FOOD AND DRUG ADMINISTRATION
Center for Biologics Evaluation and Research
Office of Tissues and Advanced Therapies
Division of Clinical Evaluation and Pharmacology/Toxicology
Pharmacology/Toxicology Branch

BLA NUMBER:	STN #125646.000
DATE RECEIVED BY CBER:	February 2, 2017
DATE REVIEW COMPLETED:	June 2, 2017; amended July 11, 2017 and July 20, 2017
PRODUCT:	Tisagenlecleucel (KYMRIAH™, CTL019)
APPLICANT:	Novartis Pharmaceuticals Corporation
PROPOSED INDICATION:	KYMRIAH™ is a CAR-T cell product indicated for the treatment of pediatric and young adult patients with relapsed/refractory (r/r) B-cell acute lymphoblastic leukemia (ALL)
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EXECUTIVE SUMMARY:

Tisagenlecleucel (KYMRIAH™, CTL019) gene transfer is accomplished via *ex vivo* transduction with a replication-deficient human lentiviral vector (LV) encoding the transgene. The expression of the transgene is expected to confer the transduced T cells with specificity for cells that express CD19 on the cell surface. The nonclinical studies evaluating CTL019 included the specificity of the CD19-binding domain of the transgene, the *in vitro* and *in vivo* anti-tumor activity of CTL019, and safety studies measuring the presence of uncontrolled proliferation of CTL019 and its progeny; evaluation of selected toxicology parameters and biodistribution (BD)/cell persistence in animals were also conducted. In addition, a study was conducted to determine possible insertion sites of the LV genomic integration into the host genome.

The specificity of CTL019 as determined by immunohistochemistry (IHC) and *in situ* hybridization (ISH) performed in the cerebrum and cerebellum samples of human and

cynomolgus macaque showed no detection of any tissue reactivity to CD19 protein and mRNA. Reverse transcript (RT)-qPCR on the same samples did not detect expression of any CD19 splice variants. IHC analysis to assess the potential on- or off-target binding of the human and murine anti-CD19 scFvs of the CD19 CAR construct showed plasma membrane staining of CD19-rich B cell follicular regions in normal human spleen, with staining intensity slightly greater for the murine CD19-scFv then for the human CD19-scFv. Protein array assessment for binding of murine and humanized CD19-CAR scFV to human membrane proteins showed that CD19 was identified by both biotinylated scFv fragments of human and murine, while no other human membrane protein binders were detected on the array.

A series of studies in (b) (4) mice bearing human primary pre-B ALL conducted to evaluate anti-tumor activity of CTL019 derived from different healthy human donors showed significantly improved survival in the tumor-bearing mice receiving intravenous (i.v.) injection of CTL019 at $2x10^6$ cells/mouse as compared to the mock T cell administered animals. The median leukemia-free survival time was 7 weeks with human cell engraftment, while continuing proliferation of pre-B ALL cells was present in control animals.

A genomic insertion site analysis was performed on CTL019 from 14 individual donors (2 healthy donors and 12 patients representing pediatric ALL-derived and diffuse large B-cell lymphoma (DLBCL)-derived cells). In general, no preferential insertion site of the CTL019 lentiviral vector was identified and no clonality was observed. The distribution of the integration sites to genes with known adverse effects or clonal expansions appeared to be similar to integration sites identified with stem cell samples transduced with gamma retroviral vectors in other gene therapy clinical trials. Unlike the stem cell samples, the CTL019 LV genomic integration was disfavored relative to regions with long gene widths or intergenic distances. The CTL019 LV transduced samples also exhibited well known patterns for lentiviral integration, (e.g., LV prone to integration of transcription units and to regions near a collection of sequences featuring DNAse I hypersensitive sites, CpG island and regions of high GC content).

In vitro expansion studies with CTL019 from healthy donors and patients showed no evidence for transformation and/or immortalization of T cells.

An i*n vivo* study was conducted to evaluate toxicity and BD in tumor-bearing (b) (4) mice receiving i.v. injection of CTL019 at 1, 5, and $20x10^6$ cells/mouse and observed up to Day 217 post-injection (p.i.) with three terminal sacrifices. There was no histologic evidence of systemic toxicity associated with CTL019 versus mock T cells. No abnormal cell growth was detected by clinical pathology or by PCR at any time point in any animal. There were no indications of uncontrolled cellular proliferation in the CTL019 cell groups over the mock T cell groups. A CTL019 cell dose-dependent inhibition of B-ALL tumor growth was observed.

On Day 42 p.i., CTL019 cell signals were detected in the spleen, lung, kidney, and brain in high-dose group only. On Day 56 p.i., CTL019 cell signals were detected in these same tissues and in the liver, heart, blood and bone marrow in all CTL019 groups with more frequency in the high-dose group as compared to the lower dose groups. During Days 65 to 146 p.i., cell signals were detected in the high-dose group (spleen, lung, liver, kidney, brain, heart blood and bone marrow),

and on Day 217, cell signals were detected in the spleen, liver, kidney, and bone marrow in the mid-dose group.

Classical genotoxicity assays and carcinogenicity assessment in rodent models were not performed for CTL019. No preclinical reproductive studies were conducted with CTL019 to assess whether it can cause fetal harm when administered to a pregnant woman. It is not known if CTL019 constitutes a risk to pregnant women or fetuses.

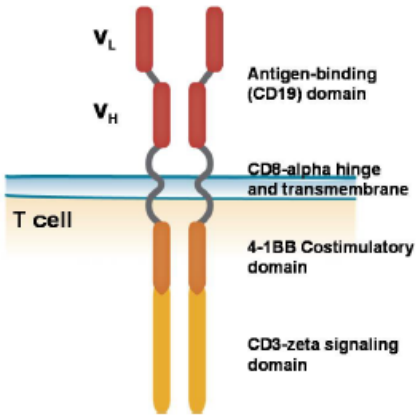
PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:
There were no nonclinical deficiencies identified in this submission. There are no requests for any further nonclinical testing of Tisagenlecleucel (CTL019) at this time. Based on the review of the submitted toxicology and pharmacology data, this original biological application STN 125646/0 is recommended for approval for the treatment of pediatric and young adult patients with relapsed/refractory B-cell acute lymphoblastic leukemia.

