYP enzymes

Summary of results: *In vitro* evaluation of MMAE as an inhibitor of human CYP enzymes

		Direct inhibition		Time-dependent inhibition		
		Zero-minute preincubation		30-minute preincubation		Potential for time-dependent inhibition [c]
Enzyme	CYP reaction	IC_{50} (μM) [a]	Maximum inhibition at 100 μM (%) [b]	IC_{50} (μM) [a]	Maximum inhibition at 100 μM (%) [b]	
CYP1A2	Phenacetin *O*-deethylation	>100	17	>100	19	Little or no
CYP2B6	Bupropion hydroxylation	>100	2.6	>100	NA	Little or no
CYP2C8	Amodiaquine *N*-dealkylation	>100	NA	>100	NA	Little or no
CYP2C9	Diclofenac 4′-hydroxylation	>100	NA	>100	2	Little or no
CYP2C19	*S*-Mephenytoin 4′-hydroxylation	>100	17	>100	18	Little or no
CYP2D6	Dextromethorphan *O*-demethylation	>100	10	>100	10	Little or no
CYP3A4/5	Testosterone 6β-hydroxylation	>100	27	0.6	92	Yes[d, e, f]
CYP3A4/5	Midazolam 1′-hydroxylation	10	89	0.4	97	Yes

a Average data (i.e., percent of control activity) obtained from duplicate samples for each test article concentration were used to calculate IC_{50} values. IC_{50} values were calculated with XLFit.

b Indicates Maximum inhibition (%) is calculated with the following formula and data for the highest concentration of test article evaluated (results are rounded to two significant figures): Maximum inhibition (%) = 100% – Percent solvent control.

c Time-dependent inhibition was determined by comparison of IC_{50} values with and without preincubation, by comparison of the maximum inhibition (%) with and without preincubation and by visual inspection of the IC_{50} plot.

d Time dependent inhibition of CYP3A4/5 as measured by testosterone 6β-hydroxylation was found to be partially reversed with microsomal re-isolation and fully reversed with treatment by potassium ferricyanide prior to re-isolation.

e MMAE was found to be a mechanism based inhibitor of CYP3A4/5 as measured by testosterone 6β-hydroxylation with a K_I of 1.12 μM and a k_{inact} of 0.10 (min^{-1}).

f MMAE was found to form a Metabolite Inhibitory Complex (MIC) with CYP3A4/5.

NA Not applicable. No value was obtained as the rates at the highest concentration of MMAE evaluated (100 μM) were higher than the control rates.

Best Available Copy

Cross-species comparison of MMAE metabolism and excretion (amended, Study (b) (4)-SGN35-001 by Settle Genetics)

Study Design: This non-GLP study was to investigate the metabolism and excretion of MMAE in humans following a single IV dose of SGN-35 and in comparison to data in vivo and in vitro across species (rat, monkey, and human). This study was based on 3 studies:1) (b) (4)084007 – in vitro metabolism of MMAE, 2) 420501 – excretion profile of MMAE (in feces), and 3) 8219-193: metabolism and excretion from patients with $CD30^+$ malignancies following a single IV dose of SGN-35.

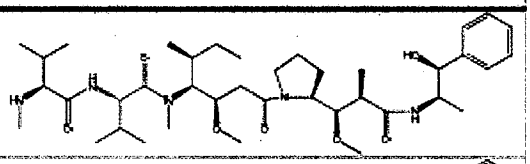
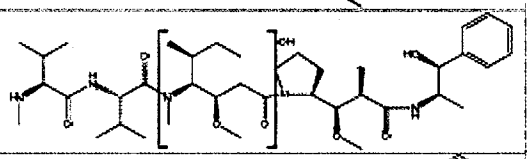
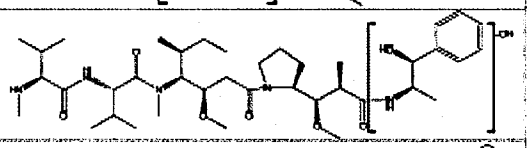
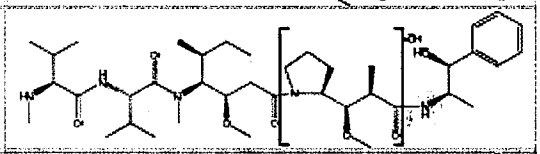
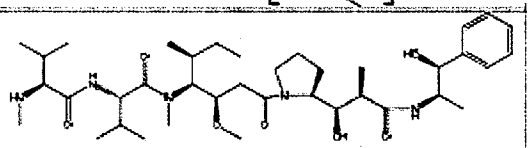
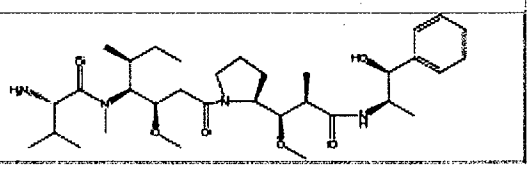
Findings:

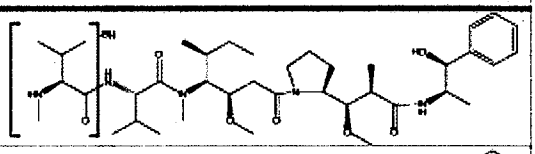
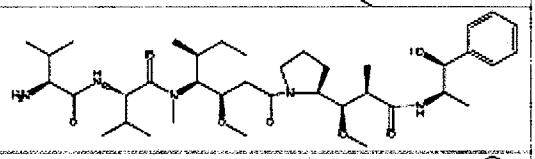
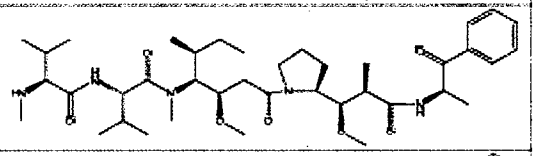
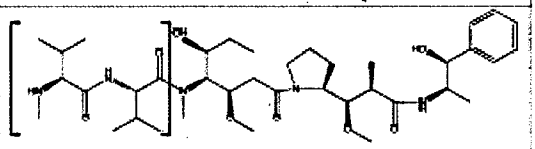
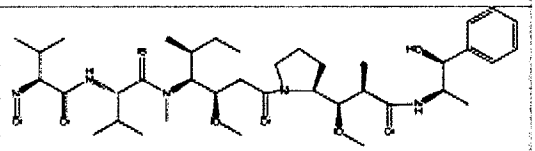
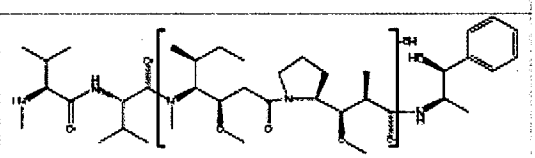
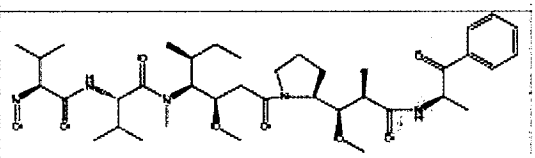
- Across all studies, 13 metabolites were identified.
- All twelve metabolites were formed by monkey hepatocytes, nine of which were formed by rat hepatocytes and a different set of nine were formed by human hepatocytes (see detailed review under Study No (b) (4)084007 above)

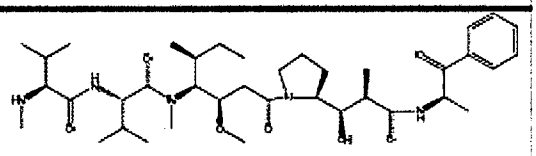
- The major route of elimination in rats after IV bolus dose was via feces (see detailed review under Study No 420501 below).
- Based on in vivo studies and in vitro studies across species, 1) the primary metabolic pathway in human was proposed to involve N- and O- demetylation, dehydrogenation, amide hydrolysis, and oxidation, and 2) the primary clearance mechanism for MMAE in human was likely via fecal/biliary excretion of the intact parent molecule (Clinical Study Report 8219-193).

Table 18. Metabolism and Excretion Profiles of MMAE across species (rat, monkey, human)

Metabolite assignments, and proposed metabolic transformation and structure for 13 metabolites of MMAE observed *in vitro* (study number (b)(4)084007), *in vivo* (study number 420501), and in human clinical samples (report number 8218-193).

Metabolite Assign-ment	m/z	Change in Mass (amu) from Parent	Proposed Transfor-mation from MMAE	Proposed Structure
MMAE (parent)	718	N/A	N/A	
C1	734	16	hydroxylation	
C2	734	16	hydroxylation	
C3	734	16	hydroxylation	
C4	704	−14	demethylation	
C5	605	−113	amide hydrolysis	

Metabolite Assign-ment	m/z	Change in Mass (amu) from Parent	Proposed Transfor-mation from MMAE	Proposed Structure
C6	734	16	hydroxylation	
C7	704	−14	demethylation	
C8	716	−2	loss of H_2	
C9	734	16	hydroxylation	
C10	718	0	demethylation and oxidation	
C11	734	16	hydroxylation	
C12	716	−2	C8 and C10	

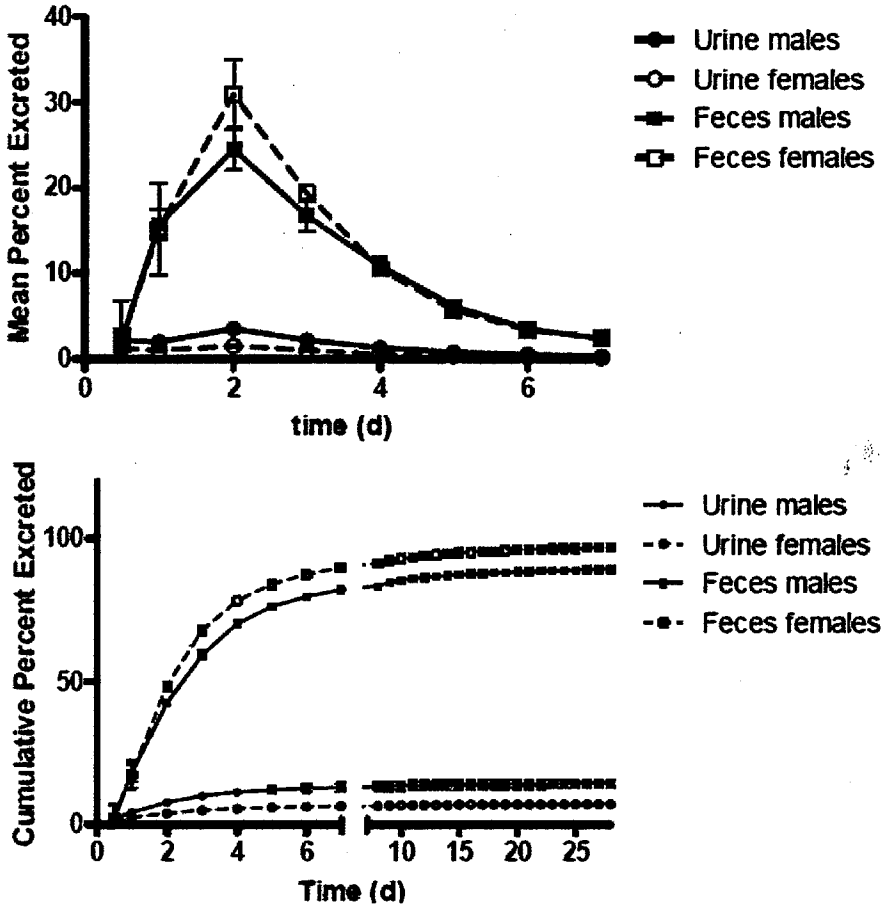
Metabolite Assign-ment	m/z	Change in Mass (amu) from Parent	Proposed Transfor-mation from MMAE	Proposed Structure
C13	702	−16	C4 and C8	

Cross-species comparison of MMAE metabolites identified *in vitro* (study number (b)(4)084007) and in human clinical samples (report number 8218-193).

Metabolite assignment	Rat hepatocytes	Monkey hepatocytes	Human hepatocytes	Human urine	Human feces
MMAE	X	X	X	X	X
C1	X	X			
C2		X			
C3	X	X	X		
C4	X	X	X		X
C5	X	X	X	X	X
C6	X	X	X	X	
C7	X	X	X	X	
C8	X	X	X	X	X
C9	X	X	X		
C10	X	X	X	X	
C11		X			
C12		X	X	X	
C13				X	X

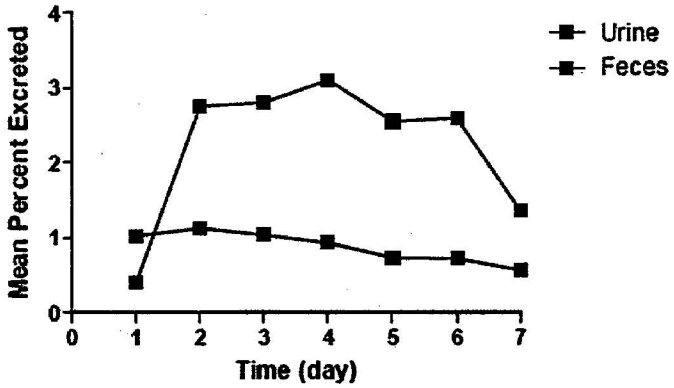
Figure 17. Excretion Profiles of MMAE across species (rat and human)

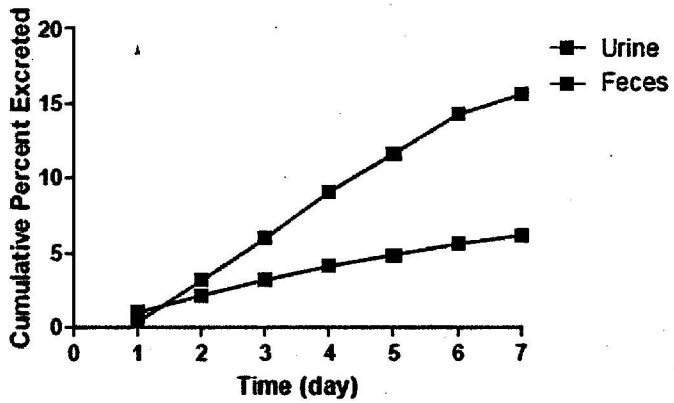
Mean and cumulative percent excretion of SGN-35 in rats.



Mean and cumulative percent excretion of SGN-35 recovered as MMAE in human urine and feces.

Note: Points with higher percent excretion (red color in graphs below) represent fecal excretion





Excretion

Excretion, Mass Balance and Pharmacokinetics of Radioactivity in Sprague-Dawley Rats Following a Single Intravenous Bolus Dose of cAC10-vc-^{3}H-MMAE or ^{3}H MMAE (Study 420501 by CRO: (b) (4))

Study Design: This GLP study was conducted to determine the main route of excretion of SGN-35 and to identify the species excreted. Rats were given a single IV bolus dose of 3 mg/kg cAC10-vc-^{3}H-MMAE (ADC, Lot No 1082:116 with 97.7% purity) or 0.056 mg/kg ^{3}H-MMAE (Batch Lot No 646-062-0258 with 99.9% purity. Note: Lot No for MMAE: 2002E with 93.8% purity). The urine and feces were collected at 0-12, 12-24, and at 24 hr intervals (until 672 hrs postdose ≈ 28 days). The concentration of radioactivity in blood, plasma, urine, feces, and carcass, were measured by LS. The radioactive species in urine and feces were measured using an HPLC radiometric/UV detection method, and the major metabolite identified by LC-MS/MS.

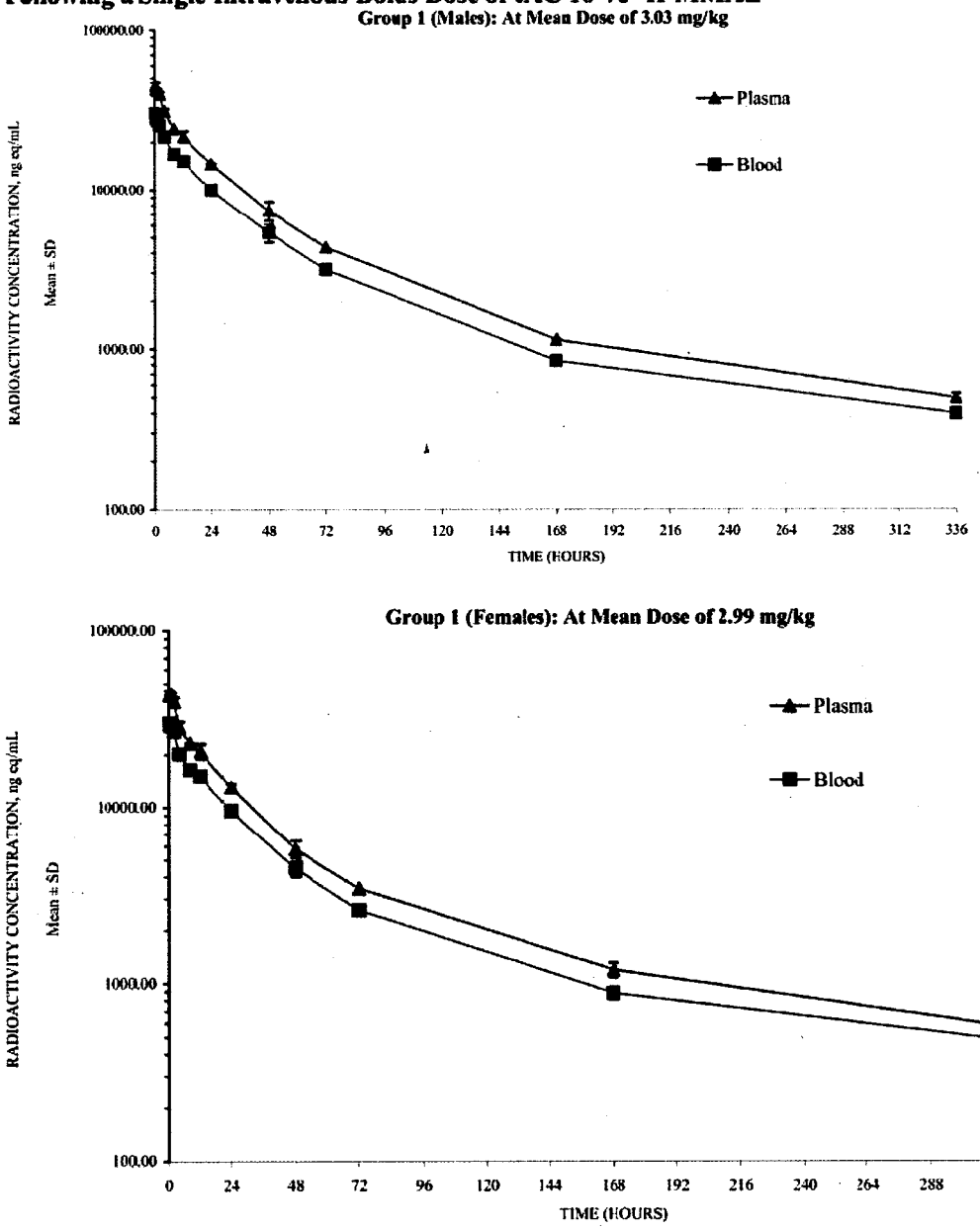
Group Number	Test Articles	Dose Level (mg/kg)	Concentration (mg/mL)	Dose Volume (mL/kg)	Animal Numbers	
					Males	Females
1	cAC10-vc-^{3}H-MMAE	3	0.6	5	1001-1012	1501-1512
2	cAC10-vc-^{3}H-MMAE	3	0.6	5	2001-2004	2501-2504
3	^{3}H-MMAE	0.056	0.0112	5	3001-3012	3501-3512
4	^{3}H-MMAE	0.056	0.0112	5	4001-4004	4501-4504

Findings:

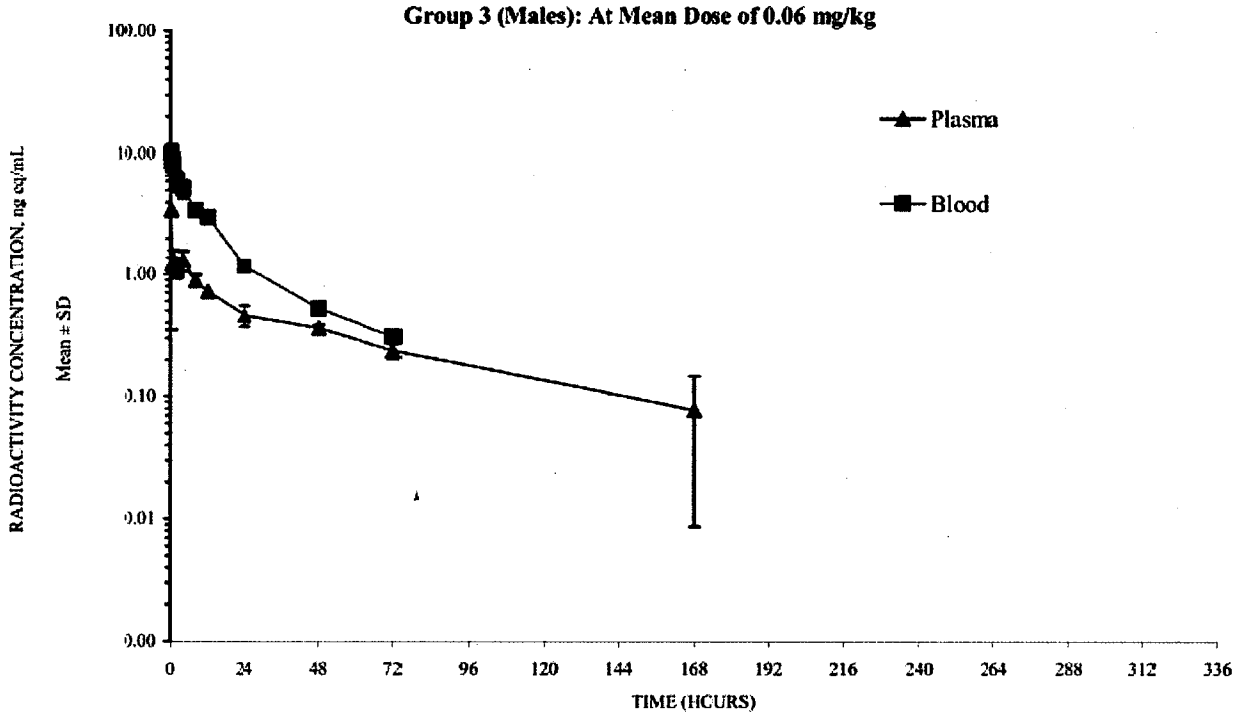
- The major route of elimination for both cAC10-vc-^{3}H-MMAE and ^{3}H-MMAE in rats was via feces, suggesting biliary excretion with some recovery in urine.
- For cAC10-vc-^{3}H-MMAE, approximately 89% (males) or 97% (females) of the radiolabeled material was detected in feces. Approximately 14% (males) or 7% (females) of the radiolabeled materials was detected in urine.
- For ^{3}H-MMAE, approximately 97% (males) or 102%(females) of the radiolabeled material was detected in feces. Approximately 15% (males) or 9% (females) of the radiolabeled materials was detected in urine.
- No radioactivity was recovered in the carcass (with ^{3}H-MMAE) at 28 days post dose, suggesting that elimination was complete at this time after a single dose administration.
- In the 1st 48 hr postdose, approximately 50% cAC10-vc-^{3}H-MMAE was excreted in feces and urine, whereas approximately 90-95% ^{3}H-MMAE was excreted during this period, suggesting more rapid elimination of free drug from the system than when conjugated to an antibody. The half-life of cAC10-vc-^{3}H-MMAE was 8.5-10.7 days in rats whereas the half-life of ^{3}H-MMAE was 1-2.3 days.
- The majority of radiolabeled component seen in urine and feces was identified as ^{3}H MMAE. Smaller peaks were seen in excreta, which were identified as C4 (predominant metabolite of the parent compound in the feces).
- With cAC10-vc-^{3}H-MMAE (ADC), the findings displayed the PK characteristics of an antibody, resulting in higher concentrations of radiolabeled material in circulation, slower clearance, and a longer half-life, and a smaller volume of distribution, when compared to ^{3}H-MMAE (small free drug molecule).

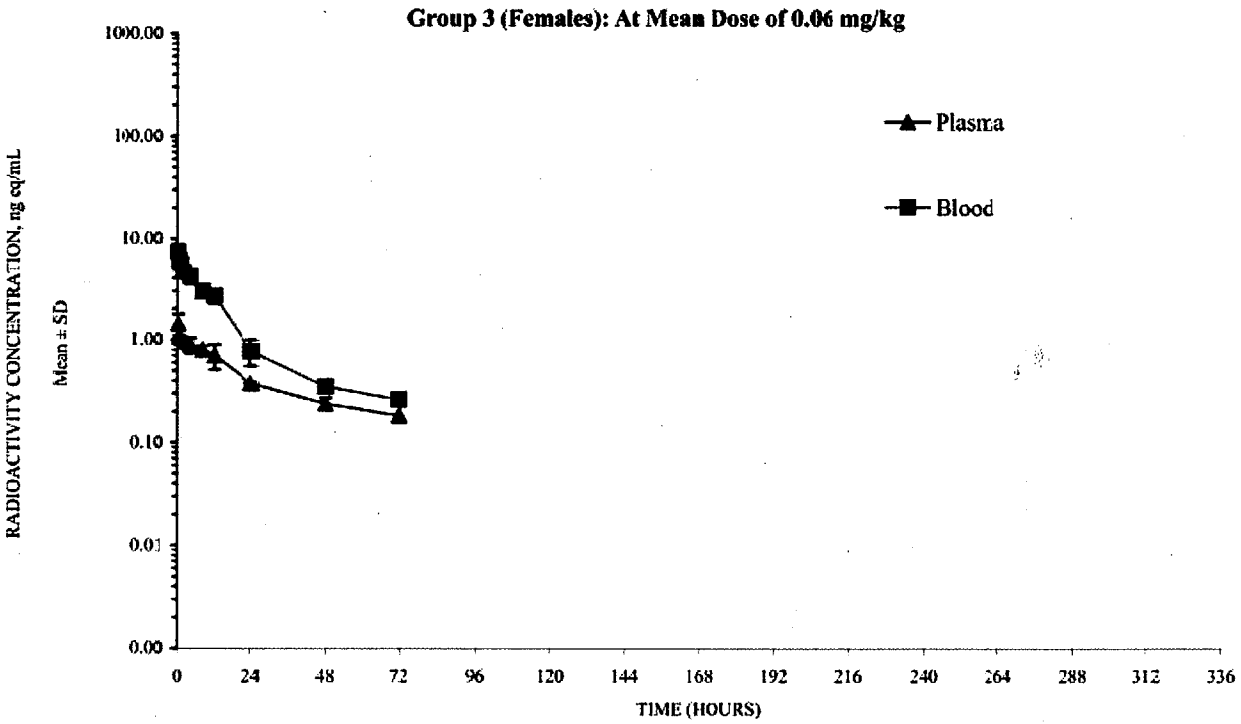
Figure 18. Radioactivity in Plasma and Blood of Rats after a Single IV Bolus Dose

Group Mean Concentrations of Radioactivity in Plasma and Blood of Sprague-Dawley Rats Following a Single Intravenous Bolus Dose of cAC 10-vc-^{3}H-MMAE



Group Mean Concentrations of Radioactivity in Plasma and Blood of Sprague-Dawley Rats Following a Single Intravenous Bolus Dose of ^{3}H-MMAE





Plasma Stability of cAC10-vcMMAE4 (SGN-35) (Study TRN-0512-A (by Settle Genetics)

Study Design: This non-GLP study investigated the stability of cAC10-vcMMAE (SGN-35) in native plasma. The concentration of released MMAE in various biological matrixes was measured by LC-MS/MS (Note: Lot No 1010-01)

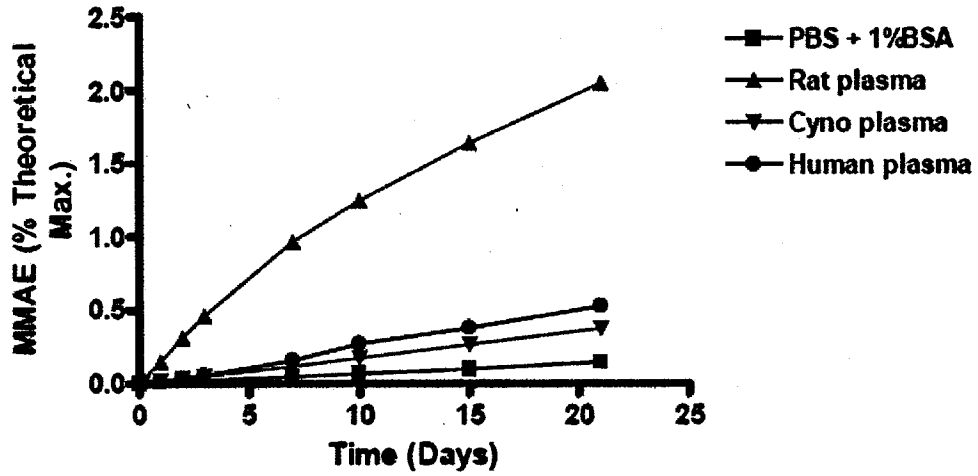
Findings:

- SGN-35 was stable, generating no more than 2% of the maximum theoretical amount of MMAE over 21 days.
- SGN-35 was least stable in rat plasma and most stable in Cynomolgus monkey and human plasma, and PBS with 1% BSA.

