I concur with this review. M. Serabian 9/09/20
I concur with this review. S. Sanduja 9/09/20

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

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DATE REVIEW COMPLETED:	April 10, 2020; amended June 22, 2020; amended August 4, 2020; amended August 8, 2020; amended September 9, 2020
PRODUCT:	Lisocabtagene maraleucel (BREYANZI®; JCAR017)
APPLICANT:	Juno Therapeutics, Inc., a Celgene Company
PROPOSED INDICATION:	Treatment of adult patients with relapsed or refractory (R/R) large B-cell lymphoma after at least 2 prior therapies.
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EXECUTIVE SUMMARY:

BREYANZI® (lisocabtagene maraleucel; JCAR017) is a cell suspension consisting of autologous human CD4+ and CD8+ T cells that are engineered with a lentiviral vector encoding a chimeric antigen receptor (CAR) consisting of an anti-CD19 single-chain variable fragment (scFv) linked to 4-1BB and CD3ζ t cell activating domains. JCAR017 also includes a non-functional truncated epidermal growth factor receptor (EGFRt) that is co-expressed on the cell surface with the CD19-specific CAR.

In vitro pharmacology studies support the purported mechanism of action of JCAR017 by showing cytokine release, CAR T cell expansion, and tumor cell cytotoxicity following exposure to CD19-expressing cancer cells. Additional *in vitro* studies showed that JCAR017 in combination with various small molecule drugs, such as (b) (4), may enhance CAR T cell expansion and anti-tumor activity. Dose-dependent anti-tumor activity and improved animal survival were demonstrated in immunocompromised rodent models engrafted with CD19-expressing tumor cells following administration of JCAR017.

Animal studies to assess the long-term safety of JCAR017 were limited because the JCAR017 does not survive in immunocompetent rodents and induces graft-versus-host disease in immunocompromised mice. (b) (4) studies and (b) (4) against a panel of normal human tissues reflected the antigen specificity of JCAR017, with on-target/off-tumor and aspecific recognition against off-target tissues of low safety concern. *In vivo* studies in tumor-bearing murine models showed that JCAR017 is significantly reduced following administration of cetuximab (ERBITUX®, a commercially available anti-EGFR antibody). These data provide support for administration of commercially available antibodies targeting the truncated EGFR domain on the surface of JCAR017 to reduce/eliminate the CAR T cells *in vivo*, if necessary.

The risk of insertional mutagenesis with lentiviral transduction of the T cells, leading to malignant transformation, was studied using unbiased, genome-wide bioinformatics methods. The resulting data show that the lentivirus used to manufacture JCAR017 does not preferentially integrate at specific genomic sites of concern for oncogenic transformation. In addition, long-term cellular growth assays showed that the purity and identity profile of JCAR017 was comparable to the profile on Day 1, suggesting that no shift in cellular phenotype due to clonal expansion occurs over time. These data support the conclusion that any insertional events resulting from lentiviral transduction methods used to generate JCAR017 have minimal risk for oncogenic transformation.

Traditional genotoxicity assays and carcinogenicity assessments were not performed for JCAR017. Due to the lack of survival of JCAR017 in immunocompetent rodents, traditional animal developmental and reproductive toxicity (DART) studies were not conducted. However, Section 8.1 of the proposed label describes the potential risks of JCAR017 to the developing embryo or fetus if the transduced cells cross the placenta following administration in women of childbearing potential.

PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:

There are no nonclinical deficiencies identified in this submission. There are no outstanding requests for additional nonclinical data for evaluation of JCAR017. The nonclinical information provided in the BLA submission supports approval of the licensure application.

Formulation and Chemistry:

JCAR017 consists of autologous CD8+ and CD4+ T cell components that are separately activated and genetically modified using a replication incompetent lentiviral vector to express a CD19-specific chimeric antigen receptor (CAR). The CAR is comprised of a single chain variable fragment (scFv) binding domain derived from a murine CD19-specific monoclonal antibody (mAb; FMC63), an IgG4 hinge region, CD28 transmembrane domain, 4-1BB (CD137) costimulatory domain, and the CD3ζ activation domain. In addition, JCAR017 includes a non-functional EGFRt that is co-expressed on the cell surface with the CD19-specific CAR.

The transduced T cells are expanded in cell culture, washed, formulated into a suspension, and cryopreserved as separate CD8 and CD4 component vials (each containing (b) (4) CAR+ viable T cells/mL) that together constitute a single dose of BREYANZI®. Refer to the CMC review memos for more details regarding the drug substance and drug product.

(b) (4)

(b) (4)

(b) (4)

Abbreviations

4-1BB	CD137; member of the TNF-receptor superfamily
ALL	acute lymphoblastic leukemia
anti-EGFR	antibody to extracellular portion of EGFR
AP-1	activator protein-1
CAR	chimeric antigen receptor
CD	cluster of differentiation
CLL	chronic lymphocytic leukemia
DLBCL	diffuse large B cell lymphomas
E:T	effector-to-target
EGFRt	truncated human epidermal growth factor receptor
FL	follicular lymphoma
FMC63	CD19-scFv binder found in JCAR017
GLP	good laboratory practice

IDO	indoleamine 2,3-doxygenase I
IFN-γ	interferon-gamma
IL-2	interleukin-2
IL-2Rγ	interleukin-2 receptor gamma
ITAM	immunoreceptor tyrosine-based activation motif
K_d	dissociation constant
mAb	monoclonal antibody
MCL	Mantle cell lymphoma
NFAT	nuclear factor of activated T-cells
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NHL	non-Hodgkin’s lymphoma
NOD	non-obese diabetic
PD	pharmacodynamic
PD-1/L1	programmed death receptor 1/programmed death ligand 1
PHKB	phosphorylase kinase regulatory subunit beta
PK	pharmacokinetic(s)
PMBCL	primary mediastinal B-cell lymphoma
R/R	relapsed/refractory
RNA	ribonucleic acid
scFv	single-chain variable fragment
SCID	severe combined immune deficient
SNP	single nucleotide polymorphism
SPR	surface plasmon resonance
TNF-α	tumor necrosis factor-alpha

Related Files

IND #16506; ‘Autologous CD4+ and CD8+ T cells (JCAR017) Transduced with Self-Inactivating (SIN) Lentiviral Vector (b) (4) Encoding CD19-specific Chimeric Antigen Receptor (CAR) and Truncated Epidermal Growth Factor Receptor (EGFRt); Following Fludarabine and Cyclophosphamide (Lisocabtagene Maraleucel)’; Juno Therapeutics; r/r B-cell lymphoma; ACTIVE

IND #17501; ‘Autologous CD4+/CD8+ T Cells Expressing CD19 Chimeric Antigen Receptor(JCAR017)’; Celegene Corp.; r/r aggressive B-cell non-Hodgkin lymphoma (NHL); ACTIVE

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INTRODUCTION

Patients with B-cell malignancies often relapse or have refractory disease that remains incurable. The administration of anti-CD19 CAR T cells has resulted in efficacy and an acceptable safety profile in subjects with NHL (which includes diffuse large B cell lymphomas [DLBCL], Mantle cell lymphoma [MCL], and follicular lymphoma [FL]), chronic lymphocytic leukemia (CLL), and acute lymphoblastic leukemia (ALL).[1,2,3] NHL is the seventh most common cancer in the US[4], and results in approximately 20,000 deaths per year. CLL is a hematologic cancer characterized by accumulation of clonally-derived CD19 positive B-lymphocytes in lymphatic tissues, blood, and bone marrow. Currently, CLL is considered 'incurable' and patients will eventually relapse.[5] ALL is characterized by the proliferation of immature lymphoid cells in the bone marrow, peripheral blood, and other organs. In the US, approximately 6,000 ALL cases are diagnosed per year, with an estimated 1,400 deaths annually.[6] Long-term, disease-free survival occurs in less than 10% of patients, with a median time of survival of less than 6 months.[7,8]

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[1] Hay KA, Turtle CJ. Chimeric Antigen Receptor (CAR) T Cells: Lessons Learned from Targeting of CD19 in B-Cell Malignancies. Drugs. 2017 Mar;77(3):237-245.

[2] Brudno JN. Safety and feasibility of anti-CD19 CAR T cells with fully human binding domains in patients with B-cell lymphoma. Nat Med. 2020 Feb;26(2):270-280.

[3] Abramson JS.Anti-CD19 CAR T-Cell Therapy for B-Cell Non-Hodgkin Lymphoma. Transfus Med Rev. 2020 Jan;34(1):29-33.

[4] SEER. (2016). "Surveillance, Epidemiology, and End Results (SEER) Cancer Statistics Factsheets: Chronic Lymphocytic Leukemia." Retrieved May 2016, from http://seer.cancer.gov/statfacts/html/clyl.html.

[5] Sharma S, Rai KR. Chronic lymphocytic leukemia (CLL) treatment: So many choices, such great options. Cancer. 2019 May 1;125(9):1432-1440.

[6] ACS. Cancer Facts and Figures 2014. 2014; Atlanta, American Cancer Society.

[7] Fielding AK, Richards SM, Chopra R, et al. Outcome of 609 adults after relapse of acute lymphoblastic leukemia (ALL); an MRC UKALL12/ECOG 2993 study. Blood 2007;109(3):944-950

[8] Kantarjian HM, Thomas D, Ravandi F, et al. Outcome of adults with acute lymphocytic leukemia in second or subsequent complete remission. Leuk Lymphoma 2010;51(3):475-480

CD19 is a well-established target for selectively destroying B-cell malignancies and is not expressed on normal tissues or hematopoietic stem cells.[9,10,11] JCAR017 is an engineered CAR T cell therapy directed against CD19-expressing B cells. CAR binding to CD19 expressed on the cell surface of tumor and normal B cells induces activation and proliferation of CAR T cells, release of pro-inflammatory cytokines, and cytotoxic killing of target cells. In clinical studies, administration of CD19-targeted CAR T cells has demonstrated encouraging anti-leukemic activity in adult and pediatric patients with R/R B-cell ALL and other B-cell malignancies.[12] In addition, JCAR017 expresses a truncated EGFR on the surface. This allows for targeting of the CAR T cells with an exogenously administered anti-EGFR antibody, resulting in depletion of the cells, should significant toxicity occur.

NONCLINICAL STUDIES

PHARMACOLOGY STUDIES

Summary List of Pharmacology Studies

The following pharmacology studies were conducted to support the rationale for administration of JCAR017 to treat the proposed clinical indication.

In Vitro Studies

Study Number	Study Title	Report Number
1	*In Vitro* Analysis of Lisocabtagene Maraleucel (JCAR017) Activity Across CD19-expressing Target Cell Lines	RPT-001113
2	*In Vitro* Cytolytic Activity of Patient-Derived Lisocabtagene Maraleucel	RPT-001114
3	*In Vitro* Growth and Viability of NHL Patient-Derived Lisocabtagene Maraleucel CD4+ and CD8+ T Cells Following 6 Days of CAR Stimulation	RPT-001115
4	*In Vitro* Activity of Lisocabtagene Maraleucel Across Follicular Lymphoma-derived Cell Lines	RPT-001116
5	Activity of Lisocabtagene Maraleucel Across Primary Mediastinal B-cell Lymphoma-derived Cell Lines	RPT-001117
6	Lisocabtagene Maraleucel Chimeric Antigen Receptor Intracellular Domain Mechanism of Action	RPT-001183
7	FMC63 Binder Characterization/Epitope Analysis	RPT-001244

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[9] Davila ML, Brentjens R, Wang X, et al. How do CARs work? Early insights from recent clinical studies targeting CD19. Oncoimmunology 2012;1(9):1577-1583.

[10] Li YS, Hayakawa K and Hardy RR. The regulated expression of B lineage associated genes during B cell differentiation in bone marrow and fetal liver. Journal of Experimental Medicine 1993;178(3):951-960.

[11] Li YS, Wasserman R, Hayakawa K, et al. Identification of the earliest B lineage stage in mouse bone marrow. Immunity 1996;5(6):527-535.

[12] Davila ML and Sadelain M. Biology and clinical application of CAR T cells for B cell malignancies. Int J Hematol 2016;104(1):6-17.

Study Number	Study Title	Report Number
8	Characterization of CD19-specific CART cell Product Qualification Runs: CD19-Specific Cytolytic Activity of CD4/EGFRt-Enriched and CD8/EGFRt-enriched T Cells Transduced with the (b) (4) Batch Preparation of the CD19 scFv-lgG4 Hinge-CD28tm/4-1BB(b) (4)-EGFRt_(b) (4) Lentiviral Vector	SR0005
9	Characterization of CD19-specific CAR T Cell Product Qualification Runs: CD19-Specific Cytolytic Activity of CD4/EGFRt-Enriched and CD8/EGFRt-Enriched T Cells Transduced with the (b) (4) Batch Preparation of the CD19scFv-lgG4 Hinge-CD28tm/4-1BB-(b) (4)- EGFRt(b) (4) Lentiviral Vector Following Re-stimulation and Expansion	SR0007
10	Characterization Of CD19-Specific CAR T Cell Product Qualification Runs: Cell Surface Phenotype and Cytokine Production Analysis of CD4/EGFRt-Enriched and CD8/EGFRt-Enriched T Cells Transduced with the (b) (4) Batch Preparation Of The CD19 scFv-Lgg4 Hinge-CD28tm/4-1BB-(b) (4) EGFRt_(b) (4) Lentiviral Vector Following Re-Stimulation and Expansion	SR0008
11	JCAR017 CD19 Species Cross-Reactivity Analysis	RPT-0279
12	CD19 Protein Sequence Alignment and Homology Comparison Across Human, Non-Human Primate, and Mouse	RPT-0332
13	CD19 Expression in Human Tumors and Normal Tissues	RPT-0333
14	Assessment of FMC63 scFv-Fc Binding Profile Using a (b) (4)	RPT-0365
15	A Tissue Cross-Reactivity Study of (b) (4) in Normal Human Tissues	RPT-0479
16	*In Vitro* Pharmacology Study of (b) (4) or Lenalidomide in Combination with JCAR017 Cell Product	RPT-001118
17	*In Vitro* Functional Evaluation of Epacadostat in Combination with Lisocabtagene Maraleucel Cell Product	RPT-001119
18	Nonclinical Evaluation of Anti-PD-L1 Antibody (Durvalumab) Combination with JCAR017 Cell Product	RPT-17002
19	*In Vitro* Functional Evaluation of JCAR017 in Combination with Ibrutinib or Acalabrutinib	RPT-17004
20	Method Qualification of an (b) (4) to Quantify Cetuximab in Mouse Serum	CC-DISC-DMPK-3415
21	Epidermal Growth Factor Receptor Expression by Immunohistochemistry in JCAR017 Cells, Colorectal Carcinoma, and Normal Human Tissues	RPT-H-1195
22	(b) (4) Assay for the Detection of Human JCAR017 Cells in Mouse Whole Blood Specimens	XIBC001STU

In Vivo Studies

In Vivo Studies in Tumor Xenograft Animal Models

Study Number	Study Title	Report Number
23	Efficacy Evaluation of JCAR017 Processes (b) (4) Mice Engrafted with Raji Burkitt's Lymphoma Cells	RPT-0433
24	Characterization of CD19-Specific CAR T Cell Preclinical Production Runs: *In Vivo* Analysis of Tumor Growth and Survival Post-Treatment with EGFRt-Enriched CD4 and CD8 T Cells Transduced with the (b) (4) Batch Preparation of CD19scFV-IgG4 hinge-CD28/41BB-(b) (4)-EGFRt (b) (4)	SR0006
25	Efficacy Evaluation of JCAR017 in Combination with Ibrutinib or Acalabrutinib in (b) (4) Mice Engrafted with Nalm-6 Tumor Cells	RPT-17005

Study Number	Study Title	Report Number
26	Elimination of JCAR017 T Cells with Cetuximab and Effect on Anti-tumor Efficacy in (b) (4) Mice Engrafted with Raji Burkitt's Lymphoma Cells	RPT-001479
27	Cetuximab-Mediated Ablation of JCAR017 in (b) (4) Mice Engrafted with Raji Burkitt's Lymphoma	RPT-002100
28	Pharmacokinetics of Erbitux in Female (b) (4) Mice Following a Single Intravenous and Intraperitoneal Administration	CC-DISC-DMPK-3291

Overview of Pharmacology Studies

The lot number for each CAR T cell product administered in each nonclinical study are provided in the table below.