Formulation and Chemistry:
CTL019 is an autologous T cell product formulated as a single-dose cell suspension for infusion. The cell suspension is formulated in a defined cryopreservation medium containing a stock solution comprised of several excipients: Plasma-Lyte A for Injection, Dextrose and Sodium Chloride for Injection, Human Serum Albumin, Dextran 40 in Dextrose for Injection and Cryoserv® solution (containing DMSO as cryopreservation agent). The CTL019 final formulation contains 7.5% DMSO.

CTL019 final product is cryopreserved in (b) (4) freezing bags. Bags of two sizes can be used: (b) (4) for volumes of 10 - 30mL and (b) (4) for volumes of 30 - (b) (4) of cell suspension.

The gene transfer is accomplished via *ex vivo* transduction with a replication-deficient human lentiviral vector (LV) encoding the transgene. Under the control of an EF1α promoter, the expressed transgene is comprised of a CD8α leader sequence, a murine single chain antibody fragment (anti-CD19scFv), a CD8 hinge and transmembrane region and a 4-1BB (CD137) and CD3ζ (TCRζ) signaling domain (Figure 1). The expression of the transgene is expected to confer the transduced T cells with specificity for cells that express CD19 on cell surface.

Figure 1. Schematic of the chimeric antigen receptor (CAR) structure on the surface of atisagenlecleucel



Abbreviations

ALL	Acute lymphoblastic leukemia
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BD	Biodistribution
CAR-T, CART	Chimeric antigen receptor T cells
CLL	Chronic lymphocytic leukemia
GvHD	Graft-versus-host disease
HCL	Hairy cell leukemia
IHC	Immunohistochemistry
ISH	*In situ* hybridization
LV	Lentiviral vector
MOI	Multiplicity of infection
NOD	Non-obese diabetic
(b) (4)	
(b) (4)	
p.i.	Post injection
scFV	Single-chain variable fragment
SCID	Severe combined immunodeficiency
SIN	Self-inactivating
TU	Infectious Titer Unit
UPenn	University of Pennsylvania
xGvHD	Xenograft Graft-versus-host disease

Note:
The term ‘CART-19 cell’ is used in preclinical study reports interchangeably with CTL019.

Related File(s)

IND #13960: University of Pennsylvania; Autologous T Cells Cultured with Anti-CD3 and Anti-CD28 Coated Magnetic Beads; Transduced with Lentiviral Vector (CART-19, Lentigen) Expressing CD19 Chimeric Antigen Receptor, for treatment of refractory CD19+ leukemia and lymphoma; ACTIVE

IND #16130: Novartis Pharmaceuticals Corp.; Autologous T Cells Elutriated or Cultured with Anti-CD3 and Anti-CD28 Coated Magnetic Beads; Transduced with Lentiviral Vector Expressing CD19 Chimeric Antigen Receptor, for treatment of acute lymphoblastic leukemia (ALL); ACTIVE

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the malignant proliferation of lymphoid progenitor cells in the bone marrow, characterized by an excess of malignant lymphoblasts. The majority of ALL malignancies are of B-cell origin, and although ALL can occur at any age, it is more commonly seen in children with approximately 60% of the cases occurring in patients aged younger than 20 years, with a peak incidence between 2 to 5 years; the incidence rises again after the age of 60 years.[1] Approximately 3000 children in the US and 5000 children in the EU are diagnosed with ALL per year.[2]

[1] Howlader N et al., SEER Cancer Statistics Review 1975–2013 National Cancer Institute, Bethesda, MD. Available at: http://seer.cancer.gov/csr/1975_2013/. Accessed based on November 2015 SEER data submission.
[2] Orphanet. The portal for rare diseases and orphan drugs. Available from: http://www.orpha net/consor/cgi-bin/OC_Exp.php?Lng=GB&Expert=513. Accessed 10-Dec-2016.

Treatment options for patients with relapsed/refractory (r/r) B-cell malignancies include high dose chemotherapy with subsequent allogeneic stem cell transplantation, standard chemo-immunotherapy, targeted treatment with small molecule pathway inhibitors, or supportive care with non-curative palliative goals. The currently approved therapeutic agents include chemotherapies (e.g., the combination of clofarabine with cyclophosphamide and etoposide). In the US, the bi-specific antibody blinatumomab was approved in 2016 for the treatment of pediatric patients with Philadelphia chromosome (Ph)-negative r/r B-cell precursor ALL. Treatment of patients with r/r B-cell ALL after failing 2 prior regimens of therapy is challenging and the prognosis of patients with r/r B-cell ALL disease remains poor, as no effective treatment regimens exist for this patient population.

NON-CLINICAL STUDIES

PHARMACOLOGY STUDIES

Summary List of Pharmacology Studies

The following pharmacology studies were conducted to support the rationale for the administration of CTL019 to treat the proposed clinical indication.

In Vitro Studies

Study Number	Study Title / Publication Citation	Report Number
1	Assessment of CD19 mRNA and protein expression in normal human and cynomolgus macaque brain sections	1420055
2	Detection of CD19 in human and cyno brain by RT-PCR	1420059
3	Assessment of human membrane protein off-target binding of CD19 scFv using (b) (4) array	1470028
4	CD19 EXP 081407	pcs-rsir08-009
5	CD19 EXP 080108	pcs-rsir08-016
6	CD19 EXP 102108	pcs-rsir09-003
7	CD19 EXP111308	pcs-rsir09-004

In Vivo Studies

***In Vivo* Studies in Tumor Xenograft Animal Models**

Study Number	Study Title / Publication Citation	Report Number
8	CART-19 Preclinical Animal Studies	pcs-rcart-19
9	CART-19 Preclinical Animal Studies Final Report Amendment No.1	dmpk-rcart-19

Overview of Pharmacology Studies

Overview of In Vitro Studies

Study #1

This study was conducted by Novartis to determine whether CD19 protein or mRNA expression was present in the brain (cerebrum and cerebellum) samples of normal human (n=15) and cynomolgus macaque (cyno monkey, n=8). Immunohistochemistry (IHC) and *in situ* hybridization (ISH) was performed to detect the levels of the CD19 protein and mRNA, respectively. The IHC analysis included an irrelevant antibody control and known negative and positive tissues as control. No reactivity was detected in human and monkey brain samples. The ISH analysis included positive and negative control probes. CD19 mRNA levels were not detected in human and monkey brain samples.