Figure 19. Plasma Stability of SGN-35

Concentration of free MMAE over time.
SGN-35 was incubated in citrated plasma from rat, cyno, or human or PBS + 1% (w/v) BSA at 37 °C for up to 21 days. The d8-MMAE internal standard was added and the samples processed by solid-phase extraction and analyzed by LC-MS/MS.

TRN-0512-A Page 12 o



Overall ADME Conclusions:

Brentuximab vedotin was referred as SGN-35 or cAC10-vcMMAE in these ADME studies.

The distribution (i.e. a limited volume of distribution) and clearance (i.e. low serum clearance) characteristics of SGN-35 were similar to a monoclonal antibody product. Exposure (i.e. C_{max} and AUC) to SGN-35 was approximately dose proportional in rats (Study Nos R-PK-08 and R-PK-14) and approximately dose-proportional or greater than dose-proportional in monkeys (Study No 8201-470). Total Ab exposure was generally greater than exposures to cAC10-vcMMAE. After the cAC10-vcMMAE administration, MMAE exposures were approximately dose-proportional in rats and monkeys, and

MMAE t_{max} ranged from 1-3 days. On the other hand, MMAE exhibited higher clearance and large volume of distribution after MMAE administration.

The immunogenicity of cAC10-vcMMAE was measured in monkeys (Study No 8201-470) and rats (Study Nos (b) (4)-08 and (b) (4)-14). Anti-therapeutic antibodies (ATA) to cAC10-vcMMAE were detected in both species.

In *in vitro* plasma protein binding assay (Study No XS-0025) at concentrations tested, MMAE was 70-80% bound to plasma proteins in human, similar to results obtained for rat (70% bound). Plasma protein binding was species-dependent with less binding (~20-30%) in mouse and monkey plasma.

The MMAE metabolism was similar in rat, monkey and human hepatocytes and metabolites formed in human hepatocytes were similar to those formed in rat and monkey hepatocytes. In addition, MMAE was also found to be a quasi-irreversible CYP3A4/5 inhibitor and the primary in vitro metabolites were formed by CYP3A4.

Excretion study was conducted in rats after a single IV dose of SGN-35 or MMAE (Study No 420501). The main route of excretion in rats was via feces with some urinary excretion. MMAE was the predominant compound being excreted. In a stability study (Study No TRN 0512A), SGN-35 was stable for up to 21 days and the rates of MMAE formation in rat, monkey, and human plasma was similar to the uncatalyzed rate measured in PBS+1%BSA.

5.2 Toxicokinetics

TK studies are reviewed in corresponding toxicity studies.

6 General Toxicology

6.1 Single-Dose Toxicity

Not reviewed.

6.2 Repeat-Dose Toxicity

Repeat-dose toxicity and toxicokinetic studies were conducted in rats and monkeys.

Four week (once every week, 4 doses in total) study in rats and three month (once every three week, 4 doses in total) study in monkeys were reviewed under IND 71,634 by Dr. Haleh Saber

---

Study Title: A 4-Week Intravenous Injection Toxicity and Toxicokinetic Study of SGN-35, SGD-1010, and SGN-30 in Rats with a 4-Week Recovery

Key study findings: Toxicities were similar after SGN-35 and MMAE treatments. Hematopoietic, hepatobiliary, and male reproductive system toxicities were the main treatment-related findings. Hepatobiliary toxicity was more pronounced for SGN-35 than for MMAE treatment.

Study no.: (b) (4) Number 7646-118
Volume #, and page #: electronic submission
Conducting laboratory and location: (b) (4)

Date of study initiation: 9/21/2005
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity:

Test Article 1	Lot/Batch No.	Storage	Purity
SGD-1010	SGD-1010-0-09	In a freezer, set to maintain -10 to -30°C with desiccant	97.35%
Test Article 2			
SGN-35	P02905	In a freezer, set to maintain -60 to -80°C	93.4%
Test Article 3			
SGN-30	STW001	In a refrigerator, set to maintain 2° to 8°C	98.4%

Methods

Doses: on Days 1, 8, 15, and 22

Group	Treatment	No. of Animals Male	No. of Animals Female	Dose Level (mg/kg/dose)[a]	Dose Concentration (mg/mL)[a]	Terminal Sacrifice Study Day 26 (No./sex)	Recovery Sacrifice Study Day 51 (No./sex)
Toxicity Animals							
1	(Control Article 1)[b,c]	15	15	0	0	10	5
2	SGD-1010 (low)	10	10	0.0097	0.0019	10	-
3	SGD-1010 (mid)[c]	15	15	0.097	0.0194	10	5
4	SGD-1010 (high)[c]	15	15	0.194	0.0388	10	5
5	(Control Article 2)[b,c]	15	15	0	0	10	5
6	SGN-35 (low)	10	10	0.5	0.1	10	-
7	SGN-35 (mid)[c]	15	15	5	1	10	5
8	SGN-35 (high)[c]	15	15	10	2	10	5
9	SGN-30[c]	15	15	10	2	10	5
Toxicokinetic Animals[d]							
10	(Control Article 1)[b]	10	10	0	0	-	-
11	SGD-1010 (low)	10	10	0.0097	0.0019	-	-
12	SGD-1010 (mid)	10	10	0.097	0.0194	-	-
13	SGD-1010 (high)	10	10	0.194	0.0388	-	-
14	(Control Article 2)[b]	10	10	0	0	-	-
15	SGN-35 (low)	10	10	0.5	0.1	-	-
16	SGN-35 (mid)	10	10	5	1	-	-
17	SGN-35 (high)	10	10	10	2	-	-
18	SGN-30	10	10	10	2	-	-

a Animals were dosed at a volume of 5 mL/kg.
b Groups 1 and 10 received Control Article 1 only, and Groups 5 and 14 received Control Article 2 only.
c Animals designated for recovery sacrifice (five animals/sex) from all toxicity groups except Groups 2 and 6 underwent 4 weeks of recovery following 4 weeks of dosing.
d Toxicokinetic animals were included solely for the purpose of toxicokinetic and immunogenicity blood sample collections.

The mean concentrations of SGD-1010 dose preparations for dosing of ♀s on Day 1 ranged from 85.0% to 101%, and those for dosing of ♂s on Day 22 from 80.7% to 95.1%, of the intended concentrations. The mean concentrations of SGN-35 dose preparations for dosing of ♀s on Day 1 ranged from 89.9% to 98.8%, and those for dosing of ♂s on Day 22 from 84.9% to 102 %, of the intended concentrations. The mean concentrations of SGN-30 dose preparations for dosing of ♀s on Day 1 and those for dosing of ♂s on Day 22 were 103% and 98.3%, respectively, of the intended concentrations.

Species/strain: SD rats
Number/sex/group or time point (main study): see Table above
Route, formulation, volume, and infusion rate: i.v., bolus, solution, 5 mL/kg
Satellite groups used for toxicokinetics or recovery:
Age: not specified
Weight: ~200 g (♀s) and 300 g (♂s)

Observation and Times:
Clinical signs: twice daily
Body weights: once during the pre-dose phase, prior to dosing on the first day of the dosing period and weekly thereafter during the dosing and recovery periods
Food consumption: weekly

Ophthalmoscopy: once during the predose and once during Week 4 of the dosing period (Groups 1 through 9) using an indirect ophthalmoscope. The eyes were dilated with a mydriatic agent prior to examination.
EKG:
Hematology and coagulation: on Days 26 and 51 from animals in Groups 1-9 and unscheduled sacrifices (fasted for scheduled clinical pathology). Blood was collected via a jugular vein.
Serum chemistry: on Days 26 and 51 from animals in Groups 1-9 and unscheduled sacrifices (fasted for scheduled clinical pathology). Blood was collected via a jugular vein.
Urinalysis: on Days 26 and 51, Groups 1-9; overnight collection.
Gross pathology:

Unscheduled sacrifices (Group 3 ♀s)
Day 26 (10/sex/group)
Day 51 (all surviving)

Organ weights: at scheduled sacrifices

adrenal (2)	**pituitary gland**
brain	**prostate**
epididymis (2)	**salivary gland [mandibular (2)**
heart	**seminal vesicle**
kidney (2)	**spleen**
liver	**testis (2)**
lung	**thymus**
ovary (2)	**uterus**

Histopathology:
Performed for control groups (1 and 5), HD animals (Groups 4 and 8), and SGN-30 (Group 9). In addition, unscheduled sacrifices and macroscopic lesions (LD and MD) were examined microscopically.

The following were preserved in 10% NBF, unless otherwise indicated.

adrenal (2)	ovary (2)
aorta	pancreas
brain	pituitary gland
cecum	prostate
colon	rectum
duodenum	salivary gland [mandibular (2)]
epididymis (2)[a]	sciatic nerve
esophagus	seminal vesicle
eye (2)[a]	skeletal muscle (thigh)
femur with bone marrow (articular surface of the distal end)	skin
	spinal cord (cervical, thoracic, and lumbar)
Harderian gland[a]	spleen
heart	sternum with bone marrow
ileum	stomach
injection site(s)	testis (2)[a]
jejunum	thymus
kidney (2)	thyroid (2 lobes) with parathyroid
lesions	tongue
liver	trachea
lung with large bronchi	urinary bladder
lymph node (mesenteric)	uterus
mammary gland (females)	vagina
optic nerve (2)[a]	

[a] *Preserved in modified Davidson's fixative.*
Bone marrow smears were made but not read.

TK and immunogenicity:

Blood for toxicokinetic analyses was for SGD-1010 (MMAE), SGN-35, and SGN-30.

Blood samples for MMAE TK were collected once during acclimation and on Days 1, 2, 8, 15, 22 and 23. Blood samples for TK from Groups 14-18 (SGN-35) were collected once during acclimation and on Days 1, 3, 8, 10, 15, 17, 22, and 24.

Total Ab
The serum concentration of the antibody component of SGN-35, irrespective of the presence of the SGD-1010 conjugated drug was determined for animals in Groups 14-18 on the following sample time-points:
Day 1, within 15 minutes of dose administration
Day 8, predose
Day 8, within 15 minutes of dose administration
Day 15, predose
Day 15, within 15 minutes of dose administration
Day 22, predose
Day 22, within 15 minutes of dose administration

The method that was validated and used was ELISA-0186. The assay format used anti-ID-30 (anti-idiotypic murine monoclonal antibody to cAC10, clone 30.16) to coat plates and utilized biotinylated anti-ID30 for detection.

ADC

The serum concentration of the SGN-35 with at least one SGD-10101 drug moiety conjugated to the antibody was determined for animals in Groups 14-17 on the following sample time-points:

Day 1, within 15 minutes of dose administration
Day 8, predose
Day 8, within 15 minutes of dose administration
Day 15, predose
Day 15, within 15 minutes of dose administration
Day 22, predose
Day 22, within 15 minutes of dose administration

The method that was validated and used for the sample testing was ELISA-0184. The assay format was a "bridging" ELISA in which anti-MMAE (SGD-1010) antibody was coated on the plate and biotinylated anti-ID30 antibody was used for detection.

SGD-1010

The serum concentration of free SGD-1010 was determined for animals treated with SGD-1010 (Groups 11-13) and SGN-35 (Groups 15-17) on the following sample time-points:

Day 1, 15 min
Day 1, 30 min
Day 1, 2 h
Day 1, 8 h
Day1, 24 h
Day 8, predose
Day 8, within 15 minutes
Day 15, predose
Day 15, within 15 minutes
Day 22, predose
Day 22, within 15 minutes
Day 22, 30 min
Day 22, 2 h
Day 22, 8 h
Day 22, 24 h

Analyzed using liquid chromatography (LC) with tandem mass spectrometric detection (MS/MS).

Immunogenicity assay:

Immunogenicity analysis for anti-SGN-35 antibodies was performed on samples collected from animals in Groups 14 through 18 treated with Control Article 1 (Group 14), SGN-35 (Groups 15-17) and SGN-30 (Group 18) on the following time-points:

Acclimation
Day 8
Day 15
Day 22

The method that was validated was ELISA-0185. The assay format used SGN-35 to coat plates and used biotinylated SGN-35 for detection.

Results:

Mortality: 2 animals died after blood collection, Animal No. B99214 (Group 18 ♂, Day 8 of dosing) and Animal No. B99360 (Group 3 ♀, Day 26 of dosing). Both deaths were evaluated as accidental. All other animals survived until the day of scheduled sacrifice.

Clinical signs: No noteworthy clinical signs

Body weights:
BW loss or ↓BW gain in SGD-1010 (MMAE) HD ♂s, during the treatment period.
Periods of ↓BW gain in SGD-10101 (MMAE) MD ♂s, during the treatment period.

Food consumption: ↓ during week 1 in SGD-1010 HD ♂s, corresponding to reduced weight in this group

Ophthalmoscopy: No drug-related effect
One control and one MD SGN-35 had chorioretinal scars

EKG: not done

Hematology: effects were reversible

- No drug related findings for SGN-30, LD SGD-1010 (MMAE), and LD SGN-35.
- The most pronounced effects were seen in HD SGD-1010 and HD SGN-35: ↓erythropoiesis and hepatobiliary injury

Day 26 data

	RBC		HGB		HCT		Retic	
	♂	♀	♂	♀	♂	♀	♂	♀
MMAE-MD	—	—	—	—	—	—	↓45%*	↓35%*
MMAE-HD	↓50%*	↓35%*	↓35%*	↓25%*	↓35%*	↓25%*	↓90%*	↓95%*
SGN35-MD	—	—	—	—	—	—	↓55%*	↓35%*
SGN35-HD	↓35%*	↓35%*	↓30%*	↓25%*	↓30%*	↓25%*	↓90%*	↓75%*

	Platelet		WBC		Neut		Lymph	
	♂	♀	♂	♀	♂	♀	♂	♀
MMAE-MD	—	—	—	—	—	—	—	—
MMAE-HD	—	—	↓35%*	↓30%*	↓35%*	↓80%*	↓35%*	↓20%
SGN35-	↓20%*	—	↓15%*	—	↓35%*	—	↓15%*	—

MD								
SGN35-HD	↓20%*	↓35%*	↓50%*	↓35%*	↓50%*	↓30%	↓45%*	↓35%*

	Mono		Eos	
	♂	♀	♂	♀
MMAE-MD	—	—	↓45%*	—
MMAE-HD	↓50%*	↓50%*	↓70%*	↓85%*
SGN35-MD	—	—	↓65%*	↓50%*
SGN35-HD	↓50%*	↓30%*	↓80%*	↓50%*

* statistically significant.
—: not toxicologically significant or no effect.

Clinical chemistry:
Although effects were generally reversible, some hepato-biliary findings were toxicologically significant at the end of the recovery period (recovery 26)
D26 clinical chemistry

	Cholesterol		Total Bil		AST	
	♂	♀	♂	♀	♂	♀
MMAE-MD	↑40%* (↑35%*)	↑20% (—)	—	—	—	—
MMAE-HD	↑60%* (↑50%*)	↑30%* (↑15%)	↑100%* (—)	—	↑100%* (↑35%*)	—
SGN35-MD	↑30%* (—)	↑20% (↑45%)	—	—	—	—
SGN35-HD	120%* (↑35%*)	↑100%* (↑55%)	↑4-fold* (—)	↑2-fold* (—)	↑3-fold* (—)	↑2-fold* (↑50%)

	ALT		GGT	
	♂	♀	♂	♀
MMAE-MD		—	—	—
MMAE-HD	↑100% (—)	—	↑9-fold* (100%)	↑100%* (↑100%)
SGN35-MD		—	—	—
SGN35-HD	↑3.5-fold* (—)	↑4-fold* (↑2-fold)	↑8-fold* (—)	↑2-fold* (↑2-fold)

* Statistically significant.
—: not toxicologically significant or no effect; (): recovery data.

Urinalysis: No test article-related effect

Gross pathology:
End of dosing
ADC: antibody-drug conjugate (SGN-35)

	C	ADC LD	ADC MD	ADC HD	free Ab
	-- Males --				
Group:	5	6	7	8	9
Number in group:	10	10	10	10	10
Testis					
Small	0	0	0	4	0
Soft	0	0	0	3	0
Total:	0	0	0	7	0

Recovery

	-- Males --				
Group:	5	6	7	8	9
Number in group:	5	0	5	5	5
Testis					
Small	0	0	2	3	0
Soft	0	0	0	2	0
Total:	0	0	2	5	0

Organ weights:

	Heart		Liver		Kidney		Lung	
	♂	♀	♂	♀	♂	♀	♂	♀
MMAE-MD	—	—	—	—	—	—	—	—
MMAE-HD	↑18%*	↑20%*	↑17%*	↑9%*	↑10%*	—	↑15%*	↑10%*
SGN35-MD	—	—	—	—	—	—	—	—
SGN35-HD	—	—	↑10%*	↑12%*	—	—	↑10%*	—

	Spleen		Epididymis		Thymus		Testis	
	♂	♀	♂	♀	♂	♀	♂	♀
MMAE-MD	—	—	—	NA	↓25%*	↓25%	—	NA
MMAE-HD	↑15%*	—	—	NA	↓85%*	↓40%*	—	NA
SGN35-MD	—	—	—	NA	↓10%*	↓30%*	↓23%*	NA
SGN35-HD	—	—	↑20%*	NA	↓50%*	↓60%*	↓40%*	NA

	Mand Salivary G	
	♂	♀
MMAE-MD	—	—
MMAE-HD	—	—
SGN35-MD	—	—
SGN35-HD	↑15%*	—

Histopathology:
MMAE Groups (2-4)
Treatment data

Controls from group(s): 1 / Tissues With Diagnoses	Animal sex: / Dosage group: / No. in group:	-- Animals Affected -- Males: Ctls	2	3	4	-- Females: Ctls	2	3	4
		10	10	10	10	10	10	9	10
Thyroid	Number examined:	10	0	0	10	10	0	0	10
Cyst, Ultimobranchial	->	8	0	0	8	8	0	0	6
	1>	2	0	0	2	2	0	0	3
	2>	0	0	0	0	0	0	0	1
	Total Incidence of Finding Observed:	2	0	0	2	2	0	0	4
Liver	Number examined:	10	10	10	10	10	0	0	10
Necrosis, Coagulative	->	9	10	10	7	10	0	0	10
	1>	1	0	0	3	0	0	0	0
	Total Incidence of Finding Observed:	1	0	0	3	0	0	0	0
Spleen	Number examined:	10	0	0	10	10	0	0	10
Fibrosis	->	10	0	0	10	10	0	0	9
	1>	0	0	0	0	0	0	0	1
	Total Incidence of Finding Observed:	0	0	0	0	0	0	0	1
Lung									
Infiltrate, Macrophages, Alveolus	->	10	0	0	10	10	0	0	9
	1>	0	0	0	0	0	0	0	1
	Total Incidence of Finding Observed:	0	0	0	0	0	0	0	1
Thymus									
Depletion, Lymphocytes	->	10	10	10	1	10	10	9	2
	1>	0	0	0	1	0	0	0	3
	2>	0	0	0	4	0	0	0	3
	3>	0	0	0	4	0	0	0	2
	Total Incidence of Finding Observed:	0	0	0	9	0	0	0	8
Necrosis, Lymphocytes	->	10	10	10	10	10	10	9	8
	1>	0	0	0	0	0	0	0	2
	Total Incidence of Finding Observed:	0	0	0	0	0	0	0	2
Kidney	Number examined:	10	0	0	10	10	0	0	10
Hydronephrosis	->	10	0	0	9	10	0	0	10
	3>	0	0	0	1	0	0	0	0
	Total Incidence of Finding Observed:	0	0	0	1	0	0	0	0
Cyst	->	10	0	0	9	10	0	0	10
	1>	0	0	0	1	0	0	0	0
	Total Incidence of Finding Observed:	0	0	0	1	0	0	0	0
Rectum	Number examined:	10	0	0	10	10	0	0	10
Infiltrate, Lymphocytes	->	7	0	0	6	7	0	0	7
	1>	3	0	0	4	3	0	0	3
	Total Incidence of Finding Observed:	3	0	0	4	3	0	0	3
LN, Mesenteric	Number examined:	10	0	0	10	10	0	0	10
Depletion, Lymphocytes	->	10	0	0	9	10	0	0	10
	2>	0	0	0	1	0	0	0	0
	Total Incidence of Finding Observed:	0	0	0	1	0	0	0	0
Pancreas	Number examined:	10	0	0	10	10	0	0	10
Hyperplasia, Islet Cell	->	10	0	0	9	10	0	0	10
	2>	0	0	0	1	0	0	0	0
	Total Incidence of Finding Observed:	0	0	0	1	0	0	0	0

Prostate ..... Number examined:	10	0	0	10						
Inflammation, Subacute										
->	9	0	0	8						
1>	1	0	0	2						
..... Total Incidence of Finding Observed:	1	0	0	2						

Testis

Tubules With Decreased Spermatocytes										
->	10	0	10	4						
1>	0	0	0	6						
..... Total Incidence of Finding Observed:	0	0	0	6						
Marrow, Femur ..... Number examined:	10	10	10	10		10	10	9	10	
Hypocellular										
->	10	10	10	1		10	10	9	7	
1>	0	0	0	2		0	0	0	2	
2>	0	0	0	2		0	0	0	1	
4>	0	0	0	5		0	0	0	0	
..... Total Incidence of Finding Observed:	0	0	0	9		0	0	0	3	
Marrow, Sternum ..... Number examined:	10	10	10	10		9	10	9	10	
Hypocellular										
->	10	10	10	1		9	10	9	2	
1>	0	0	0	1		0	0	0	4	
2>	0	0	0	1		0	0	0	3	
3>	0	0	0	5		0	0	0	1	
4>	0	0	0	2		0	0	0	0	
..... Total Incidence of Finding Observed:	0	0	0	9		0	0	0	8	

Recovery data

Testis ..... Number examined:	5	0	5	5	
Syncytial Cells					
->	5	0	5	5	
..... Total Incidence of Finding Observed:	0	0	0	0	
Degeneration, Seminiferous Tubule					
->	5	0	5	1	
2>	0	0	0	3	
3>	0	0	0	1	
..... Total Incidence of Finding Observed:	0	0	0	4	
Vacuolation, Sertoli Cells					
->	5	0	5	2	
1>	0	0	0	3	
..... Total Incidence of Finding Observed:	0	0	0	3	
Epididymis ..... Number examined:	5	0	5	5	
Aspermia					
->	5	0	5	2	
1>	0	0	0	3	
..... Total Incidence of Finding Observed:	0	0	0	3	

SGN-35 Groups 6-8 and SGN-30 (Group 9)

Thyroid ..... Number examined:	10	0	0	10	10	10	0	0	10	9
Cyst, Ultimobranchial										
->	10	0	0	8	8	10	0	0	9	7
1>	0	0	0	2	2	0	0	0	1	2
..... Total Incidence of Finding Observed:	0	0	0	2	2	0	0	0	1	2

Liver

Necrosis, Coagulative										
->	10	9	10	7	10	10	0	0	9	
1>	0	0	0	3	0	0	0	0	0	
2>	0	0	0	0	0	0	0	0	1	
..... Total Incidence of Finding Observed:	0	0	0	3	0	0	0	0	1	
Spleen ..... Number examined:	10	0	0	10	10	10	0	0	10	10
Fibrosis										
->	10	0	0	10	10	10	0	0	9	10
1>	0	0	0	0	0	0	0	0	1	0
..... Total Incidence of Finding Observed:	0	0	0	0	0	0	0	0	1	0
Thymus ..... Number examined:	10	10	10	10	10	10	10	10	10	10
Depletion, Lymphocytes										
->	10	10	10	1	10	10	10	3	10	
1>	0	0	0	6	0	0	0	0	0	
2>	0	0	0	2	0	0	0	0	0	
3>	0	0	0	1	0	0	0	6	0	
4>	0	0	0	0	0	0	0	1	0	
..... Total Incidence of Finding Observed:	0	0	0	9	0	0	0	7	0	
Necrosis, Lymphocytes										
->	10	10	10	9	10	10	10	5	10	
1>	0	0	0	1	0	0	0	4	0	
2>	0	0	0	0	0	0	0	1	0	
..... Total Incidence of Finding Observed:	0	0	0	1	0	0	0	5	0	
Testis ..... Number examined:	10	10	10	10	10					
Syncytial Cells										
->	10	10	10	6	10					
1>	0	0	0	4	0					
..... Total Incidence of Finding Observed:	0	0	0	4	0					
Degeneration, Seminiferous Tubule										
->	10	10	10	7	10					
1>	0	0	0	1	0					
2>	0	0	0	1	0					
3>	0	0	0	1	0					
..... Total Incidence of Finding Observed:	0	0	0	3	0					
Vacuolation, Sertoli Cells										
->	10	10	9	8	10					
1>	0	0	1	2	0					
..... Total Incidence of Finding Observed:	0	0	1	2	0					

Testis ..... Number examined:	10	10	10	10	10						
Tubules With Decreased Spermatocytes											
->	10	10	3	1	10						
1>	0	0	7	8	0						
2>	0	0	0	1	0						
..... Total Incidence of Finding Observed:	0	0	7	9	0						
Epididymis ..... Number examined:	10	10	10	10	10						
Aspermia											
->	10	10	10	7	10						
1>	0	0	0	1	0						
2>	0	0	0	1	0						
4>	0	0	0	1	0						
..... Total Incidence of Finding Observed:	0	0	0	3	0						

Uterus

Hemorrhage											
->							10	0	0	10	9
1>							0	0	0	0	1
..... Total Incidence of Finding Observed:							0	0	0	0	1
Marrow, Femur ..... Number examined:	10	10	10	10	10		10	10	10	10	10
Hypocellular											
->	10	10	2	0	10		10	10	7	0	10
1>	0	0	8	0	0		0	0	3	3	0
2>	0	0	0	0	0		0	0	0	7	0
3>	0	0	0	1	0		0	0	0	0	0
4>	0	0	0	9	0		0	0	0	0	0
..... Total Incidence of Finding Observed:	0	0	8	10	0		0	0	3	10	0
Hemorrhage											
->	10	10	10	10	10		10	10	10	9	10
2>	0	0	0	0	0		0	0	0	1	0
..... Total Incidence of Finding Observed:	0	0	0	0	0		0	0	0	1	0
Marrow, Sternum ..... Number examined:	10	10	10	10	10		10	10	10	10	10
Hypocellular											
->	10	10	6	0	10		10	10	9	0	10
1>	0	0	4	0	0		0	0	1	3	0
2>	0	0	0	2	0		0	0	0	2	0
3>	0	0	0	1	0		0	0	0	5	0
4>	0	0	0	7	0		0	0	0	0	0
..... Total Incidence of Finding Observed:	0	0	4	10	0		0	0	1	10	0

Recovery data

Thyroid ..... Number examined:	5	0	0	5	5		5	0	0	5	5
Cyst, Ultimobranchial											
->	5	0	0	5	3		3	0	0	4	4
1>	0	0	0	0	2		2	0	0	1	1
..... Total Incidence of Finding Observed:	0	0	0	0	2		2	0	0	1	1
Heart ..... Number examined:	5	0	0	5	5		5	0	0	5	5
Inflammation, Subacute											
->	4	0	0	4	2		5	0	0	5	4
1>	1	0	0	1	3		0	0	0	0	1
..... Total Incidence of Finding Observed:	1	0	0	1	3		0	0	0	0	1

Liver

Hemorrhage											
->	5	0	5	5	5		5	0	0	4	5
1>	0	0	0	0	0		0	0	0	1	0
..... Total Incidence of Finding Observed:	0	0	0	0	0		0	0	0	1	0
Hyperplasia, Bile Duct											
->	5	0	5	4	5		5	0	0	5	5
1>	0	0	0	1	0		0	0	0	0	0
..... Total Incidence of Finding Observed:	0	0	0	1	0		0	0	0	0	0
Fibrosis, Capsule											
->	5	0	5	5	4		5	0	0	5	5
1>	0	0	0	0	1		0	0	0	0	0
..... Total Incidence of Finding Observed:	0	0	0	0	1		0	0	0	0	0
Testis ..... Number examined:	5	0	5	5	5						
Syncytial Cells											
->	5	0	5	5	5						
..... Total Incidence of Finding Observed:	0	0	0	0	0						
Degeneration, Seminiferous Tubule											
->	5	0	0	0	5						
2>	0	0	2	1	0						
3>	0	0	3	3	0						
4>	0	0	0	1	0						
..... Total Incidence of Finding Observed:	0	0	5	5	0						
Vacuolation, Sertoli Cells											
->	5	0	1	0	5						
1>	0	0	4	3	0						
2>	0	0	0	2	0						
..... Total Incidence of Finding Observed:	0	0	4	5	0						
Epididymis ..... Number examined:	5	0	5	5	5						
Aspermia											
->	5	0	0	0	5						
2>	0	0	2	1	0						
3>	0	0	3	3	0						
4>	0	0	0	1	0						
..... Total Incidence of Finding Observed:	0	0	5	5	0						

Toxicokinetics:

Since concentrations for ♂s and ♀s were similar, results were combined.

