BLA 125646

tisagenlecleucel

Novartis Pharmaceuticals Corporation

Reviewers
Xiaobin Victor Lu, Ph.D
Andrew Byrnes, Ph.D.
Kimberly Schultz, Ph.D.
Elena Gubina, Ph.D
Thomas Finn, Ph.D.

BC Reviewer
Denise Gavin, Ph.D.
Gene Therapies Branch
Division of Cellular and Gene Therapies

CMC Review

1. **BLA#:** STN 125646

2. **REVIEW DATE:** August 29, 2017

3. **PRIMARY REVIEW TEAM:**
Medical Officer: Maura O'Leary, MD, and Donna Przepiorka, MD, PhD
Pharm/Tox: Ying Huang, PhD
Product Quality Team: Xiaobin (Victor) Lu, PhD, Andrew Byrnes, PhD, Kimberly Schultz, PhD, Elena Gubina, PhD, and Tom Finn, PhD
Facilities: Randa Melhem, PhD, Joan Johnson, MS,
Statistics: Xue (Mary) Lin, PhD
Labeling: Dana Jones, PharmD
RPM: Erica Giordano

4. **COMMUNICATIONS WITH APPLICANT:**

Communication/Document	Date
Teleconference/batch analysis data in electronic format	2/24/2017
Teleconference/CMC discussion with the applicant to clarify established conditions	3/2/2017
Teleconference /discuss the CMC section of the AC meeting	5/3/2017
Teleconference/Mycoplasma Testing at (b) (4)	5/31/2017
Teleconference/ FMO control/ lot release specification/ Plans for OOS lots/VCN/appearance test/	7/6/2017
Teleconference/(b) (4) deviation GMP2491: storage of sterility samples at (b) (4).	07/17/2017

5. **SUBMISSION(S) REVIEWED:**

Submission	Date Received	Review Completed (Yes/No)
Original submission	2/2/2017	Yes
Amendment #6	3/14/2017	Yes
Amendment #8	3/15/2017	Yes
Amendment #12	4/3/2017	Yes
Amendment #14	4/7/2017	Yes
Amendment #15	4/14/2017	Yes
Amendment #18	4/27/2017	Yes
Amendment #20	5/1/2017	Yes
Amendment #23	5/5/2017	Yes
Amendment #24	5/10/2017	Yes
Amendment #25	5/11/2017	Yes
Amendment #26	5/12/2017	Yes
Amendment #30	5/30/2017	Yes
Amendment #31	6/6/2017	Yes

Amendment #32	6/16/2017	Yes
Amendment #34	6/21/2017	Yes
Amendment #36	6/21/2017	Yes
Amendment #39	7/7/2017	Yes
Amendment #42	7/11/2017	Yes
Amendment #44	7/18/2017	Yes
Amendment #45	7/25/2017	Yes
Amendment #46	7/25/2017	Yes
Amendment #48	8/2/2017	Yes
Amendment #50	8/4/2017	Yes
Amendment #51	8/10/2017	Yes
Amendment #52	8/11/2017	Yes
Amendment#55	8/23/2017	Yes
Amendment#56	8/25/2017	Yes
Amendment#57	8/28/2017	Yes
Amendment#61	8/28/2017	Yes

6. **DRUG PRODUCT NAME/CODE/TYPE:**
   a. Proprietary Name: KYMRIAH
   b. Trade Name: KYMRIAH
   c. Non-Proprietary/USAN: tisagenlecleucel
   d. CAS name: Not established
   e. Common name: Chimeric antigen receptor (CAR) T cells against CD19 (CAR-19)
   f. INN Name: tisagenlecleucel
   g. Compendial Name: Not established
   h. OBP systematic name: N/A
   i. Other Names: CTL019
   j. FDA UNII number: Q6C9WHR03O

7. **PHARMACOLOGICAL CATEGORY:** CD19-directed genetically modified autologous T cell immunotherapy