IHC analysis was also performed to assess the potential on- or off-target binding of the human and murine anti-CD19 scFvs contained within the CAR constructs. Examination of normal human spleen demonstrated discrete plasma membrane staining of CD19 rich B cell follicular regions. Staining intensity was slightly greater for the muCD19-scFv than for the huCD19-scFv. In each case the antibody controls demonstrated no non-specific signal. Antibodies were then used to stain normal frozen human cerebrum and cerebellum. No reactivity was detected in the sections stained with either huCD19-scFv or muCD19-scFv chimeric antibody reagents.

Study #2

This study, conducted by Novartis, was to determine whether CD19 splice variants not detected by ISH or IHC could be expressed in brain tissue. Commercially-available mRNA samples of healthy human brain tissues and cyno monkey brain tissues obtained in-house were analyzed by reverse transcript (RT)-PCR along with positive control human tissues. RT-PCR primer sets were designed to detect splice variants based on the sequences for the five splice variants of human CD19 mRNA, and three splice variants of cyno monkey CD19 mRNA reported in an NCBI database.

The results showed no detectable expression of any CD19 splice variants in any human or cyno monkey brain samples tested. Thus, CD19 is not expressed in the human and cyno monkey brain.

Study #3

This study, conducted by (b) (4), was to assess binding of murine and humanized CD19-CAR scFV to human membrane proteins that are present in the (b) (4) plasma membrane protein array (Annex) manufactured by (b) (4). This array is reported to cover approximately 3550 full human membrane proteins expressed on HEK293 cells. Two biotinylated scFv fragments (anti-hCD19-AVI-His murine [IC-57-WH78], and anti-hCD19-AVI-His humanized [ME-55-PP72]) were used as the specific targets to the array at three concentrations (0.4, 1 and 4 μg/ml). The results showed: 1) CD19 was identified as target for both IC-57-WN78 and ME-55-PP72, and 2) no other human membrane protein binders were detected on the array.

Study #4

This study was conducted by the University of Pennsylvania (UPenn) to assess lentiviral transduction efficiency *in vitro*. Lymphocytes from a healthy donor were transduced with the Lentigen vector expressing αCD19ζ (LTG118) or αCD19-4-1BB-ζ (LTG119) at MOIs of 4, 10, 20, or 40 infectious titer unit (TU/cell). Cells were incubated for approximately 48 hours. On day 2 of culture, the vector was washed off and the cells were seeded (small scale) and cultured for approximately 38 days. Non-transduced cultures (vector storage buffer alone) were used as the controls. The results showed:

a) Transduction efficiencies (measured by percent scFv expression level) were ≤48% and the level of scFv expression increased with increasing multiplicity of infection (MOI).
b) Vector copy number was initially <2 copies/cell, but increased as the MOI increased, from 4 to 40 for both LTG118 and LTG119, as shown in the table below.

Culture Conditions	LTG119			LTG118		
	41BB D4	41BB D7	41BB D10	Zeta D4	Zeta D7	Zeta D10
C1 (LTG118 MOI 4)	n/a	n/a	n/a	0.18	0.14	0.16
C2 (LTG118 MOI 10)	n/a	n/a	n/a	**0.42**	**0.36**	**0.34**
C3 (LTG118 MOI 20)	n/a	n/a	n/a	**0.82**	**0.67**	**0.64**
C4 (LTG118 MOI 40)	n/a	n/a	n/a	**1.76**	**1.40**	**1.16**
C5 (LTG119 MOI 4)	0.19	0.13	0.19	0.02	0.02	0.02
C6 (LTG119 MOI 10)	**0.67**	**0.31**	**0.38**	0.05	0.06	0.03
C7 (LTG119 MOI 20)	**1.02**	**0.60**	**0.80**	0.10	0.08	0.08
C8 (LTG119 MOI 40)	**1.95**	**1.10**	**1.10**	0.19	0.14	0.12

Cells transduced with MOI of 4-10 TU/cell of LTG118 or LTG119 had a similar growth rate. These cells also grew better than cells transduced at MOI of 40 TU/cell, as well as the non-transduced controls. Cultures transduced with LTG119 grew for 38 days and cells transduced with LTG118 grew for 28 days. This increasing number of cells is due to a delay in the cell death rate as opposed to enhanced proliferation. This was correlated only with increased persistence of CD8+ T cells, as CD4+ T cells stopped expanding around Week 2, as was observed in other cultures.

Study #5

This study was conducted by UPenn to assess lentiviral transduction efficiency in vitro. Lymphocytes from a CLL patient were transduced with the LTG119 (α-CD19-ζ-4-1BB) lentiviral vector at an MOI of 25 TU/cell. Cells were incubated for approximately 48 hours followed by washing to remove the vector; the cells remained in culture for approximately 38 days. Non-transduced cultures were carried out as an expansion control. Transduction efficiency was >70% in the transduced culture as measured by CART-19 expression by flow cytometry.

The results showed that cultures transduced with LTG119 had a similar fold expansion to control cultures and survived about 4 days longer in culture in the absence of re-stimulation.

Study #6

This experiment was carried out by UPenn to evaluate the transduction efficiency of LTG119 and expansion potential of transduced T cells isolated from subjects that meet the enrollment

criteria of the planned clinical trial. Primary human lymphocytes were obtained from a CLL cancer patient and were transduced with the LTG 119 lent/viral vector (α-CD19-ζ-4-1BB, DOM06101/2007, Research Grade, (b) (4) at a total MOI of 25 TU/cell, equal to an MOI of 12.5 TU/cell per day on Day 0 and on Day 1. On Day 3 of culture, the vector was washed off and the cells were seeded into small scale and cultured for approximately 28 days to follow the cells until they were no longer proliferating. A non-transduced culture was set up as a control. Detection of scFv expression via flow cytometry was performed on Days 7, 9 and 12. Transduction efficiency was measured by QT-PCR assay for 4-1BB sequences.