Concentrations (ng/mL) of SGN-35 (♂ and ♀ Combined)

Dose Group	Dose Level (mg/kg/day)		Day 1 Minute 15	Day 8 Predose	Day 8 Minute 1 or 2	Day 15 Predose	Day 15 Minute 1 or 2	Day 22 Predose	Day 22 Minute 15
14	0	Mean	0	0	0	0	0	0	0
		SD	NA	NA	NA	NA	NA	NA	NA
		RSD (%)	NA	NA	NA	NA	NA	NA	NA
15	0.5	Mean	11105	357	12475	537	NA	633	15625
		SD	1593	54	1607	52	NA	74	3662
		RSD (%)	14	15	13	10	NA	12	23
16	5	Mean	108050	3575**	136333	7820	122500	8180	138250
		SD	25594	NA	15044	722	20984	632	11026
		RSD (%)	24	NA	11	9	17	8	8
17	10	Mean	230500	8938	261750	16875	241333*	16875	219250
		SD	37251	4900	55145	3350	15044	2546	26788
		RSD (%)	16	55	21	20	6	15	12

Concentrations (ng/mL) of SGN-35 and SGN-30 Total Antibody (♂ and ♀ Combined)

Dose Group	Dose Level (mg/kg/day)		Day 1 Minute 15	Day 8 Predose	Day 8 Minute 1 or 2	Day 15 Predose	Day 15 Minute 1 or 2	Day 22 Predose	Day 22 Minute 15
14	0	Mean	0	0	0	0	0	0	0
		SD	NA	NA	NA	NA	NA	NA	NA
		RSD (%)	NA	NA	NA	NA	NA	NA	NA
15	0.5	Mean	10313	579**	10840	1397*	11625	1181**	11950
		SD	2221	NA	1293	90	1245	NA	1380
		RSD (%)	22	NA	12	6	11	NA	12
16	5	Mean	122250	10265	108475	19900	127450	18350	153250
		SD	31637	1503	32894	6183	58000	2551	12842
		RSD (%)	26	15	30	31	46	14	8
17	10	Mean	333750	21468	257500	31375	384000	34850	228750
		SD	187548	9447	83958	3305	254489	1515	57221
		RSD (%)	56	44	33	11	66	4	25
18	10	Mean	188550	57800	292500	105875	288250	115850	338500
		SD	99686	5802	74858	9681	43500	22703	40485
		RSD (%)	53	10	26	9	15	20	12

Note: Values below the limit of quantitation are treated as zero in descriptive statistic calculations. Values represent the mean data for 2 males and 2 females except as noted.
* n=3. **n=2. NA reported for the mean values indicates no data were obtained

- SGN-35 did not elicit anti-SGN-35 antibodies
- Increase in SGN-35 concentrations was generally dose-proportional from 0.5 to 10 mg/kg (Groups 15-17)
- The post-dose concentrations of SGN-35 were relatively constant throughout the study, whereas the pre-dose trough SGN-35 serum concentrations increased slightly toward the end of the dosing period, suggesting accumulation of SGN-35 after multiple dosing.
- Increase in the concentrations of total Ab was dose proportional (Groups 15-17). Post-dose plasma concentrations were relatively constant over the 4-week dosing period, whereas pre-dose trough concentrations appeared to plateau after the 2nd or 3rd. For Group 18, dosed with 10 mg/kg of SGN-30, post-dose serum concentrations of total antibody were similar to Group 17 administered 10 mg/kg of SGN-35. In contrast, the pre-dose trough concentrations of total antibody were about 3-fold lower in animals treated with an equivalent dose of SGN-35. These results suggest that, in rats, conjugation of drug to antibody may enhance the clearance.

Dose Group	Dose Level (mg/kg/day)	Sex	C_{max} (pg/mL)	T_{max} (hr)	AUC_{0-t} (pg•hr/mL)	AUC_{0-24} (pg•hr/mL)	$AUC_{0-\infty}$ (pg•hr/mL)	$t_{1/2}$ (hr)	V_d (mL/kg)	CL (mL/hr/kg)
Day 1										
11	0.0097	M	593	0.250	2610	2610	2747	5.76	29327	3531
		F	495	0.250	2567	2567	2686	5.65	29425	3612
12	0.097	M	3530	0.250	25698	25698	32293	10.9	47223	3004
		F	3750	0.250	28591	28591	NC	NC	NC	NC
13	0.194	M	5795	0.250	49414	49414	NC	NC	NC	NC
		F	12550	0.500	91009	91009	NC	NC	NC	NC
Day 22										
11	0.0097	M	698	0.250	3984	3984	4123	5.17	17559	2353
		F	840	0.250	3067	3067	3140	4.68	20848	3089
12	0.097	M	5490	0.250	52445	52445	NC	NC	NC	NC
		F	4835	0.250	28600	28600	NC	NC	NC	NC
13	0.194	M	11050	0.250	70993	70993	NC	NC	NC	NC
		F	7005	0.250	49184	49184	NC	NC	NC	NC

- Exposure to SGD-1010 increased as the dose level increased from 0.0097 to 0.194 mg/kg/day.
- Mean concentrations of SGD-1010 were generally similar on Days 1 and 22. Serum levels collected on Days 8 and 15 at the protocol nominal time of 15 minutes post-dose (actual collection time of 1 minute post-dose) were markedly higher (approximately 2 orders of magnitude) than those on Days 1 and 22. However, those samples were collected within 1-2 min post-dose rather than 15 minutes post-dose.
- After IV administration at the low dose level of 0.0097 mg/kg/day, SGD-1010 serum levels generally declined in a bi-exponential manner with a slow elimination phase. Elimination half-life ($t_{1/2}$) values were ~5 hrs on Days 1 and 22. No marked increases in $t_{1/2}$ were observed after multiple dosing (similar $t_{1/2}$ on Day 1 and Day 22)
- After dosing at the mid and high dose levels of 0.097 and 0.194 mg/kg/day, the concentration-time curves became flat indicating that the terminal elimination phase has not been reached within 24 hours of dosing. Due to the absence of a distinct elimination phase in the mid and high dose groups, estimation of $t_{1/2}$ was not attempted, with the exception of male animals in Group 12 (MD) on Day 1 which showed a $t_{1/2}$ of 10.9 hours.
- Estimation of $t_{1/2}$ was not possible for the HD groups due to a limited collection.
- Exposures were generally higher on Day 22 than Day 1, suggesting accumulation after multiple dosing.
- No marked (>2-fold) gender differences were observed in Cmax and AUC0-24 values with the exception of Day 1, Group 13 high dose males, which showed markedly (>2-fold) lower Cmax and AUC0-24 values than females.

Dose Proportionality Ratios for SGD-1010 in Male and Female Rat Serum

	Proportional Dose	Males		Females	
Interval	Level Increase	C_{max}	AUC_{0-24}	C_{max}	AUC_{0-24}
Day 1	1:10:20-fold	1.0:6.0:9.8-fold	1.0:9.8:19-fold	1.0:7.6:25-fold	1.0:11:35-fold
Day 22	1:10:20-fold	1.0:7.9:16-fold	1.0:13:18-fold	1.0:5.8:8.3-fold	1.0:9.3:16-fold

Summary of the study:
The mean values of the Day 22 low concentration preparations for SGD-1010 and SGN-35 were out of the 15% specification.

SD rats were dosed with MMAE (SGD-1010), SGN-35, or SGN-30. Two unscheduled deaths occurred during the study, one in group 3 (MD MMAE) and one in group 18 (TK SGN-30). Both were considered accidental.

Treatment-related toxicities were seen mainly for MD and HD MMAE, and for MD and HD SGN-35. Toxicities were mainly to the hematopoietic system, liver, and male reproductive system.

The types of toxicities were in general similar for MMAE and SGN-35 treatment and included the following changes in the clinical pathology parameters: ↓reticulocyte (up to 90%), ↓RBC and lineages, ↓WBC and differentials, ↑cholesterol, bilirubin, AST, ALT, and GGT (up to 9-fold). Hepatobiliary toxicities were more profound in the SGN-35 group (HD) than in the MMAE group (HD). Hepatobiliary toxicity was further confirmed by liver hypertrophy and histopathology findings (coagulative necrosis) at HD MMAE and SGN-35.

In conclusion, once-weekly intravenous injection of MMAE for 4 weeks caused decreased body weights and food consumption; reduced erythropoiesis; clinical pathology changes consistent with liver injury; decreased thymus, epididymides, and testes weights; bone marrow hypocellularity and irreversible seminiferous tubule degeneration; Sertoli cell vacuolation; and reduced spermatogenesis and epididymal aspermia.

SGN-35 caused reduced erythropoiesis; clinical pathology changes consistent with liver injury; decreased thymus, epididymides, and testes weights; bone marrow hypocellularity and irreversible seminiferous tubule degeneration; Sertoli cell vacuolation; and reduced spermatogenesis and epididymal aspermia.

SGN-30 did not cause clear or toxicologically relevant adverse effects when administered at 10 mg/kg/dose.

Study title: An 11-Week Repeat-Dose Intravenous Infusion Toxicity Study of SGN-35 and SGD-1010 (MMAE) in Cynomolgus Monkeys with a Five-Week Recovery Period

Key study findings:

- Myelotoxicity was the DLT.
- Unscheduled deaths at the high dose (HD) SGN-35 were mainly due to severe neutropenia
- This study demonstrated a steep dose-toxicity curve after treatment with SGN-35. The 6 mg/kg dose level was severely toxic whereas a two-fold lower 3 mg/kg dosage was well tolerated

Study no.: (b) (4).163.16
Volume #, and page #: electronic document
Conducting laboratory and location: (b) (4)

Date of study initiation: June 2, 2005
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: SGN-35, lot# P02905
SGD-1010 (MMAE), lot # SGD-1010-0-09
Vehicle: buffered 0.9% saline

Methods

Doses: Q3W (once every 3 weeks on Days 1, 22, 43, and 64) x 4 cycles
Main animals were sacrificed on Day 71 (7 days after the last dose)
Recovery animals were sacrificed on R29 (36 days after the last dose)

Group	No. of Animals (M/F)	Treatment	Dose Level (mg/kg)	Terminal Sacrifice Day 71		Recovery Sacrifice Day R29	
				Subset 1	Subset 2	Subset 1	Subset 2
1	6/6	Vehicle	0	3/3	1/1	2/2	-
2	3/3	SGN-35 (low)	1	3/3	-	-	-
3	5/5	SGN-35 (mid)	3	2/2	1/1	1/1	1/1
4	8/8	SGN-35 (high)	6	3/4	2/2	2/1	1/1
5	5/5	SGD-1010	0.058	3/3	-	2/2	-

SGD1010 (MMAE) dose in Group 5 is equivalent to the amount used in Group 3. Initially, 42 animals were placed on study (Subset 1) and an additional 12 animals were added to the study (Subset 2).

Species/strain: Cynomolgus monkeys
Number/sex/group: see Table above

Route, formulation, volume, and infusion rate: i.v. 1-hr infusion, 10 mL/kg
Age: information not found
Weight: ~3 kg (at the start of the study)
Unique study design: An antibiotic treatment regimen was implemented for animals in Groups 4 and 5 to reduce the risk of opportunistic bacterial infection during periods when neutrophil count was diminished. Animals in Subset 1 (Groups 4 and 5 only) were treated with Cefazolin (25-50 mg/kg) b.i.d., beginning on D12 through D35, and D41 through D71 (terminal group) or through R18 (recovery group). Animals in Subset 2 (Group 4 only) were treated with Cefazolin (25-50 mg/kg; b.i.d.) and Baytril (5 mg/kg; s.i.d.) beginning on D5 and ending on D71 (terminal group) or through R17 (recovery group). Note: because Subset two animals in Group 4 were treated with Baytril, but the Subset 1 animals were not, the two Group 4 subsets were separated in the statistical analysis

Observation and Times:
Clinical signs: daily for mortality
Clinical observations on dosing days: 3 times/day (once before, twice after dosing)
Clinical observation on non-dosing days: once a day
Body weights: Twice during acclimation and once weekly on D7, D14, D21, D28, D35, D42, D49, D56, D63, D70, R7, R14, R21, R28.
Food consumption: daily
Ophthalmoscopy: Ophthalmology was performed once during acclimation and once during the week prior to terminal necropsy (D65 through D70). Ophthalmology recordings were not obtained during the recovery period because there were no findings during the dosing period.

ECG: Respiration rate, heart rate, and rectal temperature were recorded once during acclimation and once on D1 (6 hours post-dose). ECG was performed once during acclimation and once during the week prior to terminal necropsy (Days D65 through D70). ECG recordings were not obtained from recovery animals because no test article-related effects occurred during the dosing phase.

Hematology, Coagulation
Hematology: prior to treatment, D1 (within 20 minutes post-dose), D8, D15, D20 D22, D29, D36 D43, D50, D57 D64, and D70, and from recovery animals on R7, R14, R22 and R28.

Coagulation: prior to treatment, D8, D22, and D70, and also on R22 for recovery animals.

Serum Chemistry: prior to treatment, D8, D22, and D70; also on R22 for the recovery animals.

Urinalysis: Samples were collected once on the day of necropsy (D71 for the terminal group and R29 for the recovery group).

Gross pathology: at necropsy (D71 for main animals and R29 for recovery animals)

Organ weights: At necropsy

Adrenals	Pituitary
Brain (cerebrum, cerebellum and brain stem)	Prostate/Seminal vesicle
Epididymides	Spleen
Heart	Submandibular glands
Kidneys	Thyroids (including parathyroids)
Liver	Testes
Lungs (including bronchi)	Thymus
Ovaries	Uterus

Paired organs were weighed together as a pair.

Histopathology: The following tissues were collected for examination:

Adrenals**	Large Intestine -cecum -colon -rectum	Small Intestine -duodenum -ileum -jejunum
Aorta (thoracic)	Liver	Spinal Cord (thoracic)
Bone * -femur/knee joint ** -sternum	Lungs** (with bronchi**)	Spleen
Bone marrow* -sternum -femur/knee joint** -bone marrow cores -femur (left, non decalcified)		
Brain -brain stem -cerebellum -cerebrum	Lymph Nodes -mesenteric -mandibular***	Stomach -fundus -pylorus
Epididymides**	Mammary Gland***	Submandibular Glands**
Esophagus (thoracic)	Ovaries**	Testes**
Eye Balls/ Optic Nerves**	Pancreas	Thymus
Gall Bladder	Pituitary	Thyroids with Parathyroids(if possible)**
Gross Lesions	Prostate	Tongue
Heart	Sciatic Nerve***	Trachea
Injection Site of last dose (D64))	Seminal Vesicle	Urinary Bladder
Kidneys**	Skeletal Muscle*** (quadriceps femoris)	Uterus(body and cervix)
Lacrimal Glands***	Skin*** (gluteal area)	Vagina

* Bone and bone marrow was examined as decalcified specimens. Bone marrow smears were also prepared from the sternum.

** Both left and right organs were examined.

*** Both left and right organs were collected. If there were no gross lesions, only the left was examined.

Tissues were fixed and preserved in 10% neutral buffered formalin. The formalin fixed tissues were vacuum-packed and stored at room temperature (the eyes were fixed in a mixture solution of formaldehyde and glutaraldehyde and testes were fixed in Bouin's solution) for histopathology examination.

Toxicokinetics (SGN-35, total antibody, and MMAE levels):
Once during acclimation, on D1 (within 20 minutes post-dose), D8, D15, D22 (pre-dose and within 20 minutes post-dose), D25, D29, D43 (pre-dose and within 20 minutes post-dose), D46, D50, D64 (pre-dose and within 20 minutes post-dose), D67, and D71. Blood from recovery animals was collected on R7, R14, and R28.

The following pre and post dose samples were analyzed for serum concentrations of SGN-35 and Total antibody:

- Day 1, within 20 minutes of dose end
- Day 22, pre-dose and within 20 minutes of dose end
- Day 43, pre-dose and within 20 minutes of dose end
- Day 64, pre-dose and within 20 minutes of dose end
- Day 71

The following samples were analyzed for serum concentration of SGD-1010:

- Day 1, within 20 minutes of dose end
- Day 22, pre-dose and within 20 minutes of dose end
- Day 25
- Day 43, pre-dose and within 20 minutes of dose end
- Day 46
- Day 64, predose and Day 64, within 20 minutes of dose end
- Day 67
- Day 71

TK techniques:
SGN-35 levels: the serum concentration of the SGN-35 (ADC) was determined for animals in Groups 1-4 by SGN-35 ELISA. The assay format used anti-MMAE to coat plates and utilized biotinylated anti-ID30 for detection.

Total Antibody levels in serum: The total amount of cAC10 antibody, either unconjugated or as SGN-35 was determined for animals in Groups 1-4 by using the "total antibody" ELISA. The assay format used anti-ID-30 (anti-idiotypic murine monoclonal antibody to cAC10, clone 30.16) to coat plates and utilized biotinylated anti-ID30 for detection.

SGD-1010 levels in serum: The serum concentration of free SGD-1010 (MMAE) was determined by (b) (4) via HPLC with MS/MS detection (Appendix 12) for animals treated with vehicle (Group 1), SGN-35 (Groups 2-4) and SGD-1010 (Group 5). Free MMAE and the internal standard (D8-MMAE) were extracted from samples using solid-phase extraction (SPE).

Immunogenicity
Immunogenicity analysis for anti-SGN-35 antibodies was performed on samples collected from animals in Groups 1 through 4. The assay format used SGN-35 to coat plates and used biotinylated SGN-35 for detection.

Blood for immunogenicity analysis was collected during acclimation, prior to dosing on D22, D43, and D64 and on D70 for the terminal group only and at the end of the recovery period.

Flow Cytometry and immunophenotyping
Blood was collected once during acclimation for all animals and on D70 for terminal group animals, and on R22 for recovery period animals.

Results:

Mortality: 3 unscheduled sacrifices in HD SGN-35 (6 mg/kg)

Each incident occurred prior to the second dosage of SGN-35. Early deaths were attributed to sepsis, consistent with the panleukopenia and specifically the neutropenia noted for those animals able to be sampled.

Unscheduled deaths-HD	Day of dosing	Findings
♀ (#SSAN31)	D11	• Found dead
♀ (#SSAN29)	D15	• Severely neutropenic on D15 with a count of 140 /µL- animal sacrificed
♂ (#SSAN 60)	D12	• Clinical signs of distress during week two. • Moderate salivation and mild but obvious petecchial hemorrhages on the medial aspect of the right thigh on D12 • Severely neutropenic on D12 with a count of 10/µL, was febrile with temperatures of 105.4 and 104.4 °C, and was in a declining condition- animal sacrificed

Anatomic pathology findings for each animal indicated that a severe bacterial infection was either the cause of death or led to the moribund state. Animals 29 and 60 had received prophylactic antibiotic treatment starting on Day D5 and D12, respectively. SSAN 31 did not receive any prophylactic antibiotic treatment.

Clinical signs:

Group 4 (HD SGN-35) Observations

SSAN	Sex	Observation	Period	Resolution
26	M	• Hunched posture Anorexic condition	D13-D17 D12-D13, D16-D18	Recovered
29	F	• Hunched posture	D10-D15	Euthanized (D15)
31	M	-	-	Found Dead (D11)
54	M	• Lying down • Anorexic condition • Hunched posture	D15 D16-D19 D15-D16	Recovered

		• Febrile		
60	M	• Unresponsive • Febrile • Petecchial hemorrhage	D12	Euthanized (D12)

Other observations:

- Lameness of the hands for animal 24 (SGN-35 6 mg/kg) on Days D10-14. This animal was observed to be normal following this episode.
- Occasions of liquid or soft feces, loss of fur. These were either noted across all dose groups and/or, according to the sponsor, are common findings for cynomolgus monkeys.

Body weights: No test article-related effect

Food consumption: Reduced food consumption occurred during week 3 in males treated with 6 mg/kg (HD) of SGN-35.

Ophthalmoscopy: no test article-related findings

ECG:
No effects related to treatment with either SGN-35 or SGD-1010 occurred on diastolic or systolic blood pressure, body temperature, heart rate, or respiration when measured 6 hours after the first dose. The elevated heart rate noted for male animal #34 (SGD-1010) on D68 was considered to be incidental.

Electrocardiograms were qualitatively and quantitatively normal for cynomolgus monkeys.

Hematology:
D70 (main) data in ♂s

	Eosin	Lymph	Mono	Neut	RBC	Ret	WBC
SGN-35 (LD)	↓50%	—	—	—	↓6%	—	↓8%
SGN-35 (MD)	↓85% (R28)	—	—	↓23% (R22)	↓15% (R14)	↓55% (R7)	↓14% (R14)
SGN-35 (HD)	↓85% (R28)	↓45% (R7)	↓50% (R14)	↓50% (R14)	↓20% (R14)	↓85% (R7)	↓45% (R14)
MMAE	↓85% (R22)	↓45% (R7)	↓50% (R7)	↓45% (R14)	↓10% (R7)	↓85% (R7)	↓45% (R14)

No recovery data for SGN-35 LD.
(): indicates when during the recovery period, parameters started to reverse.

D70 (main) data in ♀s

	Eosin	Lymph	Mono	Neut	RBC	Ret	WBC
SGN-35 (LD)	↓65%	—	—	—	—	—	—
SGN-35 (MD)	↓75% (R14)	—	—	—	↓8% (R14)	↓80% (R7)	—
SGN-35 (HD)	↓95% (R28)	↓45% (R14)	↓20% (R14)	↓30% (R22)	↓25% (R14)	↓80% (R7)	↓40% (R22)
MMAE	↓85% (R14)	↓45% (R14)	↓25% (R7)	↓65% (R14)	↓8% (R8%)	↓80% (R7)	↓50% (R14)

No recovery data for SGN-35 LD.
(): indicates when during the recovery period parameters started to reverse.

Coagulation: no relevant changes

Clinical chemistry: results were inconclusive

	ALT		ALP		AST		GGT		CK		Cholesterol		Triglycerides	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
SGN-35 (LD)	↓20%	—	↓12%	—	—	↑30%	↓19%	—	—	↑1.5 Fold	—	—	—	↓30%
SGN-35 (MD)	↓20%	—	↓30%	—	—	—	↓8%	—	—	—	↑15%	—	—	—
SGN-35 (HD)	↓20%	—	↓18%	—	↑80%	↑30%	—	—	↑80%	—	↑20%	—	↓30%	↓20%
MMAE	↓50%	—	—	—	↑14%	—	↓13%	—	↑30%	—	—	—	—	↓40%

Urinalysis: no test article-related findings

Gross pathology: Test article-related findings consisted of

- Paleness of the bone marrow: 2 ♀s dosed with LD and MD SGN-35
- Atrophy of the white pulp of the spleen: 1 ♀ dosed with SGD-1010 (MMAE)
- Enlarged spleen: 1 ♂ at HD SGN-35 (histopathology showed extensive deposition of brown pigment consistent in appearance with hemosiderin in the red pulp)

Findings were reversible.

Organ weights:

- Main sacrifice: ↓ thymus (75%) in ♂s treated with HD SGN-35

Histopathology:
At the end of the treatment period, test article-related histopathology findings were observed in the:

- Hematopoietic tissues and in the lymphoid organs (hypocellularity, hemorrhage, congestion, and necrosis); see Table below. This was the major product-related finding and resulted in death in monkeys (severe neutropenia).
- Injection site
- Glomerulosclerosis with capsular fibrosis (2/4 HD SGN-35 ♂s)
- Mononuclear cell infiltration in multiple tissues, including liver, kidney, thoracic spinal cord/epineurium of nerve, and GI (mainly at HD SGN-35); this was generally of low incidence.

Findings in sternal bone marrow (main sacrifice):

Treatment	Vehicle		SGN-35						SGD-1010	
Dose (mg/kg)	0		1		3		6		0.058	
Group	1		2		3		4		5	
Sex	M	F	M	F	M	F	M	F	M	F
Hypercellularity										
All hematopoietic cells	0	0	0	0	0	0	0	0	0	0
Megakaryocytes	0	0	0	2	1	2	2	2	1	2
Granulocyte precursors	0	0	0	1	3	2	0	0	0	0
Erythroid precursors	0	0	0	0	0	0	0	0	1	2
Hematopoietic progenitors	0	0	0	0	0	0	0	1	1	0
Hypocellularity										
All hematopoietic cells	0	0	0	0	1	1	4	4	3	2
Megakaryocytes	0	0	0	0	0	0	1	0	0	0
Granulocyte precursors	0	0	0	0	0	0	3	3	3	3
Erythroid precursors	0	0	0	0	0	0	2	0	0	0
Hematopoietic progenitors	0	0	0	0	1	0	3	2	0	1
Necrosis/cell debris	0	0	0	0	2	1	4	4	2	3
Hemorrhage/congestion	0	0	0	0	1	1	4	3	2	2

Main sacrifice

Organ	Drug-related findings
Thymus	Mild hypocellularity and/or apoptosis of cortical lymphocytes: • 2/4 ♂s and 1/4 ♀s at HD SGN-35 • 2/2 ♀s dosed with SGN-1010 (MMAE).
Spleen	Hypocellularity of lymphocytes in the germinal centers and periarteriolar lymphoid sheath (PALS) of the spleen in MMAE group: mild to marked severity • 1/4 Vehicle ♀ • 1/3 ♀ at LD SGN-35 • 2/4 ♀s and 1/4 ♂s at HD SGN-35 • 3/3 ♀s and 2/3 ♂s in MMAE group (Group 5)

Toxicokinetics:

Immunogenicity: Anti-SGN-35 Antibodies

No animals in the vehicle group showed detectable anti-SGN-35 antibody concentrations. For groups treated with 1, 3, or 6 mg/kg of SGN-35, 5/6 (83%), 5/10 (50%), or 1/13 (8%) animals developed anti-SGN-35 antibodies during the study.