8. **DOSAGE FORM:** Cell suspension

9. **STRENGTH/POTENCY:** Up to 2.5×10^8 CAR positive viable T cells

10. **ROUTE OF ADMINISTRATION:** Intravenous infusion

11. **INSPECTIONAL ACTIVITIES:** PLI inspections completed 5/25/2017

14. CONSULTS REQUESTED: N/A

16. PRECEDENTS: First-in-class

17. ADMINISTRATIVE

A. Signature Block

Name and Title	Signature and Date
Reviewed by	
Xiaobin Victor Lu, Ph.D., CQA Chair, Review Committee Biologist, OTAT, CBER	
Andrew Byrnes, Ph.D. Supervisory Microbiologist, OTAT, CBER	
Kimberly LW Schultz, Ph.D. Biologist, OTAT, CBER	
Elena Gubina, Ph.D. Biologist, OTAT, CBER	
Thomas Finn, Ph.D. Microbiologist, OTAT, CBER	
Branch Concurrence	
Denise Gavin, Ph.D. Branch Chief, Gene Therapy Branch. OTAT, CBER	
Division Sign-off	
Steven Oh, Ph.D., Acting Deputy Director, Division of Cellular and Gene Therapies, OTAT, CBER	
Raj Puri, M.D., Ph.D., Director, Division of Cellular and Gene Therapies, OTAT, CBER	

SUMMARY OF QUALITY ASSESSMENTS

I. Primary Reviewer Summary Recommendation

This biological license application (BLA) provides an adequate description of the manufacturing process and characterization of the new drug product, tisagenlecleucel. The CMC review team has concluded that the manufacturing process, along with associated test methods and control measures, is capable of yielding a product with consistent quality characteristics. This information, along with post-marketing commitment (PMC) from Novartis Pharmaceuticals Corporation (for additional validation study for mycoplasma test method performed by (b) (4) for the vector (b) (4) , will satisfy the CMC requirements for biological product licensure per the provisions of section 351(a) of the Public Health Service (PHS) Act controlling the manufacture and sale of biological products.

II. List of Deficiencies To Be Communicated

All CMC related deficiencies identified during the BLA review or during the pre-license inspections have been fully addressed by Novartis Pharmaceuticals Corporation and its subcontractors. There are no outstanding CMC deficiencies with one CMC postmarket commitment (PMC) for the revalidation of the PCR based mycoplasma test method used for the vector (b) (4) lot release.

III. Review of Common Technical Document-Quality overall summary Module 2

The common technical document- Quality overall summary was reviewed. This section contains an overview of all aspects of the Module 3: Quality including the eCTD structure. Specific CMC issues were addressed within each section under Module 3 throughout this review memorandum.

IV. Environmental Assessment or Claim of Categorical Exclusion

The applicant is claiming a categorical exclusion under 21 CFR 25.31 (c) from the need to prepare an environmental assessment. The applicant provided the following justifications: (1) the application does not significantly alter the concentration or distribution of the substance, its metabolites, or degradation products in the environment; (2) the cells have stringent nutritional requirements for survival and replication and are not viable in the environment, and are degraded into naturally occurring substances; (3) T cells are terminally differentiated cells unable to proliferate or survive outside of the human body unless they are in highly controlled, tissue culture conditions; (4) potential for release of the lentiviral construct in the environment can also be considered negligible due to the low probability of free viral particle carry over in the final drug product; and (5) no extraordinary circumstances exist, which may significantly affect the quality of the human environment and would thus require the preparation of at least an Environmental Assessment.

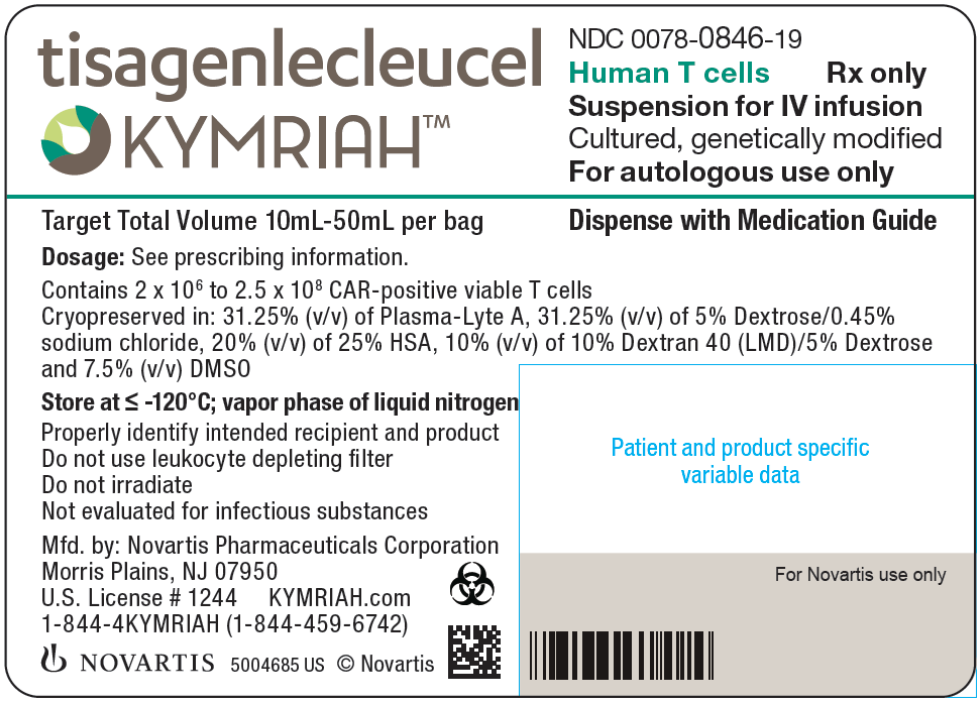
Reviewer comment: The applicant's justifications and the rationale for claiming categorical exclusion under 21CFR 25.31 (c) from the need to prepare an environmental assessment are acceptable.