The results showed that transduction efficiency was greater than 70% and expression was stable from Days 7 through 12 of culture. As observed in prior experiments, cells transduced with the 4-1BB containing construct expanded for a longer period of time following a single stimulation and rested down at 4 weeks compared to 3 weeks in the control cultures. Longer survival of the LTG119 transduced cells did not result in differences in population doublings between the cultures.

Study #7

This experiment was carried out by UPenn to evaluate the transduction efficiency of LTG119 and expansion potential of transduced T cells isolated from subjects with hematologic cancer. Lymphocytes from a patient with hairy cell leukemia (HCL) were transduced with the clinical lot LTG119 (α-CD19-ζ-4-1BB) lentiviral vector at an MOI of 10 or 20. Cells were incubated for approximately 48 hours followed by washing to remove the vector; the cells remained in culture for approximately 38 days. Non-transduced cultures (vector storage buffer alone) were also expanded and used as the controls.

The results showed:

a) The transduction efficiency was 29% and 25% for MOI 20 and 10, respectively, as measured by CART-19 surface expression by flow cytometry.
b) Cultures transduced with LTG119 at an MOI of 20 had a similar fold expansion to control culture. Cultures transduced at an MOI of 10 grew better than the control culture, although the viability of these transduced cells was no different than the control cultures. This indicates that the culture transduced at an MOI of 10 appeared to have a greater doubling rate than the other culture, but did not have a greater survival rate.

Overview of In Vivo Studies

***In Vivo* Studies in Tumor Xenograft Animal Models:**

Study #8

This study contained a series of three preclinical animal studies conducted at the UPenn Abramson Cancer Center. Different human donors were used for each experiment except for one repeat donor. The purpose of the studies was to evaluate the *in vivo* efficacy of four CART-19 receptor constructs: αCD19-ζ, αCD19-BB-ζ, αCD19-28-ζ, and αCD19-28-BB-ζ. These data were

used to support the selection of αCD19-BB-ζ construct for clinical development based on cell survival *in vivo*, anti-tumor effects and animal survival.

Methods:
CART cell preparation: Peripheral blood lymphocytes were isolated from healthy donors, and CD4+ and CD8+ T cells were purified by negative selection using the (b) (4) method for purification of CD4+ or CD8+ T cells (b) (4) Primary pre-B ALL cells were derived from patients with acute preB-ALL. T cells were transduced with self-inactivating (SIN) lentiviral vectors encoding a mouse CD8-human CD28 chimeric protein and eGFP to generate CART-19.

Study animal and design: (b) (4)[1], 6-12 week old, supplied by (b) (4) or bred in house; 4-10 mice/group, animals were i.v. injected with 0.5-2 x 10^6 viable primary ALL cells. At 35 days after tumor implantation animals were injected with T cells expressing various CARs. Animals were monitored for signs of GvHD as evidenced by >10% weight loss, loss of fur, and/or diarrhea; peripheral blood was collected and ALL and T cell engraftment was determined by flow cytometry. CD19, CD3, CD4, and/or CD8 expression was detected by immunocytochemistry staining; CAR+ T cells were identified by GFP expression using lentiviral vectors in which the CAR was linked to eGFP-2A.

Comment:

- (b) (4) reported that (b) (4) mice are the early generation of (b) (4) mice. This strain does not allow engraftment of human T cells. However, this model is acceptable for the pharmacology study.

Results: A data summary of each study is provided below.

CART-A1 Studies:
In this study series, only the CART-19 receptor αCD19ζ was used to determine CART-19 specific anti-tumor activity and identify an optimal dose for subsequent studies where various signaling constructs could be compared. T cells that were transduced with vectors containing a non-functional truncated signaling chain (αCD19:$\zeta^{truncated}$ or αCD19Δζ), CART-19, or mock transduced cells were injected i.v. into the B cell-ALL-bearing mice at 1x, 5x and 10x10^6 αCD19Δζ expressing cells/mouse and 5x10^6 αCD19 expressing cells/mouse. Blood was collected at 8, 9 and 10 weeks post-ALL implantation (3, 4 and 5 weeks post cell injection) to assess tumor cell burden and T cell engraftment. Subsequently, another study was conducted to determine the dose-response of CART-19 cells in the B cell-ALL-bearing mice. The results showed that 5x10^6 cells/mouse elicited anti-tumor activity to B-ALL and slightly improved survival. A follow-on study was carried out to determine the dose-dependent effect of the αCD19-ζ constructs in this tumor-bearing model. A dose of 5 x 10^6 cells was able to delay onset of B-ALL and prevent B-ALL-related death in these animals. Lower doses still had an anti-tumor effect which was proportional to the dose administered, indicating an antitumor effect in a

[3] (b) (4)

dose-dependent manner, and the positive effect of CART-19 cells on animal survival. The study suggests that a dose between 2-5 x 10^6 cells per mouse is optimal for the antitumor activity.

CART-A2 Studies:
This study included the i.v. administration of αCD19-ζ CART-19 cells (αCD19-BB-ζ, or αCD19-ζ, each at 2x10^6 cells/mouse). Other groups in the study included the administration of mock transduced T cells at a dose four times higher than the αCD19-ζ cells. The ALL blast cells were counted on days 21, 28 and 35 post-T-cell injection. The results show that both constructs were effective and the bipartite αCD19-BB-ζ cells seemed to have a slight improvement in antitumor activity, but these results are not conclusive from this study. A follow on study with administration of bipartite and tripartite αCD19-ζ CART-19 cells (αCD19-ζ, αCD19-BB-ζ, αCD19-28-ζ, or αCD19-28-BB-ζ; each at 2x10^6 cells/mouse) confirmed the cell dose level at 2x10^6 cells/mouse was effective across all the CART-19 cells evaluated. There were no significant differences in the level of cell engraftment (CD45/CD4 and CD45/CD8) between CART-19 and mock injected animals.