The development of anti-SGN-35 antibodies may have impacted accurate assessment of serum SGN-35 concentrations at the pre-dose trough sampling, in particular at the low dose levels.

Serum SGN-35 concentrations

Male animals/ SGN-35 ELISA/ (ng/mL)

Group		D1 Dose End (20 min)	D22 Pre	D22 Dose End (20 min)	D43 Pre	D43 Dose End (20 min)	D64 Pre	D64 Dose End (20 min)	D71
Vehicle	N	6	6	6	5	6	6	6	6
	Mean	< 12.5	< 12.5	< 12.5	< 12.5	< 12.5	< 12.5	< 12.5	< 12.5
	SD	NA	NA	NA	NA	NA	NA	NA	NA
	RSD (%)	NA	NA	NA	NA	NA	NA	NA	NA
SGN-35 (1 mg/kg)	N	3	3*	3	3	3	3	3	3
	Mean	30167	17	21667	< 12.5	17817	< 12.5	15947	< 12.5
	SD	13696	7	10220	NA	9463	NA	11721	NA
	RSD (%)	45%	40%	47%	NA	53%	NA	74%	NA
SGN-35 (3 mg/kg)	N	5	5*	5	5*	5	5*	5	5*
	Mean	80440	301	70900	852	79700	1319	66216	5047
	SD	8983	604	20554	1147	25889	1809	36647	4887
	RSD (%)	11%	200%	29%	135%	32%	137%	55%	97%
SGN-35 (6 mg/kg)	N	8	7*	7	7*	7	6*	7	7
	Mean	161500	1007	131057	1766	162429	3105	158429	14729
	SD	32249	1354	28206	1957	42801	1840	28646	3164
	RSD (%)	20%	134%	22%	111%	26%	59%	18%	21%

Female animals/ SGN-35 ELISA/ (ng/mL)

Group		D1 Dose End (20 min)	D22 Pre	D22 Dose End (20 min)	D43 Pre	D43 Dose End (20 min)	D64 Pre	D64 Dose End (20 min)	D71
Vehicle	N	6	6	6	6	6	6	6	6
	Mean	< 12.5	< 12.5	< 12.5	< 12.5	< 12.5	< 12.5	< 12.5	< 12.5
	SD	NA	NA	NA	NA	NA	NA	NA	NA
	RSD (%)	NA	NA	NA	NA	NA	NA	NA	NA
SGN-35 (1 mg/kg)	N	3	3*	3*	3*	3	3*	3	3*
	Mean	21633	65	12204	88	19810	100	19264	772
	SD	11279	74	8806	107	12551	124	14710	1074
	RSD (%)	52%	114%	72%	121%	63%	124%	76%	139%
SGN-35 (3 mg/kg)	N	5	5*	5	5*	5	5*	5	5
	Mean	83120	63	78480	256	89580	761	87200	4822
	SD	12616	89	15117	544	31393	1259	15637	4253
	RSD (%)	15%	142%	19%	213%	35%	166%	18%	88%
SGN-35 (6 mg/kg)	N	8	6	6	6	6	6	6	6
	Mean	179375	1549	168000	3330	173000	4905	172000	17250
	SD	30161	1657	28934	1350	35009	1275	36943	3860
	RSD (%)	17%	107%	17%	41%	20%	26%	21%	22%

- No marked differences were observed between genders.
- Increases in the SGN-35 levels were approximately dose-proportional. Post-dose serum concentrations at the MD (3 mg/kg) level were greater than 3-fold higher than serum concentrations in the LD (1 mg/kg) animals, and SGN-35 serum concentration at HD (6 mg/kg) were twice those of the MD (3 mg/kg).
- Peak post-dose serum SGN-35 concentrations in the MD SGN-35 group were generally similar following each dosage. Trough SGN-35 concentrations gradually increased with successive doses, indicating accumulation.
- Average peak SGN-35 concentrations in the HD SGN-35 group were relatively consistent across each day of dosage. Trough SGN-35 concentrations gradually increased with successive doses, indicating accumulation.

Serum Total cAC10 Antibody Concentration

Male cAC10 serum levels by the Total Antibody ELISA (ng/mL)

Group		D1 Dose End (20 min)	D22 Pre	D22 Dose End (20 min)	D43 Pre	D43 Dose End (20 min)	D64 Pre	D64 Dose End (20 min)	D71
Vehicle	N	6	6	6	6	6	6	6	6*
	Mean	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	28.3
	SD	NA	NA	NA	NA	NA	NA	NA	6
	RSD (%)	NA	NA	NA	NA	NA	NA	NA	20%
SGN-35 (1 mg/kg)	N	3	3	3	3	3	3	3	3
	Mean	23800	<25.0	19633	NA	17477	<25.0	14826	<25.0
	SD	6165	NA	2650	NA	7927	NA	12463	NA
	RSD (%)	26%	NA	13%	NA	45%	NA	84%	NA
SGN-35 (3 mg/kg)	N	5	5*	5	5*	5	5*	5	5*
	Mean	80000	1145	70540	1812	74640	2041	61526	10237
	SD	16875	1540	17368	2456	21467	2699	36996	9723
	RSD (%)	21%	135%	25%	136%	29%	132%	60%	95%
SGN-35 (6 mg/kg)	N	8	7*	7	7*	7	7*	7	7
	Mean	146875	2055	121401	3565	137886	6799	142071	29434
	SD	17780	2707	54977	3436	60583	3652	56072	10173
	RSD (%)	12%	132%	45%	96%	44%	54%	39%	35%

Female cAC10 serum levels by the Total Antibody ELISA (ng/mL)

Group		D1 Dose End (20 min)	D22 Pre	D22 Dose End (20 min)	D43 Pre	D43 Dose End (20 min)	D64 Pre	D64 Dose End (20 min)	D71
Vehicle	N	6	6	6	6	6	6	6	6
	Mean	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0
	SD	NA	NA	NA	NA	NA	NA	NA	NA
	RSD (%)	NA	NA	NA	NA	NA	NA	NA	NA
SGN-35 (1 mg/kg)	N	3	3*	3	3*	3	3*	3	3*
	Mean	23400	184	13275	184	16657	319	18977	1710
	SD	5071	275	11479	275	8632	509	17198	2919
	RSD (%)	22%	150%	86%	150%	52%	160%	91%	171%
SGN-35 (3 mg/kg)	N	5	5*	5	5*	5	5*	5	5
	Mean	76300	228	83840	472	73420	999	71880	9021
	SD	24746	428	41143	1000	23454	1523	25222	7407
	RSD (%)	32%	187%	49%	212%	32%	152%	35%	82%
SGN-35 (6 mg/kg)	N	8	6	6	6	6	6	6	6
	Mean	168500	3406	161833	6137	180167	8527	166500	30183
	SD	22501	3361	28937	2250	19426	2245	28183	5427
	RSD (%)	13%	99%	18%	37%	11%	26%	17%	18%

- Numbers for the total Ab levels (end of dosing) were comparable to those for the SGN-35 levels, suggesting that at this time point, all antibodies are in form of

SGN-35 (conjugated). However, the trough (pre-dose) values are greater for the total Ab, indicating that during the 3 week period some of the MMAE has come off, resulting in free antibody.

Trough ratios (free cAC10: SGN35)

	Day 22	Day 43	Day 64
MD- ♂	3.8	2.2	1.5
MD- ♀	3.6	2	1.3
HD- ♂	2	2	2.2
HD- ♀	2.3	1.8	1.7

Serum SGD-1010 Concentration

Male animals/ MMAE LC-MS/ (ng/mL)

Group		D1 Dose End	D22 Predose	D22 Dose End	D43 Predose	D43 Dose End	D64 Predose	D64 Dose End	D71
Vehicle	N	6	6	6	6	6	6	6	6
	Mean	< 10.0	< 10.0	< 10.0	< 10.0	< 10.0	< 10.0	< 10.0	< 10.0
	SD	NA	NA	NA	NA	NA	NA	NA	NA
	RSD (%)	NA	NA	NA	NA	NA	NA	NA	NA
SGN-35 (1 mg/kg)	N	3*	3	3	3	3	3	3	3
	Mean	11.0	< 10.0	22.8	< 10.0	22.5	< 10.0	31.8	15.8
	SD	0.9	NA	11.6	NA	11.7	NA	26.8	6.2
	RSD (%)	8%	NA	51%	NA	52%	NA	84%	39%
SGN-35 (3 mg/kg)	N	5	5	5	5	5	5*	5	5
	Mean	35.3	< 10.0	160.6	< 10.0	54.2	13.5	76.6	72.2
	SD	9.3	NA	140.3	NA	21.4	7.8	60.6	64.6
	RSD (%)	26%	NA	87%	NA	40%	58%	79%	89%
SGN-35 (6 mg/kg)	N	8	7*	7	7	7	7*	7	7
	Mean	65.2	10.1	214.7	< 10.0	155.9	11.3	82.0	96.3
	SD	15.9	0.2	125.3	NA	212.9	1.7	13.4	16.3
	RSD (%)	24%	2%	58%	NA	137%	15%	16%	17%
SGD-1010	N	5	5	5	5	5	5	5	5
	Mean	2020.0	< 10.0	4954.0	< 10.0	5300.0	< 10.0	3240.0	15.9
	SD	369.4	NA	2740.4	NA	3088.4	NA	1427.9	5.4
	RSD (%)	18%	NA	55%	NA	58%	NA	44%	34%

Female animals/ MMAE LC-MS/ (ng/mL)

Group		D1 Dose End	D22 Predose	D22 Dose End	D43 Predose	D43 Dose End	D64 Predose	D64 Dose End	D71
Vehicle	N	6	6	6	6	6	6	6	6
	Mean	< 10.0	< 10.0	< 10.0	< 10.0	< 10.0	< 10.0	< 10.0	< 10.0
	SD	NA	NA	NA	NA	NA	NA	NA	NA
	RSD (%)	NA	NA	NA	NA	NA	NA	NA	NA
SGN-35 (1 mg/kg)	N	3*	3	3	3*	2	3	3	3*
	Mean	12.0	< 10.0	1750.4	10.2	22.5	< 10.0	43.0	12.5
	SD	2.0	NA	2996.1	0.3	8.8	NA	41.8	4.3
	RSD (%)	16%	NA	171%	3%	39%	NA	97%	35%
SGN-35 (3 mg/kg)	N	5	5	5	5	5	5	5	5
	Mean	34.1	< 10.0	78.5	< 10.0	40.3	< 10.0	41.6	33.0
	SD	6.8	NA	64.7	NA	17.7	NA	12.4	4.8
	RSD (%)	20%	NA	82%	NA	44%	NA	30%	15%
SGN-35 (6 mg/kg)	N	8	6	6	6	6	6*	6	6
	Mean	72.9	< 10.0	92.4	< 10.0	70.5	10.2	69.2	75.2
	SD	30.8	NA	41.4	NA	17.2	0.6	14.3	14.4
	RSD (%)	42%	NA	45%	NA	24%	6%	21%	19%
SGD-1010	N	5	5	5	5	5	5	5	5*
	Mean	1724.0	< 10.0	4297.1	< 10.0	2976.0	< 10.0	5616.0	12.6
	SD	297.0	NA	3293.1	NA	1630.7	NA	3802.2	3.6
	RSD (%)	17%	NA	77%	NA	55%	NA	68%	28%

- Average peak serum SGD-1010 levels for animals given 0.058 mg/kg SGD- 1010 ranged from 1872 to 4626 pg/mL immediately after each dose. Average peak levels were similar following the second through fourth dose and were approximately double the concentration resulting from the first dose. Serum SGD-1010 levels decreased over 40 to 80-fold within 3 days of administration (ranging from 52 to 120 pg/mL) and fell to low or undetectable levels one week after dosing.
- Peak SGD-1010 serum concentrations for animals given the drug conjugate SGN-35 were considerably lower than the peak concentrations following administration of the unconjugated (free) SGD-1010 drug. The SGD-1010 drug levels generally peaked 3 days after administration of SGN-35 in contrast to immediately post-dose after IV administration of the free drug.
- Average SGD-1010 levels in animals given 1 mg/kg SGN-35 ranged from 12 to 37 pg/mL up to 3 days post-dose.
- Average SGD-1010 levels in the 3 mg/kg SGN-35 group ranged between 34 and 120 pg/mL post-dose and generally peaked in most animals 3 days after dose administration, in particular, toward the end of the dosing phase..
- Animals dosed with 6 mg/kg SGN-35 had average post-dosing peak SGD-1010 concentrations of between 167 and 180 pg/mL and the peak concentrations generally occurred three days after dosage.
- Pre-dose SGD-1010 levels measured 3 weeks after dose administration were generally low or undetectable for all SGN-35 treated groups.

Accumulation of SGN-35, Total Antibody, and MMAE

The ELISA assay used to detect SGN-35 was subject to interference by anti-SGN-35 antibodies. Consequently animals displaying formation of anti-SGN-35 antibodies were not evaluated for evidence of accumulation of either SGN-35, total antibody, or MMAE.

Trough values for each analyte were evaluated over the course of successive doses as an indicator of accumulation. The following table presents the number of animals per group that showed accumulation of either SGN-35, total antibody, or MMAE.

		Evidence of Accumulation[2]		
Treatment Group	Assessable[1]	SGN-35	Total Antibody	MMAE
1 (vehicle)	12/12	0/12	0/12	0/12
2 (SGN-35 1 mg/kg)	1/6	1/1	1/1	0/1
3 (SGN-35 3 mg/kg)	5/10	5/5	5/5	0/5
4 (SGN-35 6 mg/kg)[3]	12/13	12/12	12/12	4/12
5 (SGD-1010)	-	-	-	0/10

[1]) Incidence: number of animals with undetectable levels of anti-SGN-35 antibodies / total number of animals per group

[2]) Incidence: animals with that showed evidence of accumulation / number of animals that were able to be assessed. To allow assessment, animals could not have detectable levels of anti-SGN-35 antibodies. Accumulation is identified as a progressive increase in analyte concentration with successive dosages. Measurements occurred immediately prior to administration of doses 2, 3, and 4.

[3]) Unscheduled deaths are excluded from analysis

- Apparent accumulation of MMAE only occurred in some animals treated with 6 mg/kg of SGN-35; it is unclear whether quantifiable MMAE concentrations represent accumulation or continued release of MMAE from resident SGN-35 concentrations.
- Accumulation of antibody (SGN-35 and total antibody) occurred in all animals allowing assessment (no detectable anti-SGN-35 antibody concentrations). This was evidenced in all treated groups.

Immunophenotyping

Flow cytometry results and analysis were not reviewed as these data will not be used to make a safety decision for the starting dose.

According to the sponsor:

The high within-group variability in cell counts, the treatment-related cyclic lymphocytopenia (as noted above), the lack of a consistent dose-related response, and the small number of sampling time points limit the ability to draw definitive conclusions about responses in lymphocyte subsets as a consequence of treatment.

Potentially, test-article related changes occurred in the groups treated with either 6 mg/kg of SGN-35 or 0.058 mg/kg of SGD-1010 and were associated with decreased absolute counts of B-lymphocytes (CD3-CD20+). Decrements in B-lymphocytes persisted throughout the recovery period. It was unclear if decreases in counts of

activated (i.e. CD30+) B- and T-lymphocytes (CD20+CD30+ and CD3+CD30+), and monocyte counts (CD3-/CD14+) in the 6 mg/kg SGN-35, and SGD-1010 groups were related to the test articles.

The activation marker, CD30, was not highly endogenously expressed on T- or B-lymphocytes (less than 1% of the lymphocytes), and no increases in responsive cell populations occurred during the study.

Summary of the study
Cynomolgus monkeys were treated with either SGN-35 (1, 3, or 6 mg/kg/dose) or MMAE (SGD-1010, 0.058 mg/kg/dose), once every 3 weeks for 3 cycles.

All groups treated with SGN-35 showed formation of anti-SGN-35 antibodies, inversely related to the dose level. Across groups treated with SGN-35, post-dose serum concentrations of SGN-35, total antibody, and SGD-1010 showed dose-proportional increases. Serum concentration trough values (taken immediately pre-dose across successive occasions of dosage) showed evidence of accumulation for SGN-35 and total antibody for groups treated with SGN-35. Some animals given 6 mg/kg of SGN-35 showed increasing trough values of SGD-1010.

SGN-35 or SGD-1010 treatment did not result in changes in the body weight, blood pressure, heart rate, respiration, body temperature, ophthalmology, electrocardiogram, coagulation and urinalysis.

The main SGN-35-related toxicity was moderate to marked myelotoxicity. Animals treated with the high dose SGN-35 (6 mg/kg) showed decreases in most hematopoietic cell components of the bone marrow, and experienced leukopenia with severe neutropenia 1 and 2 weeks post-dose. Hematological changes correlated with reduced thymic weights and histopathology findings of bone marrow hypocellularity and lymphoid depletion in thymus and spleen. Treatments with SGD-1010 (0.058 mg/kg/dose) elicited responses similar to those from animals given 6 mg/kg SGN-35, including severe neutropenia as well as anatomic involvement of hematopoietic tissues and lymphoid organs. Findings were reversible, following the 5-week recovery period.

Study title: 6-Month Chronic Toxicity Study of SGN-35 in Cynomolgus Monkeys with a 6-Week Recovery Phase

Study no.:	(b) (4) Study Number 8216375
Study report location:	M4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	14 September 2009
GLP compliance:	Yes*
QA statement:	Yes
Drug, lot #, and % purity:	Brentuximab Vedotin (SGN-35), lot #,

SDD002 (clinical lot), and purity, 95.3%, 55.9 mg/vial

* The following statement was included in the compliance statement.

Serum analysis for SGN-35 total antibody, SGN-35 antibody-drug conjugate, and anti-SGN-35 antibody was performed by Seattle Genetics, Inc. under GLP conditions.

Statistical analysis of MMAE exposure (AUC) and C_{max} versus immunogenicity titers were performed by the sponsor under non-GLP conditions.

Key Study Findings

Toxicity

Dose- and time-related decreases in red and white cell parameters with the most decrease in neutrophil counts, more profound after the first dose; at least partially recovered during dosing phase and recovery phase

TK

- Dose-related exposure, either dose proportional or slightly more than dose proportional
- Significant MMAE accumulation but not Ab-drug conjugate (ADC) or total Ab (TAb) accumulation
- No significant gender differences

Anti-drug Ab (ADA)

ADA detected in all animals in drug groups except for one animal in the 3 mg/kg group, ADA detected as early as Day 22 for majority of animals and lasted until scheduled sacrifices with increased titers during dosing phase

Methods

Doses:	See Table 19.
Frequency of dosing:	Once every 3 weeks (Q3wk) for a total of nine doses (Days 1, 22, 43, 64, 85, 106, 127, 148 and 169 of the dosing phase)
Route of administration:	Intravenous infusion over approximately 30 minutes with a dose rate of 10 mL/kg/hour
Dose volume:	5 mL/kg/dose
Formulation/Vehicle:	Lyophilized formulations: SGN-35 or SGN-35 Placebo for Injection Water for Injection for reconstitution and 0.9% Sodium Chloride for Injection as diluent
Species/Strain:	Monkeys/naïve cynomolgus (*Macaca*

fascicularis) from (b) (4)

Number/Sex/Group: Main study: 3/sex in the control group and 6/sex in the SGN-35 group
Recovery study: 2/sex in the control group and 4/sex in the SGN-35 group
Age: 2 to 6 years old at initiation of dosing
Weight: 2.5 to 6.2 kg for males and 2.1 to 4.2 kg for females at initiation of dosing
Satellite groups: No
Unique study design: No

Table 19. Summary of the Study Design

Group	No. of Animals Male	No. of Animals Female	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)
1 (Control)[a]	5	5	0	0
2 (Low)[b]	10	10	1.0	0.2
3 (High)[b]	10	10	3.0	0.6

[a] Group 1 received SGN-35 Placebo (lyophilized formulation buffer, composed of 20 mM citrate, 63 mg/mL trehalose, and 0.2 mg/mL Tween-80, pH 6.6, Lot #: DEVSYB-1). Upon completion of the dosing phase, two animals/sex remained on study for at least 6 weeks for recovery.

[b] All animals in Groups 2 and 3 received SGN-35. Upon completion of the dosing phase, four animals/sex/group remained on study for at least 6 weeks for recovery.

The doses were chosen based on doses tested in a previous q3wk x 4 toxicity study where 1 mg/kg was the NOAEL, 3 mg/kg caused nonsevere toxicity, but 6 mg/kg was lethal to 3 out of 16 animals.

Observations and Results

Mortality

All animals survived to the scheduled sacrifice.

Clinical Signs

One animal (Animal No. 102399) given 3 mg/kg was noted being hunched and hypoactive after administration of the sixth dose and having pale tongue, gums, and face during administration of the seventh dose. Although diphenhydramine was administered before all subsequent doses, vomitus and paleness were still noted. This reaction was likely drug-related.

Alopecia was noted in males given 1 (3/10) or 3 (4/10) mg/kg but not in control males (0/5). However, the incidence of alopecia was comparable in females in all groups.

Higher incidence of food-containing vomitus and discolored feces was noted in animals given 1 mg/kg (vomitus: 2/10, 8/20, or 2/20 in 0, 1.0 or 3.0 mg/kg groups, respectively).

Body Weights

There was no remarkable drug-related alteration in body weight or body weight gain.

Feed Consumption

There was no remarkable drug-related alteration.

Ophthalmoscopy

There was no abnormal ophthalmic observation.

ECG

No remarkable drug-related changes were observed during Week 25 of the dosing phase or Week 5 of the recovery phase.

A ventricular premature complex was noted during Week 5 of the recovery phase in one female given 3 mg/kg (Animal No. I02429). According to the study report, a single ventricular premature complex in this animal was considered an incidental finding and not attributed to SGN-35 (reviewer's comment: the finding was an isolated event noted during the recovery phase only, therefore, the finding may be not drug-related.)

Blood Pressure, Heart Rate, and Respiratory Rate

No remarkable, consistent, drug-related changes were observed.

Hematology

Dose-related decreases in red blood cell counts (more sensitive in females), HGB (more sensitive in males, 8% or 12% decreases in the 1 or 3 mg/kg groups, respectively, on Day 15), and HCT were noted and the effects remained after the recovery period (see Table 20 using HGB as an example).

Table 20. Summary of the Effect of SGN-35 on HGB in Males**

Group/ Sex		HGB g/dL PRED 4	DSNG 1	DSNG 15	DSNG 22	DSNG 36	DSNG 43	DSNG 57	DSNG 64
1M	Mean	13.7	13.1	12.4	12.5	13.3	13.6	13.4	14.1
	SD	0.80	0.91	0.46	0.37	0.42	0.25	0.29	0.60
	N	5	5	5	5	5	5	5	5
2M	Mean	13.4	13.3	11.4*	12.2	12.9	13.3	13.1	13.7
	SD	0.49	0.46	0.60	0.55	0.60	0.55	0.57	0.61
	N	10	10	10	10	10	10	10	10
3M	Mean	13.3	12.9	10.9*	11.8	12.0*	12.5*	12.3	12.8*
	SD	1.01	1.21	0.88	0.94	0.71	0.72	1.14	0.97
	N	10	10	10	10	10	10	10	10

Group/ Sex		HGB g/dL DSNG 78	DSNG 85	DSNG 99	DSNG 106	DSNG 120	DSNG 127	DSNG 141	DSNG 148
1M	Mean	13.5	13.4	13.3	13.4	13.6	13.5	13.6	13.8
	SD	0.55	0.71	0.60	0.54	0.40	0.55	0.49	0.51
	N	5	5	5	5	5	5	5	5
2M	Mean	13.4	13.3	13.7	13.7	13.7	13.5	13.7	13.7
	SD	0.67	0.66	0.54	0.53	0.57	0.60	0.57	0.47
	N	10	10	10	10	10	10	10	10
3M	Mean	12.3*	12.4	12.6	12.8	12.4	12.9	12.3*	12.9
	SD	1.11	0.93	1.39	1.14	1.40	1.18	1.23	1.28
	N	10	10	10	10	10	10	10	10

Group/ Sex		HGB g/dL DSNG 162	DSNG 169	DSNG 176	RECO 15	RECO 29	RECO 36
1M	Mean	13.8	14.0	12.4	13.5	14.7	14.6
	SD	0.63	0.50	0.67	0.57	0.07	0.07
	N	5	5	5	2	2	2
2M	Mean	13.7	13.7	12.3	13.2	14.0	13.8
	SD	0.54	0.46	0.59	0.49	0.40	0.57
	N	10	10	10	4	4	4
3M	Mean	12.3*	12.5*	10.9*	12.4	13.3*	13.2*
	SD	1.34	1.02	1.23	0.95	0.34	0.38
	N	10	10	10	4	4	4

**1M, 2M or 3M: males in 0, 1.0, or 3.0 mg/kg groups, respectively. PRED: Predose phase, DSGN: Dosing phase, and RECO: Recovery phase
* P< or =0.05

Dose- and dosing duration-related decreases in white blood cell counts and neutrophils were noted (see Table 21 and Table 22, respectively, using males as examples). The decreases were most profound after the first dose (on Day 15 of dosing phase, approximately 2 or 4 folds decreases in white blood cell counts or neutrophils, respectively, in males given 3 mg/kg). Cell counts were partially recovered during the dosing phase but remained lower than the values of controls after the recovery period.

Dose- and dosing duration-related decrease in lymphocytes with similar pattern as white blood cell counts was also noted (approximately 1.6 folds decrease in lymphocytes in males of 3 mg/kg group on Day 15 of dosing phase, after the first dose) and the values in drug groups were either higher than (males) or comparable to (females) the values in controls at the end of recovery phase. Dose-related decreases in monocytes and eosinophils were noted only after the first dose (approximately 2 folds decrease in males of 3 mg/kg group on Day 15 of dosing phase) and the values were comparable among the groups since Day 22 of dosing phase.