(b) (4)

Reviewer's Comments:

- The version numbers are listed above as 'vX.Y' (X and Y = 0, 1, 2, 3, or 4). These numbers reflect the 'version' of the process used to manufacture the product. Please refer to the CMC review memos regarding product manufacturing details. A summary comparison between different versions of JCAR017 is also provided in Table 3 (page 15) in Module 2.3.P.CD8.

- Designation of ‘N/A’ for a lot number means that the study did not evaluate JCAR017. The *in vitro* studies focused on mechanistic aspects of various elements of JCAR017 or were *in silico*-based.

- The reports for *Studies #8-11* and *Study #24* did not identify the version(s) of JCAR017 studied.

Overview of In vitro Studies

Study #1 (RPT-001113)
In Vitro Analysis of Lisocabtagene Maraleucel (JCAR017) Activity Across CD19-expressing Target Cell Lines; conducted by Juno Therapeutics

JCAR017 was co-incubated with multiple tumor cell lines expressing different levels of cognate antigen CD19. The cancer cell lines chosen were derived from patients with human chronic myelogenous leukemia, Burkitt’s lymphoma, acute lymphoblastic leukemia, Non-Hodgkin’s Lymphoma, and mantle cell lymphoma. Negative control lines included mock transduced T cells and non-CD19 expressing tumor cell lines. CD19 was also genetically engineered into K562 cells (human chronic myelogenous leukemia), which does not naturally express CD19, providing a positive control.

Results demonstrated that JCAR017 was activated when co-incubated with the CD19-expressing cells, regardless of level of expression of CD19. Activation of JCAR017 was confirmed by measuring IFNγ, IL-2, and TNF-α levels using immuno-based cytokine assays, and by assessing upregulation of activation markers CD25 and CD69 by (b) (4) .

Study #2 (RPT-001114)
In Vitro Cytolytic Activity of Patient-Derived Lisocabtagene Maraleucel; conducted by Juno Therapeutics

NHL tumor cell lines (CD19+ and CD19-) were co-incubated with JCAR017 at effector-to-target (E:T) ratios of 2.5:1 and 5:1. The cytolytic function of CAR T cells was assessed by live cell imaging over the course of 6 days. Day 4 results showed JCAR017 cytolytic activity, killing 93% and 98% of CD19+ tumor cells at E:T ratios of 2.5:1 and 5:1, respectively. JCAR017 had no cytolytic activity on CD19- tumor cells.

Note: The data are consistent with the mechanism of action and function of the JCAR017.

Study #3 (RPT-001115)
In Vitro Growth and Viability of NHL Patient-Derived Lisocabtagene Maraleucel CD4+ and CD8+ T Cells Following 6 Days of CAR Stimulation; conducted by Juno Therapeutics

CD4+ and CD8+ cells obtained from two patients with NHL. The transduced cells were cultured alone (i.e., CAR expressing CD4+ cells only or CAR expressing CD8+ cells only) or in a 1:1 ratio, in the presence of an anti-idotype antibody to activate the CAR. Proliferation was assessed over 6 days by cell counting. Data were consistent across the patients: a 1:1 ratio of the transduced cells (i.e., JCAR017) resulted in maximal proliferation of CAR expressing cells compared to either transduced cell subset alone.

Study #4 (RPT-001116)
In Vitro Activity of Lisocabtagene Maraleucel Across Follicular Lymphoma-derived Cell Lines; conducted by Juno Therapeutics

This study design was similar to *Study #1*, with assessment of JCAR017 activity against five different follicular lymphoma (FL) cell lines. In addition, E:T ratios ranged from 0:1 to 10:1. Results showed that JCAR017 had cytolytic activity against the FL cell lines, which was not dependent on the CD19 expression level. In addition, the cytolytic activity generally correlated with increasing E:T ratio. Increased levels of IFN-γ, IL-2, and TNF-α were also observed.

Reviewer's Comment:
- Results from *Study #1* and *Study #4* imply that only a low threshold of CD19 expression on target cells is needed to induce JCAR017 cytolytic activity.

Study #5 (RPT-001117)
Activity of Lisocabtagene Maraleucel Across Primary Mediastinal B-cell Lymphoma-derived Cell Lines; conducted by Juno Therapeutics

JCAR017 was co-incubated with a panel of primary mediastinal B-cell lymphoma (PMBCL) tumor cell lines. The resulting data mirrored *Study #1* and *Study #4*, with measurable cytokine release, activation markers for JCAR017 (CD25 and CD69), and cytolytic activity at varying E:T ratios.

Study #6 (RPT-001183)
Lisocabtagene Maraleucel Chimeric Antigen Receptor Intracellular Domain Mechanism of Action; conducted by Juno Therapeutics

Experiments were conducted to confirm the putative mechanism of action of JCAR017. JCAR017 endodomains (CD3(b) (4) and 4-1BB) and the CD19-specific single-chain variable fragment (scFv) binding domain derived from the murine CD19-specific monoclonal antibody FMC63 were expressed in (b) (4)

(b) (4) was analyzed by (b) (4).

Data obtained showed that the CD3(b) (4) motifs are responsible for activation of (b) (4)

Note: The data are consistent with the expected mechanism of action of the CAR and endodomain constructs,[13,14,15] and demonstrates that JCAR017 coordinates both TCR and co-stimulatory pathway signaling, resulting in activity against CD19-expressing targets.

Study #7 (RPT-001244)
FMC63 Binder Characterization/Epitope Analysis; conducted by Juno Therapeutics

Anti-human (b) (4) were used to capture the FMC63 scFv Fc molecule that comprises the binding domain of JCAR017. Interaction of the binding domain was then assessed against recombinant CD19 by (b) (4). Results showed an estimated $K_d = 0.9 - 21$ nM, characterizing the binding affinity of the CAR towards CD19. Epitope mapping studies using (b) (4) were conducted to identify the CD19 binding epitope for FMC63. FMC63 bound to the amino acid sequences (b) (4)

***Reviewer's Comment*:**

- These results show that the binding domain of the TCR engineered into JCAR017 cells has high affinity towards the intended epitope.

Study #8 (SR0005)
Characterization of CD19-specific CART cell Product Qualification Runs: CD19-Specific Cytolytic Activity of CD4/EGFRt-Enriched and CD8/EGFRt-enriched T Cells Transduced with the (b) (4) Batch Preparation of the CD19 scFv-lgG4 Hinge-CD28tm/4-1BB-(b) (4 EGFRt_(b) (4) Lentiviral Vector; conducted by Seattle Children's Hospital

JCAR017 generated using GMP qualification runs, using a patient-derived source of T cells was evaluated for cytolytic activity against CD19-expressing tumor cells. Resulting data showed

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[13] Ashouri, JF, Weiss, A. Endogenous Nur77 Is a Specific Indicator of Antigen Receptor Signaling in Human T and B Cells. J Immunol 2017; 198: 657-668.

[14] Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. Nat Rev Immunol. 2013; 13: 227-242.

[15] Sanchez-Paulete AR, Labiano S, Rodriguez-Ruiz ME, Azpilikueta A, Etxeberria I, Bolaños E, et al. Deciphering CD137 (4-1BB) signaling in T-cell costimulation for translation into successful cancer immunotherapy. Eur J Immunol. 2016; 46: 513-522.

[16] Geysen M, et al (1984). Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid. PNAS, July 1, 1984 vol. 81 no. 13 3998-4002.

similar *in vitro* activity as previously demonstrated in *Study #1* and *Study #5* in which JCAR017 that was generated using laboratory scale manufacturing.

Study #9 (SR0007)
Characterization of CD19-specific CAR T Cell Product Qualification Runs: CD19-Specific Cytolytic Activity of CD4/EGFRt-Enriched and CD8/EGFRt-Enriched T Cells Transduced with the (b) (4) *Batch Preparation of the CD19scFv-lgG4 Hinge-CD28tm/4-1BB-*(b) (4) *EGFRt_*(b) (4) *Lentiviral Vector Following Re-stimulation and Expansion; conducted by Seattle Children's Hospital*

This study design was similar to *Study #8*, except that the transduced cells were (b) (4) (CD4+ and CD8+ cells): 1) JCAR017 CD8+ cells were (b) (4) in the presence of IL-2/IL-15 and 2) JCAR017 CD4+ cells were (b) (4) with IL-7/IL-15. Results showed that the CAR T cells displayed cytolytic activity against CD19-expressing tumor cells.

Reviewer's Comments:

- The JCAR017 manufacturing method used in *Study #9* differed from the method used for *Study #8*, due to the (b) (4). Presumably this change was intended to (b) (4) of JCAR017 in each lot. It is unclear why different (b) (4) were used for CD4 and CD8 cells. According to Table (page 15) in Module 2.3.P.CD8, these cytokines are used in the clinical manufacturing process.

- The T cells used to generate JCAR017 product tested in *Study #8* and *Study #9* were obtained from the same (b) (4).

Study #10 (SR0008)
Characterization Of CD19-Specific CAR T Cell Product Qualification Runs: Cell Surface Phenotype and Cytokine Production Analysis of CD4/EGFRt-Enriched and CD8/EGFRt-Enriched T Cells Transduced with the (b) (4) *Batch Preparation Of The CD19 scFv-Lgg4 Hinge-CD28tm/4-1BB-*(b) (4)*-EGFRt_*(b) (4) *Lentiviral Vector Following Re-Stimulation and Expansion; conducted by Seattle Children's Hospital*

JCAR017 was manufactured similar to the method used in *Study #9*. Resulting data showed that JCAR017 retained transgene expression, cytolytic potency, and cytokine release characteristics when (b) (4) with CD19-expressing cells.

Note: The study concluded that the cryopreserved JCAR017 retained their desired activity and phenotype following thawing, expansion, and re-stimulation.

Study #11 (RPT-0279)
JCAR017 CD19 Species Cross-Reactivity Analysis; conducted by Juno Therapeutics

JCAR017 was (b) (4) with CD19-expressing cells collected from healthy humans, mice, and nonhuman primates (NHPs). Cytotoxicity data showed that JCAR017 was cytotoxic only against human CD19. (b) (4) data correlated with the cytotoxicity results, demonstrating that the recognition domain of the CAR specifically recognized human CD19. CD-19-expressing cells obtained from NHPs and mice were not recognized. In addition, a competition assay using a monoclonal antibody specific for human CD19 was performed. Data from competition assays showed that scFv binding to human CD19 was nearly abrogated when in competition with an excess of anti-CD19 mAb.

Reviewer's Comment:

- The results for this study imply that studies in immune competent mice and NHPs to assess the safety profile of JCAR017 are not relevant. The high specificity of JCAR017 for human CD19 likely restricts conduct of *in vivo* animal studies to evaluation in an immunocompromised rodent model engrafted with a human tumor cell line expressing CD19.

Study #12 (RPT-0332)
CD19 Protein Sequence Alignment and Homology Comparison Across Human, Non-Human Primate, and Mouse; conducted by Juno Therapeutics

Protein sequence alignments of CD19 across human, mouse, and NHP mouse were compared using an informatics based approach. Human and mouse sequences were found to be 67% identical overall and 58% identical in the extracellular domain. NHP sequences were 92% identical to the human sequence and 88% identical to the human extracellular domain. Results correlate with the data in *Study #11* and suggest that the human CD19-targeting scFv of JCAR017 will not bind to NHP or mouse CD19.

Study #13 (RPT-0333)
CD19 Expression in Human Tumors and Normal Tissues; conducted by Juno Therapeutics

Expression (b) (4) variant assessments for CD19 were evaluated in 50 normal tissues using (b) (4) and informatics analyses. Overall, CD19 was expressed at low levels in most hematopoietic cells and in other normal tissues with the exception of B-lymphocytes. Additional informatics assessments using publicly available sets of data re-confirmed that: 1) CD19 expression is present on B-cell lymphomas and 2) there does not appear to be tumor associated isoforms of CD19 that would potentially diminish CAR T cell recognition due to antigen loss. (b) (4) analysis also suggested that CD19 expression is not altered by germline or somatic cell mutations that may alter antigen presentation. No specific SNPs were statistically enriched from the tumor data sets.

Reviewer's Comment:

- The informatics results suggest that there does not appear to be evidence that selective pressures leading to tumor resistance would be a significant problem (i.e. selection of tumor cells with (b) (4) in CD19 that block recognition by JCAR017), nor does there appear to be specific subpopulations of patients harboring B cell lymphomas that would have a higher probability of not responding to JCAR017.

Study #14 (RPT-0365)
Assessment of FMC63 scFv-Fc Binding Profile Using a (b) (4) ; conducted by (b) (4)

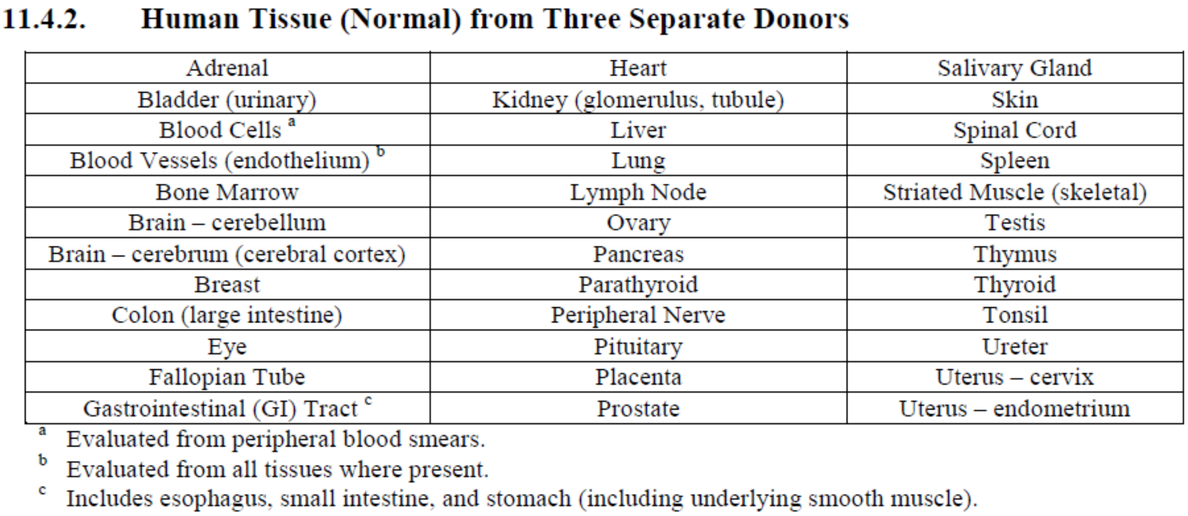
The scFv was screened against 4417 (b) (4) to look for evidence of potential off-target binding of the CAR recognition domain. Only one off-target was identified (phosphorylase kinase regulatory subunit beta; PHKB) as having weak-to-moderate interaction with the scFv.