CART-A3 Studies:
This study further evaluated the effectiveness of each bipartite derivative of the CART-19 cells (αCD19-BB-ζ and αCD19-28-ζ) compared to the parental αCD19-ζ CART-19 T cells. Mice were i.v. injected with 8x10^6 bulk T cells/mouse (adjusted to 50% CAR+ T cells in order to follow the fate of CAR+ vs. CAR- cells) at 3 weeks after establishment of leukemia. To track the transduced cells *in vivo,* CART-19 T cells were engineered to express GFP, as well as the CAR. The results showed that increased engraftment was observed only in mice injected with αCD19-BBζ CART-19 T cells, indicating engraftment of the cells *in vivo*. Similarly, survival was evident only in mice injected with αCD19-BBζ CART-19 T cells. The sponsor's analysis indicates that the median leukemia-free survival time was increased by 7 weeks, in contrast to the doubling time of 2.7 days for pre-B ALL cells. This 7-week delay in the onset of leukemia represented a reduction in the leukemia burden of >10^5-fold as compared to mice injected with αCD19ζ or αCD19-28-ζ cells.

Conclusion: The data support the selection of αCD19-BB-ζ expressing CART-19 cell.

Study #9
This study report, provided by Novartis, describes minor modifications to Study #8:

1. Text in Section 5.1 (p. 6) inaccurately refers to Figure 5.0-1. Correct reference should be Figure 5.1-1. (The indicated figure is in the original report, not shown in this memo.)

2. Text in Section 5.3 (p. 10) indicates "10 million bulk T cells" were injected into mice which should read "8 million bulk T cells", as described in the figure legend for Figure 5.3-2. (The correct dose level is indicated in the review for Study #8.)

3. The schematic in Figure 5.3-1 (p. 11) suggests the T cells were infused on Day 14 after primary ALL. The actual day of infusion was Day 21, or 3 weeks post-ALL infusion, as described in the figure legend for Figure 5.3-2. (The correct interval is indicated in the review for Study #8.)

Comment:

- The modifications in Study #9 has no impact on the conclusions in Study #8.

SAFETY PHARMACOLOGY STUDIES:
None

PHARMACOKINETIC STUDIES

Summary List of Pharmacokinetics Studies
The following biodistribution study was conducted in conjunction with the toxicology study.

In Vivo Studies

Study Number	Study Title / Publication Citation	Report Number
11	Summary Report and Data Analysis: Evaluation of CART-19 Biotoxicity and Biodistribution in Human Immune-reconstituted (b) (4) Mice Protocol# (b) (4)	pcs-racgt-10
12	Evaluation of CART-19 Biotoxicity and Biodistribution in Human Immune reconstituted (b) (4) Mice Protocol# (b) (4) , final report amendment no.1	dmpk-pcs-racgt-10-01

TOXICOLOGY STUDIES

Summary List of Toxicology Studies
The following toxicology studies were conducted to evaluate the safety of CART-19 following administration in animals.

Toxicology Studies:

Study Number	Study Title / Publication Citation	Report Number
11	Summary Report and Data Analysis: Evaluation of CART-19 Biotoxicity and Biodistribution in Human Immune-reconstituted (b) (4) Mice Protocol# (b) (4)	pcs-racgt-10
12	Evaluation of CART-19 Biotoxicity and Biodistribution in Human Immune reconstituted (b) (4) Mice Protocol# (b) (4) , final report amendment no.1	dmpk-pcs-racgt-10-01

Developmental and Reproductive Toxicology Studies: None

Genotoxicity Studies: Classical genotoxicity studies were not conducted. The provided LV integration site analysis may be relevant for evaluation of potential genotoxicity.

Study Number	Study Title / Publication Citation	Report Number
10	Lentivirus integration site analysis characterization of CTL019	1620234

Carcinogenicity/Tumorigenicity Studies: None

Other Safety/Toxicology Studies: None

Toxicology Studies

Study #10

This study was conducted by Novartis to evaluate genomic insertion sites with focuses on integration site distribution (genomic location) and their relative abundance (polyclonality). GMP-manufactured human T-cell batches including pediatric ALL-derived (n=6) and diffuse large B-cell lymphoma (DLBCL)-derived (n=6) were transduced *ex vivo* using the CTL019 GMP-manufactured batches, and DNA prepared after 9 to 10 days of expansion in culture.

- Transduction efficiency evaluated by qPCR (average DNA copies per cell) and by flow cytometry (relative % of viable cells expressing the CAR protein on cell surface) showed:
    - In the ALL samples, 0.1-0.3 copies / cell and 3.7-29.1 % transduction.
    - In the DLBCL samples, 0.2-0.71 copies / cell and 21.6-42.3 % transduction.

- The locations of integrated vectors were analyzed on the 12 GMP-manufactured batches described above and an additional two batches of non-GMP healthy donor-derived T cells transduced with the clinical lentiviral vector.

- Methods and Results
    - 13/14 samples yielded >6,690 integration sites. One sample (pMPAABG1) was consistently lower, yielding 979 unique sites. All samples showed relatively high values of the Shannon diversity index. This method is a mathematical measure of species diversity in a community, and was used here to indicate polyclonal nature of the identified integration sites.

    - To evaluate the occurrence of a single clone with an integration site near a cancer associated gene that had grown out to represent a substantial fraction of the population, 4 statistical methods summarizing population structure were used (Gini index, Shannon index, population size/Chao estimate and UC50).
        - To generate the UC50 statistic, all clones were ranked from highest to lowest abundance using the SonicAbundance method, and then the numbers of clones comprising the top 50% are scored to yield the UC50 value. For all samples except pMPAABG1 this number was high (in the thousands). For pMPAABG1 this number was 282.
        - Similar results were obtained for the Gini index (range 0.1039-0.2917; highest value for pMPAABG1), Shannon index (range 6.7302-9.341; lowest value for pMPAABG1) and Chao estimate of population size (2759- 30231; lowest value for pMPAABG1).