Table 21. Summary of the Effect of SGN-35 on WBC in Males**

Group/ Sex		WBC E3/uL PRED 4	DSNG 1	DSNG 15	DSNG 22	DSNG 36	DSNG 43	DSNG 57	DSNG 64
1M	Mean	15.79	16.98	15.73	14.51	13.30	14.94	15.68	15.64
	SD	7.419	5.955	6.234	6.475	3.827	3.923	7.218	4.736
	N	5	5	5	5	5	5	5	5
2M	Mean	14.55	13.05	12.51	11.67	14.21	12.75	14.01	13.35
	SD	3.202	5.063	3.136	4.068	3.330	4.088	2.630	3.419
	N	10	10	10	10	10	10	10	10
3M	Mean	11.34	10.76	7.56*	9.90	9.98	9.48*	9.10*	11.64
	SD	3.625	3.017	1.782	4.494	2.739	3.030	2.300	4.755
	N	10	10	10	10	10	10	10	10

Group/ Sex		WBC E3/uL DSNG 78	DSNG 85	DSNG 99	DSNG 106	DSNG 120	DSNG 127	DSNG 141	DSNG 148
1M	Mean	15.46	12.07	16.05	16.35	15.47	15.70	15.83	17.07
	SD	7.087	3.104	3.203	4.541	3.585	3.670	4.099	4.622
	N	5	5	5	5	5	5	5	5
2M	Mean	14.04	12.05	14.73	15.21	13.14	13.51	13.02	13.10
	SD	3.982	3.493	4.413	4.424	3.176	3.623	3.677	4.027
	N	10	10	10	10	10	10	10	10
3M	Mean	7.86*	8.68	9.60*	14.93	8.99*	12.23	8.88*	13.26
	SD	2.235	4.945	3.110	4.465	3.190	4.131	3.459	5.556
	N	10	10	10	10	10	10	10	10

Group/ Sex		WBC E3/uL DSNG 162	DSNG 169	DSNG 176	RECO 15	RECO 29	RECO 36
1M	Mean	14.53	13.86	14.17	14.16	13.49	15.86
	SD	3.421	1.959	5.963	3.380	3.055	7.757
	N	5	5	5	2	2	2
2M	Mean	13.31	12.90	12.90	11.71	11.99	12.68
	SD	4.082	4.350	3.361	2.523	2.497	1.813
	N	10	10	10	4	4	4
3M	Mean	8.76*	13.51	9.66	11.48	14.89	13.45
	SD	3.209	5.798	4.826	1.706	1.342	2.157
	N	10	10	10	4	4	4

**1M, 2M or 3M: males in 0, 1.0, or 3.0 mg/kg groups, respectively. PRED: Predose phase, DSGN: Dosing phase, and RECO: Recovery phase

* P< or =0.05

Table 22. Summary of the Effect of SGN-35 on Neutrophils in Males**

Group/ Sex		NEUT E3/uL PRED 4	DSNG 1	DSNG 15	DSNG 22	DSNG 36	DSNG 43	DSNG 57	DSNG 64
1M	Mean	5.62	7.68	5.38	5.06	4.05	5.45	5.96	6.01
	SD	3.276	4.301	3.659	4.021	1.311	1.231	5.392	3.192
	N	5	5	5	5	5	5	5	5
2M	Mean	6.06	4.90	4.06	3.20	4.08	4.87	3.58	5.09
	SD	3.306	3.958	2.344	2.555	2.017	2.922	1.534	2.887
	N	10	10	10	10	10	10	10	10
3M	Mean	3.61	4.21	1.36*	2.49	3.11	3.14	2.29	4.30
	SD	2.715	2.360	0.536	3.051	2.325	2.201	1.888	3.927
	N	10	10	10	10	10	10	10	10

Group/ Sex		NEUT E3/uL DSNG 78	DSNG 85	DSNG 99	DSNG 106	DSNG 120	DSNG 127	DSNG 141	DSNG 148
1M	Mean	7.05	4.69	5.65	5.65	4.63	4.95	5.93	7.24
	SD	5.071	2.364	2.754	3.548	2.621	2.904	3.569	4.433
	N	5	5	5	5	5	5	5	5
2M	Mean	5.07	4.63	5.08	5.85	4.25	4.37	3.97	6.15
	SD	3.448	2.605	2.682	3.337	2.126	2.115	1.983	3.297
	N	10	10	10	10	10	10	10	10
3M	Mean	1.81*	2.55	2.36*	6.28	2.22	3.46	1.86*	5.73
	SD	0.991	3.153	1.502	4.976	2.442	2.867	1.151	5.749
	N	10	10	10	10	10	10	10	10

Group/ Sex		NEUT E3/uL DSNG 162	DSNG 169	DSNG 176	RECO 15	RECO 29	RECO 36
1M	Mean	4.85	4.29	6.08	6.06	4.78	7.55
	SD	2.988	1.769	5.199	4.130	3.550	6.986
	N	5	5	5	2	2	2
2M	Mean	4.33	4.77	4.73	3.71	2.63	3.76
	SD	2.124	2.145	3.107	2.644	0.838	1.820
	N	10	10	10	4	4	4
3M	Mean	1.38*	5.06	3.19	1.50*	2.20	2.16
	SD	0.707	5.055	4.082	0.506	0.399	0.959
	N	10	10	10	4	4	4

**1M, 2M or 3M: males in 0, 1.0, or 3.0 mg/kg groups, respectively. PRED: Predose phase, DSGN: Dosing phase, and RECO: Recovery phase
* P< or =0.05

No drug-related PT and APTT changes were observed (tested on Days 1, 64, 148, and 176 of the dosing phase or on Day 36 of the recovery phase).

Clinical Chemistry

No remarkable drug-related changes were noted.

Urinalysis

No remarkable drug-related change was noted (tested on Day 176 of the dosing phase or on Day 36 of the recovery phase, a large number of samples were missing).

Gross Pathology

No remarkable drug-related change was observed.

Organ Weights

Dose-related decrease in mean absolute or relative uterus weight was noted at the terminal and recovery sacrifices but changes were more profound at the recovery sacrifice (Table 23). There was no correlating macroscopic or microscopic findings; therefore, this weight increase may not be drug-related.

Table 23. Summary of absolute or relative uterus weight (recovery sacrifice)**

Group/ Sex		Terminal Body weight (Kg)	Uterus Unadjusted (g)	Uterus Ratio (%)
1F	Mean	3.1	9.215	0.296
	SD	0.14	2.0075	0.0513
	N	2	2	2
2F	Mean	3.2	6.065	0.187*
	SD	0.71	2.3142	0.0533
	N	4	4	4
3F	Mean	3.5	6.019	0.170*
	SD	1.09	2.4271	0.0140
	N	4	4	4

** 1F, 2F or 3F: Females in 0, 1.0, or 3.0 mg/kg groups, respectively, * P< or =0.05

Histopathology

Adequate Battery: yes

Peer Review: no

Histological Findings: No remarkable drug-related change was observed.

Special Evaluation

N/A

Toxicokinetics

Free MMAE Analysis

Toxicokinetic parameters are summarized in Table 24. The increases in mean C_{max} and AUC were roughly dose proportional on Day 1. Gender differences were generally less than 2-fold based on mean C_{max} and AUC values.

Significant MMAE accumulation was noted. Mean MMAE $AUC_{168\text{-}189d}$ to $AUC_{0\text{-}21d}$ ratios ranged from approximately 15 to 162 fold.

Table 24. Summary of Toxicokinetic Parameters for MMAE *

SGN-35 Dose Group	SGN-35 Dose Level (mg/kg)	Sex		First Dose (Day 1) C_{max} (ng/mL)	First Dose (Day 1) T_{max} [a,b] (day)	First Dose (Day 1) $AUC_{0\text{-}21d}$ (ng·day/mL)	First Dose (Day 1) $AUC_{0\text{-}last}$ (ng·day/mL)	Last Dose (Day 169) C_{max} (ng/mL)	Last Dose (Day 169) T_{max} [a,b] (day)	Last Dose (Day 169) $AUC_{0\text{-}21d}$ (ng·day/mL)	Last Dose (Day 169) $AUC_{0\text{-}last}$ (ng·day/mL)	Accumulation Ratio[c] C_{max}	Accumulation Ratio[c] AUC
2	1	M	Mean	0.0369	2.0208 (1.0208,	0.183	0.152	3.72	168.0625 (168.0278,	2.94	2.38	93.7	15.0
			SD	0.0109	3.0208)	0.050	0.047	5.55	168.2708)	3.53	3.09	132	16.9
			N	10	10	10	10	10	10	9	10	10	9
		F	Mean	0.0238	2.0208 (1.0208,	0.105	0.0798	6.21	168.0625 (168.0278,	14.0	7.02	292	162
			SD	0.0053	3.0208)	0.038	0.0391	13.62	169.0208)	23.0	16.97	679	285
			N	10	10	10	10	10	10	5	10	10	5
		Combined	Mean	0.0303	2.0208 (1.0208,	0.144	0.116	4.97	168.0625 (168.0278,	6.91	4.70	193	67.5
			SD	0.0107	3.0208)	0.059	0.056	10.20	169.0208)	14.15	12.11	487	175
			N	20	20	20	20	20	20	14	20	20	14
3	3	M[d]	Mean	0.103	2.0208 (1.0208,	0.663	0.634	7.51	169.0208 (168.0278,	12.1	9.38	67.8	17.0
			SD	0.042	3.0208)	0.239	0.242	16.76	171.0208)	24.1	20.95	146.9	31.9
			N	9	9	9	9	9	9	7	9	9	7
		F	Mean	0.0654	2.0208	0.367	0.327	4.87	168.2917 (168.0278,	7.53	4.61	84.7	19.8
			SD	0.0095	(1.0208, 3.0208)	0.063	0.076	11.91	170.0208)	14.76	11.57	216	39.6
			N	10	10	10	10	10	10	6	10	10	6
		Combined	Mean	0.0831	2.0208 (1.0208,	0.508	0.472	6.12	168.5208 (168.0278,	10.0	6.87	76.7	18.3
			SD	0.0345	3.0208)	0.225	0.231	14.05	171.0208)	19.6	16.37	181.8	34.1
			N	19	19	19	19	19	19	13	19	19	13
		Animal No. I02398[e]		0.0915	2.0208	0.482	0.457	0.101	170.0208	NC	0.478	1.10	NC

* Times were normalized to Day 1 dose administration; $AUC_{168\text{-}189d}$ was not reported if the % AUC extrapolated value was >20%; NC: Not calculated.
[a] Tmax values presented to 4 decimal places for clarity.
[b] Median (Min, Max) reported for Tmax.
[c] Accumulation ratio calculated as Cmax Day 169/Cmax Day 1 or $AUC_{168\text{-}189d}/AUC_{0\text{-}21d}$.
[d] Animal I02398 was excluded from descriptive statistics since it was the only animal that did not have positive ADA results.
[e] Individual values for Animal I02398 are presented for informational purposes.

Antibody-Drug Conjugate (ADC) and Total Antibody (TAb) Analysis

Toxicokinetic parameters of ADC and TAb are summarized in Table 25 and Table 26, respectively. ADC and TAb serum exposures generally increased with the increase in SGN-35 dose level from 1 to 3 mg/kg and TAb exposures were higher than ADC. The increases in mean C_{max} on Day 1 were roughly dose proportional while the increases in $AUC_{0\text{-}21d}$ were slightly higher than dose proportional. No apparent sex differences were observed based on C_{max} and AUC values on Day 1. There appeared to be no accumulation in serum ADC or TAb concentrations following multiple doses of SGN-35.

Table 25. Summary of Toxicokinetic Parameters for ADC*

Dose Group	SGN-35 Dose Level (mg/kg)	Sex		First Dose (Day 1) C_{max} (µg/mL)	First Dose (Day 1) T_{max}[a,b] (day)	First Dose (Day 1) AUC_{0-21d} (µg·day/mL)	First Dose (Day 1) AUC_{0-last} (µg·day/mL)	Last Dose (Day 169) C_{max} (µg/mL)	Last Dose (Day 169) T_{max}[a,b] (day)	Last Dose (Day 169) $AUC_{168-189d}$ (µg·day/mL)	Last Dose (Day 169) AUC_{0-last} (µg·day/mL)	Accumulation Ratio[c] C_{max}	Accumulation Ratio[c] AUC
2	1	M	Mean	27.2	0.0278 (0.0278, 0.06250)	47.6	47.1	0.137	168.0278 (168.0278, 168.0278)	0.0128	0.0121	0.00512	0.000268
			SD	5.5		4.3	4.3	0.259		0.0250	0.0241	0.00928	0.000515
			N	10	10	10	10	10	7	6	6	10	6
		F	Mean	26.1	0.0278 (0.0278, 0.2708)	44.0	43.8	9.82	168.0278 (168.0278, 168.0278)	12.6	17.8	0.365	0.339
			SD	4.6		7.7	7.6	15.79		19.4	22.6	0.607	0.545
			N	10	10	10	10	10	7	6	7	10	6
		Combined	Mean	26.6	0.0278 (0.0278, 0.2708)	45.8	45.4	4.98	168.0278 (168.0278, 168.0278)	6.29	9.60	0.185	0.169
			SD	5.0		6.3	6.2	11.95		14.61	18.48	0.457	0.408
			N	20	20	20	20	20	14	12	13	20	12
3	3	M[d]	Mean	86.6	0.0625 (0.0278, 0.2708)	189	187	57.6	168.0278 (168.0278, 168.2708)	167	163	0.656	0.764
			SD	16.0		42	40	54.8		157	130	0.633	0.679
			N	9	9	9	9	9	8	6	7	9	6
		F	Mean	81.4	0.0278 (0.0278, 0.0625)	162	161	53.3	168.0278 (168.0278, 168.0625)	126	101	0.667	0.729
			SD	14.8		24	25	55.7		91	85	0.713	0.527
			N	10	10	10	10	10	7	7	8	10	7
		Combined	Mean	83.8	0.0278 (0.0278, 0.2708)	175	173	55.4	168.0278 (168.0278, 168.2708)	145	130	0.662	0.745
			SD	15.2		36	35	53.8		122	109	0.657	0.575
			N	19	19	19	19	19	15	13	15	19	13
		Animal No. I02398[e]		74.9	0.0625	154	154	84.0	168.0278	162	130	1.12	1.05

* Times were normalized to Day 1 dose administration; Cmax and AUC were treated as zero and Tmax was treated as missing for all animals with no measurable concentrations above the lower limit of quantitation in the profile; If there were measurable concentrations in the profile, at least three measurable concentrations above the lower limit of quantitation were needed to calculate AUC; $AUC_{168-189d}$ was not reported if the % AUC extrapolated value was >20%.

[a] Tmax values presented to 4 decimal places for clarity.

[b] Median (Min, Max) reported for Tmax.

[c] Accumulation ratio calculated as Cmax Day 169/Cmax Day 1 or $AUC_{168-189d}/AUC_{0-21d}$.

[d] Animal I02398 was excluded from descriptive statistics since it was the only animal that did not have positive ADA results.

[e] Individual values for Animal I02398 are presented for informational purposes.

Table 26. Summary of Toxicokinetic Parameters for TAb*

Dose Group	SGN-35 Dose Level (mg/kg)	Sex	Animal Number	First Dose (Day 1) C_{max} (µg/mL)	First Dose (Day 1) T_{max}[a,b] (day)	First Dose (Day 1) AUC_{0-21d} (µg·day/mL)	First Dose (Day 1) AUC_{0-last} (µg·day/mL)	Last Dose (Day 169) C_{max} (µg/mL)	Last Dose (Day 169) T_{max}[a,b] (day)	Last Dose (Day 169) $AUC_{168-189d}$ (µg·day/mL)	Last Dose (Day 169) AUC_{0-last} (µg·day/mL)	Accumulation Ratio C_{max}	Accumulation Ratio AUC[c]
2	1	M	Mean	26.7	0.0278 (0.0278, 0.0625)	61.4	60.2	0	NC	0	0	0	0
			SD	4.9		7.3	7.3	0	NC	0	0	0	0
			N	10	10	10	10	10	0	10	10	10	6
		F	Mean	25.2	0.0278 (0.0278, 0.2708)	58.3	57.7	7.29	168.0278 (168.0278, 168.0278)	10.7	14.2	0.284	0.177
			SD	4.8		8.7	8.5	12.08		18.4	19.7	0.483	0.304
			N	10	10	10	10	10	5	7	8	10	7
		Combined	Mean	25.9	0.0278 (0.0278, 0.2708)	59.8	59.0	3.65	NC	4.42	6.31	0.142	0.0728
			SD	4.8		8.0	7.8	9.12	NC	12.50	14.57	0.363	0.2064
			N	20	20	20	20	20	5	17	18	20	13

3	3	M[d]	Mean	83.5	0.0625 (0.0278,	244	239	49.4	168.0278 (168.0278,	163	158	0.606	0.573
			SD	13.1	0.5208)	53	50	47.9	168.2708)	187	154	0.599	0.615
			N	9	9	9	9	9	6	7	8	9	7
		F	Mean	75.3	0.0278 (0.0278,	196	194	40.1	168.0278 (168.0278,	82.0	83.3	0.550	0.402
			SD	15.2	0.0625)	23	25	43.3	168.0625)	89.2	79.5	0.614	0.444
			N	10	10	10	10	10	6	8	9	10	8
		Combined	Mean	79.2	0.0278 (0.0278,	218	216	44.5	168.0278 (168.0278,	120	118	0.577	0.482
			SD	14.4	0.5208)	46	44	44.5	168.2708)	144	123	0.591	0.518
			N	19	19	19	19	19	12	15	17	19	15
		Animal No. I02398[e]		75.3	0.0625	224	224	68.7	168.0278	NR	139	0.912	NC

* Times were normalized to Day 1 dose administration; Cmax and AUC were treated as zero and Tmax was treated as missing for all animals with no measurable concentrations above the lower limit of quantitation in the profile; If there were measurable concentrations in the profile, at least three measurable concentrations above the lower limit of quantitation were needed to calculate AUC; $AUC_{168-189d}$ was not reported if the % AUC extrapolated value was >20%; Descriptive statistics were not calculated for parameters when N<5 for males and females or N<10 for combined sex; NR: Not reported; and NC: Not calculated.

[a] Tmax values presented to 4 decimal places for clarity.

[b] Median (Min, Max) reported for Tmax.

[c] Accumulation ratio calculated as Cmax Day 169/Cmax Day 1 or $AUC_{168-189d}/AUC_{0-21d}$.

[d] Animal I02398 was excluded from descriptive statistics since it was the only animal that did not have positive ADA results.

[e] Individual values for Animal I02398 are presented for informational purposes.

Anti-Drug Antibody (ADA)

A positive immunogenicity response was noted in all animals in the 1 mg/kg group and in 19 of the 20 animals in the 3 mg/kg group (except for a male, # 102398). The positive immunogenicity response was detected as early as Day 22 for majority of animals with increased titers along with additional dosing. For example, males in the 1 mg/kg group had titers <16 to 64 on Day 22, 1024 to 131072 on Day 176, and 4096 to 65536 on Day 211. ADA remained until Days 176 or 211 (last testing for animals in terminal or recovery sacrifice groups, respectively).

The positive immunogenicity responses appeared to reduce the concentrations of ADC and TAb after multiple doses, and conversely increased the concentrations of MMAE after multiple doses in most of the animals.

According to the clinical overview presented by the Applicant, approximately 7% of patients with HL and ALCL developed positive antibodies (>2 positive samples). The presence of ADA did not correlate with a substantial reduction in serum brentuximab vedotin levels and did not result in a decrease in the efficacy of SGN-35. The ADA response was always directed against the antibody portion of the ADC and typically was detected for the first time in the pre-dose Cycle 2 sample. Of 58 patients with ADA post baseline, 36 (62%) were positive for the presence of neutralizing antibodies, 18 (31%) were negative for the presence of neutralizing antibodies, and 4 (7%) were of unknown status due to insufficient sample.

Stability and Homogeneity

Stability

According to the stability study report, the ongoing studies for lot SDD002 demonstrated that it was stable for at least 12 months at 2-8°C.

Dose preparation was stable for 7 hours at room temperature (all dose preparations were used within 7 hours of completion of preparation).

Concentration Verification

Duplicate samples (1.00 mL each) were taken from the formulation prepared for dosing during Weeks 1, 4, 13, and 25 of the dosing phase. The first set of samples was stored at room temperature and analyzed on the day of preparation and the second set of samples stored in a refrigerator (2 to 8°C) until analyzed or discarded upon receipt of acceptable analytical results.

All formulations were within ±10% of the target concentrations (mean concentrations ranging from 94.5 to 109% of theoretical ones).

Reviewer's comment

The recommended dose of SGN-35 is 1.8 mg/kg. According to the clinical overview presented by the Applicant, at this dose level, AUCs were 79.41 day•micg/mL for SGN-35 and 37.03 day•ng/mL for MMAE (SGD-1010). Most common AEs were peripheral neuropathy (53%, sensory 44 % and motor 9%), fatigue (42 %), nausea (41 %), diarrhea (34 %), pyrexia (31 %), upper respiratory tract infection (28 %), neutropenia (21 %), vomiting (20 %), and thrombocytopenia (10%). Monkeys had lower systemic exposure to MMAE despite of higher SGN-35 exposure. At 3 mg/kg, the highest AUC was 10 day•ng/mL for MMAE while 175 day•micg/mL for SGN-35.

7 Genetic Toxicology

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

Study title: Bacterial Reverse Mutation Assay: SGD-1010

Study no.:	AA66EH.503.BTL
Study report location:	M4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	03 October 2002
GLP compliance:	Yes*
QA statement:	Yes
Drug, lot #, and % purity:	SGD-1010 (ALB 10787), lot #: RIL-B-

114(8), and purity: 98.7% (by HPLC)
* The stability of the drug and homogeneity, concentration, and stability of the drug preparations were not determined.

Key Study Findings

Negative

Methods: Plate incorporation

Strains:	*Salmonella typhimurium* histidine auxotrophs TA98, TA100, TA1535, and TA1537 and the *Escherichia coli* tryptophan auxotroph WP2*uvr*A
Concentrations in definitive study:	75, 200, 600, 1800, and 5000 mcg per plate in the presence or absence of S9
Basis of concentration selection:	Range finding assay at up to 5000 mcg/plate
Negative control:	Dimethylsulfoxide (DMSO)
Positive control:	See Table 27
Formulation/Vehicle:	DMSO
Incubation & sampling time:	48 to 72 hours

Table 27. Summary of Positive Control Agents*

Assay	Chemicals	Concentration (mcg/plate)	Responding strains
Nonactivation	2-nitrofluorene (2NF)	1.0	TA 98
	Sodium azide (SA)	1.0	TA 100, 1535
	9-aminoacridine	75	TA 1537
	Methyl methanesulfonate (MMS)	1,000	WP2uvrA
Activation	2-aminoanthracene (2-AA)	1.0	TA 98, 100, 1535, 1537
	2-aminoanthracene (2-AA)	10	WP2uvrA

*Prepared by the reviewer based on the submission.

Study Validity

1. Concentration selection was acceptable because recommended maximum concentration, 5000 mcg per plate, was used.
2. The negative control counts fell within the historic control ranges.
3. The positive controls induced a greater than 3-fold increase in mean revertant colony numbers over that of the vehicle control.
4. Triplicate cultures.

Results

Under the conditions of this study, SGD-1010 did not cause a positive increase in the mean number of revertants per plate with any of the tester strains either in the presence or absence of S9 (Table 28) .

Table 28. Summary of Results

Average Revertants Per Plate ± Standard Deviation					
Liver Microsomes: None					
Dose (µg/plate)	TA98	TA100[a]	TA1535	TA1537	WP2 uvrA
Vehicle	13 ± 2	125 ± 11	13 ± 1	12 ± 2	11 ± 1
75	11 ± 1	133 ± 26	13 ± 1	11 ± 1	13 ± 2
200	11 ± 1	132 ± 21	12 ± 1	11 ± 1	11 ± 2
600	11 ± 1	133 ± 13	13 ± 4	11 ± 1	11 ± 1
1800	12 ± 1	140 ± 11	12 ± 1	11 ± 1	11 ± 2
5000	12 ± 2	129 ± 14	12 ± 1	10 ± 1	10 ± 1
Positive	152 ± 19	549 ± 22	506 ± 61	1087 ± 122	208 ± 21
Liver Microsomes: Rat liver S9					
Dose (µg/plate)	TA98	TA100[a]	TA1535	TA1537	WP2 uvrA
Vehicle	16 ± 4	136 ± 14	11 ± 1	8 ± 3	10 ± 1
75	15 ± 1	146 ± 7	10 ± 2	5 ± 2	12 ± 2
200	11 ± 2	130 ± 6	8 ± 3	10 ± 1	12 ± 1
600	13 ± 2	138 ± 17	10 ± 3	8 ± 1	11 ± 1
1800	13 ± 2	141 ± 17	10 ± 2	10 ± 2	11 ± 1
5000	11 ± 1	129 ± 9	11 ± 2	10 ± 2	11 ± 1
Positive	890 ± 85	828 ± 90	171 ± 54	1471 ± 114	376 ± 56

7.2 *In Vitro Mammalian Cell Gene Mutation Assays*

Study title: L5178Y TK+/- Mouse Lymphoma Forward Mutation Assay with a Confirmatory Assay

Study no.:	(b) (4) Study Number 8204155
Study report location:	M4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	20 April 2009
GLP compliance:	Yes*
QA statement:	Yes
Drug, lot #, and % purity:	SGD-1010, lot #: 2002E, and purity: 95.8 % (by HPLC)

* Exceptions: The stability and concentration of the dosing preparations were not analyzed.

Key Study Findings

Negative

Methods: Soft Agar Version, colonies were counted with an automated colony counter

Cell line:	L5178Y/TK $^{+/-}$ Mouse Lymphoma
Concentrations in definitive study:	4-hour treatment, 0.05, 0.1, 0.5, 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 ng/mL with S9, and 0.05, 0.1, 0.5, 1, 2.5, 5, 7.5, 10, 12.5, 15, 20, 25, 30, 40, and 50 ng/mL without S9 Confirmatory: 4-hour treatment: 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, 10, 20, 30, 40, 50, 60, and 70 ng/mL with S9 24-hour treatment: 0.001, 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 ng/mL without S9
Basis of concentration selection:	Range-finding assay at concentrations of 0.197, 0.393, 0.785, 1.57, 3.13, 6.25, 12.5, 25, 50, and 100 ng/mL
Negative control:	0.9% saline for injection
Positive control:	Methyl methanesulfonate (MMS) in the absence of S9 at concentrations of 10, 15, and 20 mcg/mL (4-hour treatment), or 6.5, 10, and 15 mcg/mL (24-hour treatment) Methylcholanthrene (MCA) in the presence of S9 at concentrations of 1.5, 2, and 4 mcg/mL
Formulation/Vehicle:	Initially dissolved in 0.01N HCl (1 mg/mL) then diluted in 0.9% saline for injection
Incubation & sampling time:	2 or 3 days (expression period) + 13 or 14 days

Study Validity

The study validity is evaluated using criteria recommended by the Mouse Lymphoma Assay Workgroup of the International Workshop on Genotoxicity testing.

- Negative controls
  - Absolute cloning efficiency (ACE): within 65-120% range
  - Suspension growth (SG): > 8- or 32-fold for the 2- or 3-day expression periods
  - Mutant frequency (MF): majority within 35 to 140 x 10^{-6}, a few less than 35 x 10^{-6} but around 30 x 10^{-6}
- Positive controls:
  - MF: at least 300 x 10^{-6} with at least 40% of which is from small colonies in at least one concentration (two used)

  - Relative total growth (RTG) >10% in at least one concentration
- Small colony detection: >40% small colonies in majority of cultures
- Concentration selection:
  - RTG: 16.6% at 50.0 ng SGD-1010/mL with S9 and 22.1% at 7.50 ng/mL without S9 in the initial assay with a 4-hour treatment
  - RTG: 24.8% at 30.0 ng/mL with S9 (4-hour treatment) and 17.8% at 2.50 ng/mL without S9 (24-hour treatment) in the confirmatory assay
  - RTG; 15.7% at 50.0 ng/mL with S9 and 27.0% at 5.00 ng/mL without S9 in the re-test
- At least 8 analyzable concentrations for single cultures, re-tested when there were < 8 analyzable concentrations
- Single cultures in all SGD-1010 and positive control concentrations and triplicate in the vehicle controls
- The results evaluated using global evaluation factor (GEF): positive as induced MF> the GEF (background MF + 90) and statistically significant response

Although MF was less than 35 x 10^{-6} (but around 30 x 10^{-6}) in a few cultures of negative controls and some data points were evaluated at greater than 20% RTG (but >25% in one assay only, should be in the approximately 10-20% range). The study was considered as acceptable considering the totality of study.

Results

In the initial assay, MF in cultures treated with SGD-1010 ranged from 63.6 to 80.7 TFT^r mutants/10^6 clonable cells with S9 (4 analyzable cultures) and 39.8 to 69.3 TFT^r mutants/10^6 clonable cells without S9 (7 analyzable cultures, Table 29).

In the confirmatory assay, MF in cultures treated with SGD-1010 ranged from 31.3 to 74.4 TFT^r mutants/10^6 clonable cells with S9 (Table 30 and Table 31) and 27.2 to 92.5 TFT^r mutants/10^6 clonable cells without S9 (Table 32 and Table 33).