Reviewer's Comment:

- The identified protein is an intracellular kinase, thus the risk for off-target CAR recognition towards PHKB is theoretically low. The investigator that conducted the (b) (4) stated that this finding was likely an artefactual result of the study.

Study#15 (RPT-0479)
A Tissue Cross-Reactivity Study of (b) (4) in Normal Human Tissues; conducted by (b) (4)

A GLP-compliant study was conducted to assess the potential for off-target cross-reactivity of: 1) (b) (4) , which are CD19-specific single chain variable region antibody fragments expressed as Fc fusion proteins, and 2) FMC63 against cryosections of the normal human tissues listed in Table 11.4.2:



11.4.2. Human Tissue (Normal) from Three Separate Donors

Adrenal	Heart	Salivary Gland
Bladder (urinary)	Kidney (glomerulus, tubule)	Skin
Blood Cells [a]	Liver	Spinal Cord
Blood Vessels (endothelium) [b]	Lung	Spleen
Bone Marrow	Lymph Node	Striated Muscle (skeletal)
Brain – cerebellum	Ovary	Testis
Brain – cerebrum (cerebral cortex)	Pancreas	Thymus
Breast	Parathyroid	Thyroid
Colon (large intestine)	Peripheral Nerve	Tonsil
Eye	Pituitary	Ureter
Fallopian Tube	Placenta	Uterus – cervix
Gastrointestinal (GI) Tract [c]	Prostate	Uterus – endometrium

[a] Evaluated from peripheral blood smears.
[b] Evaluated from all tissues where present.
[c] Includes esophagus, small intestine, and stomach (including underlying smooth muscle).

Source: Report No. RPT-0479, located in Section 11.4 in Module 4.2.1.2.rpt-0479 of the BLA.

Reviewer's Comment:

- Per the study report, the tissues tested in this study included all of the tissues on the "suggested list of human tissues to be used for immunohistochemical or cytochemical investigations of cross reactivity of monoclonal antibodies" in Annex I of the EMA document, *Guideline on Development, Production, Characterisation and Specifications for Monoclonal Antibodies and Related Products*, adopted by the Committee for Medicinal Products for Human Use (CHMP) (https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-development-production-characterisation-specification-monoclonal-antibodies-related_en.pdf), and all of the tissues recommended in the FDA Center for Biologics Evaluation and Research (CBER) document, *Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use* (https://www.fda.gov/media/76798/download).

Cross-reactivity evaluation was conducted using immunohistochemistry and scored by a board-certified veterinarian pathologist. (b) (4) , and FMC63 test articles were (b) (4) against CD19-positive K562 cells and non-CD19 expressing K562 cells (positive and negative controls, respectively).

(b) (4) test articles (b) (4) mononuclear leukocytes in the tissues with a morphology and/or localization consistent with B cells. (b) (4) in the epithelium of the adrenal cortex, placental trophoblasts, luteal cells of the ovary, decidual cells in the placenta, and interstitial cells of the testis, was also observed.

(b) (4) for FMC63 was also observed in the mononuclear leukocytes in the tissues with a morphology and/or localization consistent with B cells. However, (b) (4) FMC63 in liver hepatocytes, thymic Hassall's corpuscles, squamous epithelium in the esophageal mucosa, tonsil mucosa and crypts, and cervical mucosa, as well as extracellular material in the prostate, was also observed with weak-to-moderate or weak-to-strong intensity .

Reviewer's Comment:

- Per the study report (page 12), (b) (4) appear to be included in this study to support potential inclusion in a future investigational product. FMC63 is the focus for this BLA. Results suggest that there may be some off-target tissue cross-reactivity for FMC63. However, the data generated in *Studies #13-15* indicate a high selectivity towards CD19-expressing cells of the B cell lineage. Additionally, the truncated EGFR expressed extracellularly on JCAR017 can be targeted with exogenously administered anti-EGFR antibodies to deplete JCAR017 should toxicities occur.

Study #16 (RPT-001118)
In Vitro Pharmacology Study of (b) (4) or Lenalidomide in Combination with JCAR017 Cell Product; conducted by Juno Therapeutics

This study was performed to investigate the effect of the immunomodulating agents (b) (4) and lenalidomide on JCAR017 activity, phenotype, and expansion *in vitro*. (b) (4)

(b) (4). Lenalidomide is approved for the treatment of patients with multiple myeloma. Published literature data suggest that combination strategies with lenalidomide and CAR T cells may have pleiotropic effects beneficial for B-cell malignancies[17,18,19].

(b) (4)-derived T cells obtained from (b) (4) individuals were used to produce JCAR017. JCAR017 was (b) (4) or lenalidomide *in vitro*. Assessments showed the following changes compared to JCAR017 alone: 1) decreased expression of the protein (b) (4), 2) increased cytokine production, 3) inconsistent enhancement in cytolytic activity against CD19-expressing tumor cells, 4) general increase in expression of markers for activation and effector differentiation for both short-term and long-term cultures, and 5) addition of (b) (4) or lenalidomide improved cell counts in (b) (4) in the (b) (4) (b) (4) compared to controls with no small molecule conditioning.

Study #17 (RPT-001119)

In Vitro Functional Evaluation of Epacadostat in Combination with Lisocabtagene Maraleucel Cell Product; conducted by Juno Therapeutics

This study was performed to examine whether tryptophan depletion and kynurenine accumulation, through the indoleamine 2,3-dioxygenase pathway (IDO), inhibits JCAR017 proliferation[21,22,23]. A549.CD19 cells (a lung carcinoma cell line expressing CD19 and IDO-1) were co-cultured with JCAR017 Results showed that proliferation of JCAR017 was significantly inhibited. However, addition of epacadostat (a small molecule inhibitor of IDO-1[24,25]) to the co-culture restored JCAR017 proliferation, suggesting that tumors expressing IDO-1 may escape cytotoxicity by abrogating CAR T cell proliferation.

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[17] Sabrina Bertilaccio MT, Tettamanti S, Giordano Attianese GM, Galletti G, Arcangeli S, Rodriguez TV, et al. Low-dose lenalidomide improves CAR-based immunotherapy in CLL by reverting T-cell defects *in vivo* [abstract]. Blood. 2013; 122(21): 4171.

[18] Kuramitsu S, Ohno M, Ohka F, Shiina S, Yamamichi A, Kato A, et al. Lenalidomide enhances the function of chimeric antigen receptor T cells against the epidermal growth factor receptor variant III by enhancing immune synapses. Cancer Gene Ther. 2015; 22(10): 487-95.

[19] Wang X, Walter M, Urak R, Weng L, Huynh C, Lim L, et al. Lenalidomide enhances the function of CS1 chimeric antigen receptor redirected-T cells against multiple myeloma Clin Cancer Res. 2018; 24(1): 106-119.

[20] Ikaros is a transcription factor, that when inhibited, reduces thresholds for T cell activation, increases markers for T-cell activation, enhances survival of T cells, and increases cytokine production.

[21] Théate I van Baren N, Pilotte L, Moulin P, Larrieu P, Renauld JC, et al. Extensive profiling of the expression of the indoleamine 2, 3-dioxygenase 1 protein in normal and tumoral human tissues. Cancer Immunol Res 2015;3(2):161-172.

[22] Uyttenhove C, Pilotte L, Théate I, Stroobant V, Colau D, Parmentier N, et al. Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. Nat Med 2003;9(10):1269-1274.

[23] Vacchelli E, Aranda F, Eggermont A, Sautès-Fridman C, Tartour E, Kennedy EP, et al. Trial watch: IDO inhibitors in cancer therapy. Oncoimmunology 2014;3(10):e957994.

[24] Gangadhar TC, Hamid O, Smith DC, Bauer TM, Wasser JS, Olszanski AJ, et al., Epacadostat plus pembrolizumab in patients with advanced melanoma and select solid tumors: Updated phase 1 results from ECHO-202/KEYNOTE-037. Annals of Oncology, 1 October 2016; Volume 27, Issue suppl_6.

[25] Komiya T and Huang CH. Updates in the Clinical Development of Epacadostat and Other Indoleamine 2,3-Dioxygenase 1 Inhibitors (IDO1) for Human Cancers. Front Oncol. 2018 Oct 4;8:423.

Study #18 (RPT-17002)
Nonclinical Evaluation of Anti-PD-L1 Antibody (Durvalumab) Combination with JCAR017 Cell Product; conducted by Juno Therapeutics

Programmed death receptor 1(PD-1) is a T cell checkpoint receptor, that reduces T cell activation to inhibit T cell functionality. Tumors commonly express the ligand for PD-1 (PD-L1), enabling evasion of immune detection. (b) (4) of JCAR017 with (b) (4).CD19 cells (a tumor cell line expressing CD19 and high levels of PD-L1) resulted in reduced cytokine secretion and an alteration of cell surface activation markers on the CAR T cells. However, addition of durvalumab (an anti-PD-L1 monoclonal antibody) to the co-culture restored cytokine production and cell surface markers on JCAR017.

Reviewer's Comment:

- Data from this study suggest that JCAR017 can potentially be inactivated by the PD-1/PD-L1 pathway, and that tumors expressing PD-L1 may escape cytotoxicity when exposed to JCAR017. The results also imply that there may be enhanced effectiveness following administration of the combination of JCAR017 and an immune checkpoint inhibitor such as durvalumab.

Study #19 (RPT-17004)
In Vitro Functional Evaluation of JCAR017 in Combination with Ibrutinib or Acalabrutinib; conducted by Juno Therapeutics

Burton tyrosine kinase inhibitors (BTKi), such as ibrutinib and acalabrutinib, are common therapeutics used to treat B-cell malignancies.[26,27,28] JCAR017 (derived from (b) (4) was (b) (4) with (b) (4).CD19 cells and (b) (4) of ibrutinib or acalabrutinib for (b) (4). Resulting data showed enhanced long-term function (assessed by cytolytic activity and cytokine secretion out to (b) (4) of JCAR017 in the presence of the BTKi. Additionally, (b) (4) with the BTKi had no significant impact on JCAR017 proliferation.

Reviewer's Comments:

- Although not stated by the applicant, *Studies #16-19* were likely conducted to identify any potential synergism between JCAR017 and various small molecules that are either approved for treatment of various cancers or are under investigation for the treatment of B-cell malignancies.

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[26] Byrd JC, Harrington B, O'Brien S, Jones JA, Schuh A, Devereux S, et al. Acalabrutinib (ACP-196) in relapsed chronic lymphocytic leukemia. N Engl J Med. 2016; 374(4):323-332

[27] Davids MS, Brown JR. Ibrutinib: a first in class covalent inhibitor of Bruton's tyrosine kinase. Future Oncol. 2014; 10(6):957-967.

[28] Wilson WH, Young RM, Schmitz R, Yang Y, Pittaluga S, Wright G, et al. Targeting B cell receptor signaling with ibrutinib in diffuse large B cell lymphoma. Nat Med. 2015; 21(8):922-926.

- There are reported instances of B-cell malignancies capable of escaping cytotoxicity from anti-CD19 CAR T cell therapy because of developed resistance.[29,30] These studies provide insight into possible combination therapies to potentially administer when tumor resistance in patients is encountered.

Study #20 (CC-DISC-DMPK-3415)

Method Qualification of an (b) (4) to Quantify Cetuximab in Mouse Serum; conducted by (b) (4)

An (b) (4)-based assay for quantitation of cetuximab (anti-EGFR antibody) was developed for use in detection of this antibody in mouse serum samples. The assay was qualified for quantification of cetuximab levels of 7.81 ng/mL to 1000 ng/mL in mouse serum.

Study #21 (RPT-H-1195)

Epidermal Growth Factor Receptor Expression by Immunohistochemistry in JCAR017 Cells, Colorectal Carcinoma, and Normal Human Tissues; conducted by Celgene

Epidermal growth factor receptor (EGFR) expression in JCAR017 and in normal human peripheral blood mononuclear cells (PBMCs; negative control), colorectal cancer (CoCA) tissue samples, a multi-cancer (breast, colorectal, and lung carcinoma; positive control) block sample, and a normal human tissue microarray (consisting of (b) (4) tissues) (b) (4)) was determined using immunohistochemistry (IHC). EGFR (b) (4) in normal tissues was evaluated using a semiquantitative scoring criteria in which 1+ was considered weak (b) (4), 2+ moderate, and 3+ strong (b) (4), while CAR T cells and CoCA samples were evaluated by (b) (4) (b) (4) . The IHC results showed positive EGFR (b) (4) in JCAR017, no (b) (4) in the PBMCs, and (b) (4) consistent with EGFR expression in CoCA samples. EGFR (b) (4) in the normal human tissues was consistent with patterns of (b) (4) reported in the literature.[31]

Reviewer's Comments:

- In an appropriately designed study, JCAR017 (b) (4) positive for EGFR, providing evidence that this product is capable of expressing the truncated EGFR domain.

- No data were reported for the multi-cancer block. These samples, however, served as positive controls, and it is this reviewer's assessment that omission of these data does not notably impact overall study interpretation because cetuximab is administered to deplete JCAR017. In addition, *in vivo* studies described below (e.g. *Study #27*) demonstrate that cetuximab depletes JCAR017.

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[29] Shah NN and Fry TJ. Mechanisms of Resistance to CAR T Cell Therapy. Nat Rev Clin Oncol. 2019 Jun;16(6):372-385

[30] Jacoby E, Shahani SA, Shah NN. Updates on CAR T-Cell Therapy in B-Cell Malignancies. Immunol Rev. 2019 Jul;290(1):39-59.

[31] Yano S, Kondo K, Yamaguchi M, Richmond G, Hutchison M, Wakeling A, et al. Distribution and function of egfr in human tissue and the effect of egfr tyrosine kinase inhibition. Anticancer Res. 2003;23(5A):3639-3650.

Study #22 (XIBC001STU)
(b) (4) *Assay for the Detection of Human JCAR017 Cells in Mouse Whole Blood Specimens; conducted by* (b) (4)

The objective of this study was to detect JCAR017 using (b) (4) in blood samples collected from mice that were administered cetuximab. Twenty blood samples were collected at from two groups: 1) mice that received JCAR017 alone (Group 2) and 2) mice that received JCAR017 plus cetuximab (Group 3). Per the study report, the overall results showed that administration of cetuximab reduced the levels of JCAR017.

Reviewer's Comment:

- This study is a companion analysis to RPT-002100 (*Study #27*) in which (b) (4) mice received a single injection of 2x10^6 JCAR017 on Day 1. Cetuximab (0.25 mg/mouse; intravenous administration) was administered on Days 5, 8, 11, and 14. Blood was collected on Days 5 (prior to cetuximab administration) and 15 for (b) (4) analysis.