    - The distribution of the integration sites was determined using non-restrictive ligation-mediated PCR (nrLAM-PCR) then analyzed using the INSPIIRED

pipeline as described by Sherman et al.[4] and Berry et al.[5] This analysis included the targeting preferences of the lentiviral vector and features of the transduced cell populations, such as population structure, and specific queries of integration site density near chromosomal features, sites of epigenetic marks, and cancer-associated genes.

- Relative abundance of cell clones by stacked bar graphs and integration sites by Word bubbles indicated that all samples were highly polyclonal including pMPAABG1.
- Analysis of integration sites near genes that have been associated with adverse events (LMO2, CCND2, and MECOM) or clonal expansions (IKZF1, HMGA2) in a distance within the 100kb window to the nearest oncogene 5' end showed the detection of integration sites for 2/14 samples ((pMPAAB21 and pMPAABB1) near IKZF1 and CCND2, mainly downstream of transcription starting site and with coverage equal or lower than 5 reads, the estimated absolute abundance limit for binning as low abundance sites.
- No multi-hit sites with higher abundance than unique sites for any given sample were detected.
- Relationship between integration sites and genomic annotation was analyzed by a comparison of integration frequency to chromosomal features including cancer-associated genes. Two additional samples were added for comparison: one gamma retroviral transduction of a stem cell sample from the SCID2 trial; the second is from transduction of a stem cell sample with a retroviral vector from WAS gene therapy study.
- The results presented by a heat map indicated that the CTL019 transduced samples resemble well-known patterns for lentiviral integration (e.g., LV prone to integration of transcription units and to regions near a collection of sequences featuring DNAse I hypersensitive sites, CpG island and regions of high GC content).
- Integration was slightly favored with 100kb of cancer-associated genes as similar to the samples from SCID2 or WAS cell samples. Unlike the SCID2 and WAS cell samples, integration is disfavored relative to regions with long gene widths or intergenic distances.
- There were some differences in the integration site patterns from T-cell samples versus the long-term repopulating stem cell samples, such that the integration site sequences appear to reflect a result of evolution of HIV for replication in T cells, while such patterns were not often observed in the stem cell samples.

Conclusions: The 14 samples from the CTL019 project looked like conventional lentiviral integration sites in T-cells. There was no evidence for preferential integration near genes of concern, or preferential outgrowth of cells harboring integration sites of concern.

[4] Sherman E et al., INSPIIRED: A Pipeline for quantitative analysis of sites of new DNA integration in cellular genomes. Mol Ther Methods and Clin Dev. 4:39-49, 2016.

[5] INSPIIRED: Quantification and visualization tools for analyzing integration site distributions. Mol Ther Methods and Clin Dev. 4:17-26, 2016.

Study #11

Report Number		pcs-racgt-10
Date Report Signed		February 16, 2009
Title		Summary Report and Data Analysis: Evaluation of CART-19 Biotoxicity and Biodistribution in Human Immune-reconstituted (b) (4) Mice Protocol (b) (4)
GLP Status		No
Testing Facility		University of Pennsylvania
Objective(s)		To evaluate CART-19 biotoxicity and biodistribution
Study Animals	**Strain/Breed**	(b) (4) bearing systemic human acute B cell lymphocytic leukemia (B-ALL 240 cells)
	Species	mice
	Age	adult
	Body Weight	Not provided
	#/sex/group	8 mice/sex/group or otherwise specified
	Total #	104 mice
Test Article(s)*		RAT-19 cells: 1:1 mixture of LTG118- and LTG119 lentiviruses transduced human CD4+ and CD8+ T cells derived from a leukapheresis of a healthy donor, with 70-80% transduction efficiency and 0.2-5 copies/cell
Control Article(s)		Mock-transfected T cells
Route of Administration		Intravenous route of administration
Description of the Disease/Injury Model and Implant Procedure		N/A
Study Groups and Dose Levels		RAT-19 cells to B-ALL tumor-bearing mice: Group 1 – $20x10^6$ cells/mouse Group 2 – $5x10^6$ cells/mouse Group 3 – $1x10^6$ cells/mouse Mock cells to B-ALL tumor-bearing mice: Group 4 - $20x10^6$ cells/mouse Group 5 - $5x10^6$ cells/mouse Group 6 - $1x10^6$ cells/mouse Control Groups: Group 7 - Uninjected B-ALL tumor-bearing mice (n=4) Group 8 - RAT-19 ($20x10^6$ cells/mouse) injected to non-tumor-bearing mice (n=4)
Dosing Regimen		Single administration
Randomization		Yes
Description of Masking		Not provided
Scheduled Sacrifice Time Points		At 3 and 5 weeks post-T cell implantation. Later sacrifice time points occurred once T cells were not detected in blood samples or animal became moribund

Note: The term 'RAT-19 cells' was used interchangeably with CART-19 cells which later became CTL019. LTG118 expresses the scFv aCD19-CD3-s chimeric immunoreceptor, while LTG119 expresses the scFv aCD19-CD3s-4-1BBL chimeric immunoreceptor. The exact clinical construct was used in the studies and the vectors were research grade. Three vector lots were combined and used to transduce T cells. Greater than 95% of cultures at day 3 and at the end of culture were CD4/CD8 T cells, with 85% and 79% expressing the two constructs, respectively, and vector copy number >3 copies/cell.

Comment:

- Study #12 states that "The vendor described animals as (b) (4) ." Thus, the study animals should be (b) (4) mice.

Key Evaluations and Assessments:
Mice were monitored twice weekly for weight, general appearance, and behavioral abnormalities. Starting at day 70 post-T cell implantation, blood samples were collected biweekly to evaluate peripheral engraftment of human cells by flow cytometry and PCR. Clinical pathology and macroscopic pathology on 13 tissues were conducted upon sacrifice. Microscopic pathology, immunohistochemistry (IHC, for CD3+ cells), and PCR (sensitivity ≤ 50 copies/μg DNA) for biodistribution (BD) were conducted on samples of inguinal lymph nodes, lung, heart, kidney, bone marrow, liver, gut, skin, blood (by flow cytometry for CD3+ cells), spleen, gonads and brain collected at sacrifice.