Table 29. Initial Mutation Assay without Activation (4-h treatment)

Test Condition	Daily Cell Density/mL (x 10^5)		Cumulative RSG[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (x 10^{-6})[d]
	Day 1	Day 2								
Nonactivation Controls[e]				AVG VC				AVG VC		
Vehicle Control	12.4	9.9	13.6		97	511	85.2		91.4	38.1
Vehicle Control	12.1	12.7	17.1		123	449	74.9		100.6	54.9
Vehicle Control	13.7	10.7	16.3	15.7	107	500	83.4	81.1	106.8	42.7
MMS 15.0 µg/mL	8.9	8.0	7.9		296	158	26.3		16.4	375.9[f]
MMS 20.0 µg/mL	7.9	6.3	5.5		249	106	17.6		7.6	471.9[f]
Test Article (ng/mL)			Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
0.0500	14.0	9.3	92.3		147	492	101.1		93.3	59.8
0.100	14.7	10.7	111.5		96	435	89.4		99.7	44.1
0.500	13.1	10.5	97.5		108	475	97.6		95.3	45.4
1.00	13.8	7.7	75.4		128	595	122.2		92.1	43.0
2.50	11.0	9.6	74.9		107	536	110.1		82.4	39.8
5.00	8.3	7.3	43.0		101	340	69.9		30.0	59.2
7.50	7.3	6.4	33.1		113	325	66.7		22.1	69.3
10.0	5.2	4.7	17.3		[h]	204	41.9		7.3	[i]
12.5	3.9[g]	4.9	10.4		[h]	157	32.1		3.4	[i]
15.0	4.0	3.7	10.5		[h]	154	31.7		2.5	[i]

Table 30.Confirmatory Mutation Assay with Activation (4-h treatment)

Test Condition	Daily Cell Density/mL (x 10^5)		Cumulative RSG[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (x 10^{-6})[d]
	Day 1	Day 2								
Activation Controls[e]				AVG VC				AVG VC		
Vehicle Control	11.4	12.3	15.6		108	433	72.1		86.4	49.9
Vehicle Control	11.6	12.9	16.6		121	526	87.7		112.1	45.9
Vehicle Control	14.5	10.2	16.4	16.2	117	485	80.8	80.2	102.1	48.4
MCA 1.50 µg/mL	5.7	8.2	5.2		490	234	38.9		15.5	419.3[f]
MCA 2.00 µg/mL	4.3	8.5	4.1		395	231	38.5		12.0	341.5[f]
Test Article (ng/mL)			Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
0.0500	14.3	10.5	102.9		65	416	86.5		89.0	31.3
0.250	13.5	10.5	97.1		100	394	81.8		79.4	50.6
0.500	12.1	10.5	87.1		98	345	71.7		62.4	57.0
2.50	13.4	9.9	90.9		77	333	69.2		62.9	46.3
5.00	12.0	8.2	67.4		129	567	117.7		79.4	45.6
10.0	12.3	10.1	85.1		90	455	94.6		80.5	39.6
20.0	8.5	9.1	53.0		111	366	76.1		40.3	60.8
30.0	6.7	6.1	28.0		159	427	88.7		24.8	74.4
50.0	5.1	6.1	21.3		[g]	219	45.6		9.7	[h]
60.0	4.3	6.0	17.7		[g]	186	38.7		6.8	[h]

[a]RSG = (Day 1 Count)/3 x (Day 2 Count)/3 (or Day 1 Count if not subcultured)
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100
[c]Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency)/100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2×10^{-4})
[e]Vehicle Control = 10% Saline, 0.9% Sodium Chloride injection USP
Positive Control: MCA = Methylcholanthrene
[f]Mutagenic. Exceeds Minimum Criterion of 138.1 x 10^{-6}
[g]Not scored due to excessive cytotoxicity.
[h]Insufficient data for calculations.

Table 31. Sizing Data for Confirmatory Mutation Assay with Activation (4-h treatment)

Test Condition	Conc.	Cum. RSG (%)[a]		Cloning Efficiency[b]		Relative Growth[c]	Mutant Frequency (x 10^{-6})[d]		
		Day 1	Day 2	Abs %	Rel %	(%)	Total	Small	Large
Vehicle Control[e]									
	10%	91.2	96.1	72.1	89.9	86.4	49.9	18.1	31.8
	10%	92.8	102.5	87.7	109.4	112.1	45.9	14.9	31.1
	10%	116.0	101.4	80.8	100.7	102.1	48.4	12.7	35.7
MCA[f] (µg/mL)									
	1.50	45.6	32.0	38.9	48.5	15.5	419.3	202.0	217.3
	2.00	34.4	25.0	38.5	48.0	12.0	341.5	163.1	178.5
Test Article (ng/mL)									
	0.050	114.4	102.9	69.4	86.5	89.0	31.3	6.8	24.5
	0.250	108.0	97.1	65.6	81.8	79.4	50.6	12.7	38.0
	0.500	96.8	87.1	57.5	71.7	62.4	57.0	11.0	46.0
	2.50	107.2	90.9	55.5	69.2	62.9	46.3	17.1	29.2
	5.00	96.0	67.4	94.5	117.7	79.4	45.6	16.7	28.9
	10.0	98.4	85.1	75.9	94.6	80.5	39.6	12.0	27.6
	20.0	68.0	53.0	61.1	76.1	40.3	60.8	21.4	39.5
	30.0	53.6	28.0	71.1	88.7	24.8	74.4	24.4	50.0

[a] Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100
[c]Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency)/100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x10^{-4}), Expressed as Total Mutant Frequency, Small Colony Mutant Frequency and Large Colony Mutant Frequency
[e]Vehicle Control = 10% Saline, 0.9% Sodium Chloride injection USP
[f]Positive Control: MCA = Methylcholanthrene
Mutagenic. Exceeds Minimum Criterion of 138.1 x 10^{-6}
[g]Not scored due to excessive cytotoxicity.
[h]Insufficient data for calculations.

Table 32. Confirmatory Mutation Assay without Activation (24-h treatment)

Test Condition	Daily Cell Density/mL (x 10^5)			Cumulative RSG[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (x 10^{-6})[d]
	Day 1	Day 2	Day 3		AVG VC				AVG VC		
Nonactivation Controls[e]											
Vehicle Control	15.5	11.4	8.4	55.0		84	375	62.4		75.5	44.9
Vehicle Control	16.2	10.0	10.9	65.4		72	493	82.2		118.3	29.3
Vehicle Control	17.0	10.9	9.2	63.1	61.2	74	470	78.3	74.3	108.7	31.3
MMS 6.50 µg/mL	14.0	10.2	7.6	40.2		177	173	28.9		25.5	204.1[f]
MMS 10.0 µg/mL	13.4	8.3	8.3	34.2		229	142	23.7		17.8	321.7[f]
Test Article (ng/mL)				Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
0.00500	18.0	9.7	11.4	120.5		66	298	66.8		80.5	44.6
0.0100	18.9	9.7	10.8	119.9		49	273	61.2		73.3	35.7
0.100	19.3	10.7	9.9	123.8		47	346	77.7		96.1	27.4
0.500	20.0	10.1	9.5	116.2		62	370	83.0		96.4	33.3
0.750	20.9	8.9	9.7	109.2		65	379	85.1		93.0	34.4
1.00	17.1	11.5	9.7	115.5		41	305	68.4		78.9	27.2
1.50	16.1	11.1	10.4	112.5		55	243	54.5		61.4	44.9
2.00	15.0	9.7	9.5	83.7		89	267	59.8		50.1	66.7
2.50	10.1	6.2	9.5	36.0		102	221	49.5		17.8	92.5
3.00	6.4	6.6	7.0	17.9		[g]	225	50.5		9.0	[h]

[a] RSG = [Treatment termination (Day 1) cell density/3] x [Day 2 cell density/3 or Day 1 density if not split back] x [Day 3 cell density/3 or Day 2 density if not split back]
[b] Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100
[c] Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100
[d] Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10-4)
[e] Vehicle Control = 10% Saline, 0.9% Sodium Chloride injection USP
Positive Control: MMS = Methyl methanesulfonate
[f] Mutagenic. Exceeds Minimum Criterion of 125.2 x 10^{-6}
[g] Not scored due to excessive cytotoxicity.
[h] Insufficient data for calculations.

Table 33. Sizing Data for Confirmatory Mutation Assay without Activation (24-h treatment)

Test Condition	Conc.	Cum. RSG (%)[a] Day 1	Day 2	Day 3	Cloning Efficiency[b] Abs %	Rel %	Relative Growth[c] (%)	Mutant Frequency (x 10^{-6}) Total	Small	Large
Vehicle Control[e]										
	10%	95.5	101.2	89.9	62.4	84.0	75.5	44.9	12.0	32.9
	10%	99.8	92.7	106.9	82.2	110.6	118.3	29.3	7.2	22.1
	10%	104.7	106.1	103.2	78.3	105.3	108.7	31.3	12.1	19.2
MMS[f] (µg/mL)										
	6.50	86.2	81.8	65.7	28.9	38.8	25.5	204.1	109.6	94.5
	10.0	82.5	63.7	55.9	23.7	31.9	17.8	321.7	173.3	148.3
Test Article (ng/mL)										
	0.00500	110.9	100.0	120.5	49.6	66.8	80.5	44.6	9.6	35.1
	0.0100	116.4	105.0	119.9	45.4	61.2	73.3	35.7	8.7	27.0
	0.100	118.9	118.2	123.8	57.7	77.7	96.1	27.4	6.2	21.2
	0.500	123.2	115.6	116.2	61.7	83.0	96.4	33.3	9.0	24.4
	0.750	128.7	106.5	109.2	63.2	85.1	93.0	34.4	6.9	27.5
	1.00	105.3	112.6	115.5	50.8	68.4	78.9	27.2	3.9	23.3
	1.50	99.2	102.3	112.5	40.5	54.5	61.4	44.9	10.7	34.1
	2.00	92.4	83.3	83.7	44.5	59.8	50.1	66.7	17.8	48.9
	2.50	62.2	35.9	36.0	36.8	49.5	17.8	92.5	21.5	71.0

[a] Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth
[b] Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100
[c] Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100
[d] Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10-4)
Expressed as Total Mutant Frequency, Small Colony Mutant Frequency and Large Colony Mutant Frequency
[e] Vehicle Control = Saline, 0.9% Sodium Chloride injection USP
[f] Positive Control: MMS = Methyl methanesulfonate

Due to excessive cytotoxicity in the initial assay, fewer than eight cultures were available for analysis. Therefore, SGD-1010 was re-evaluated using a 4-hour treatment. MF in cultures treated with SGD-1010 ranged from 27.9 to 100.0 TFT^r mutants/10^6 clonable cells with S9 (Table 34 and Table 35) and 24.7 to 69.6 TFT^r mutants/10^6 clonable cells without S9 (Table 36 and Table 37).

Table 34. Re-test Mutation Assay with Activation (4-h treatment)

Test Condition	Daily Cell Density/mL (x 10^5)		Cumulative RSG[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (x 10^{-6})[d]
	Day 1	Day 2		AVG VC				AVG VC		
Activation Controls[e]										
Vehicle Control	12.5	13.2	18.3		72	411	68.6		91.9	35.2
Vehicle Control	12.1	11.8	15.9		142	528	87.9		102.0	53.9
Vehicle Control	12.0	10.2	13.6	15.9	147	606	101.0	85.8	100.4	48.5
MCA 1.50 µg/mL	4.7	9.3	4.9		447	254	42.3		15.0	352.3[f]
MCA 2.00 µg/mL	3.8[g]	8.2	2.7		558	282	47.0		9.4	395.8[f]
Test Article (ng/mL)			Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
0.250	10.8	11.3	85.1		154	626	121.6		103.5	49.2
0.500	11.1	11.4	88.2		164	651	126.4		111.5	50.3
1.00	11.7	12.0	97.9		83	594	115.3		112.9	27.9
5.00	9.9	14.4	99.4		119	475	92.3		91.8	49.9
10.0	8.8	14.9	91.4		101	443	86.1		78.7	45.5
20.0	7.2	15.3	76.8		91	470	91.2		70.0	38.9
30.0	6.7	12.0	56.1		115	404	78.5		44.0	56.9
40.0	4.5	10.4	32.6		74	285	55.3		18.0	51.7
50.0	4.7	8.4	27.5		147	294	57.1		15.7	100.0
60.0	3.3[g]	6.3	13.2		[h]	250	48.6		6.4	[i]

[a] RSG = (Day 1 Count)/3 x (Day 2 Count)/3 (or Day 1 Count if not subcultured)
[b] Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100
[c] Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100
[d] Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10^{-4})
[e] Vehicle Control = 10% Saline, 0.9% Sodium Chloride injection USP
Positive Control: MCA = Methylcholanthrene
[f] Mutagenic. Exceeds Minimum Criterion of 135.9 x 10^{-6}
[g] Not subcultured.
[h] Not scored due to excessive cytotoxicity.
[i] Insufficient data for calculations.

Table 35. Sizing Data Re-test Mutation Assay with Activation (4-h treatment)

Test Condition	Conc.	Cum. RSG (%)[a] Day 1	Cum. RSG (%)[a] Day 2	Cloning Efficiency[b] Abs %	Cloning Efficiency[b] Rel %	Relative Growth[c] (%)	Mutant Frequency (x 10^{-6}) Total	Mutant Frequency (x 10^{-6}) Small	Mutant Frequency (x 10^{-6}) Large
Vehicle Control[e]									
	10%	102.5	115.1	68.6	79.9	91.9	35.2	9.8	25.4
	10%	99.2	99.6	87.9	102.5	102.0	53.9	16.2	37.8
	10%	98.4	85.4	101.0	117.7	100.4	48.5	14.5	34.1
MCA[f] (µg/mL)									
	1.50	38.5	30.5	42.3	49.3	15.0	352.3	187.9	164.5
	2.00	31.1	17.2	47.0	54.8	9.4	395.8	216.0	179.8
Test Article (ng/mL)									
	0.250	88.5	85.1	104.3	121.6	103.5	49.2	17.8	31.4
	0.500	91.0	88.2	108.5	126.4	111.5	50.3	13.5	36.8
	1.00	95.9	97.9	99.0	115.3	112.9	27.9	11.2	16.8
	5.00	81.1	99.4	79.2	92.3	91.8	49.9	16.5	33.4
	10.0	72.1	91.4	73.9	86.1	78.7	45.5	14.4	31.0
	20.0	59.0	76.8	78.3	91.2	70.0	38.9	16.2	22.7
	30.0	54.9	56.1	67.4	78.5	44.0	56.9	19.9	37.0
	40.0	36.9	32.6	47.4	55.3	18.0	51.7	15.8	35.8
	50.0	38.5	27.5	49.0	57.1	15.7	100.0	40.3	59.7

[a] Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth
[b] Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100
[c] Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100
[d] Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2×10^{-4})
Expressed as Total Mutant Frequency, Small Colony Mutant Frequency and Large Colony Mutant Frequency
[e] Vehicle Control = Saline, 0.9% Sodium Chloride injection USP
[f] Positive Control: MCA = Methylcholanthrene

Table 36. 4-Hour Re-test Mutation Assay without Activation

Test Condition	Daily Cell Density/mL (x 10^5)		Cumulative RSG[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (x 10^{-6})[d]
	Day 1	Day 2		AVG VC				AVG VC		
Nonactivation Controls[e]										
Vehicle Control	13.4	9.8	14.6		97	455	75.9		81.2	42.7
Vehicle Control	12.6	11.0	15.4		85	470	78.3		88.4	36.4
Vehicle Control	12.5	12.2	16.9	15.6	92	644	107.3	87.1	133.4	28.7
MMS 15.0 µg/mL	8.3	9.8	9.0		322	180	30.0		19.9	357.9[f]
MMS 20.0 µg/mL	7.6	8.7	7.3		211	81	13.4		7.2	523.5[f]
Test Article (ng/mL)			Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
0.0100	13.5	10.4	99.7		66	385	73.7		73.5	34.5
0.0500	13.8	9.3	91.1		53	411	78.7		71.7	25.9
0.100	13.6	10.6	102.4		57	461	88.2		90.3	24.7
0.250	14.4	10.4	106.4		72	411	78.7		83.7	35.2
0.500	12.9	11.3	103.5		64	420	80.3		83.1	30.5
0.750	10.0	12.1	85.9		65	349	66.7		57.3	37.4
2.50	11.2	11.9	94.7		82	341	65.3		61.8	47.9
5.00	5.8	10.7	44.1		111	320	61.2		27.0	69.6
7.50	5.7	5.1	20.6		[g]	237	45.3		9.4	[h]
10.0	4.1	4.5	13.1		[g]	106	20.2		2.6	[h]

[a] RSG = (Day 1 Count)/3 x (Day 2 Count)/3 (or Day 1 Count if not subcultured)
[b] Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100
[c] Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100
[d] Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10^{-4})
[e] Vehicle Control = 10% Saline, 0.9% Sodium Chloride injection USP
Positive Control: MMS = Methyl methanesulfonate
[f] Mutagenic. Exceeds Minimum Criterion of 125.9 x 10^{-6}
[g] Not scored due to excessive cytotoxicity.
[h] Insufficient data for calculations.

Table 37. Sizing Data Re-test Mutation Assay without Activation

Test Condition	Conc.	Cum. RSG (%)[a] Day 1	Cum. RSG (%)[a] Day 2	Cloning Efficiency[b] Abs %	Cloning Efficiency[b] Rel %	Relative Growth[c] (%)	Mutant Frequency (x 10^{-6})[d] Total	Small	Large
Vehicle Control[e]									
	10%	104.4	93.3	75.9	87.1	81.2	42.7	8.9	33.9
	10%	98.2	98.4	78.3	89.8	88.4	36.4	12.1	24.2
	10%	97.4	108.3	107.3	123.1	133.4	28.7	11.8	16.9
MMS[f] (µg/mL)									
	15.0	64.7	57.8	30.0	34.5	19.9	357.9	240.8	117.1
	20.0	59.2	47.0	13.4	15.4	7.2	523.5	320.6	202.9
Test Article (ng/mL)									
	0.0100	105.2	99.7	64.2	73.7	73.5	34.5	12.9	21.5
	0.0500	107.5	91.1	68.6	78.7	71.7	25.9	9.8	16.1
	0.100	106.0	102.4	76.9	88.2	90.3	24.7	4.6	20.1
	0.250	112.2	106.4	68.6	78.7	83.7	35.2	9.8	25.4
	0.500	100.5	103.5	70.0	80.3	83.1	30.5	8.5	22.0
	0.750	77.9	85.9	58.1	66.7	57.3	37.4	9.5	27.9
	2.50	87.3	94.7	56.9	65.3	61.8	47.9	14.6	33.3
	5.00	45.2	44.1	53.4	61.2	27.0	69.6	14.8	54.8

[a] Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth
[b] Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100
[c] Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100
[d] Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10^{-4})
Expressed as Total Mutant Frequency, Small Colony Mutant Frequency and Large Colony Mutant Frequency
[e] Vehicle Control = Saline, 0.9% Sodium Chloride injection USP
[f] Positive Control: MMS = Methyl methanesulfonate

None of cultures treated with SGD-1010 induced a dose-dependent increase in MF, or a net increase greater than background plus 90 TFT^r mutants/10^6 clonable cells. Therefore, the results indicated that SGD-1010 was negative in the L5178Y TK+/- mouse lymphoma forward mutation assay under the conditions of this study.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: *In Vivo* Rat Bone Marrow Micronucleus Assay

Study no.: (b) (4) Study Number 8204151
Study report location: M4
Conducting laboratory and location: (b) (4)
Date of study initiation: 28 April 2009
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: SGD-1010, lot #: 2002E, and purity: 95.8

% (by HPLC)

Key Study Findings

SGD-1010 was positive for genotoxicity under the conditions of this assay with an aneugenic mechanism.

Methods

Doses in definitive study:	0, 0.01, 0.1 and 0.2 mg/kg
Frequency of dosing:	Twice approximately 24 hours apart
Route of administration:	Intravenous Injection
Dose volume:	10 mL/kg for the positive control groups and 5 mL/kg for other groups
Formulation/Vehicle:	3.9% 0.01N Hydrochloric Acid/96.1% 0.9% Sodium Chloride for Injection
Species/Strain:	Rat/CD® (SD), bone marrow
Number/Sex/Group:	5 males for MN, 3 males for antikinetochore analysis
Satellite groups:	N/A
Basis of dose selection:	Findings in a previous micronucleus assay ((b) (4) study number 1398/86). Adverse clinical signs were not observed in this study in either male or female rats dosed up to 2000 mg/kg/day. Based on these results, males only were tested in the present test.
Negative control:	3.9% 0.01N Hydrochloric Acid/96.1% 0.9% Sodium Chloride for Injection
Positive control:	Cyclophosphamide (CP, 60 mg/kg, oral gavage) and carbendazim (CBZ, 1250 or 1500 mg/kg, oral gavage)

Study Validity

The PCEs with micronuclei in vehicle controls (Table 38 and Table 39) were within the historical control range (0.00 to 0.25 for 24 hour harvest and 0.00 to 0.3 for 48 hour harvest).

Cyclophosphamide induced a statistically significant increase in micronucleated PCEs (Table 38 and Table 39) compared to the vehicle control, which was consistent with historical positive control data.

The micronuclei in animals treated with the clastogen (CP) were mainly centromere-negative (72-84%), while micronuclei in animals treated with the aneugen (CBZ) mainly centromere-positive (68%).

Results

Results of concentration verification analyses showed that all formulations were within 97-107 % of target concentrations, with the exception of the 0.01 mg/kg (low dose) group (129 % of target concentration).

There was no mortality or adverse clinical sign observed.

SGD-1010 was cytotoxic to the bone marrow at the dose of 0.2 mg/kg (Table 38).

SGD-1010 induced statistically significant increases in micronucleated PCEs at doses of 0.1 and 0.2 mg/kg (Table 38).

Table 38. Summary of Micronucleus Assay

Treatment	Dose	Harvest Time	% Micronucleated PCEs Mean ± SD Male	Ratio PCE:NCE Mean ± SD Male
Controls				
Vehicle	HCl/Saline 5 mL/kg	24	0.07 ± 0.07	0.70 ± 0.11
		48	0.08 ± 0.09	0.82 ± 0.21
Positive	CP 60 mg/kg	24	1.77 ± 0.99*	0.69 ± 0.10
Test Article	0.01 mg/kg	24	0.12 ± 0.15	0.70 ± 0.18
	0.1 mg/kg	24	1.18 ± 0.33*	0.66 ± 0.16
	0.2 mg/kg	24	1.34 ± 0.46*	0.55 ± 0.07
		48	1.59 ± 0.33*	0.38 ± 0.07**

* Significantly greater than the corresponding vehicle control, $p \leq 0.01$.
** Significantly less than the corresponding vehicle control, $p \leq 0.01$.
PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte

Confirmatory Micronucleus Assay

Similarly there was no mortality or adverse clinical sign observed.

Consistent with the initial study, SGD-1010 was cytotoxic to the bone marrow and induced increases in micronucleated PCEs (Table 39).

Table 39. Summary of Confirmatory Micronucleus Assay*

Treatment	Dose	Harvest Time	% Micronucleated PCEs Mean ± SD Male	Ratio PCE:NCE Mean ± SD Male
Controls				
Vehicle	HCl/Saline 5 mL/kg	24	0.16 ± 0.10	0.72 ± 0.12
Positive	CP 60 mg/kg	24	2.13 ± 0.33	0.46 ± 0.05
	CBZ 1250 mg/kg/day[a]		0.48 ± 0.39	0.19 ± 0.04
	CBZ 1500 mg/kg/day[a]		0.51 ± 0.46	0.43 ± 0.08
Test Article	0.1 mg/kg	24	1.23 ± 0.53	0.48 ± 0.15
	0.2 mg/kg	24	0.93 ± 0.46	0.23 ± 0.10

* CP: cyclophosphamide, CBZ: carbendazim

The centromere+ micronuclei were 16-28% in positive controls given cyclophosphamide (clastogenic) while 68% in positive controls given carbendazim (CBZ, aneugenic) and 60-76% in animals given SGD-1010. SGD-1010 predominantly induced the formation of micronuclei with centromere, which indicated an aneugenic mode of action.

7.4 Other Genetic Toxicity Studies

N/A

8 Carcinogenicity

Not conducted. No carcinogenicity study is needed for the proposed indications.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Not conducted. No FEED study is required for drugs treating patients with advanced cancers according to ICH S9.

9.2 Embryonic Fetal Development

EFD study was conducted in rats only and reviewed below.

Study title: Intravenous Injection Study for Effects on Embryofetal Developmental and Toxicokinetics with SGN-35 and SGD-1010 in Rats

Study no:	(b) (4) Study Number 8204397
Study report location:	M4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	09 September 2009
GLP compliance:	Yes*
QA statement:	Yes
Drug, lot #, and % purity:	SGN-35, lot #, SDD002, purity: 95.3% Monomer SGD-1010 (MMAE); lot #, 2002E, purity: 95.8% (HPLC)

* The stability testing for SGN-35 placebo and the characterization of SGD-1010 were performed by Seattle Genetics under non-GLP conditions.

Key Study Findings

SGN-35 and SGD-1010 had marked adverse effects on embryonic and/or fetal development. The followings were noted.

- Marked post-Implantation loss (99% or 100% in the 3 or 10 mg/kg SGN-35 groups, respectively, 27% in the 0.2 mg/kg SGD-1010 group)
- No viable fetuses in 22/24 or 25/25 dams given 3 or 10 mg/kg SGN-35, respectively, mainly due to early resorptions (99 or 100%, respectively); this effect was observed in 1/24 dams given 0.2 mg/kg SGD-1010
- Decreased fetal weight associated with SGN-35 treatment
- Early delivery in 1/24 females in the 0.2 mg/kg SGD-1010 group
- Higher preimplantation loss in SGN-35 and SGD-1010 groups (9-10%)
- Fetal external malformations in one fetus (umbilical hernia and malrotated hindlimbs) in the 3 mg/kg SGN-35 group and 3 fetuses (in 3 separate litters, protruding tongue, malrotated hindlimbs, gastroschisis, and agnathia) in the 0.2 mg/kg SGD-1010 group
- Fetal soft tissue malformations in 0.3/kg (situs inversus in one fetus) and 1 mg/kg (reduced testis size in one fetus) SGN-35 and 0.2 mg/kg SGD-1010 (situs inversus in one fetus) groups (none in the historical control data)
- Fetal skeletal malformations in two fetuses (in 2 separate litters) in the 0.2 mg/kg SGD-1010 group only, including malformed mandible, misaligned, fused and/or absent caudal vertebrae, split sternebrae, and shortened long bone (only shortened long bone recorded in the historical control data).
- SGN-35 and SGD-1010 were transferred across the placenta.

Methods

Doses:	Table 40
Frequency of dosing:	Twice: once each on GD 6 and GD 13*
Dose volume:	5 mL/kg
Route of administration:	Intravenous injection
Formulation/Vehicle:	SGN-35 Placebo (lyophilized formulation, excipients were composed of 20 mM citrate, 63 mg/mL trehalose, 0.2 mg/mL Tween 80, pH 6.6); SGN-35 and SGN-35 placebo were reconstituted with sterile water for injection; The diluent for SGN-35 was 0.9% sodium chloride for injection; The diluent for SGD-1010 was 3.9% 0.01N hydrochloric acid/96.1% 0.9% sodium chloride for injection (HCl/saline).
Species/Strain:	Rat/Crl:CD(SD)
Number/Sex/Group:	Table 40
Satellite groups:	Table 40
Study design:	Table 40
Deviation from study protocol:	Recorded in the submissions

* The day of confirmation was designated as GD 0 and the females were received prior to GD 4.

Table 40. Summary of Study Design*

Group[a]	No. of Animals Mated Female	Dose Level (mg/kg/dose)	Dose Concentration[b] (mg/mL)
Main Study Animals			
1 (Control)[a]	25	0	0
2 (Low – SGN-35)	25	0.3	0.06
3 (Mid-Low – SGN-35)	25	1	0.2
4 (Mid-High – SGN-35)	25	3	0.6
5 (High – SGN-35)	25	10	2.0
6 (High – SGD-1010)	25	0.2	0.04
Toxicokinetic Animals			
7 (Control)[a]	9	0	0
8 (Low – SGN-35)[b]	9	0.3	0.06
9 (Mid-Low – SGN-35)[b]	9	1	0.2
10 (Mid-High – SGN-35)[b]	9	3	0.6
11 (High – SGN-35)[b]	9	10	2.0
12 (High – SGD-1010)	9	0.2	0.04

[a] Groups 1 and 7 received SGN-35 placebo.
[b] Concentrations were based on the test articles as supplied.
* The molar dose of SGD-1010 is equivalent in groups 5 and 6; According to the study report, the first 10 animals dosed in Group 6 (on 14 or 15 September 2009) were removed from study, sacrificed, and discarded without necropsy due to the dose concentration error (~230% of the target concentration). These animals were replaced.