V. Primary Container Labeling Review

In the original BLA submission, the primary container label lacked patient specific information, expiration date and did not comply with 21CFR 610.62(a) and (b). In response to FDA information requests (*on 3/13/2017, 8/4/2017, 8/16/2017*), the applicant submitted amendments #18 (4/27/2017), #52

(8/11/2017), and #56 (8/24/2017). In amendment #58 (8/28/2017), the applicant submitted the final revised primary container label as shown below.

Figure 1 Primary container label



Review comment: The final revised primary container label is in compliance with 21CFR 610.62(a) and (b) and contains the necessary product and patient specific information including proper name, trade name, ranges of cell dose and volume of tisagenlecleucel in the (b) (4) *infusion bag, patient identifier information, expiration date. The applicant clarified that there will be only one bag for each batch of tisagenlecleucel shipped to an infusion center. The revised primary container label is acceptable.*

There is no secondary carton label for tisagenlecleucel. The cryopreserved tisagenlecleucel infusion bag is stored in a (b) (4) in a vapor phase liquid nitrogen freezer. For shipping from the manufacturing facility to an infusion center, tisagenlecleucel within the (b) (4) is transferred from the freezer to a dry shipper charged with liquid nitrogen. Refer to section 3.2.S.2.2.4 "Filling, Storage, and Transportation" in this review for details of the secondary shipping packaging.

VI. Review of Common Technical Document-Quality Module 3.2

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

EXECUTIVE SUMMARY

Tisagenlecleucel is composed of autologous T cells that are genetically modified with a lentiviral vector (CTL019 (murine) HIV-1) encoding a chimeric antigen receptor (CAR) that specifically recognizes the CD19 protein present on CD19+ B lineage tumor cells as well as normal B cells. Tisagenlecleucel is a rationally designed immunotherapy, and the presumed mechanism of action is understood. Briefly, T cell activation begins with scFv binding to CD19 which physically brings the CAR T cells to the CD19+ tumor cells. The interaction of the CAR and CD19 results in formation of immune synapses, similar to the natural T cell activation pathways. Formation of immune synapses triggers a cascade of T cell signaling that leads to T cell activation. Upon activation, T cells produce cytokines for autocrine and paracrine signaling. The T cells may also produce perforin and granzymes to initiate direct cytolytic tumor cell killing. Activation of the CAR T cells also promotes the cell expansion and differentiation.

Tisagenlecleucel is manufactured at the Novartis Gene Therapy Manufacturing Facility located in Morris Plains, New Jersey, USA. The manufacturing process starts with the receipt of an autologous leukapheresis product from national or international apheresis centers that have been certified or qualified by Novartis. Depending upon the cellular composition of the received leukapheresis product, it is enriched for T cells with two alternative manufacturing pathways. The enriched T cells are pre-stimulated with CD3/CD28 antibody-conjugated beads and then transduced with the CTL019 (murine) HIV-1 vector. The CAR-expressing autologous T cells are then expanded in culture for up to (b) (4) to reach sufficient number of transduced T cells. The cells are washed to remove impurities (including the CD3/CD28 antibody conjugated beads) and formulated with infusion media for cryopreservation in a vapor-phase liquid nitrogen freezer. After successful quality control testing by the Novartis quality control (QC) unit, the final product is released by the Novartis quality assurance (QA) unit. Upon request from the clinical center, the released tisagenlecleucel is shipped in a vapor phase liquid nitrogen dry shipper (Dewar) to the clinical infusion center by a qualified courier. The chain-of-identity (COI) of the entire process from leukapheresis to infusion and throughout all manufacturing steps is strictly controlled by a computer based system termed (b) (4) to ensure the product's identity and product traceability.