Overview of In vivo Studies

In Vivo Studies in Tumor-bearing Xenograft Models

Study #23

Report Number		RPT-0433
Date Report Signed		1/15/19
Title		Efficacy Evaluation of JCAR017 Processes 3.0 and 4.0 in (b) (4) Mice Engrafted with Raji Burkitt's Lymphoma Cells
GLP Status		No
Testing Facility		Juno Therapeutics
Objective(s)		To assess the efficacy of JCAR017 T cells produced with Process (b) (4) and Process (b) (4) in immune-deficient mice engrafted with Raji tumors.
Study Animals	**Strain/Breed**	(b) (4)
	Species	Mouse
	Age	8 weeks old
	Body Weight	18.0-25.6 g
	#/sex/group	5 or 8 females/group
	Total #	193
Test Article(s)		JCAR017 (b) (4)
Control Article(s)		Mock transduced T cells
Route of Administration		Intravenous injection
Description of the Disease/Injury Model and Implant Procedure		Immune-deficient mice were engrafted with 5x10^5 Raji Burkitt's lymphomas cells/mouse that were modified to express either (b) (4) **Note:** The Raji cells are a human cell line Mice were engrafted with tumor cells on Day 0, followed by administration of JCAR017 on Day 6.

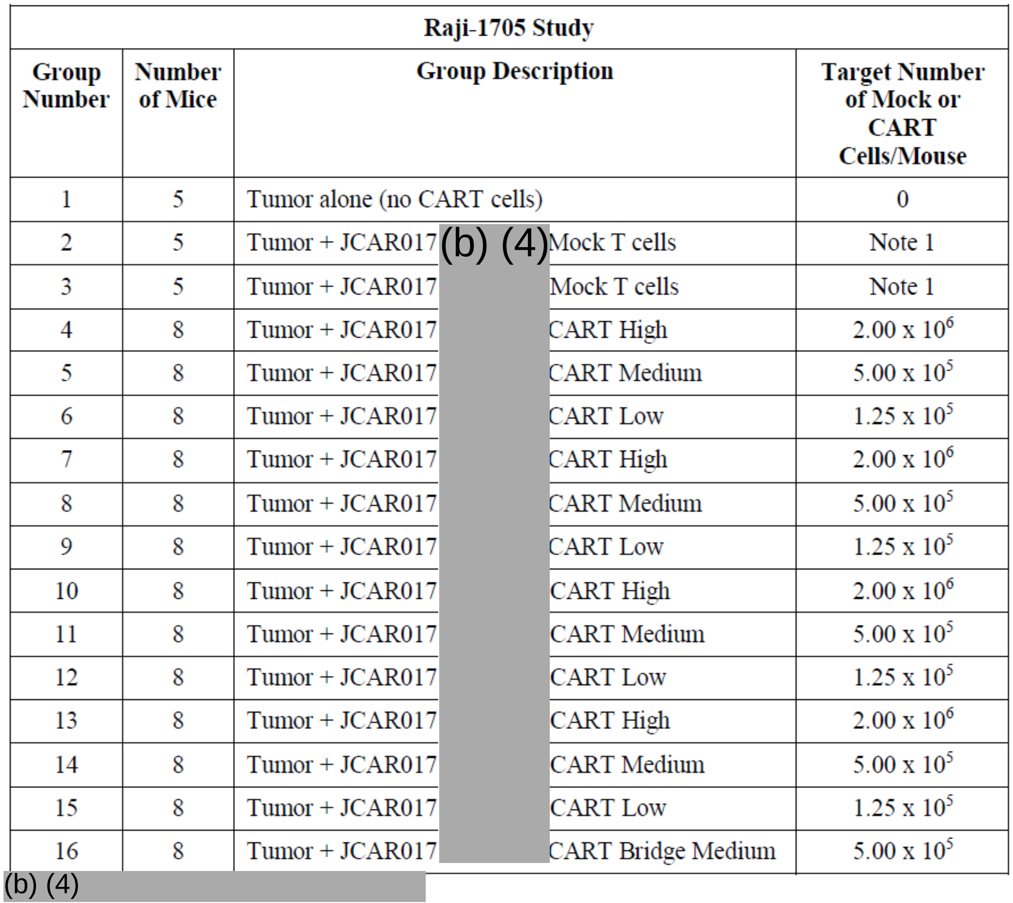
Study Groups and Dose Levels	See Tables 1 and 2 below
Dosing Regimen	Single administration
Randomization	Yes; based on tumor burden
Description of Masking	Not specified
Scheduled Sacrifice Time Point	Day 100

Reviewer's Comments:

- 'JCAR017 (b) (4)' is product generated using a pre-commercial process and 'JCAR017 (b) (4)' is product generated using the proposed commercial process. The T cells used to manufacture JCAR017 were obtained from a total of (b) (4) – (b) (4) patients with diffuse large B-cell lymphoma (b) (4)

- The study groups and dose levels listed in Tables 1 and 2 below are for two sub-studies (Raji-1705 and Raji-1706). Due to the large number of groups, the study was split into two sub-studies separated one day apart, which were labeled as 'Raji-1705' and 'Raji-1706'.

Table 1: Dose Groups for Raji-1705 Study



Raji-1705 Study			
Group Number	**Number of Mice**	**Group Description**	**Target Number of Mock or CART Cells/Mouse**
1	5	Tumor alone (no CART cells)	0
2	5	Tumor + JCAR017 (b) (4) Mock T cells	Note 1
3	5	Tumor + JCAR017 Mock T cells	Note 1
4	8	Tumor + JCAR017 CART High	2.00×10^6
5	8	Tumor + JCAR017 CART Medium	5.00×10^5
6	8	Tumor + JCAR017 CART Low	1.25×10^5
7	8	Tumor + JCAR017 CART High	2.00×10^6
8	8	Tumor + JCAR017 CART Medium	5.00×10^5
9	8	Tumor + JCAR017 CART Low	1.25×10^5
10	8	Tumor + JCAR017 CART High	2.00×10^6
11	8	Tumor + JCAR017 CART Medium	5.00×10^5
12	8	Tumor + JCAR017 CART Low	1.25×10^5
13	8	Tumor + JCAR017 CART High	2.00×10^6
14	8	Tumor + JCAR017 CART Medium	5.00×10^5
15	8	Tumor + JCAR017 CART Low	1.25×10^5
16	8	Tumor + JCAR017 CART Bridge Medium	5.00×10^5

(b) (4)

Note 1: Mock T-cell doses were matched to the highest total cell number in administered in the respective CART high dose groups for each donor.

Source: Report No. RPT-0433, located in Section 4.5 in Module 4.2.1.1.rpt-0433 of the BLA.

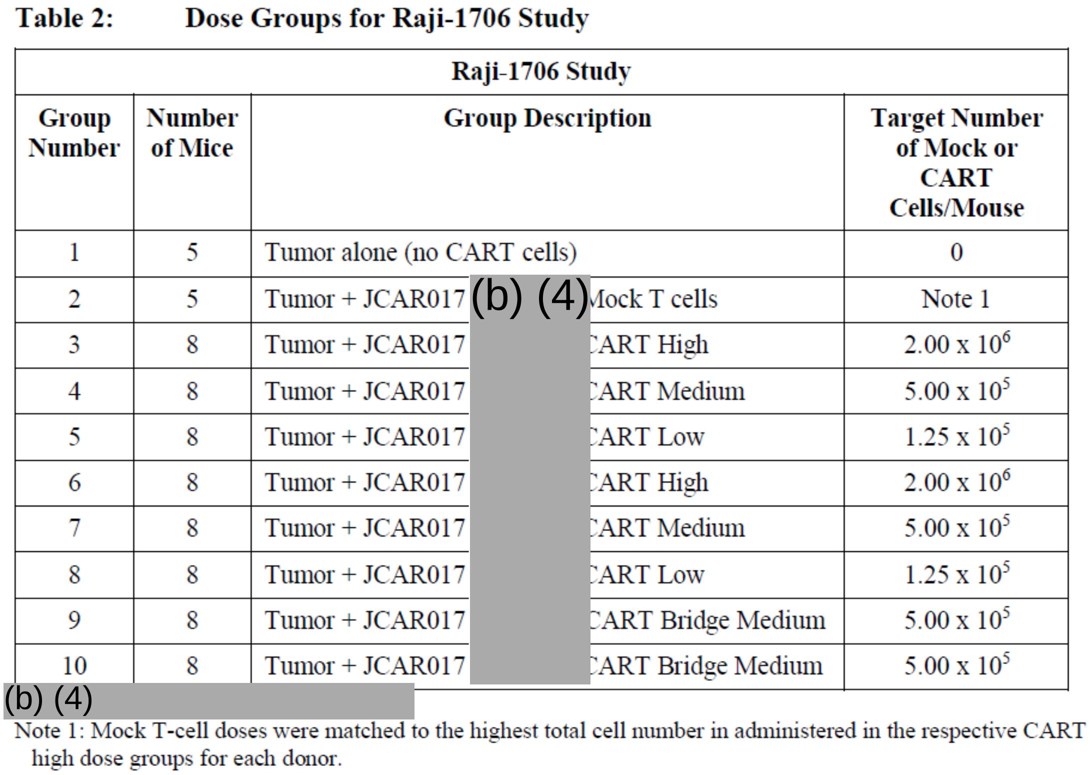


Table 2: Dose Groups for Raji-1706 Study

Raji-1706 Study			
Group Number	**Number of Mice**	**Group Description**	**Target Number of Mock or CART Cells/Mouse**
1	5	Tumor alone (no CART cells)	0
2	5	Tumor + JCAR017 (b) (4) Mock T cells	Note 1
3	8	Tumor + JCAR017 [redacted] CART High	2.00 x 10^6
4	8	Tumor + JCAR017 [redacted] CART Medium	5.00 x 10^5
5	8	Tumor + JCAR017 [redacted] CART Low	1.25 x 10^5
6	8	Tumor + JCAR017 [redacted] CART High	2.00 x 10^6
7	8	Tumor + JCAR017 [redacted] CART Medium	5.00 x 10^5
8	8	Tumor + JCAR017 [redacted] CART Low	1.25 x 10^5
9	8	Tumor + JCAR017 [redacted] CART Bridge Medium	5.00 x 10^5
10	8	Tumor + JCAR017 [redacted] CART Bridge Medium	5.00 x 10^5

(b) (4)

Note 1: Mock T-cell doses were matched to the highest total cell number in administered in the respective CART high dose groups for each donor.

Source: Report No. RPT-0433, located in Section 4.5 in Module 4.2.1.1.rpt-0433 of the BLA.

Key Evaluations and Assessments:
Tumor burden was quantified by (b) (4)
JCAR017 survival and expansion were characterized by analyzing blood samples collected on Days 8, 14, 21, and 28 using (b) (4) (CAR$^+$CD4$^+$ T cells). Sera IFN-γ levels were also assessed by (b) (4). Body weights were recorded prior to dosing and on Days 9, 13, 14, 16, 20, 24, 27, 30, 34, 36, 41 and 45.

Key Results:

Survival:
Results showed:

- Median survival times of 11 to 13 days were observed for mice that were untreated or administered mock transduced T cells (regardless of donor). Mice injected with JCAR017 had median survival times ranging from 15 to 95 days following tumor engraftment (Table 5).

- Survival depended on both the JCAR017 dose level and the donor source of the T cells. The data were independent of the manufacturing process.

Anti-Tumor Activity:
(b) (4) assessments to quantify tumor burdens in mice generally showed the following:

- Anti-tumor activity was only discernable at >1.25x10^5 JCAR017/mouse.

- Dose level-dependent decreases in group mean tumor burdens were observed in mice regardless of T cell donor source or manufacturing process. Compared to concurrent controls, notably decreased group mean tumor burdens were observed by Days 7-10 in mice dosed at 5x10^5 JCAR017/mouse and by Day 5 in mice dosed with 2x10^6 JCAR017/mouse. Reduced tumor burdens plateaued from approximately Days 10-25 in the mice injected with 2x10^6 JCAR017/mouse, but increased thereafter.

- Anti-tumor potency of JCAR017 cells is dependent on donor source.

Reviewer's Comment:

- The effect of JCAR017 on tumor burden appears to be transient and there is donor-to-donor variability in the activity of the test article. The proof-of-concept data, however, demonstrated a dose-dependent anti-tumor effect following JCAR017 administration.

Blood JCAR017 Levels:
The mean amount of circulating JCAR017 peaked at Day 8 and gradually decreased between Days 14-28.

Reviewer's Comments:

- It is difficult to discern any additional conclusions due to a large variance in the data and a limited number of samples for each group.

- Circulating JCAR017 levels appear to correlate with anti-tumor activity.

- These data are not reflective of the clinical scenario; however, JCAR017 was administered to mouse xenograft models. Xenotransplantation of a human-derived product into mice limits the longer-term survival of the CAR T cells. JCAR017 is manufactured from an autologous source for human subjects, thus these cells are anticipated to have longer survival times and increased potential for expansion following administration in humans.

Plasma IFN-γ Levels:
The highest concentrations of IFN-γ were detected on Days 8 and 14 across all donors and dose groups. However, no other consistent patterns that suggest differences in human IFN-γ production following administration of JCAR017 produced using Process (b) (4) or Process (b) (4)

Reviewer's Comment:

- The strongest conclusion that can be drawn from the data is that mean plasma IFN-γ levels in mice receiving JCAR017 were higher when compared to control mice receiving the mock transduced T cells or vehicle control. No other consistent observations related to the JCAR017 dose level, manufacturing process, or the T cell donor source can be made. Based on this reviewer's regulatory experience, assay results for determination of cytokine levels (to evaluate CAR T cell activation) in blood samples collected from rodents often have large variance and low absolute cytokine quantities that can make consistent quantitation difficult. Nonetheless, the results from this study support the administration of JCAR017 to treat B-cell lymphoma.

Body Weight Changes:

Mice injected with vehicle, mock transduced T cells, or a low dose level of JCAR017 (1.25x10^5 cells/mouse) exhibited rapid weight loss and death by Day 14. Mice dosed at 5x10^5 or 2x10^6 JCAR017/mouse displayed increased percent body weight changes compared to the control groups. No consistent pattern in mean body weight changes related to the T cell donor source or the manufacturing process were observed.

Reviewer's Comment:

- An amended study Report No. RPT-0433 was submitted on June 1, 2020 (Amendment #046) containing updated information in the 'Materials and Methods' section. The resulting data in the amended report did not change.

Study #24 (SR0006)

Characterization of CD19-Specific CAR T Cell Preclinical Production Runs: In Vivo Analysis of Tumor Growth and Survival Post-Treatment with EGFRt-Enriched CD4 and CD8 T Cells Transduced with the (b) (4) *Batch Preparation of CD19scFV-IgG4 hinge-CD28/41BB-*(b) (4)*-EGFRt_*(b) (4) *conducted by Seattle Children's Hospital*

The objective of this *in vivo* study was to evaluate the anti-tumor activity of cryopreserved preclinical lots of JCAR017 (b) (4) produced from several preclinical production runs (page 3 in the study report). The cells were thawed and expanded according to a protocol that mimics the post-transduction manipulation process for BREYANZI®.

(b) (4) mice (6-10 weeks old) were IV injected on Day 0 with 0.5x10^6 Raji tumor cells expressing luciferase. The transduced T cells were thawed, washed, and re-constituted on Day 7. JCAR017 dose levels of 1.25, 2.5, 5, and 10x10^6 cells/mouse or vehicle control were IV injected in the tumor-bearing mice on Day 7. Tumor burden was assessed using (b) (4) on Days 14, 20, and 28.

Reviewer's Comments:

- It is unclear from the report what 'version' and lot of JCAR017 were used in this study. The 'Materials and Methods' section only identify the cells as (b) (4) .

- The composition of the vehicle was not provided; however, this reviewer does not anticipate that this omission significantly impacts interpretation of the results.