Observations and Results

Mortality

SGN-35 Groups
All animals survived until scheduled sacrifice.

SGD-1010 Group
All animals survived until scheduled sacrifice except one dam (B57447), who delivered on GD 21 and was sacrificed.

Clinical Signs

SGN-35 Groups
SGN-35-related red/black vaginal discharge was noted in dams in the 3 and 10 mg/kg/dose groups.

SGD-1010 Group
SGD-1010-related paleness (both ears and entire body), red/black vaginal discharge, and red fluid in cage pan were noted.

Body Weight

The body weight data are summarized in Table 41 to Table 43. SGN-35 dose-related decreases in maternal body weights were noted as early as on GD 8 and progressed through GD 21. On GD 21, there were 25.0 or 26.4 % decreases in the 3 or 10 mg /kg SGN-35 groups, respectively, when compared to that in controls. This decrease was, at least partly, attributed to a decrease in uterine weights (e.g. increased postimplantation loss), as further shown by significant reductions in gravid uterine weights at 3 or 10 mg/kg SGN-35 (reduced 97-98%; see Table 43).

Of interest, SGD-1010-related decrease in maternal body weights was also noted as early as on GD 8, but body weights were recovered through GD 21.

Table 41. Mean Maternal Body Weights During Gestation (g)*

	DOSE LEVEL	GROUP 1 0 MG/KG/DOSE	GROUP 2 0.3 MG/KG/DOSE	GROUP 3 1 MG/KG/DOSE	GROUP 4 3 MG/KG/DOSE	GROUP 5 10 MG/KG/DOSE	GROUP 6 0.2 MG/KG/DOSI
DAY 0	MEAN	211.0	211.4	211.1	212.2	210.8	209.3
	S.D.	11.5	11.3	10.6	13.2	10.4	13.7
	N	25	25	23	24	25	24
DAY 4	MEAN	230.6	231.9	230.7	231.1	229.6	229.1
	S.D.	14.6	16.0	14.4	14.3	12.3	16.9
	N	25	25	23	24	25	24
DAY 6	MEAN	247.0	249.0	248.3	246.0	245.2	243.8
	S.D.	13.4	19.1	14.0	16.5	14.6	17.0
	N	25	25	23	24	25	24
DAY 8	MEAN	254.5	258.2	256.8	252.0	247.5	247.6
	S.D.	14.0	20.0	14.4	14.5	14.1	17.1
	N	25	25	23	24	25	24
DAY 10	MEAN	269.4	273.7	273.7	265.4	260.6	250.2
	S.D.	12.3	21.0	16.2	15.7	17.8	18.3
	N	25	25	23	24	25	24
DAY 12	MEAN	284.6	290.3	289.3	276.9	271.2*1	264.1
	S.D.	11.7	23.5	15.7	15.9	19.2	18.6
	N	25	25	23	24	25	24
DAY 14	MEAN	298.4	304.8	305.8	282.0&1	274.0&1	273.0
	S.D.	12.4	22.3	15.3	16.3	18.0	19.7
	N	25	25	23	24	25	24
DAY 16	MEAN	320.6	327.4	325.7	285.7&1	277.0&1	294.3&5
	S.D.	13.2	23.6	15.3	18.7	20.2	22.9
	N	25	25	23	24	25	24
DAY 18	MEAN	351.2	357.4	357.0	284.9&1	277.4&1	317.1&5
	S.D.	16.5	27.2	16.7	21.3	21.3	28.9
	N	25	25	23	24	25	24
DAY 21	MEAN	379.4	386.3	388.2	284.6&1	279.2&1	350.4&5
	S.D.	15.5	34.2	21.8	23.0	22.3	38.6
	N	25	25	23	24	25	23

* GD0 body weights were provided by the supplier
*1 or &1: significantly different from group 1 with P ≤ 0.05 or P≤ 0.01, respectively
*5 or &5: significantly different from group 5 with P ≤ 0.05 or P≤ 0.01, respectively

Table 42. Mean Maternal Body Weight Changes During Gestation (g)

	DOSE LEVEL	GROUP 1 0 MG/KG/DOSE	GROUP 2 0.3 MG/KG/DOSE	GROUP 3 1 MG/KG/DOSE	GROUP 4 3 MG/KG/DOSE	GROUP 5 10 MG/KG/DOSE	GROUP 6 0.2 MG/KG/DOSE
DAYS 4 TO 6	MEAN	16.4	17.1	17.5	14.9	15.6	14.7
	S.D.	4.8	6.4	4.8	6.5	6.0	6.3
	N	25	25	23	24	25	24
DAYS 6 TO 8	MEAN	7.5	9.2	8.5	6.0	2.2&1	3.8
	S.D.	4.6	4.7	3.5	4.2	4.4	8.2
	N	25	25	23	24	25	24
DAYS 8 TO 10	MEAN	14.9	15.5	16.9	13.4	13.1	2.6&5
	S.D.	5.6	6.3	6.0	6.4	6.8	9.9
	N	25	25	23	24	25	24
DAYS 10 TO 12	MEAN	15.2	16.6	15.7	11.5	10.7*1	13.9*5
	S.D.	6.1	5.5	4.8	6.1	5.7	3.9
	N	25	25	23	24	25	24
DAYS 12 TO 14	MEAN	13.8	14.5	16.4	5.2&1	2.8&1	8.9&5
	S.D.	6.0	5.8	5.1	8.1	5.5	5.8
	N	25	25	23	24	25	24
DAYS 14 TO 16	MEAN	22.2	22.6	20.0	3.7&1	3.0&1	21.3&5
	S.D.	3.3	5.1	5.4	8.2	7.5	7.9
	N	25	25	23	24	25	24
DAYS 16 TO 18	MEAN	30.5	30.0	31.3	-0.8&1	0.3&1	22.8&5
	S.D.	7.0	5.4	5.2	11.0	6.4	9.0
	N	25	25	23	24	25	24
DAYS 18 TO 21	MEAN	28.3	28.9	31.1	-0.2&1	1.9&1	33.3&5
	S.D.	11.9	13.2	11.8	7.0	8.0	13.4
	N	25	25	23	24	25	23

*1 or &1: significantly different from group 1 with P ≤ 0.05 or P≤ 0.01, respectively
*5 or &5: significantly different from group 5 with P ≤ 0.05 or P≤ 0.01, respectively

Table 43. Summary of Uterine and Net Body Weights

	DOSE LEVEL	GROUP 1 0 MG/KG/DOSE	GROUP 2 0.3 MG/KG/DOSE	GROUP 3 1 MG/KG/DOSE	GROUP 4 3 MG/KG/DOSE	GROUP 5 10 MG/KG/DOSE	GROUP 6 0.2 MG/KG/DOSE
GRAVID UTERUS	MEAN	97.73	97.75	99.48	2.61&1	1.84&1	74.20&5
	S.D.	11.93	13.28	9.91	2.26	0.66	27.40
	N	25	25	23	24	25	24
CORRECTED WEIGHT	MEAN	281.71	288.53	288.69	282.01	277.40	273.20
	S.D.	17.90	31.60	22.19	21.57	21.97	28.85
	N	25	25	23	24	25	23
NET CHANGE FROM DAY 4	MEAN	51.15	56.61	57.95	50.89	47.76	43.94
	S.D.	17.97	23.03	20.42	15.22	17.37	20.11
	N	25	25	23	24	25	23

*1 or &1: significantly different from group 1 with P ≤ 0.05 or P≤ 0.01, respectively
*5 or &5: significantly different from group 5 with P ≤ 0.05 or P≤ 0.01, respectively
Corrected weight = terminal body weight minus gravid uterine weight
Net weight change from Day 4 = corrected weight minus Day 4 body weight

Feed Consumption

Feed consumption was recorded at GDs 4, 6, 8, 10, 12, 14, 16, 18, and 21. SGN-35- or SGD-1010-related decreases in feed consumption were noted (11%, 17%, or 12% decrease from GDs 6 to 21 in 3 or 10 mg /kg SGN-35 or 0.2 mg /kg SGD-1010 groups, respectively, when compared with that in controls). There was no remarkable change in feed consumption in 0.3 or 1 mg /kg SGN-35 groups.

Toxicokinetics

Blood samples were collected from toxicokinetic animals via a jugular vein prior to, at 5 minutes (±1 minute), and 24 hours (± 30 minutes) postdose on GDs 6 and 13 (3 animals/time point). In addition, blood samples were collected from all surviving animals on GD 18 prior to pregnancy status determination. SGN-35 analysis (TAb or ADC) was conducted by Seattle Genetics, Inc. and SGD-1010 by (b) (4). The lower limits of quantitation were 0.0122 mcg/mL or 0.0100 ng/mL for SGN-35 or SGD-1010, respectively.

Amniotic fluid was collected from all pregnant toxicokinetic animals on GD 18. Maximum amniotic fluid was collected from the amniotic sac of each live fetus, and samples were pooled per litter.

Fetuses were removed from the uteri of all pregnant toxicokinetic animals on GD 18. Maximum fetal blood samples were collected from each fetus via cardiac puncture, and samples were pooled per litter.

Control group

All maternal serum, fetal serum, and amniotic fluid concentrations of SGN-35 and TAb in the control group were below the lower limit of quantitation (BLQ).

Maternal serum

SGN-35 groups

In the SGN-35 groups, concentrations of SGN-35 (ADC) and SGD-1010 (free drug) in maternal serum were measured. Toxicokinetic parameters are summarized in Table 44 (SGN-35) and Table 45 (SGD-1010). C_{max} and $AUC_{0\text{-}1d}$ for SGN-35 and SGD-1010 increased in an approximate dose proportional manner from 0.3 to 10 mg/kg. No accumulation of SGN-35 or SGD-1010 was observed.

Table 44. Mean TK Parameters for SGN-35 (Maternal serum, SGN-35 groups)*

Dose Group	SGN-35 Dose Level (mg/kg/dose)	N[a]	Gestational Day	C_{max} (µg/mL)	DN C_{max} (µg/mL)/(mg/kg/dose)	T_{max} (day)	$AUC_{0\text{-}last}$ (µg·day/mL)	$AUC_{0\text{-}1d}$ (µg·day/mL)	DN $AUC_{0\text{-}1d}$ (µg·day/mL)/(mg/kg/dose)
8[b]	0.3	9	6	6.80	22.7	0.00347	8.55	4.06	13.5
			13	4.96	16.5	0.00347	5.82	3.10	10.3
8[c]	0.3	8	6	6.80	22.7	0.00347	8.55	4.06	13.5
			13	4.96	16.5	0.00347	6.01	3.13	10.4
9	1	9	6	21.0	21.0	0.00347	30.4	13.1	13.1
			13	17.2	17.2	0.00347	22.7	11.2	11.2
10	3	9	6	50.8	16.9	0.00347	83.3	32.8	10.9
			13	53.6	17.9	0.00347	80.2	35.8	11.9
11	10	9	6	283	28.3	0.00347	446	181	18.1
			13	238	23.8	0.00347	405	167	16.7

* DN Cmax: Dose normalized Cmax calculated as Cmax/Dose level
DN $AUC_{0\text{-}1d}$: Dose normalized $AUC_{0\text{-}1d}$ calculated as $AUC_{0\text{-}1d}$/Dose level
[a] Total number of animals. Blood was collected from 3 animals/time point.
[b] All animals were included in TK analysis.
[c] One animal (B57487) was excluded from TK analysis due to positive ADA.

Table 45. Mean TK Parameters for SGD-1010 (Maternal serum, SGN-35 groups)*

Dose Group	SGN-35 Dose Level (mg/kg/dose)	N[a]	Gestational Day	C_{max} (ng/mL)	DN C_{max} (ng/mL)/(mg/kg/dose)	T_{max} (Day)	$AUC_{0\text{-}last}$ (ng·day/mL)	$AUC_{0\text{-}1d}$ (ng·day/mL)	DN $AUC_{0\text{-}1d}$ (ng·day/mL)/(mg/kg/dose)
8[b]	0.3	9	6	0.0115	0.0383	1.00	NR	NR	NA
			13	0.0140	0.0467	1.00	NR	NR	NA
8[c]	0.3	8	6	0.0111	0.0370	1.00	NR	NR	NA
			13	0.0152	0.0507	1.00	NR	NR	NA
9	1	9	6	0.0410	0.0410	1.00	0.0295	0.0295	0.0295
			13	0.0544	0.0544	1.00	0.149	0.0374	0.0374
10	3	9	6	0.138	0.0460	1.00	0.517	0.0901	0.0300
			13	0.154	0.0513	1.00	0.469	0.105	0.0350
11	10	9	6	0.463	0.0463	1.00	1.89	0.317	0.0317
			13	0.708	0.0708	1.00	2.14	0.442	0.0442

* DN Cmax: Dose normalized Cmax calculated as Cmax/Dose level
DN $AUC_{0\text{-}1d}$: Dose normalized $AUC_{0\text{-}1d}$ calculated as $AUC_{0\text{-}1d}$/Dose level
Note: AUC values in Group 8 were not estimated because there were only single mean measurable concentration values after each dose.
NR: Not reportable when there was no agreement (within 20%) among analyses
NA: Not applicable
[a] Total number of animals. Blood was collected from 3 animals/time point.
[b] All animals were included in TK analysis.
[c] One animal (B57487) was excluded from TK analysis due to positive ADA.

SGD-1010 group

In the SGD-1010 group, the concentration of SGD-1010 (free drug) in maternal serum was measured. Toxicokinetic parameters are summarized in Table 46. Serum concentrations of SGD-1010 on GD13 were approximately 60% higher than those on GD 6.

Table 46. Mean TK Parameters for SGD-1010 (Maternal Serum, SGD-1010 group)

Dose Group	SGD-1010 Dose Level (mg/kg/dose)	N[a]	Gestational Day	C_{max} (ng/mL)	DN C_{max} (ng/mL)/(mg/kg/dose)	T_{max} (Day)	$AUC_{0\text{-last}}$ (ng·day/mL)	$AUC_{0\text{-1d}}$ (ng·day/mL)	DN $AUC_{0\text{-1d}}$ (ng·day/mL)/(mg/kg/dose)
12	0.2	9	6	29.7	148	0.00347	16.2	16.2	80.9
			13	50.2	251	0.00347	25.6	25.6	128

* DN Cmax: Dose normalized Cmax calculated as Cmax/Dose level
DN $AUC_{0\text{-1d}}$: Dose normalized $AUC_{0\text{-1d}}$ calculated as $AUC_{0\text{-1d}}$/Dose level
[a] Total number of animals (3 animals/time point)

Reviewer's comments:

Although the molar doses of SGD-1010 in 0.2 mg/kg SGD-1010 and 10 mg/kg SGN-35 groups are equivalent, concentration profiles of the free SGD-1010 were different (Figure 20, Table 45, Table 46, Table 47 and Table 48), which may at least in part, contribute to the different pregnancy outcomes in the two groups. SGD-1010 concentrations peaked at 5 min postdose then rapidly decreased within 24 hours in 0.2 mg/kg SGD-1010 group while were detectable at 5 min postdose but increased within 24 hours in 10 mg/kg SGN-35 group. SGD-1010 concentrations were higher at earlier time points (5 min and 24h postdose) after the first dose in 0.2 mg/kg SGD-1010 group than those in 10 mg /kg SGN-35 group; the concentration was below the limit of quantification on Day 5 postdose in 0.2 mg/kg SGD-1010 group while remained detectable on Day 7 in 10 mg /kg SGN-35 group. The results indicated that animals in 10 mg/kg SGN-35 group were continuously exposed to the free SGD-1010 through the organogenesis period while animals in 0.2 mg/kg SGD-1010 group were exposed in a shorter pulse period.

Figure 20. Concentrations (Mean±SD ng/mL) of SGD-1010 in maternal serum following administration of SGN-35 (Groups 8-10) or SGD-1010 (Group 12) on GDs 6 and 13

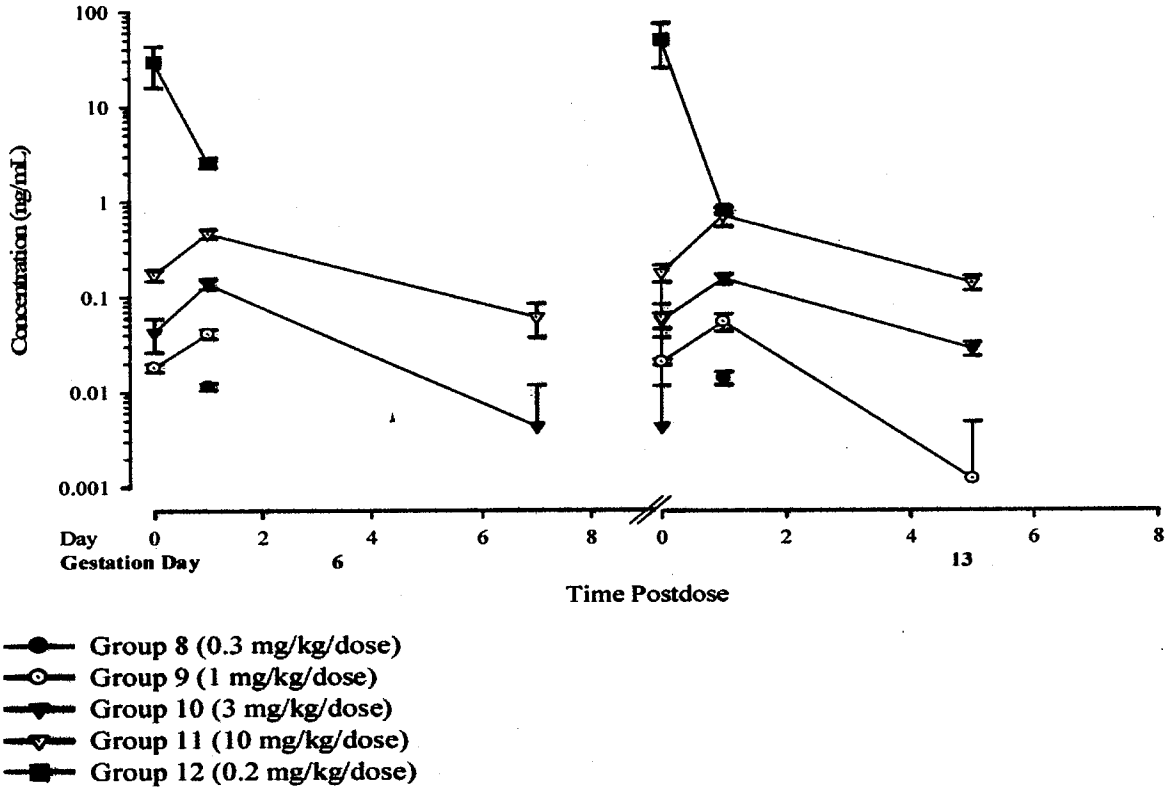


Table 47. Individual and Mean Concentrations (ng/mL) of SGD-1010 in Maternal Serum Following SGN-35 Administration

Dose Group	SGN-35 Dose Level (mg/kg/dose)	Gestational Day	Animal Number	Nominal Time Postdose (Day) 0	0.0034722	1	5[b]	7[a]
11	10	6	B57506	BLQ<(0.0100)				0.0529
			B57507	BLQ<(0.0100)				0.0869
			B57508	BLQ<(0.0100)				0.0420
			B57509		0.200			
			B57510		0.158			
			B57511		0.159			
			B57512			0.494		
			B57513			0.491		
			B57514			0.405		
			Mean	0	0.172	0.463		0.0606
			SD	0	0.024	0.051		0.0234
			CV%	NA	14	11		39
		13	B57506	0.0529			0.146	
			B57507	0.0869			0.186	
			B57508	0.0420			0.134	
			B57509		0.212		0.116	
			B57510		0.184		0.121	
			B57511		0.140		0.121	
			B57512			0.578	0.133	
			B57513			0.650	0.166	
			B57514			0.897	0.124	
			Mean	0.0606	0.179	0.708	0.139	
			SD	0.0234	0.036	0.167	0.024	
			CV%	39	20	24	17	

Notes: Values below the lower limit of quantitation (BLQ<(0.0100) ng/mL) were treated as zeros in descriptive statistics and toxicokinetic analysis.
[a] 7 days postdose on GD 6 (equivalent to predose on GD 13)
[b] 5 days postdose on GD 13 (equivalent to the time prior to pregnancy determination on GD 18)
NA: Not applicable
NR: Not reportable when there was no agreement (within 20%) among analyses

Table 48. Individual and Mean Concentrations (ng/mL) of SGD-1010 in Maternal Serum Following SGD-1010 Administration

Dose Group	SGD-1010 Dose Level (mg/kg/dose)	Gestational Day	Animal Number	Nominal Time Postdose (Day) 0	0.0034722	1	5[b]	7[a]
12	0.2	6	B57515	BLQ<(0.0100)				BLQ<(0.0100)
			B57516	BLQ<(0.0100)				BLQ<(0.0100)
			B57517	BLQ<(0.0100)				BLQ<(0.0100)
			B57518		27.7			
			B57519		17.1			
			B57520		44.2			
			B57521			2.95		
			B57522			2.33		
			B57523			2.49		
			Mean	0	29.7	2.59		0
			SD	0	13.7	0.32		0
			CV%	NA	46	12		NA
		13	B57515	BLQ<(0.0100)			BLQ<(0.0100)	
			B57516	BLQ<(0.0100)			BLQ<(0.0100)	
			B57517	BLQ<(0.0100)			BLQ<(0.0100)	
			B57518		41.5		BLQ<(0.0100)	
			B57519		30.9		BLQ<(0.0100)	
			B57520		78.1		BLQ<(0.0100)	
			B57521			0.755	BLQ<(0.0100)	
			B57522			0.860	BLQ<(0.0100)	
			B57523			0.783	BLQ<(0.0100)	
			Mean	0	50.2	0.799	0	
			SD	0	24.8	0.054	0	
			CV%	NA	49	7	NA	

Notes: Values below the lower limit of quantitation (BLQ<(0.0100) ng/mL) were treated as zeros in descriptive statistics and toxicokinetic analysis. BLQ values at predose were excluded from analysis to allow for back extrapolation.
a 7 days postdose on GD 6 is equivalent to predose on GD 13.
b 5 days postdose on GD 13 is equivalent to the time prior to pregnancy determination on GD 18.
NA Not applicable.

Placenta transfer

SGN-35 groups

Concentrations of SGN-35 and SGD-1010 in maternal serum, fetal serum and amniotic fluid were measured in SGN-35 groups on GD 18. The results indicated that SGN-35 and SGD-1010 were transferred across the placenta. SGN-35 concentrations in fetal serum and amniotic fluid were lower than those in maternal serum at respective dose levels on GD 18 (Table 49). For example, in the 1 mg /kg SGN-35 group, mean SGN-35 concentration in fetal serum was 0.100 (0.0505 to 0.145) micg/mL while the concentration in maternal serum was 0.574 micg/mL (0.425 to 0.704).

Table 49. Ratio of SGN-35 Concentrations in Amniotic Fluid and Fetal Serum to Maternal Serum on GD 18

Dose Group	SGN-35 Dose Level (mg/kg/dose)	Animal Number	Ratio Amniotic Fluid:Maternal Serum	Ratio Fetal Serum:Maternal Serum
8	0.3	B57479	NA	0.246
		B57480	NA	0.293
		B57481	NA	0.207
		B57482	NA	0.262
		B57483	NA	0.332
		B57484	NA	0.135
		B57485	NA	0.183
		B57486	NA	0.111
		B57487	NA	NA
9	1	B57488	NA	0.0723
		B57489	NA	0.254
		B57490	NA	0.176
		B57491	NA	0.184
		B57492	0.0252	0.144
		B57493	NA	0.201
		B57494	NA	0.185
		B57495	0.0193	0.206
		B57496	NA	0.174
10	3	B57500	0.0199	NA
		B57504	0.00899	NA

NA: Not applicable, the observed concentrations were below the lower limit of quantitation.

In contrast, SGD-1010 concentrations in amniotic fluid and fetal serum were higher than those in maternal serum at respective dose levels on GD 18 (Table 50). For example, in the 1 mg /kg SGN-35 group, SGD-1010 concentrations were below the limit of quantitation (<0.0122 mcg/mL) in majority of maternal serum while mean concentrations were 0.205 or 0.188 ng/mL in amniotic fluid and fetal serum, respectively.

Table 50. SGD-1010 Concentrations in Maternal Serum, Amniotic Fluid and Fetal Serum on GD 18

SGN-35 (mg/kg)	Maternal Serum (ng/mL) Mean* (range)	Amniotic Fluid (ng/mL) Mean (range)	Fetal Serum (ng/mL) Mean (range)
0.3	<0.0100 (BLQ)	0.0189 (0.0131 to 0.0323)[a]	0.0376 (0.0221 to 0.0534)
1	0.0108 in B57489[b]	0.205 (0.0247 to 0.540)	0.188 (0.141 to 0.233)
3	0.0280 (0.0226 to 0.0357)	1.16 (0.155 or 2.16)[c]	-

Prepared by the reviewer based on the data submitted
* n=9, [a] BLQ in one animal, [#b] BLQ in all other 8 animals, [c] only two samples available, - no sample available

SGD-1010 group

Concentrations of SGD-1010 in maternal serum, fetal serum and amniotic fluid were measured in the SGD-1010 group on GD 18. SGD-1010 concentrations in amniotic fluid and fetal serum were higher than those in maternal serum (Table 51), indicating that SGD-1010 was highly transferrable from maternal serum to fetus. For example, SGD-1010 concentrations in maternal serum were below the limit of quantitation in all animals administered 0.2 mg/kg SGD-1010 while the concentrations in fetal serum ranged from 0.0132 to 0.0180 ng/mL and ranged from 0.0151 to 0.0205 ng/mL in amniotic fluid.

Table 51. Ratio of SGD-1010 Concentration in Amniotic Fluid and Fetal Serum to Maternal Serum on GD 18

Dose Group	SGD-1010 Dose Level (mg/kg/dose)	Animal Number	Ratio Amniotic Fluid:Maternal Serum	Ratio Fetal Serum:Maternal Serum
12	0.2	B57515	NA	NA
		B57516	NA	0.0137:BLQ
		B57517	NA	0.0180:BLQ
		B57518	0.0151:BLQ	0.0141:BLQ
		B57519	0.0205:BLQ	NA
		B57520	NA	NA
		B57521	0.0192:BLQ	0.0132:BLQ
		B57522	NA	NA
		B57523	NA	NA

NA Not applicable.

Immunogenicity

Blood samples were collected from all toxicokinetic animals prior to dosing on GD 6 (baseline) and prior to GD 18.

Immunogenicity was noted in one female administered 0.3 mg/kg SGN-35 on GD18, which had no significant impact on toxicokinetic parameters (Table 44).

Stability and Homogeneity

Stability

The SGD-1010 preparations from 0.002 to 0.06 mg/mL were confirmed stable for 4 days at room temperature under (b) (4) Study No. 8204151.

The SGN-35 preparations from 0.1 to 2.0 mg/mL were confirmed stable for 7 hours at room temperature under (b) (4) Study No. 7646-118 and the SGN-35 preparations from 0.0300 and 0.1 mg/mL for 4 hours under (b) (4) Study No. 8201470.

Concentration Verification

Duplicate samples were taken from all SGN-35 dose preparations. These samples were stored protected from light under refrigeration and analyzed on the day of preparation. There were four preparations having concentrations below 10% of target concentrations including one at 0.2 mg/mL on GD6 (88.5 %, for 1 mg/kg group) and three at 0.06 mg/mL on GD13 (84.3, 84.9 or 89.0%, for 0.3 mg/kg group). All others were within 10% of target concentrations.

Duplicate samples were taken from each preparation for SGD-1010. These samples were stored at room temperature and analyzed within 4 days of preparation. One preparation had a concentration far above the target concentration (230 %). As previously indicated the animals administered this preparation were sacrificed and replaced. Other 2 preparations were within ± 10% of target concentrations.