The tisagenlecleucel manufacturing process was validated, including two alternative T cell enrichment pathways. The validation acceptance criteria include meeting the predefined critical process parameters (CPPs), in-process control (IPC) tests and lot release tests. (b) (4) batches (b) (4) batches with patient cells and (b) (batches with healthy donor cells) were manufactured using the commercial manufacturing process and commercial batch product records. Deviations during the manufacturing process validation were investigated and closed. The results of the process validation (i.e., process performance qualifications) met the predefined validation acceptance criteria.

As part of the process validation, a continued process verification (CPV) protocol was established to monitor the process by trending manufacturing data, deviations, out-of-specification batches and other unexpected manufacturing quality incidents. A media fill study was also conducted to ensure the process is able to maintain aseptic conditions throughout the manufacturing procedures. The (b) (4) system was also validated to support product traceability from leukapheresis to infusion. Shipping and receiving of leukapheresis materials and the final cell product were validated for shipments between the apheresis/infusion centers and the Novartis Morris Plains manufacturing facility.

The analytical test methods for tisagenlecleucel lot release were validated for their intended purposes. All analytical test methods have been validated for sensitivity, specificity, and reproducibility. For quantitative test methods, additional parameters such as linearity, precision, accuracy and robustness were also validated. Appropriate release specifications for tisagenlecleucel were set after analysis of test results from batches that were manufactured at the Novartis Morris Plains manufacturing facility for study CCTL019B2202.

The manufacturing process for the vector used for tisagenlecleucel production can be divided into two stages: vector substance and vector product. The vector substance is manufactured by (b) (4) located in (b) (4) . Vector substance manufacturing begins with (b) (4)

(b) (4)

.

The vector product is manufactured at (b) (4), in a controlled environment under cGMP conditions. Vector product is manufactured by (b) (4), followed by (b) (4). The vector biological potency test is performed by Novartis.

The manufacturing processes for vector substance and vector product have been validated to be sufficiently robust to produce the commercial supply of vector. Analytical test methods have been validated. There is one test that was not completely validated: the mycoplasma test performed by (b) (4). The applicant and the FDA reached an agreement that an additional validation study will be completed for this mycoplasma test as a post market commitment (PMC).

All three manufacturing facilities (Morris Plains New Jersey, USA, (b) (4) have been inspected during the BLA review. Form 483 observations regarding process validation and other facility related matters were issued at the conclusion of the each pre-license inspection. The applicant addressed all issues in the 483 satisfactorily.

Therefore, tisagenlecleucel manufacturing processes at the Novartis Gene Therapy Manufacturing Facility in Morris Plains New Jersey USA, (b) (4) are considered validated for this BLA. The overall manufacturing process and controls have been shown to consistently produce pure and potent tisagenlecleucel batches.

Recommended Action: Approval

DESCRIPTION OF DRUG SUBSTANCE AND DRUG PRODUCT

S. DRUG SUBSTANCE [(b) (4), CTL019 (murine) HIV-1 vector]

(b) (4)

87 Pages determined to be not relesable: (b)(4)

(b) (4)

[redacted]

3.2. P DRUG PRODUCT (Novartis Pharmaceuticals Corporation, tisagenlecleucel)

3.2.P.1 Description and Composition of the Drug Product

Tisagenlecleucel is formulated as a single-dose cell suspension for infusion. The cell suspension contains several excipients stock solution: PlasmaLyte A for Injection, Dextrose and Sodium Chloride for Injection, Human Serum Albumin, Dextran 40 in Dextrose for Injection and Cryoserv® solution (containing DMSO as cryopreservation agent). The tisagenlecleucel final formulation contains 7.5% DMSO.

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

Tisagenlecleucel final product is a colorless to slightly yellow suspension. The composition is shown below.