Key Results:
Mortality:
There were 4/104 mice found dead; none of them were necropsied due to autolysis (2 mice each in Groups 1 and 3). A total of 46/104 mice were sacrificed due to development of GvHD, progression of B cell tumors or aging (designated as terminal sacrifice).

Comment:

- According to Study #12, 3/4 deaths occurred in Group 8 and 1/4 in Group 3.

Clinical observations:
Animals were monitored for behavior, posture, skin and general observations. The majority of observations noted were of similar incidence and severity across Groups 1-6, and were consistent with the development of xenograft GvHD (xGvHD) or tumor growth. Development of xGvHD was accompanied by hair loss, hunched posture, decreased activity and weight loss. Tumor growth was accompanied by weight gain, lethargy, and (at times) ruffled fur.

Body weights:
No significant differences in body weight gains across the study groups were observed.

T cell engraftment:
Engraftment was cell-dose dependent in each the CART-19 and Mock treated animals. Engraftment in general was greater in the mock groups, which correlated with GvHD and mononuclear infiltration as described in the histopathology report. The level was similar to engraftment of CD4+ and CD8+ T cells.

Comment:

- According to Study #12, the days for measuring cell engraftment should be 42, 56, 64, 78, 135 and 203.

Leukemia burden:
B-ALL burden was measured by (b) (4) analysis at days 35, 49, 57, 70, 135, and 203. The results showed that the lowest dose level of CART-19 cells and mock T cell (Groups 3 and 6) did

not control B-ALL proliferation. At the medium dose level, the CART-19 cell group (Group 2) reduced tumor burden by approximately 18-fold greater than the mock group (Group 5). At the highest dose level, the mock animals (Group 4) died from GvHD before any anti-tumor effect could be evaluated, while the CART-19 cell group (Group 8) survived. After day 70, control of tumor burden decreased, which appears to correlate with levels of T cell engraftment at these time points.

Comment:

- According to Study #12, the days for measuring tumor burden should be 42, 56, 64, 78, 135 and 203.

Clinical pathology:

- Statistically significant high levels of AST and ALT, low levels of cholesterol, albumin, and triglycerides were noted in Group 4 as compared to Group 1. These findings may relate to the sickness present in Group 4 such as GvHD and mononuclear infiltration.
- Increased WBCs observed in Groups 1 to 6, appear to be related to the presence of the B cell tumor and the engrafted T cells. There were no significant differences in any other hematology parameter between CART-19 and mock groups.

Comment:

- According to Study #12, the statistical analysis was likely to be invalid for the comparison between Groups 1 and 4, since the individual chemistry data includes the 3 deaths as Group 1, when they were actually Group 8 animals. In addition, measuring days noted as 35 and 49 appear to be erroneous, and should be days 42 and 56 as per the protocol.

Macroscopic pathology:
Macroscopic pathology findings were mostly related to changes in the liver and spleen relating to B-ALL growth or T cell infiltration associated with GvHD. No significant differences in the incidence were observed across study Groups 1-8.

Microscopic pathology:
The pathology report was signed by (b) (6) .
The report included four sacrifice codes: scheduled sacrifices at days 42-43 (S1) and days 55-57 (S2), unscheduled death/sacrifice (UD), and final sacrifice at termination of study (FS). Below are the most notable findings:

Group 8 (CART-19 T cell only without B-ALL, 3 males/group) –

- One male sacrificed at S1 showed infiltrations of uncharacterized mononuclear cells in the kidneys, around the vascular tracts of the lungs, in the periportal and centrilobular regions of the liver, diffusely in the spleen, and multifocally in the pancreas. The liver had lymphocytic leukocytosis, consisting of an increased population of lymphocytes within hepatic sinusoids. The heart had moderate thrombosis. These findings were considered a result of GvHD.

- Two unscheduled deaths (UD males) showed mononuclear cell infiltrations similar to the S1 sacrifice mice. A total of 1/2 males showed lymphocytic leukocytosis in the liver. Both livers had pigment accumulation and mild periportal fibrosis, indicating a chronic pathologic process. The pinna of both males had mild single-cell necrosis and their skin had single-cell necrosis with additional findings of acanthosis and escharotic exudate (inflammatory crust formation). One male had hyperkeratosis. These findings were associated with tumor cell infiltration/growth.

Group 7 (B-ALL only, 4 females/group) –

- A total of 3/4 females had minimal or mild mononuclear cell infiltration in the kidneys. All mice had moderate to severe accumulations of intravascular leukemic cells and minimal to mild infiltration of tumor cells. Severe tumor cell infiltration was present in the spleen (4/4) and the bone marrow (1/4). Minimal to mild infiltration of uncharacterized mononuclear cells were present in the pancreas (2/4), the ovary (1/4), and the pinna (1/4).

Groups 1-3 (B-ALL + CART-19 T cell) *versus Groups 4-6* (B-ALL = mock T cells) –

- S1 sacrifice: mononuclear cell infiltration in various organs was more pronounced in the mice that received the high-dose level of mock T cells than in the group that received high dose CART-19 cells. Hepatic lymphocytic leukocytosis was more pronounced in mice receiving mock T cells than those receiving CART-19 cells at the three dose levels.

- S2 sacrifice: mononuclear cell infiltration in multiple organs and hepatic lymphocytic leukocytosis was more pronounced in mice receiving mock T cells than in those receiving CART-19 cells. Tumor cell infiltration in the spleen and bone marrow and hepatic intravascular (leukemic) tumor cells were present in mice that received both mock and CART-19-T cells at the low dose level.

- UD: tumor cell infiltration in various organs was more pronounced in mice that received the low dose of CART-19 cells or mock T cells. Inflammatory lesions consistent with GvHD were more pronounced in mice that received the high dose level of both CART-19-T and mock T cells compared to the low dose groups.

- FS: infiltrating/proliferating tumor cells persisted in some organs of the surviving animals in Groups 1, 2, 4, 5 and 6.