Reduced tumor burden was observed at all dose levels compared to control mice. There were no differences in tumor burden (total flux (b) (4) reductions between the JCAR017 dose levels, indicating that a maximal anti-tumor effect was achieved at the lowest dose administered. In addition, 100% of mice dosed with JCAR017 survived for the 60-day study duration, while all control mice were euthanized on Days 21-22 due to humane reasons related to advanced tumor progression.

In conclusion, this study demonstrated that anti-tumor activity was observed in tumor-bearing mice that were administered one lot of JCAR017 manufactured and formulated according to the planned commercial process.

Study #25

Report Number		RPT-17005
Date Report Signed		9/5/19
Title		Efficacy Evaluation of JCAR017 in Combination with Ibrutinib or Acalabrutinib in (b) (4) Mice Engrafted with Nalm-6 Tumor Cells
GLP Status		No
Testing Facility		Juno Therapeutics
Objective(s)		To determine whether ibrutinib or acalabrutinib could improve the *in vivo* potency of JCAR017 T in immune-deficient mice engrafted with Nalm-6 lymphoblastic leukemia cells.
Study Animals	**Strain/Breed**	(b) (4)
	Species	Mouse
	Age	13 weeks old
	Body Weight	Not reported
	#/sex/group	5 or 8/F/group
	Total #	115
Test Article(s)		JCAR017 (Lot Nos. (b) (4) Ibrutinib (Batch No. (b) (4) Acalabrutinib (Batch No. (b) (4) **Note:** Ibrutinib is an (b) (4) that has been approved for treatment of patients with CLL and other lymphomas.[32] Acalabrutinib is a highly selective, covalent BTK inhibitor (BTKi)currently in clinical development for treatment of B-cell malignancies.[33] BTKis are becoming standard-of-care therapeutics for treatment of many B-cell malignancies. The rationale for combining BTKis with JCAR017 is based on the hypothesis that this strategy may improve CAR T potency, limit tumor environment-mediated immune dysfunction, and increase the potential for durable clinical benefit of CAR T cell therapy. Refer to *Study #19* for the rationale for administration of BTKis in combination with JCAR017.
Control Article(s)		Vehicle - (phosphate buffered saline; PBS)

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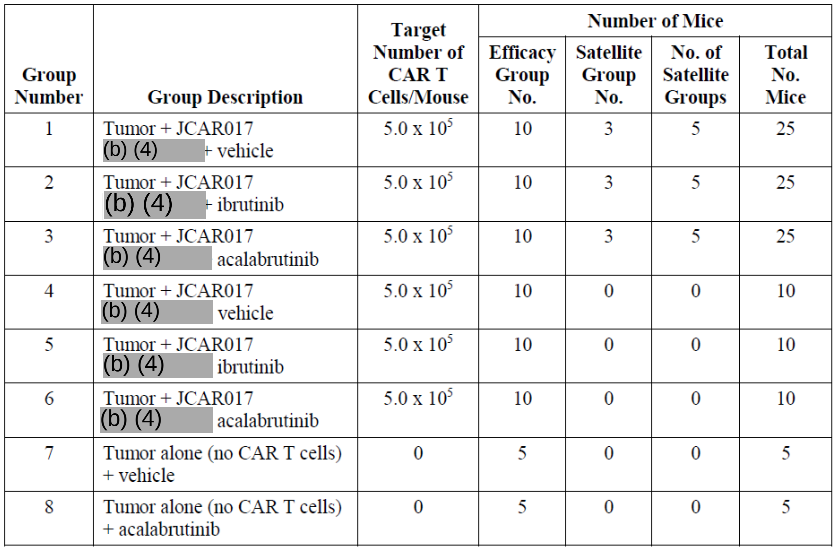
[32] Davids MS and Brown JR. Ibrutinib: a first in class covalent inhibitor of Bruton's tyrosine kinase. Future Oncol 2014;10(6):957-967.

[33] Byrd JC, Harrington B, O'Brien S, et al. Acalabrutinib (ACP-196) in relapsed chronic lymphocytic leukemia. N Engl J Med 2016;374(4):323-332.

<table>
<tr><td>Route of Administration</td><td>JCAR017 – IV injection
Ibrutinib and Acalabrutinib - oral gavage and orally through administration in drinking water, respectively.</td></tr>
<tr><td>Description of the Disease/Injury Model and Implant Procedure</td><td>Mice were engrafted (Day 0) with 5x10^5 NALM6 acute lymphoblastic leukemia cells/mouse that were modified to express red-shifted firefly luciferase (FfLuc) and GFP</td></tr>
<tr><td>Study Groups and Dose Levels</td><td>

Table 2: Dose Groups for Nalm6-1608 Study



<table>
<tr><th rowspan="2">Group Number</th><th rowspan="2">Group Description</th><th rowspan="2">Target Number of CAR T Cells/Mouse</th><th colspan="4">Number of Mice</th></tr>
<tr><th>Efficacy Group No.</th><th>Satellite Group No.</th><th>No. of Satellite Groups</th><th>Total No. Mice</th></tr>
<tr><td>1</td><td>Tumor + JCAR017
(b) (4) + vehicle</td><td>5.0 x 10^5</td><td>10</td><td>3</td><td>5</td><td>25</td></tr>
<tr><td>2</td><td>Tumor + JCAR017
(b) (4) + ibrutinib</td><td>5.0 x 10^5</td><td>10</td><td>3</td><td>5</td><td>25</td></tr>
<tr><td>3</td><td>Tumor + JCAR017
(b) (4) acalabrutinib</td><td>5.0 x 10^5</td><td>10</td><td>3</td><td>5</td><td>25</td></tr>
<tr><td>4</td><td>Tumor + JCAR017
(b) (4) vehicle</td><td>5.0 x 10^5</td><td>10</td><td>0</td><td>0</td><td>10</td></tr>
<tr><td>5</td><td>Tumor + JCAR017
(b) (4) ibrutinib</td><td>5.0 x 10^5</td><td>10</td><td>0</td><td>0</td><td>10</td></tr>
<tr><td>6</td><td>Tumor + JCAR017
(b) (4) acalabrutinib</td><td>5.0 x 10^5</td><td>10</td><td>0</td><td>0</td><td>10</td></tr>
<tr><td>7</td><td>Tumor alone (no CAR T cells)
+ vehicle</td><td>0</td><td>5</td><td>0</td><td>0</td><td>5</td></tr>
<tr><td>8</td><td>Tumor alone (no CAR T cells)
+ acalabrutinib</td><td>0</td><td>5</td><td>0</td><td>0</td><td>5</td></tr>
</table>

</td></tr>
<tr><td>Dosing Regimen</td><td>JCAR017 – Single injection

BTKis – An initial loading dose of BTKis were administered (0.625 mg ibrutinib or acalabrutinib) by oral gavage on Day 4. From study days 5-38, mice were freely allowed to consume water containing different concentrations of ibrutinib or acalabrutinib. Estimated delivery doses per day reported were 0.625 or 25 mg/kg, based on average daily water consumption.</td></tr>
<tr><td>Randomization</td><td>Yes; based tumor burden</td></tr>
<tr><td>Description of Masking</td><td>Not specified</td></tr>
<tr><td>Scheduled Sacrifice Time Point</td><td>36 days after JCAR017 administration (study Day 40)</td></tr>
</table>

<u>Reviewer’s Comments:</u>

- ‘Day 0’ refers to the timepoint at which the tumor cells were engrafted into the mice. JCAR017 cells were administered on Day 4.

- Per the study report (page 13), a suboptimal dose level of JCAR017 (5x10^5 cells/mouse) was administered in order to allow for interpretation of negative or positive effects of the BTKi combination to be detectable between JCAR017 alone and JCAR017 combined with ibrutinib or acalabrutinib and to enable evaluation of endpoints between Days 20 to 40.

- Mice consumed water ad libitum, thus the dose levels of each BTKi were calculated (mg/kg) by daily consumption of total water.[34] Mice were housed 4 to 5 per cage, thus the dose level administered per mouse on each day is likely to be widely variable. However, this reviewer presumes that this method of dosing was intended to reduce stress in the mice and to bypass the difficulties that would have been associated with handling a large number of mice for a daily gavage for 20 consecutive days if the BTKis were administered directly.

Key Evaluations and Assessments:
The primary endpoint for this study was overall survival. Tumor growth, a secondary endpoint, was monitored by (b) (4) on days -1, 4, 11, 18, 25, and 31 following JCAR017 administration. Quantitation of both JCAR017 and tumor cells in blood and bone marrow samples was performed using (b) (4). Samples were obtained at Days 0, 7, 12, 19, and 26.

Key Results:

Survival:
Survival data are summarized in Figure 1 and Table 5 below:

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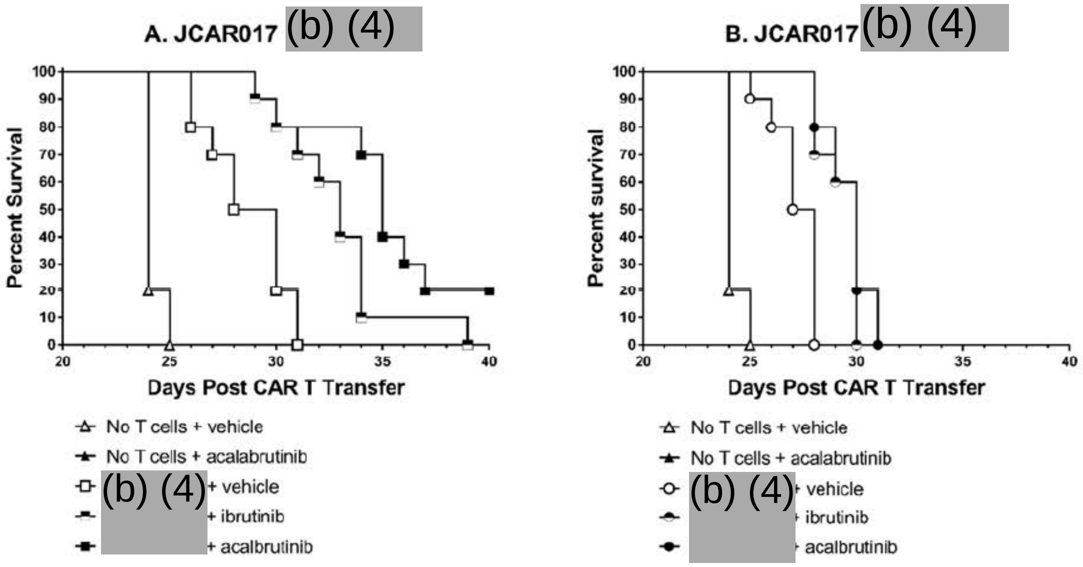
[34] Herman SEM, Montraveta A, Niemann CU, et al. The Bruton Tyrosine Kinase (BTK) Inhibitor Acalabrutinib Demonstrates Potent On-Target Effects and Efficacy in Two Mouse Models of Chronic Lymphocytic Leukemia. Clin Cancer Res 2017;23(11):2831-2841.

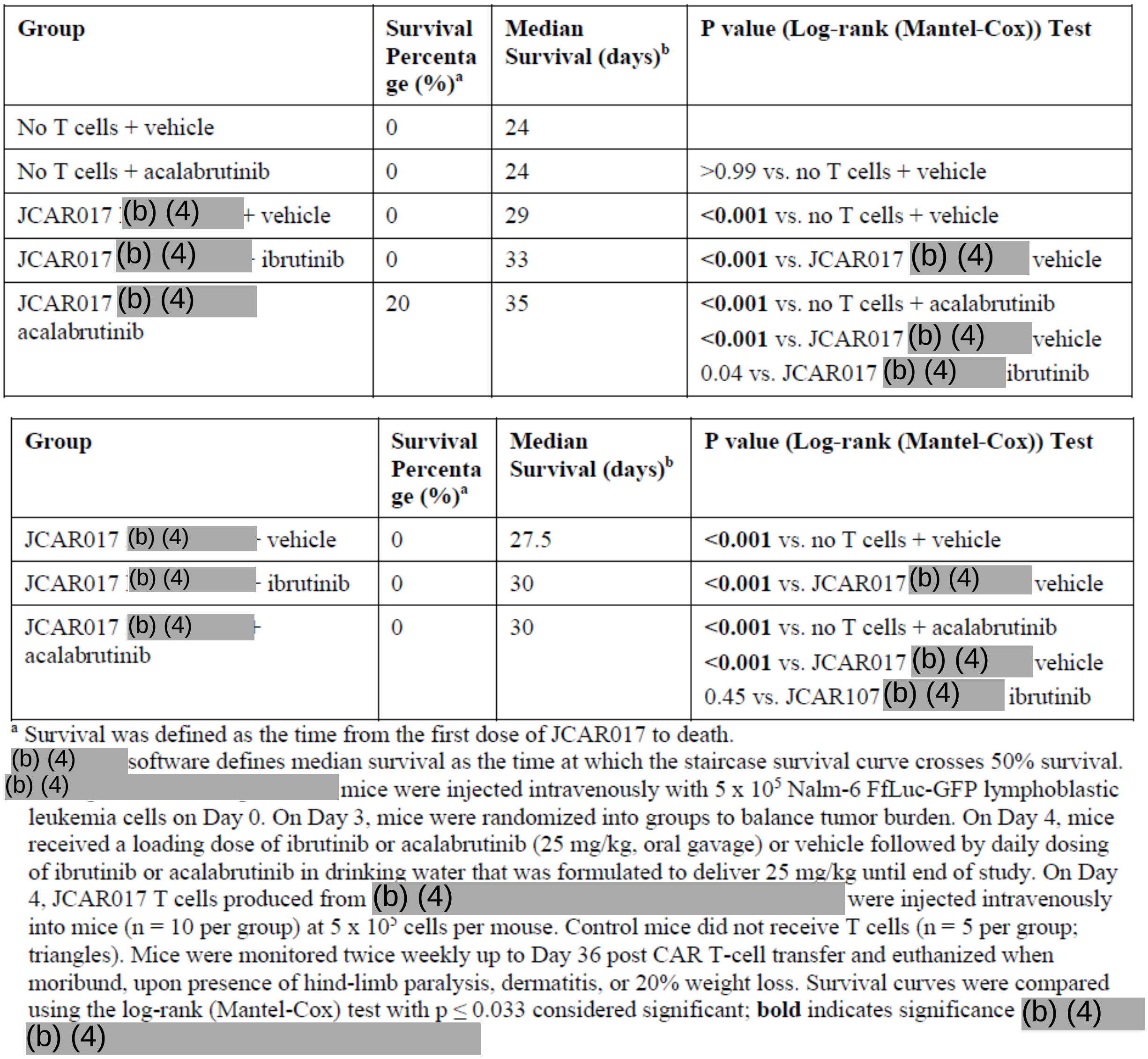
Figure 1: Survival of Mice with Nalm-6 Tumors Treated with JCAR017 T Cells in Combination with Ibrutinib or Acalabrutinib



CAR = chimeric antigen receptor; PAD = process and analytical development.
(b) (4) mice were injected intravenously with 5 x 10^5 Nalm-6 FfLuc-GFP lymphoblastic leukemia cells on Day 0. On Day 3, mice were randomized into groups to balance tumor burden. On Day 4, mice received a loading dose of ibrutinib (half-filled symbols) or acalabrutinib (filled symbols) (25 mg/kg, oral gavage) or vehicle (open symbols) followed by daily dosing of ibrutinib or acalabrutinib in drinking water that was formulated to deliver 25 mg/kg until end of study. On Day 4, JCAR017 T cells produced from (b) (4) (b) (4) were injected intravenously into mice (n = 10 per group) at 5 x 10^5 cells per mouse. Control mice did not receive T cells (n = 5 per group; triangles). Mice were monitored twice weekly up to Day 36 post CAR T-cell transfer and euthanized when moribund, upon presence of hind-limb paralysis, dermatitis, or 20% weight loss. Survival curves were plotted using the Kaplan-Meier method.