Hematology

Blood samples were collected from all surviving main study animals on GD 20; some parameters are summarized in Table 52. Both SGN-35 and SGD-1010 had effects on hematology parameters which were similar to what was seen in the general toxicology studies.

Table 52. Summary of Hematology Data during Gestation

Group/ Sex		RBC E6/uL	HGB g/dL	HCT %	PRET %	RETI E3/uL	PLT E3/uL	WBC E3/uL	NEUT E3/uL
1F	Mean	6.29	12.2	34.7	2.8	174.7	1256	10.74	3.83
	SD	0.325	0.55	1.59	0.80	51.04	193.6	1.988	0.562
	N	25	25	25	25	25	25	25	25
2F	Mean	6.27	12.2	34.8	2.8	174.5	1170	9.87	3.68
	SD	0.326	0.50	1.43	1.00	57.11	192.2	1.888	0.674
	N	25	25	25	25	25	25	25	25
3F	Mean	6.34	12.4	35.2	2.4	148.6	1147	11.10	3.58
	SD	0.653	1.19	3.72	0.81	49.33	215.0	1.921	1.078
	N	25	25	25	25	25	25	25	25
4F	Mean	7.44&1	14.6&1	41.8&1	2.9	197.7	1079&1	12.16*1	2.02&1
	SD	0.831	1.53	4.30	2.76	114.53	182.2	2.762	1.036
	N	25	25	25	25	25	25	25	25
5F	Mean	6.05	12.5	36.3&1	6.6&1	383.2&1	993&1	9.09&1	0.89&1
	SD	0.758	1.44	4.17	2.93	120.45	312.3	1.823	0.287
	N	25	25	25	25	25	25	25	25
6F	Mean	4.07&5	8.4&5	24.5&5	13.9&5	555.9*5	1152	8.68	2.27&5
	SD	0.983	1.96	5.35	3.60	124.81	332.6	1.873	0.813
	N	25	25	25	24	24	25	25	25

*1 or &1: significantly different from group 1 with P ≤ 0.05 or P≤ 0.01, respectively
*5 or &5: significantly different from group 5 with P ≤ 0.05 or P≤ 0.01, respectively

Necropsy

Large spleen was noted in 2/25 animals in Group 6 (0.2 mg/kg SGD-1010 group).

Marked SGN-35-related decreases in gravid uterus weights (Table 43) were present in the 3 or 10 mg/kg groups and correlated with the reduced live fetuses.

There were no other macroscopic observations (other than the cesarean section observations described below) noted in the females given SGN-35.

Some microscopic findings related to target organs/tissues are summarized in Table 53.

Table 53. Summary of Microscopic Observations

Controls from group(s): 1	Animal sex:	-- Animals Affected -- Females --					
Tissues With Diagnoses	Dosage group: / No. in group:	Ctls 25	2 25	3 25	4 25	5 25	6 25
Spleen	Number examined:	3	3	3	3	3	5
	Unremarkable:	2	2	3	2	0	0
Hematopoiesis, Extramedullary, Increased		1	1	0	0	0	5
Hematopoiesis, Extramedullary, Decreased		0	0	0	1	3	0
Thymus	Number examined:	3	3	3	3	3	5
	Unremarkable:	3	0	3	3	1	4
Depletion, Lymphocytes		0	0	0	0	2	1
Tingible-Body Macrophages, Increased		0	3	0	0	0	0
Uterus	Number examined:	3	3	3	3	3	5
	Unremarkable:	0	0	0	0	0	0
Placentation Site, Unremarkable		3	3	3	0	0	5
Placentation Site, Involution/Resorption		0	0	0	3	3	0
Vagina	Number examined:	3	3	3	3	3	5
	Unremarkable:	0	0	0	0	0	0
Mucification, Pregnancy-Associated		3	2	3	0	0	5
Proestrus		0	1	0	1	0	0
Estrus		0	0	0	1	0	0
Metestrus		0	0	0	1	3	0
Marrow, Sternum	Number examined:	3	3	3	3	3	5
	Unremarkable:	3	3	3	3	2	3
Hyperplasia, Erythroid		0	0	0	0	1	0
Hypercellular		0	0	0	0	0	2

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Cesarean section data are summarized in Table 54 and Table 55.

SGN-35 and SGD-1010 had marked effects on pregnancy outcomes including the followings.

- Marked post-Implantation loss (99.4% or 100% in the 3 or 10 mg/kg SGN-35 groups, respectively, 27.4 % in the 0.2 mg/kg SGD-1010 group)
- No viable fetuses in 22/24 or 25/25 dams given 3 or 10 mg/kg SGN-35, respectively, mainly due to early resorptions (99 or 100%, respectively); this effect was observed in 1/24 dams given 0.2 mg/kg SGD-1010
- Decreased fetal weight in the 3 mg/kg SGN-35 group (Table 56); this effect could not be assessed in the 10 mg/kg SGN-35 due to the 100% post-implantation loss
- Early delivery in 1/24 females in the 0.2 mg/kg SGD-1010 group

Table 54. Summary of Cesarean Section Data

DOSE LEVEL		GROUP 1 0 MG/KG/DOSE	GROUP 2 0.3 MG/KG/DOSE	GROUP 3 1 MG/KG/DOSE	GROUP 4 3 MG/KG/DOSE	GROUP 5 10 MG/KG/DOSE	GROUP 6 0.2 MG/KG/DOSE
Females Mated	N	25	25	25	25	25	25
Pregnant	N	25	25	23	24	25	24
	%	100	100	92	96	100	96
Aborted	N	0	0	0	0	0	0
	%	0.0	0.0	0.0	0.0	0.0	0.0
Died	N	0	0	0	0	0	0
	%	0.0	0.0	0.0	0.0	0.0	0.0
Delivered Early	N	0	0	0	0	0	1
	%	0.0	0.0	0.0	0.0	0.0	4.0
Pregnant at C-section	N	25	25	23	24	25	24
Dams with Viable Fetuses	N	25	25	23	2	0	23
	%	100	100	100	8.3	0.0	96
Dams with no Viable Fetuses	N	0	0	0	22	25	1
	%	0.0	0.0	0.0	92	100	4.2
Corpora Lutea	MEAN	13.8	14.5	14.4	15.1	15.2	14.2
	S.D.	2.2	2.5	2.2	3.1	2.9	2.2
	N	25	25	23	24	25	24
	TOTAL	346	362	331	362	381	342
Implantation Sites	MEAN	12.8	13.0	13.4	13.4	13.6	12.8
	S.D.	1.8	1.7	1.8	2.5	1.8	1.7
	N	25	25	23	24	25	24
	TOTAL	320	324	308	322	339	307
Preimplantation Loss	MEAN%	6.8	9.4	6.3	9.4	9.7	9.3
	S.D.	8.6	10.3	8.4	13.6	9.9	10.3

Resorptions: Total	MEAN	0.1	0.2	0.2	13.3	13.6	3.6
	S.D.	0.3	0.8	0.4	2.5	1.8	4.2
	N	25	25	23	24	25	24
	TOTAL	3	4	4	320	339	87
	MEAN%	1.0	1.5	1.2	99.4&1	100.0&1	27.4&5
	S.D.	2.9	7.3	2.7	2.2	0.0	31.6
Early	MEAN	0.1	0.2	0.2	13.3	13.6	3.0
	S.D.	0.3	0.8	0.4	2.6	1.8	3.6
	N	25	25	23	24	25	24
	TOTAL	3	4	4	319	339	72
	MEAN%	1.0	1.5	1.2	99.0&1	100.0&1	22.2&5
	S.D.	2.9	7.3	2.7	2.7	0.0	26.1
Late	MEAN	0.0	0.0	0.0	0.0	0.0	0.6
	S.D.	0.0	0.0	0.0	0.2	0.0	1.9
	N	25	25	23	24	25	24
	TOTAL	0	0	0	1	0	15
	MEAN%	0.0	0.0	0.0	0.3	0.0	5.2&5
	S.D.	0.0	0.0	0.0	1.7	0.0	16.8
Dead Fetuses	TOTAL	0	0	0	0	0	0
Postimplantation Loss	MEAN%	1.0	1.5	1.2	99.4&1	100.0&1	27.4&5
	S.D.	2.9	7.3	2.7	2.2	0.0	31.6

*1 or &1: significantly different from group 1 with P ≤ 0.05 or P≤ 0.01, respectively
*5 or &5: significantly different from group 5 with P ≤ 0.05 or P≤ 0.01, respectively

Table 55. Summary of Cesarean Section Data (Fetuses)

DOSE LEVEL		GROUP 1 0 MG/KG/DOSE	GROUP 2 0.3 MG/KG/DOSE	GROUP 3 1 MG/KG/DOSE	GROUP 4 3 MG/KG/DOSE	GROUP 5 10 MG/KG/DOSE	GROUP 6 0.2 MG/KG/DOSE
Pregnant at C-section	N	25	25	23	24	25	24
Live Fetuses	MEAN	12.7	12.8	13.2	0.1	0.0	9.2
	S.D.	1.9	2.0	1.7	0.3	0.0	3.8
	N	25	25	23	24	25	24
	TOTAL	317	320	304	2	0	220
	MEAN%	99.0	98.5	98.8	0.6&1	0.0&1	72.6&5
	S.D.	2.9	7.3	2.7	2.2	0.0	31.6
Females	MEAN	6.0	6.2	6.9	1.0		5.2
	S.D.	1.4	1.8	2.1	0.0		2.6
	N	25	25	23	2		23
	TOTAL	151	154	158	2		120
	MEAN%	47.8	48.4	51.8	100.0&1		50.0[a]
	S.D.	10.5	12.9	13.6	0.0		24.2
Males	MEAN	6.6	6.6	6.3	0.0		4.3
	S.D.	1.7	2.1	1.9	0.0		1.7
	N	25	25	23	2		23
	TOTAL	166	166	146	0		100
	MEAN%	52.2	51.6	48.2	0.0&1		50.0[a]
	S.D.	10.5	12.9	13.6	0.0		24.2
Sex Ratio M:F		52:48	52:48	48:52	0:100		45:55
Dams with Viable Fetuses	N	25	25	23	2	0	23
Resorptions: Total	MEAN	0.1	0.2	0.2	12.0		3.3
	S.D.	0.3	0.8	0.4	0.0		3.9
	N	25	25	23	2		23
	TOTAL	3	4	4	24		76
	MEAN%	1.0	1.5	1.2	92.3&1		24.3[a]
	S.D.	2.9	7.3	2.7	0.0		28.2
Early	MEAN	0.1	0.2	0.2	12.0		3.0
	S.D.	0.3	0.8	0.4	0.0		3.7
	N	25	25	23	2		23
	TOTAL	3	4	4	24		70
	MEAN%	1.0	1.5	1.2	92.3&1		22.4[a]
	S.D.	2.9	7.3	2.7	0.0		26.7
Late	MEAN	0.0	0.0	0.0	0.0		0.3
	S.D.	0.0	0.0	0.0	0.0		0.5
	N	25	25	23	2		23
	TOTAL	0	0	0	0		6
	MEAN%	0.0	0.0	0.0	0.0&1		1.9[a]
	S.D.	0.0	0.0	0.0	0.0		3.9
Dead Fetuses	TOTAL	0	0	0	0		0
Postimplantation Loss	MEAN%	1.0	1.5	1.2	92.3		24.3[a]
	S.D.	2.9	7.3	2.7	0.0		28.2

*1 or &1: significantly different from group 1 with P ≤ 0.05 or P≤ 0.01, respectively;

*5 or &5: significantly different from group 5 with P ≤ 0.05 or P≤ 0.01, respectively;
Means calculated excluding dams with no viable fetuses
[a] = statistical analysis not conducted

Table 56. Summary of Fetal Weights

DOSE LEVEL		GROUP 1 0 MG/KG/DOSE	GROUP 2 0.3 MG/KG/DOSE	GROUP 3 1 MG/KG/DOSE	GROUP 4 3 MG/KG/DOSE	GROUP 5 10 MG/KG/DOSE	GROUP 6 0.2 MG/KG/DOSE
FETAL WEIGHTS UNITS: GRAMS							
of all Viable Fetuses	MEAN	5.63	5.53	5.44	3.20		5.37
	S.D.	0.36	0.39	0.45	0.57		0.56
	N	25	25	23	2		23
Covariate Adjusted	MEAN	5.62	5.52	5.43	3.33**		5.40
of Male Fetuses	MEAN	5.78	5.68	5.61			5.47
	S.D.	0.38	0.38	0.48			0.72
	N	25	25	23			22
Covariate Adjusted	MEAN	5.78	5.68	5.61			5.48
of Female Fetuses	MEAN	5.42	5.39	5.28	3.20		5.36
	S.D.	0.36	0.45	0.43	0.57		0.39
	N	25	25	23	2		20
Covariate Adjusted	MEAN	5.43	5.40	5.30	3.04**		5.34

* = P≤0.05 ** = P≤0.01.

Offspring (Malformations, Variations, etc.)

Curly tail (as a fetal external variation) was noted in one fetus (Dam B57401, Fetus 5) in 0.2 mg/kg SGD-1010 group.

Fetal external malformation information is summarized in Table 57. Fetal external malformations were noted in one fetus in the 3 mg/kg SGN-35 group and 3 fetuses in the 0.2 mg/kg SGD-1010 group.

Table 57. Summary of Fetal External Malformations

DOSE LEVEL		GROUP 1 0 MG/KG/DOSE	GROUP 2 0.3 MG/KG/DOSE	GROUP 3 1 MG/KG/DOSE	GROUP 4 3 MG/KG/DOSE	GROUP 5 10 MG/KG/DOSE	GROUP 6 0.2 MG/KG/DOSE
Litters Evaluated	N	25	25	23	2	0	23
Fetuses Evaluated	N	317	320	304	2	0	220
Live	N	317	320	304	2		220
Dead	N	0	0	0	0		0
PROTRUDING TONGUE							
Fetal Incidence	N	0	0	0	0		1
	%	0.0	0.0	0.0	0.0		0.5
Litter Incidence	N	0	0	0	0		1
	%	0.0	0.0	0.0	0.0		4.3
MALROTATED HINDLIMBS							
Fetal Incidence	N	0	0	0	1		2
	%	0.0	0.0	0.0	50**		0.9
Litter Incidence	N	0	0	0	1		2
	%	0.0	0.0	0.0	50		8.7
UMBILICAL HERNIA							
Fetal Incidence +	N	0	0	0	1		0
	%	0.0	0.0	0.0	50**		0.0
Litter Incidence +	N	0	0	0	1		0
	%	0.0	0.0	0.0	50		0.0
GASTROSCHISIS							
Fetal Incidence	N	0	0	0	0		2
	%	0.0	0.0	0.0	0.0		0.9
Litter Incidence	N	0	0	0	0		2
	%	0.0	0.0	0.0	0.0		8.7
AGNATHIA							
Fetal Incidence	N	0	0	0	0		1
	%	0.0	0.0	0.0	0.0		0.5
Litter Incidence	N	0	0	0	0		1
	%	0.0	0.0	0.0	0.0		4.3
TOTAL FETAL EXTERNAL MALFORMATIONS							
Fetal Incidence	N	0	0	0	1		3
	%	0.0	0.0	0.0	50**		1.4
Litter Incidence	N	0	0	0	1		3
	%	0.0	0.0	0.0	50		13

* = P≤0.05 ** = P≤0.01
+ = significant positive trend

N = Number

Fetal soft tissue malformations are summarized in Table 58. Notably, fetal soft tissue malformations were noted at 0.3 and 1 mg /kg SGN-35 groups, in one fetus each only (no fetal soft tissue malformation was recorded in the historical control data).

Table 58. Summary of Fetal Soft Tissue Malformations

DOSE LEVEL		GROUP 1 0 MG/KG/DOSE	GROUP 2 0.3 MG/KG/DOSE	GROUP 3 1 MG/KG/DOSE	GROUP 4 3 MG/KG/DOSE	GROUP 5 10 MG/KG/DOSE	GROUP 6 0.2 MG/KG/DOSE
Litters Evaluated	N	25	25	23	2	0	22
Fetuses Evaluated	N	156	160	149	2	0	108
Live	N	156	160	149	2		108
Dead	N	0	0	0	0		0
SITUS INVERSUS							
Fetal Incidence	N	0	1	0	0		1
	%	0.0	0.6	0.0	0.0		0.9
Litter Incidence	N	0	1	0	0		1
	%	0.0	4.0	0.0	0.0		4.5
TESTIS(ES)-REDUCED IN SIZE							
Fetal Incidence	N	0	0	1	0		0
	%	0.0	0.0	0.7	0.0		0.0
Litter Incidence	N	0	0	1	0		0
	%	0.0	0.0	4.3	0.0		0.0
TOTAL FETAL SOFT TISSUE MALFORMATIONS							
Fetal Incidence	N	0	1	1	0		1
	%	0.0	0.6	0.7	0.0		0.9
Litter Incidence	N	0	1	1	0		1
	%	0.0	4.0	4.3	0.0		4.5

* = P≤0.05 ** = P≤0.01

Fetal skeletal malformations were noted in two fetuses (in 2 separate litters) in the 0.2 mg /kg SGD-1010 group only, including malformed mandible, misaligned, fused and/or absent caudal vertebrae, split sternebrae, and shortened long bone (only shortened long bone was recorded in the historical control data).

Reviewer's comments:

Comparison of embryo-lethality in SGN-35 and SGD-1010 groups

The results from this study demonstrated that administration of SGN-35 at 10 mg/kg/dose resulted in greater embryo-fetal toxicity than administration of SGD-1010 at an equivalent molar dose (0.2 mg/kg) using the same dose regime (GDs 6 and 13 dosing). Administering SGN-35 at 10 mg/kg to dams resulted in total resorption of implants while less than 30% in dams administered SGD-1010 at 0.2 mg/kg had the resorption. There was no viable fetus in dams given 10 mg /kg SGN-35 while this occurred in only one of 24 dams given 0.2 mg/kg SGD-1010.

The difference in PK profiles in two groups is likely responsible for this difference in toxicities. While SGD-1010 concentrations were higher immediately after dosing in animals given SGD-1010 at 0.2 mg/kg, the concentrations decreased rapidly on Day 1 post dosing and the levels were BLQ on Day 7 after the first dose or Day 5 after the second dose (Table 48). When animals were dosed with SGN-35, concentrations of free SGD-1010 were lower immediately after dosing in animals, however, the level increased on Day 1 and remained detectable throughout the study period (Table 47), resulting in sustained exposure to free SGD-1010.

Comparison of AUCs of rat and human

The recommended clinical dose is 1.8 mg/kg administered as an intravenous infusion over 30 minutes every 3 weeks (q3W). Mean (n=12) $AUC_{0-\infty}$ was 79.41 (day•micg/mL) for ADC and 37.03 (day•ng/mL) for MMAE after single dose administration at 1.8 mg/kg q3W according to the summary of clinical pharmacology; based on the study SG035-0001. The ratios of human AUC to rat AUC are summarized in Table 59. Severe embryo-fetal toxicities including embryolethality and malformations were noted in this rat study at approximately the same AUC value as in humans at the recommended clinical dose (83.3 day•micg/mL for SGN-35 in 3 mg/kg rat group vs. 79.41 day•micg/mL in humans).

Table 59. Human $AUC_{0-\infty}$ and rat AUC_{0-last}*

Dose (mg/kg)	AUC for ADC (SGN-35) (day•micg/mL)	Ratio of human AUC to rat AUC	AUC for MMAE (SGD-1010) (day•ng/mL)	Ratio of human AUC to rat AUC
Human				
1.8	79.41		37.03	
Rat				
0.3	8.55	0.11	Not reported	
1	30.4	0.38	0.0295	0.0008
3	83.3	1.1	0.517	0.0140
10	446	5.6	1.89	0.0510

* Human AUC data from the summary of clinical pharmacology based on the study SG035-0001 (single dose, q3W), rat AUC data from TK data on GD6 (single dose).

9.3 Prenatal and Postnatal Development

Not conducted. No PPND study is needed for drugs treating patients with advanced cancers according to ICH S9.

10 Special Toxicology Studies

11 Integrated Summary and Safety Evaluation

Pharmacodynamics

Brentuximab vedotin (SGN-35) is a antibody-drug conjugate (ADC). The antibody is a chimeric IgG1 directed against CD30 and the small molecule, MMAE, is a microtubule disrupting agent. CD30 is a diagnostic marker for HL and is highly expressed on subsets of NHL including ALCL. CD30 is also expressed on normal, hematopoietic cells, mainly on activated lymphocytes but is absent on most normal, non-hematopoietic cells.

Binding studies using the CD30-positive ALCL cell line (Karpas 299) and human, monkey, and murine CD30-expressing cells demonstrated that SGN-35 bound to human and monkey CD30-positive cells. The mean apparent Kd was 2.36 nM for Karpas 299 cells; similar apparent Kds were observed for activated human (0.20 nM) and cynomolgus monkey (0.19 nM) lymphocytes. SGN-35 did not bind to murine CD30-expressing cells.

Nonclinical studies demonstrated that binding of the ADC to CD30-expressing cells initiated internalization of the ADC-CD30 complex, which then trafficked to the lysosomal compartment. This was followed by MMAE release via proteolytic cleavage. MMAE inhibited microtubule polymerization with an acticity comparable to that of vinblastine (IC50: 1.00 vs. 1.41 micM) and disrupted the intracellular microtubule network. SGN-35 induced cell cycle arrest (G2/M phase cell cycle accumulation and sub-G0/G1 events), apoptosis, and cytotoxicity in CD30-positive cells but not in CD30-negative cells while MMAE had the effects on both CD30-positive and CD30-negative cells, indicating CD30 targeting nature of SGN-35. SGN-35-mediated cytotoxicity was not observed in one CD30-positive cell line which had lower intracellular MMAE concentration, suggesting the role of intracellular MMAE in cell death. The antitumor activity of SGN-35 was evaluated using xenograft models in which animals were implanted with CD30-containing human tumors. Of note, cAC10 (the antibody moiety) does not bind to rodent CD30. SGN-35 treatment significantly delayed tumor growth in HL L540cy, HL L428, or ALCL Karpas 299 tumor xenograft models in a dose-dependent manner. Treatment of animals with SGN-35 delayed tumor growth depending on tumor models tested with the effect on ALCL Karpas 299 > HL L540cy > HL L428.

General toxicity

SGN-35 bound to human and monkey CD30-positive cells with similar apparent Kds but did not bind to murine CD30-expressing cells. Therefore, monkey is considered an appropriate animal species for the general toxicity studies. Two monkey toxicity studies with 3- (0, 1, 3, or 6 mg/kg, four doses) or 6- (0, 1 or 3 mg/kg, nine doses) month durations, using the same dose regimen as in patients (once every 3 weeks) were conducted. Main toxicities were dose- and time-related hematological toxicities especially neutropenia. Toxicities resulted in three premature deaths/sacrifices in the 6 mg/kg group (all occurred prior to the second dosage of SGN-35 despite of prophylactic antibiotic treatment) with a white cell count as low as 10/mcL. The decrease in white cell counts was more profound after the first dose. This adverse effect was reversible. Hematological changes correlated with reduced thymic weights and histopathology findings of bone marrow hypocellularity and lymphoid depletion in thymus and spleen. A steep dose-toxicity was evident as severe toxicities were observed in the 6 mg/kg group while not as evident in the 3 mg/kg group.

Neurotoxicity with peripheral sensory (44%) or motor (9%) neuropathy was the main toxicity observed in clinical trials. Similarly, neurotoxicity has been observed with other microtubule inhibitors. Transient (on Days 10-14 only after the first dose, normal after this episode) lameness of hands was noted in only one monkey given 6 mg/kg SGN-35.

Drug-related hepatobiliary toxicities were noted in rats in the 10 mg/kg SGN-35 (high-dose) group only, in a 4 week, weekly dosing toxicity study). In these animals, significantly increased liver enzymes (≥ 3 folds in males) and total bilirubin (4 fold) were observed along with minimal coagulative necrosis. Effects were reversible. Considering that SGN-35 does not bind to target cells in rats, hepatobiliary toxicities in rats may be secondary to high levels of circulating SGN-35 and uptake by liver. MMAE-related toxicities, e.g. myelosuppression, were also seen in rats.

Reproductive and developmental toxicity

Fertility studies with SGN-35 or MMAE were not conducted. In a 4 week repeat-dose toxicity study in rats with weekly dosing at 0.5, 5 and 10 mg/kg SGN-35, dose-related (at 5 and 10 mg/kg, 2.8 or 5.6 fold of recommended dose of 1.8 mg/kg in patients, respectively based on body weight), persistent seminiferous tubule degeneration, Sertoli cell vacuolation, reduced spermatogenesis, and aspermia were observed. In addition, dose-related decrease in testicular weight and size and/or soft testis was also noted.

In an embryofetal toxicity study in rats, SGN-35 (when dosed once each on Pregnancy Days 6 and 13) induced dose related, marked embryofetal toxicities, including increased early resorption, pre-implantation and post-implantation loss, decreased numbers of live fetuses, and fetal external malformations (e.g., umbilical hernia and malrotated hindlimbs). Embryofetal toxicities occurred at approximately the exposure level of brentuximab vedotin (AUC) as in patients receiving the recommended dose of 1.8 mg/kg every three weeks. At this dose level (3 mg/kg), SGN-35 administration produced 99 % post-implantation loss and resulted in no viable fetuses in approximately 92% (22/24) of dams. At 10 mg/kg, there was no viable fetus in all 25 dams. MMAE, at the same dose regimen, produced similar toxicities but with less severity. This was likely due to continuous systemic exposure to free MMAE during the period of organogenesis, when conjugated to the antibody. For example, there was only 27% post-implantation loss and no viable fetus in 4% (1/24) of dams administered 0.2 mg/kg MMAE (equivalent to molar MMAE dose of 3 mg/kg SGN-35). In addition, fetal soft tissue malformations were noted in 0.3 mg/kg (situs inversus in one fetus) and 1 mg/kg (reduced testis size in one fetus) SGN-35 groups and external, soft tissue, and skeletal malformations were noted in the 0.2 mg/kg MMAE group.

Genetic toxicity

Standard genetic toxicity studies were conducted using MMAE. MMAE was not mutagenic in the bacterial reverse mutation assay and the L5178Y mouse lymphoma forward mutation assay. MMAE induced micronucleus formation via an aneugenic mechanism in rat bone marrow micronucleus study, which was consistent with the pharmacological effect of MMAE as a microtubule disrupting agent.

Comparison of toxicities of SGN-35 and MMAE alone

Hepatobiliary and embryofetal toxicities were more profound in rats given SGN-35 than in rats given MMAE at equivalent dose levels in rat studies, probably due to prolonged exposure of MMAE in the SGN-35 group. The results indicated that although ADC could have more targeted effect and lower C_{max}, ADC could prolong the exposure to MMAE and produce more pronounced toxicities especially with less frequent dose regimen.

12 Appendix/Attachments

None

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

BLA Number: 125388 **Applicant:** Seattle Genetics, Inc **Stamp Date:** 28-Feb-2011

Drug Name: Brentuximab vedotin (Adcetris) **BLA Type:** original

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section **organized in accord with current regulations** and guidelines for format and content in a manner to allow substantive review to begin?	x		
2	Is the pharmacology/toxicology section **indexed and paginated in a manner allowing** substantive review to begin?	x		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	x		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	x		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	x		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies **have been performed in accordance with the** GLP regulations (21 CFR 58) or an explanation for any significant deviations?	x		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	x		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?		x	Will communicate with the applicant and request revisions.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	x		
11	Has the applicant addressed any abuse potential issues in the submission?		x	N/A
12	**If this NDA/BLA is to support a Rx to OTC** switch, have all relevant studies been submitted?		x	N/A

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ____Yes____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

YANLI OUYANG — 3/31/11

Reviewing Pharmacologist — Date

H. Sauer — 4/1/2011

Team Leader/Supervisor — Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908