The pathology report concluded that there was no histologic evidence of systemic toxicity associated with CART-19-T as compared to mock T cells. Administration of CART-19 cells only was associated with histologic changes consistent with GvHD in multiple tissues. Administration of ALL cells only resulted in tumor cell infiltration/proliferation in multiple tissues.

Persistence of CART-19 cells in tissue by PCR:
The PCR analysis was conducted using various tissues from each group that received CART-19 cells (Groups 1-3).

Comment:

- The data analysis contains errors, for example, αCD19-ζ transduced cells were compared with the same αCD19-ζ transduced cells. Study #12 re-analyzed the data and made the correction based on Novartis' analysis. Thus, please see Study #12 for detailed results of CART-19 persistence.

Human cell infiltration by IHC:
Only sections from animals that received CART-19 cells were analyzed by IHC against human CD3. The results showed that positive IHC staining was present in multiple tissues (e.g., skin, kidney, liver, lung, spleen, bone marrow) of the mice in a dose-related pattern.

Summary of Report Conclusions:
There was no histologic evidence of systemic toxicity associated with CART-19 cells versus mock T cells. No abnormal cell growth was detected by clinical pathology or by PCR at any time point in any animals. There were no indications of uncontrolled cellular proliferation in the CART-19 cell groups over the mock T cell groups. A CART-19 cell dose-dependent control of B-ALL tumor was observed.

Study #12
Novartis provided this report, titled "Evaluation of CART-19 biotoxicity and biodistribution in Human Immune-reconstituted (b) (4) mice. Protocol (b) (4) . Final report amendment no. 1". It is a re-analysis of the resulting data from Study #11. The re-analyzed portion and the key amendment to the original information, in addition to the corrections made to specific data that have been stated along with Study #11, are provided below.

Key Evaluations and Assessments:
The amendment described errors in the:
1. Description of the study design
2. Study Day associated with certain activities
3. Individual PCR data
4. Summary Tables of the PCR data
5. Assignment in animals to Dose Group for certain analyses

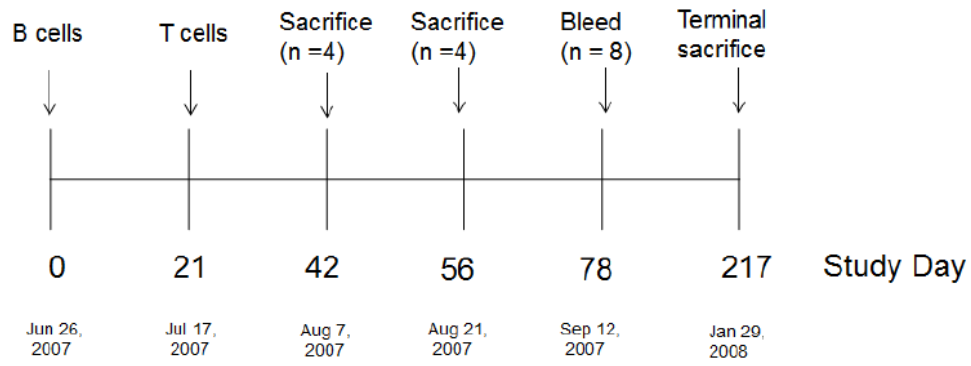
The amendment stated that, 'These changes do not alter the interpretation or conclusion of the original report in any way and is not considered to have affected the integrity of the study.'

Key Results: Other key corrections made by Novartis are described below:

Key Amendment to Section 4 (Study Methods) of the original report (OR; Study #11)

- Table 2-2 lists relationship between calendar date, nominal study day and recalculated study day for key scheduled activities.

Figure 2-1 Study timeline overview



After the second schedule sacrifice on Day 56, animals were monitored and sacrificed as required by IACUC guidelines as GVHD developed. Beginning on Day 70, remaining animals were bled periodically to evaluate persistence of vector modified cells. A final sacrifice on days 196-198 post T cell infusion was carried out due to the animal age to avoid unexpected age-related deaths that would prevent full necropsy.

- The assignment of animals to dose groups was amended.
- The staggering dates for tumor cell injection were amended.

Key Amendment to Section 5 (Executive Summary) of the OR:

- Results from the reanalysis of persistence of CART-19 cells in tissues by PCR
  - Similar patterns of biodistribution were observed by both the αCD19-BBζ and αCD19-ζ transduced cells. A preferential survival of the αCD19BBζ cells over the αCD19-ζ cells was observed.
  - On day 42 post-injection, CART-19 cell signals were detected in the spleen, lung, kidney, and brain in Group 1 mice only. On day 56 post-injection, CART-19 cell signals were detected in these same tissues plus the liver, heart, blood and bone marrow in Groups 1 through 3 with more frequency in Group 1 as compared to Groups 2 and 3. During days 65 to 146, the cell signals were detected in the Group 1 tissues (spleen, lung, liver, kidney, brain, heart blood and bone marrow). No cell signals were detected on day 203, while cell signals were detected in the spleen, liver, kidney, and bone marrow in Group 2 mice on day 217.
- Allocation of two animals to Groups 7 and 8 (control groups) were amended for the evaluation of human cell infiltration by IHC.
- The number of animals of Group 8 (total 3 instead of 4) undergoing gross pathology was amended.

Comment:

- The amended information has little impact on the study conclusion.

APPLICANT'S PROPOSED LABEL

Section 8 ('Use in Specific Populations') should be revised to comply with 21 CFR 201.56(d)(1), 201.57(c)(9), and 201.57(c)(14). Statements made regarding risks to the fetus should be removed since no preclinical, clinical, or clinical pharmacology data exists to support them.

Section 12.1 ('Mechanism of Action') should be supported by appropriate references.

Section 13 ('Nonclinical Toxicology') should be revised with appropriate wording.

CONCLUSION OF NON-CLINICAL STUDIES

Review of the non-clinical studies did not identify any safety concerns that could not be adequately addressed in labeling (see above recommendations regarding the label). The nonclinical data support approval of the license application.

KEY WORDS/TERMS

Tisagenlecleucel, KYMRIAH™, CTL019, CART-19 cells, chimeric antigen receptor