Source: Report No. RPT-17005, located in Section 5.1 in Module 4.2.1.4.rpt-17005 of the BLA.

Table 5: Summary of Survival Efficacy of Mice with Nalm-6 Tumors Treated with JCAR017 T Cells in Combination with Ibrutinib or Acalabrutinib



Group	Survival Percentage (%)[a]	Median Survival (days)[b]	P value (Log-rank (Mantel-Cox)) Test
No T cells + vehicle	0	24	
No T cells + acalabrutinib	0	24	>0.99 vs. no T cells + vehicle
JCAR017 (b) (4) + vehicle	0	29	**<0.001** vs. no T cells + vehicle
JCAR017 (b) (4) ibrutinib	0	33	**<0.001** vs. JCAR017 (b) (4) vehicle
JCAR017 (b) (4) acalabrutinib	20	35	**<0.001** vs. no T cells + acalabrutinib **<0.001** vs. JCAR017 (b) (4) vehicle 0.04 vs. JCAR017 (b) (4) ibrutinib

Group	Survival Percentage (%)[a]	Median Survival (days)[b]	P value (Log-rank (Mantel-Cox)) Test
JCAR017 (b) (4) vehicle	0	27.5	**<0.001** vs. no T cells + vehicle
JCAR017 (b) (4) ibrutinib	0	30	**<0.001** vs. JCAR017 (b) (4) vehicle
JCAR017 (b) (4) acalabrutinib	0	30	**<0.001** vs. no T cells + acalabrutinib **<0.001** vs. JCAR017 (b) (4) vehicle 0.45 vs. JCAR107 (b) (4) ibrutinib

[a] Survival was defined as the time from the first dose of JCAR017 to death.
(b) (4) software defines median survival as the time at which the staircase survival curve crosses 50% survival.
(b) (4) mice were injected intravenously with 5 x 10^5 Nalm-6 FfLuc-GFP lymphoblastic leukemia cells on Day 0. On Day 3, mice were randomized into groups to balance tumor burden. On Day 4, mice received a loading dose of ibrutinib or acalabrutinib (25 mg/kg, oral gavage) or vehicle followed by daily dosing of ibrutinib or acalabrutinib in drinking water that was formulated to deliver 25 mg/kg until end of study. On Day 4, JCAR017 T cells produced from (b) (4) were injected intravenously into mice (n = 10 per group) at 5 x 10^5 cells per mouse. Control mice did not receive T cells (n = 5 per group; triangles). Mice were monitored twice weekly up to Day 36 post CAR T-cell transfer and euthanized when moribund, upon presence of hind-limb paralysis, dermatitis, or 20% weight loss. Survival curves were compared using the log-rank (Mantel-Cox) test with $p \leq 0.033$ considered significant; **bold** indicates significance (b) (4) (b) (4)

Source: Report No. RPT-17005, located in Section 5.1 in Module 4.2.1.4.rpt-17005 of the BLA.

- No mice in the control groups (Groups 7 and 8) survived to the end of the study (Day 40).
- Median survival increased by 3 to 7 days in mice receiving JCAR017 alone (Groups 1 and 4) compared to controls (Groups 7 and 8).
- Mice administered ibrutinib (Groups 2 and 5) or acalabrutinib (Groups 3 and 6) in addition to JCAR017 displayed increased median survival times when compared to animals receiving JCAR017 only and control group animals.

- Enhanced survival times were observed in mice injected with (b) (4) different lots of JCAR017 produced from T cells obtained from (b) (4) .

- The survival curves (Figure 1) suggest anti-cancer activity differences that depend on the donor source of the T cells.

Reviewer's Comment:

- ➢ Lot-to-lot variation that is dependent on the donor source of the T cells is to be expected.

Anti-Tumor Activity of JCAR017 in Combination with BTKis:
(b) (4) data showed that mice administered a BTKi with JCAR017 exhibited significantly decreased tumor burden (compared to all control groups) at all time points tested.

Reviewer's Comment:

- ➢ Overall, the survival data correlated with the *in vitro* results for *Study #19*, suggesting that administration of a BTKi in combination with JCAR017 enhance anti-tumor activity and survival.

JCAR017 and Tumor Cell Levels in Blood and Tissues:
In general, the data showed: 1) increased JCAR017 counts in the blood (at Day 7) of mice that also received a BTKi (Groups 2 and 3) when compared to mice dosed with JCAR017 alone (Group 1) and 2) reduced tumor cell counts from Days 7-26 in the blood and bone marrow in Groups 2 and 3 mice compared to Group 1 mice.

Reviewer's Comment:

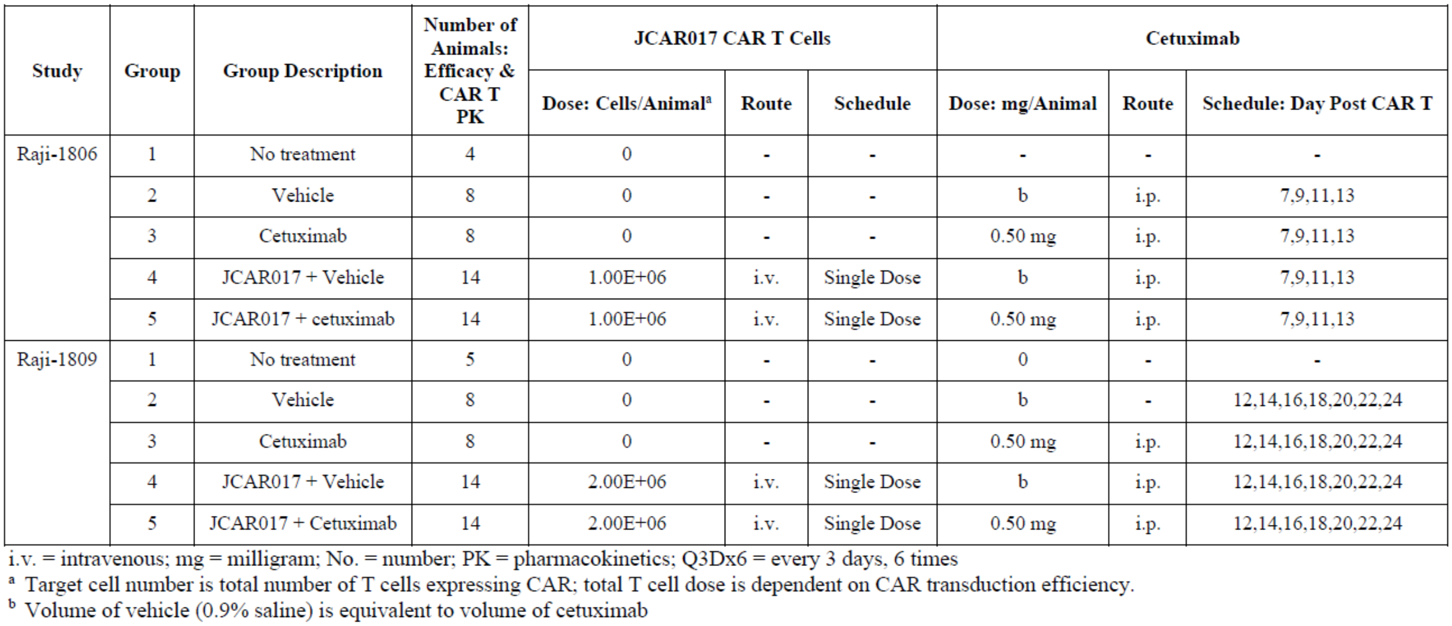
- ➢ Data are provided for mice dosed with JCAR017 from Lot No. (b) (4) only (Groups 1-3). t is this reviewer's opinion that the omission of this data has minimal impact on the over assessment of this BLA, given the totality of the preclinical data provided, and because lot-to-lot differences are expected to exist based on the donor source for T cells.

Study #26

Report Number		RPT-001479
Date Report Signed		4/29/20
Title		Elimination of JCAR017 T Cells with Cetuximab and Effect on Anti-tumor Efficacy in (b) (4) Mice Engrafted with Raji Burkitt's Lymphoma Cells
GLP Status		No
Testing Facility		Juno Therapeutics
Objective(s)		To demonstrate the *in vivo* elimination of JCAR017 after administration of cetuximab
Study Animals	**Strain/Breed**	(b) (4)
	Species	Mouse
	Age	7.5 – 9.5 weeks old
	Body Weight	Not specified
	#/sex/group	5 -14 females/group

Total #	130
Test Article(s)	JCAR017 (Lot Nos. (b) (4) Cetuximab (Lot No. (b) (4)
Control Article(s)	Vehicle (0.9% saline)
Route of Administration	JCAR017 – IV injection Cetuximab – intraperitoneal (IP) injection
Description of the Disease/Injury Model and Implant Procedure	Mice were engrafted (IV injection) with 5x10^5 Raji Burkitt's lymphoma cells modified to express (b) (4) Approximately 7-8 days later (Day 1) JCAR017 was administered.
Study Groups and Dose Levels	Refer to Table 6 below
Dosing Regimen	JCAR017 - Single administration Cetuximab - Multiple administrations per Table 6
Randomization	Yes; based on tumor burden
Description of Masking	N/A
Scheduled Sacrifice Time Point	Day 21 or Day 85

Table 6: Treatment Group Descriptions and Dosing



Study	Group	Group Description	Number of Animals: Efficacy & CAR T PK	JCAR017 CAR T Cells			Cetuximab		
				Dose: Cells/Animal[a]	Route	Schedule	Dose: mg/Animal	Route	Schedule: Day Post CAR T
Raji-1806	1	No treatment	4	0	-	-	-	-	-
	2	Vehicle	8	0	-	-	b	i.p.	7,9,11,13
	3	Cetuximab	8	0	-	-	0.50 mg	i.p.	7,9,11,13
	4	JCAR017 + Vehicle	14	1.00E+06	i.v.	Single Dose	b	i.p.	7,9,11,13
	5	JCAR017 + cetuximab	14	1.00E+06	i.v.	Single Dose	0.50 mg	i.p.	7,9,11,13
Raji-1809	1	No treatment	5	0	-	-	0	-	-
	2	Vehicle	8	0	-	-	b	-	12,14,16,18,20,22,24
	3	Cetuximab	8	0	-	-	0.50 mg	i.p.	12,14,16,18,20,22,24
	4	JCAR017 + Vehicle	14	2.00E+06	i.v.	Single Dose	b	i.p.	12,14,16,18,20,22,24
	5	JCAR017 + Cetuximab	14	2.00E+06	i.v.	Single Dose	0.50 mg	i.p.	12,14,16,18,20,22,24

i.v. = intravenous; mg = milligram; No. = number; PK = pharmacokinetics; Q3Dx6 = every 3 days, 6 times

[a] Target cell number is total number of T cells expressing CAR; total T cell dose is dependent on CAR transduction efficiency.

[b] Volume of vehicle (0.9% saline) is equivalent to volume of cetuximab

Source: Report No. RPT-001479, located in Section 4.2 in Module 4.2.1.2.rpt-001479 of the BLA.

Reviewer's Comments:

- Per the study report, the dose levels of JCAR017 were chosen based on the preclinical information obtained in *Study #23*. The dose levels, route of administration, and dosing regimen for cetuximab mimic what is described in publications for nonclinical studies in which CAR T cells and cetuximab were administered.[35,36] This reviewer agrees with this justification.

- The study report contains two sub-studies, which are designated ‘Raji-1806’ and ‘Raji-1809’. The difference between the studies is the dose levels of JCAR017 administered and the duration of the observational period (refer to Table 6).

Key Evaluations and Assessments:
The tumor burden was monitored 2-3 times per week by (b) (4), and the presence of JCAR017 in blood, spleen, and bone marrow was determined by (b) (4) on Days 6 and 13 (Study Raji-1806) or on Days 12, 19, and 26 (Study Raji-1809). Survival was monitored out to Day 21 (Study Raji-1806) or Day 85 (Study Raji-1809).
Key Results:

JCAR017 Levels:

- For Study Raji-1806, JCAR017 reached peak expansion numbers in the blood, spleen, and bone marrow by Day 13. For mice in Group 5, JCAR017 levels were significantly reduced by Day 13 (>99% reduction in the blood and spleen and >76% reduction in the bone marrow when compared to Group 4).

- For Study Raji-1809, maximal expansion of JCAR017 was observed on Day 12. At Day 19, CAR T cell expansion was significantly reduced by 84.9% in the blood and 97.1% in the spleen (Group 5 compared to Group 4), but resolved by Day 19.

Reviewer’s Comment:

- It should be noted that the JCAR017 dose level in Study Raji-1809 was 2-fold higher than in Study Raji-1806. This may explain why there was no significant reduction in JCAR017 levels in the bone marrow samples at Day 19 compared to Study Raji-1806.

Tumor Burden:
For Studies Raji-1806 and Raji-1809, the tumor burden was generally decreased in the Group 4 animals when compared to the Group 5 animals (Figures 5 and 6). Increased tumor burden correlated with decreased overall survival (Figure 7).

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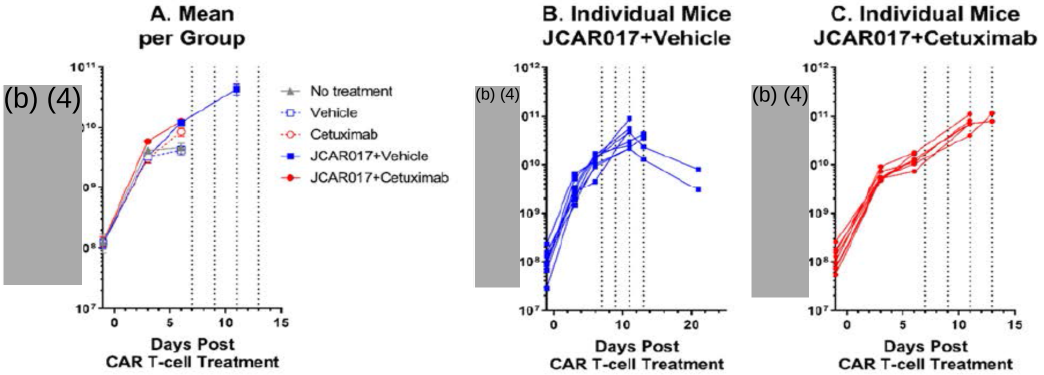
[35] Wang X, Chang WC, Wong CW, Colcher D, Sherman M, Ostberg JR, et al. A transgene-encoded cell surface polypeptide for selection, *in vivo* tracking, and ablation of engineered cells. Blood. 2011 Aug 4;118(5):1255-63.

[36] Paszkiewicz PJ, Fräßle SP, Srivastava S, Sommermeyer D, Hudecek M, Drexler I, et al. Targeted antibody-mediated depletion of murine CD19 CAR T cells permanently reverses B cell aplasia. J Clin Invest. 2016 Nov 1;126(11):4262-4272.

Figure 5: Tumor Volume in Raji Xenograft Mice After Administration of JCAR017 Followed by Four Doses of Cetuximab (Study Raji-1806)

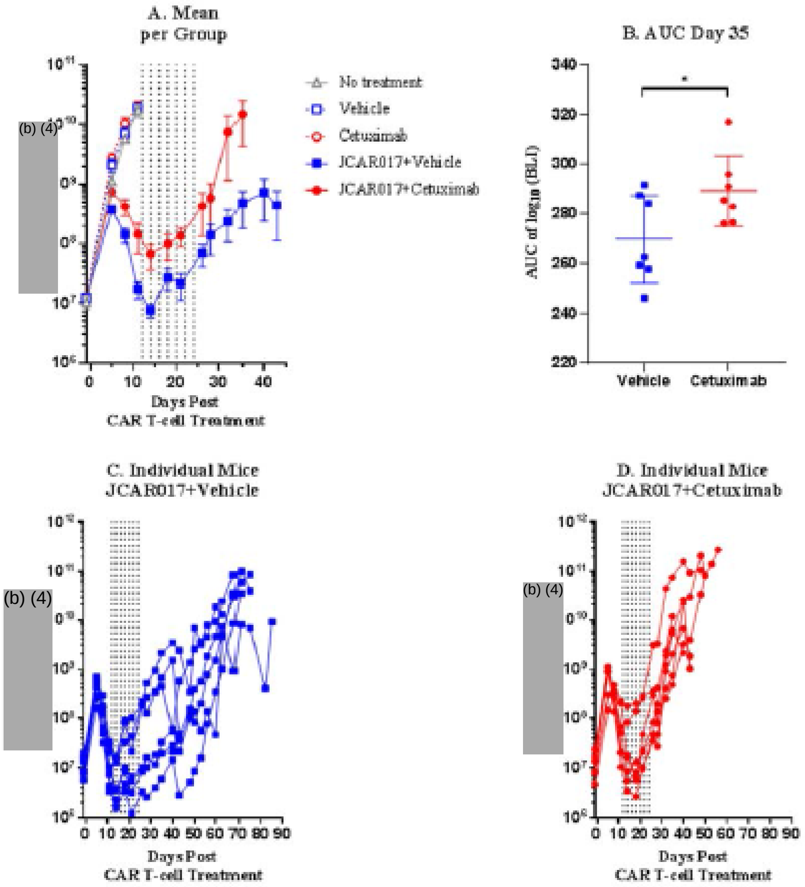


CAR = chimeric antigen receptor.

Raji-1806 study: (b) (4) mice were injected intravenously with 5.0E+05 Raji rFluc-GFP Burkitt's lymphoma cells, and 8 days later, mice were randomized into groups to balance tumor burden into the following groups: no treatment (closed gray triangles), vehicle (open blue squares), cetuximab, an anti-epidermal growth factor receptor (EGFR) antibody (open red circles), JCAR107 anti-CD19 chimeric antigen receptor (CAR) human CD4+ (0.5E+06) and CD8+ (0.5E+06) T cells with vehicle (closed blue squares) or with cetuximab (closed red circles). Mice received JCAR017 cells as an intravenous dose the day after randomization. Cetuximab (0.5 mg per mouse) or an equal volume of vehicle were administered intraperitoneally at 12, 14, 16, 18, 20, 22, and 24 days post CAR T-cell treatment. Disseminated tumor growth was assessed by (b) (4) (b) (4) Changes in (b) (4) shown for all groups (A, mean ± standard error) or for individual mice receiving JCAR017 with vehicle (B) or cetuximab (C). Vertical lines show the days that cetuximab was dosed (b) (4) (b) (4)

Source: Report No. RPT-001479, located in Section 5.2 in Module 4.2.1.2.rpt-001479 of the BLA

Figure 6: Tumor Volume in Raji Xenograft Mice After Administration of JCAR017 Followed by Seven Doses of Cetuximab (Study Raji-1809)

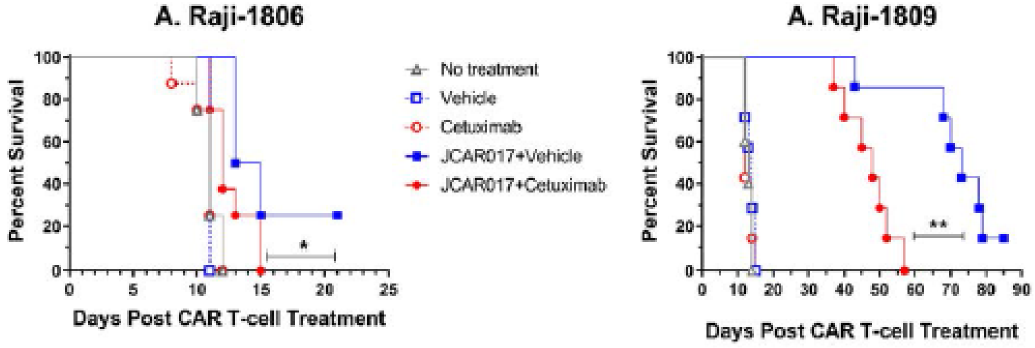


AUC = area-under-the-curve;

Raji-1809 study: (b) (4) mice were injected intravenously with 5.0E+05 Raji rFluc-GFP Burkitt's lymphoma cells, and 7 days later, mice were randomized into groups to balance tumor burden into the following groups: no treatment (open gray triangles), vehicle (open blue squares), cetuximab, an anti-epidermal growth factor receptor (EGFR) antibody (open circles), JCAR107 anti-CD19 chimeric antigen receptor (CAR) human CD4+ (1.0E+06) and CD8+ (1.0E+06) T cells with vehicle (closed blue squares) or with cetuximab (closed red circles). Mice received JCAR017 cells as an intravenous dose the day after randomization. Cetuximab (0.5 mg per mouse) or an equal volume of vehicle were administered intraperitoneally at CAR = chimeric antigen receptor.12, 14, 16, 18, 20, 22, and 24 days post CAR T-cell treatment. Disseminated tumor growth was assessed by (b) (4) Changes in (b) (4) (b) (4) are shown as mean ± standard error for all groups (A) or for individual mice receiving JCAR017 with vehicle (C) or cetuximab (D). Vertical lines show the days that cetuximab was dosed. Area-under-the-curve (AUC) analysis of log-transformed p/s measurements through Day 35 are shown for groups receiving JCAR017, with one-way ANOVA with Tukey's correction for multiple comparisons performed to compare vehicle vs. cetuximab groups (B, mean ± SE); * $p \leq 0.05$ (b) (4) (b) (4)

Source: Report No. RPT-001479, located in Section 5.2 in Module 4.2.1.2.rpt-001479 of the BLA.

Figure 7: **Survival of Raji Xenograft Mice After Administration of JCAR017 Followed by Treatment with Cetuximab (Studies Raji-1806 and Raji-1809)**



CAR = chimeric antigen receptor
(b) (4) mice were injected intravenously with 5.0E+05 Raji (b) (4) Burkitt's lymphoma cells, and 7 to 8 days later, mice were randomized into groups to balance tumor burden into the following groups: no treatment (open gray triangles), vehicle (open blue squares), cetuximab, an anti-epidermal growth factor receptor (EGFR) antibody (open circles), JCAR107 anti-CD19 chimeric antigen receptor (CAR) human CD4+ (0.5E+06 [Raji-1806] or 1.0E+06 [Raji-1809]) and CD8+ (0.5E+06 [Raji-1806] or 1.0E+06 [Raji-1809]) T cells with vehicle (closed blue squares) or with cetuximab (closed red circles). Mice received JCAR017 cells as an intravenous dose the day after randomization. Cetuximab (0.5 mg per mouse) or an equal volume of vehicle were administered intraperitoneally at 7, 9, 11, and 13 days (Raji-1806) or at 12, 14, 16, 18, 20, 22, and 24 days (Raji-1809) post CAR T-cell treatment. Mice were monitored twice weekly and euthanized when moribund, upon presence of hind-limb paralysis, dermatitis, or 20% weight loss. Survival curves were plotted using the Kaplan-Meier method, and survival curves between JCAR017 with vehicle or with cetuximab were compared using the log-rank (Mantel-Cox) test; * $p \leq 0.05$, ** $p \leq 0.01$ (b) (4)
(b) (4)

Source: Report No. RPT-001479, located in Section 5.2 in Module 4.2.1.2.rpt-001479 of the BLA.

Reviewer's Comment:

- In general, the results show that JCAR017 is depleted when the anti-EGFR antibody cetuximab was administered. This finding correlates with the imaging data that show increased tumor burden in mice receiving this combination regimen.

Study #27

Report Number		RPT-002100
Date Report Signed		4/27/20
Title		Cetuximab-Mediated Ablation of JCAR017 in (b) (4) Mice Engrafted with Raji Burkitt's Lymphoma
GLP Status		No
Testing Facility		Juno Therapeutics
Objective(s)		To demonstrate, with a validated (b) (4) assay, the *in vivo* elimination of JCAR017 after administration of cetuximab, utilizing a posology and route of administration like that in humans.
Study Animals	**Strain/Breed**	(b) (4)
	Species	Mouse
	Age	8 weeks old

	Body Weight	22.9 g
	#/sex/group	5-14 females/group
	Total #	72
Test Article(s)		JCAR017 (Lot No. (b) (4) Cetuximab (Lot No. (b) (4)
Control Article(s)		Vehicle (0.9% saline)
Route of Administration		JCAR017 and cetuximab – IV injection
Description of the Disease/Injury Model and Implant Procedure		Mice were engrafted (IV injection)on Day -7 with 5x10^5 Raji Burkitt's lymphoma cells modified to express (b) (4) . JCAR017 was administered on Day 1.
Study Groups and Dose Levels		Refer to Table 5 below.
Dosing Regimen		JCAR017 - Single administration Cetuximab - Multiple administrations per Table 5
Randomization		Yes; based on tumor burden
Description of Masking		N/A
Scheduled Sacrifice Time Point		Day 21

Table 5: Treatment Groups and Dose Levels

Group	Description	No. Animals: Efficacy & CAR T PK	No. Animals: Satellite Cetuximab PK	JCAR017 CAR T Cells			Cetuximab		
				Dose: Cells/Animal[a]	Route	Schedule	Dose: mg/Animal	Route	Schedule
1	No treatment	5	-	-	-	-	-	-	-
2	Vehicle	5	-	-	-	-	[b]	i.v.	Q3Dx6
3	Cetuximab	5	-	-	-	-	0.25 mg	i.v.	Q3Dx6
4	JCAR017 + Vehicle	15	-	3.00E+06	i.v.	Single dose	[b]	i.v.	Q3Dx6
5	JCAR017 + Cetuximab High	15	6	3.00E+06	i.v.	Single dose	0.25 mg	i.v.	Q3Dx6
6	JCAR017 + Cetuximab Low	15	6	3.00E+06	i.v.	Single dose	0.125 mg	i.v.	Q3Dx6

i.v. = intravenous; mg = milligram; No. = number; PK = pharmacokinetics; Q3Dx6 = every 3 days, 6 times

[a] Target cell number is total number of T cells expressing CAR; total T cell dose is dependent on CAR transduction efficiency.

[b] Volume of vehicle (0.9% saline) is equivalent to volume of cetuximab.

Source: Report No. RPT-002100, located in Section 4.2 in Module 4.2.1.2.rpt-002100 of the BLA.

Key Evaluations and Assessments:
Study evaluations were similar to *Study #26*. (b) (4) assessments and body weights occurred on Days -1, 4, 8, 12, 15, and 19. The levels of JCAR017 were assessed in blood collected on Days 5, 13, and 21. Serum samples were collected for quantification of cetuximab at 3 and 72 hours after the first injection and 72 hours after the fifth injection dose.

Key Results:

JCAR017 Levels:
No significant difference in JCAR017 levels in the blood were observed between any groups prior to administration of cetuximab. By Day 13, mice receiving three administrations of 0.25 or 0.125 mg cetuximab/mouse showed >94% reduction in JCAR017 levels, and remaining at reduced levels of 92% 0.25 mg/mouse) and 73.3% (0.125 mg/mouse) on Day 21.

Tumor Burden:
Increased tumor burden correlated with decreased JCAR017 levels, similar to *Study #26*.

Cetuximab Levels:
Mean cetuximab concentration in the sera are summarized in Table 9. The concentrations increased as the cetuximab dose level increased.

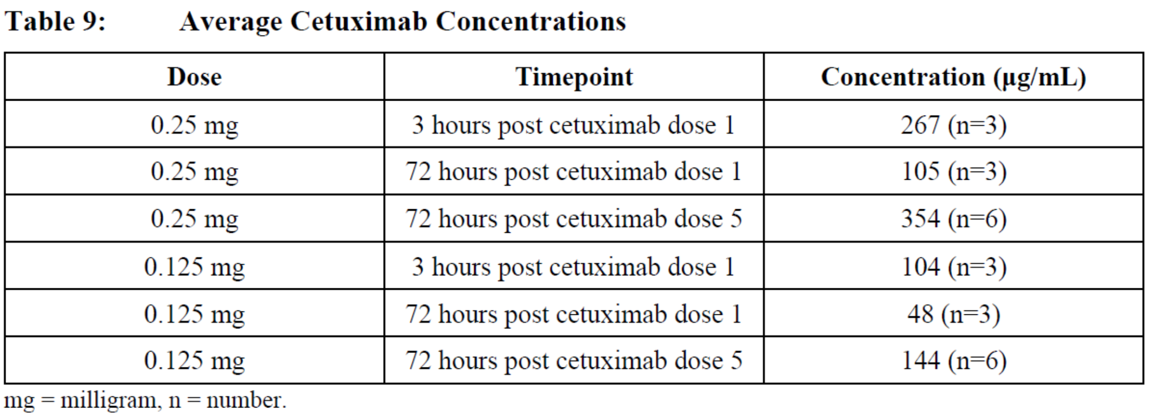


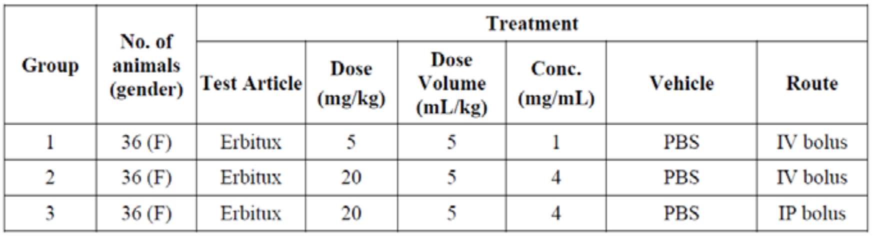
Table 9: Average Cetuximab Concentrations

Dose	Timepoint	Concentration (µg/mL)
0.25 mg	3 hours post cetuximab dose 1	267 (n=3)
0.25 mg	72 hours post cetuximab dose 1	105 (n=3)
0.25 mg	72 hours post cetuximab dose 5	354 (n=6)
0.125 mg	3 hours post cetuximab dose 1	104 (n=3)
0.125 mg	72 hours post cetuximab dose 1	48 (n=3)
0.125 mg	72 hours post cetuximab dose 5	144 (n=6)

mg = milligram, n = number.

Source: Report No. RPT-002100, located in Section 5.3 in Module 4.2.1.2.rpt-002100 of the BLA.

Study #28

Report Number		CC-DISC-DMPK-3291
Date Report Signed		4/24/20
Title		Pharmacokinetics of Erbitux in Female (b) (4) Mice Following a Single Intravenous and Intraperitoneal Administration
GLP Status		No
Testing Facility		(b) (4)
Objective(s)		To determine the pharmacokinetic (PK) properties of Erbitux in female (b) (4) mouse serum following IV or IP administration.
Study Animals	**Strain/Breed**	(b) (4)
	Species	Mouse
	Age	7-9 weeks old
	Body Weight	20.8-21.8 g
	#/sex/group	36 females/group
	Total #	108
Test Article(s)		Cetuximab (Lot No. (b) (4)
Control Article(s)		N/A
Route of Administration		IV or IP injection
Study Groups and Dose Levels		
Dosing Regimen		Single administration

Group	No. of animals (gender)	Treatment					
		Test Article	Dose (mg/kg)	Dose Volume (mL/kg)	Conc. (mg/mL)	Vehicle	Route
1	36 (F)	Erbitux	5	5	1	PBS	IV bolus
2	36 (F)	Erbitux	20	5	4	PBS	IV bolus
3	36 (F)	Erbitux	20	5	4	PBS	IP bolus

Randomization	Yes; based on tumor burden
Description of Masking	N/A
Scheduled Sacrifice Time Point	Day 14

Key Evaluations and Assessments:

Serum samples were collected for quantification of cetuximab at 0.25, 0.5, 1, 4, 8, 24, 32, 48, 96, 168, 240, and 336 hours post-dose. Levels of cetuximab were measured using (b) (4) for capture, followed by immuno-based methods.

Key Results:

Mean cetuximab concentration in the sera are summarized in Table 1; the PK profile is summarized in Table 2, below:

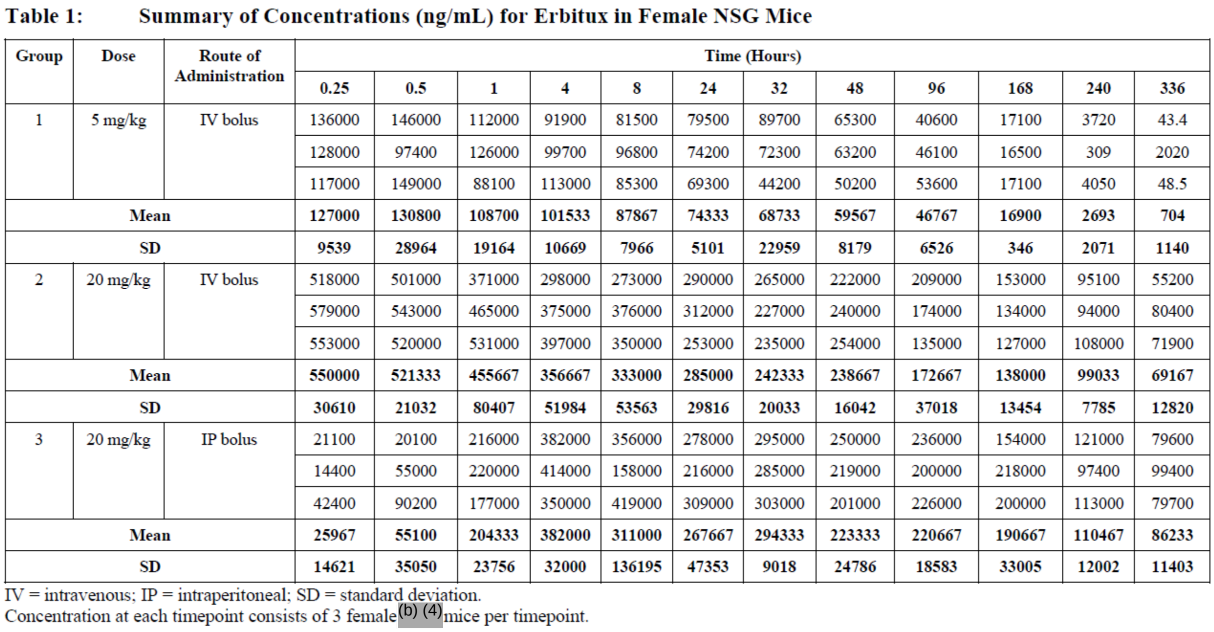


Table 1: Summary of Concentrations (ng/mL) for Erbitux in Female NSG Mice

Group	Dose	Route of Administration	Time (Hours)											
			0.25	0.5	1	4	8	24	32	48	96	168	240	336
1	5 mg/kg	IV bolus	136000	146000	112000	91900	81500	79500	89700	65300	40600	17100	3720	43.4
			128000	97400	126000	99700	96800	74200	72300	63200	46100	16500	309	2020
			117000	149000	88100	113000	85300	69300	44200	50200	53600	17100	4050	48.5
Mean			**127000**	**130800**	**108700**	**101533**	**87867**	**74333**	**68733**	**59567**	**46767**	**16900**	**2693**	**704**
SD			**9539**	**28964**	**19164**	**10669**	**7966**	**5101**	**22959**	**8179**	**6526**	**346**	**2071**	**1140**
2	20 mg/kg	IV bolus	518000	501000	371000	298000	273000	290000	265000	222000	209000	153000	95100	55200
			579000	543000	465000	375000	376000	312000	227000	240000	174000	134000	94000	80400
			553000	520000	531000	397000	350000	253000	235000	254000	135000	127000	108000	71900
Mean			**550000**	**521333**	**455667**	**356667**	**333000**	**285000**	**242333**	**238667**	**172667**	**138000**	**99033**	**69167**
SD			**30610**	**21032**	**80407**	**51984**	**53563**	**29816**	**20033**	**16042**	**37018**	**13454**	**7785**	**12820**
3	20 mg/kg	IP bolus	21100	20100	216000	382000	356000	278000	295000	250000	236000	154000	121000	79600
			14400	55000	220000	414000	158000	216000	285000	219000	200000	218000	97400	99400
			42400	90200	177000	350000	419000	309000	303000	201000	226000	200000	113000	79700
Mean			**25967**	**55100**	**204333**	**382000**	**311000**	**267667**	**294333**	**223333**	**220667**	**190667**	**110467**	**86233**
SD			**14621**	**35050**	**23756**	**32000**	**136195**	**47353**	**9018**	**24786**	**18583**	**33005**	**12002**	**11403**

IV = intravenous; IP = intraperitoneal; SD = standard deviation.
Concentration at each timepoint consists of 3 female (b) (4) mice per timepoint.

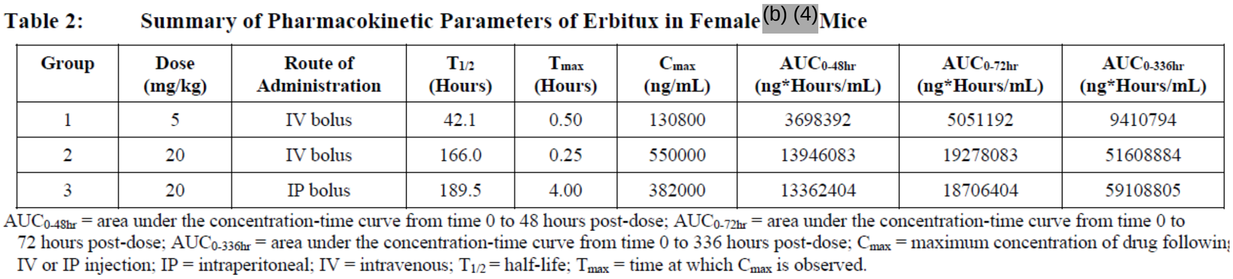


Table 2: Summary of Pharmacokinetic Parameters of Erbitux in Female (b) (4) Mice

Group	Dose (mg/kg)	Route of Administration	$T_{1/2}$ (Hours)	T_{max} (Hours)	C_{max} (ng/mL)	$AUC_{0\text{-}48hr}$ (ng*Hours/mL)	$AUC_{0\text{-}72hr}$ (ng*Hours/mL)	$AUC_{0\text{-}336hr}$ (ng*Hours/mL)
1	5	IV bolus	42.1	0.50	130800	3698392	5051192	9410794
2	20	IV bolus	166.0	0.25	550000	13946083	19278083	51608884
3	20	IP bolus	189.5	4.00	382000	13362404	18706404	59108805

$AUC_{0\text{-}48hr}$ = area under the concentration-time curve from time 0 to 48 hours post-dose; $AUC_{0\text{-}72hr}$ = area under the concentration-time curve from time 0 to 72 hours post-dose; $AUC_{0\text{-}336hr}$ = area under the concentration-time curve from time 0 to 336 hours post-dose; C_{max} = maximum concentration of drug followin IV or IP injection; IP = intraperitoneal; IV = intravenous; $T_{1/2}$ = half-life; T_{max} = time at which C_{max} is observed.

Source: Report No. CC-DISC-DMPK-3291, located in Section 11 in Module 4.2.2.7.cc-disc-dmpk-3291 of the BLA.

Reviewer’s Comments:

- Serum concentrations of cetuximab generally increased in a dose-related manner. Peak concentrations of cetuximab occurred rapidly within 0.25-4 hours post-dose, depending on the route of administration.

- Results show that, based on the calculated half-life ($T_{1/2}$) of several days, multiple administrations of cetuximab may be required to achieve a steady state level in the serum.

SAFETY PHARMACOLOGY STUDIES

No safety pharmacology studies were conducted.

PHARMACOKINETIC STUDIES (Biodistribution)

No biodistribution studies with JCAR017 were conducted.

TOXICOLOGY STUDIES

Developmental and Reproductive Toxicology(DART) Studies:

JCAR017 does not survive in immunocompetent rodents and induces graft-versus-host disease in immunocompromised mice; therefore, traditional DART studies were not conducted. However, Section 8.1 of the proposed label describes the potential risks of JCAR017 to the developing fetus, if the transduced cells cross the placenta.

Genotoxicity Studies:

Study Number	Study Title	Report Number
29	Genomic Mapping of Lentiviral Integration Sites in JCAR017 Cryopreserved Drug Product; conducted by Juno Therapeutics	RPT-00452

Lentiviral integration sites were characterized to evaluate the risk for insertional toxicities in the JCAR017. An unbiased, genome-wide, (b) (4) assay assessed viral integration events across 54 CAR T cell products derived from 34 patients that were transduced with vector to produce JCAR017.

Note: Per the study report, samples included (b) (4) patient lots manufactured under Process (b) (4) or Process (b) (4) and (b) (4) patient lots manufactured under Process (b) (4) replicates were generated from (b) (4) of the Process (b) (4) or Process (b) (4) lots, as well as from a peripheral blood mononuclear cell control lot, for a total of (b) (4) sequencing libraries.

Key study results:

- Genomic regions with low guanine-cytosine (GC) content (GC<0.3641 per window) had a reported average integration rate of 8.84 events per megabase. Regions with high GC content (GC >0.4391 per window) contained a reported average of 197.58 integrations per megabase. Insertions per megabase correlated with GC sequence content.

- Insertions were markedly higher (reported as integrations per megabase) in genomic regions with greater chromatin accessibility.[37]
- Integrations occur preferentially in regions characterized as 5'UTR exons, CDS exons, introns, and 3' UTR exons. Lower probability of insertion was determined for non-genic regions (TSS-upstream and TES-downstream regions).[38]

- JCAR017 lentiviral integration sites were comparable, in terms of nucleotide sequence preferences for target regions, to wildtype HIV-1.

- There were no differences between JCAR017 and control samples for distribution of insertion frequencies at sites encoding for tumor suppressor genes.

Reviewer's Comment:

- The study report states that lentiviral transduction methods were designed with additional features to maximize safety and to reduce the possibility of generating replication-competent viruses. The report concluded that the lentivirus used to manufacture JCAR017 integrates similarly to wildtype HIV-1. Lentiviral integrations in the samples tested correlated with GC content, gene features, and chromatin status, and with the same palindromic nucleotide bias previously reported for wildtype HIV-1. Analysis of integration rates within annotated tumor suppressor genes did not indicate preferential integration at loci associated with a potentially higher risk of transformation.

  This reviewer concurs with the study conclusion. In addition, there is no tangential data (e.g. enhanced and uncontrollable JCAR017 expansion) generated in other preclinical studies conducted with JCAR017 that suggest transformation of the T cells following transduction. However, should transformation resulting in uncontrollable expansion occur, the ability to eliminate JCAR017 (which contains the EGFRt domain) with administration of anti-EGFR antibodies (i.e., cetuximab) exists. In addition, *in vitro* expansion of JCAR017 manufactured from T cells obtained from (b) (4) showed no evidence of transformation and/or immortalization, and no preferential integration near genes of concern (*Study #30*).

Carcinogenicity/Tumorigenicity Studies:
No carcinogenicity/tumorigenicity studies were conducted.

Other Safety/Toxicology Studies:

Study Number	**Study Title / Publication Citation**	**Report Number**
30	60-Day *In Vitro* CAR T Cell Proliferation Study; conducted by (b) (4)	8398350
31	A Tissue Cross-Reactivity Study of (b) (4) in Normal Human Tissues; conducted by (b) (4)	RPT-0479

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[37] Thurman, R. E., *et al*. (2012). The accessible chromatin landscape of the human genome. Nature, 489(7414), 75–82.

[38] UTR=untranslated region; CDS = coding sequence; TSS = transcriptional start site; TES = transposable elements

An *in vitro* GLP-compliant study evaluated the IL-2 independent growth of JCAR017 to assess the risk of oncogenic transformation following transduction. Mock transduced T cells and (b) (4) of JCAR017 generated using T cells from (b) (4) in the presence or absence of IL-2 for up to (b) (4) days. Cell count and cell viability were quantified every 4 days up to Day (b) (4), and immunophenotyping of the cells from samples collected on Days 1, 9, and (b) (4) was conducted using (b) (4).

No biologically relevant differences were reported for cellular growth and viability between JCAR017 and the mock transduced T cells maintained in cell growth media lacking IL-2. Marginal differences were noted between the growth rates of JCAR017 and mock transduced T cell controls in media containing IL-2. Immunophenotyping (on Day (b) (4) of surviving cells maintained in (b) (4) containing IL-2 showed that the purity and identity profile of JCAR017 was comparable to the profile of JCAR017 on Day 1, suggesting no shift in cellular phenotype due to clonal expansion occurs over time.

These data support the conclusion that any insertional events resulting from lentiviral transduction methods used to generate JCAR017 have minimal risk for oncogenic transformation.

The study report provided for *Study #15* is identical to the report provided under the toxicology section for *Study #31*.

APPLICANT'S PROPOSED LABEL

- Subsections 8.1-8.3 of 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), as applicable.[40]

- Section 13 ('Nonclinical Toxicology') should be revised, as applicable, to accurately reflect the available nonclinical data.

CONCLUSION OF NONCLINICAL STUDIES

Review of the nonclinical studies did not identify any safety concerns that could not be addressed in the product label. The nonclinical data support approval of the license application.

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[39] The lots tested were: (b) (4)

[40] *Pregnancy, Lactation, and Reproductive Potential: Labeling for Human Prescription Drug and Biological Products – Content and Format* at: https://www fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm450636.pdf

KEY WORDS/TERMS

Lisocabtagene maraleucel, BREYANZI®, JCAR017, B-cell lymphoma, CD19 CAR T cells, Chimeric Antigen Receptor, EGFR, cetuximab, pharmacology, toxicology, lentivirus, tumor-bearing mice, (b) (4) mice