Table 33 Composition of Tisagenlecleucel Cell Suspension for Infusion

Ingredients	**Target Dose**	**Function**
tisagenlecleucel cells	• For patients ≤ 50kg: 0.2 to 5.0 x 10^6 transduced viable T cells/kg • For patients > 50kg: 0.1 to 2.5 x 10^8 transduced viable T cells	Active ingredient
Excipients	**Concentration of excipients stock solution**	
Plasma-Lyte A Injection (b) (4)	31.25% (v/v)	(b) (4)
Dextrose and Sodium Chloride Injection	31.25% (v/v)	
Albumin (Human)	20% (v/v)	
Dextran 40 in Dextrose Injection	10% (v/v)	
Cryoserv® DMSO	7.5% (v/v)	

3.2.P.2 Pharmaceutical Development

3.2.P.2.1 Components of the Drug Product

3.2.P.2.1.1 Drug Substance

Refer to the section on manufacturing process development in [3.2.S.2.6 Cells]. Compatibility of the tisagenlecleucel with the excipients has been established during clinical development and is supported by the stability studies described in [3.2.P.8.1 Cells].

3.2.P.2.1.2 Excipients

All excipients listed in table 32 above except Cryoserv® (DMSO) are either licensed drugs or USP compliant biological solutions for injection. (DMSO) has been used in FDA-licensed HPC, cord blood products to levels of up to 10%.The excipients provide the necessary stabilizing effect during cryopreservation, buffering capacity and tonicity of the formulation upon thaw.

3.2.P.2.2 Drug Product

3.2.P.2.2.1 Formulation Development

Two studies were conducted to assess the robustness of the tisagenlecleucel final formulation and to assess the suitability of the chosen excipients. The studies evaluated the impact of varying the concentration or selective removal of excipients on tisagenlecleucel viability and functionality.

In the first study, the concentration of individual excipient in the formulation was varied in a (b) (4) to accommodate potential handling differences during formulation preparation. Data were collected from

formulations used with either healthy donor or patient starting material. All tested formulations showed comparable cell cryopreservation and recovery as measured by cell viability, potency and count results. This result supports the robustness of the tisagenlecleucel final formulation intended for commercial applications.

In the second study, the removal of each of the single excipients or the removal of combinations of excipients was investigated on transduced cells from healthy donors. Some excipients (DMSO, D5 ½ NS and HSA) were shown to have a major impact on the evaluated parameters (viability, recovery and cell count, CAR expression and IFN-γ release). Therefore each of the components has its relevance in the final formulation. The study confirms the suitability of the tisagenlecleucel formulation.

3.2.P.2.2.2 Overages

Not Applicable

3.2.P.2.2.3 Physicochemical and Biological Properties

Refer to sections 3.2.S.1.3 and 3.2.S.3.1 in this BLA review.

3.2.P.2.3 Manufacturing Process Development

Refer to the section related to DS process development in section 3.2.S.2.6 of this BLA review.

3.2.P.2.4 Container Closure System

The primary packaging for the DP is (b) (4) bag, 50 mL (b) (4) or 250 mL (b) (4) . The (b) (4) or 250 mL (b) (4) freezing bag contains two sealed spike ports and one tube with two female luers. The (b) (4) bag is provided sterile by the supplier and 100% tested for leaks.

Extractables and Leachables Data Provided By the Material Vendors

(b) (4) bag is 510 (k) cleared. The (b) (4) bag was tested for resistance to (b) (4) at (b) (4) for up to (b) (4) .

Extractables And Leachables From Disposable Materials Used In tisagenlecleucel Manufacturing Process At Novartis

(b) (4)

(b) (4)

Dose reproducibility

There are losses of cells during infusion. The losses were likely due to the low volume of cells to be administered (10 mL and 30 mL for the low and high dose respectively) compared to high dead volumes in the long infusion lines. Thus it is recommended to rinse the infusion line with normal saline solution after the tisagenlecleucel infusion to ensure the full administration of the dose as described in the section 2.2 of the prescribing information for KYMRIAH.

3.2.P.2.5 Microbiological Attributes

The cell (b) (4) bags are sterile (b) (4) by the vendor). (b) (4) has been validated by the vendor for all components of the (b) (4) bag. Products were sterilized at (b) (4) or more, put through an accelerated aging study, then evaluated for change or degradation. While there was some minor discoloration of (b) (4) luer components, there was no change in physical properties. The company performing sterilization is (b) (4) inspected by FDA and EU authorities and the (b) (4) cycle is also (b) (4) qualified according to (b) (4) .

Integrity Test Performed By (b) (4)

Integrity testing is part of the validation studies submitted in the 510(k) filing.

Integrity Test Performed By Novartis

A container closure integrity test (CCIT) study was conducted for the (b) (4) bags simulating in terms of sealing during storage and until the time of use of its content. The (b) (4) test was selected as read out of the CCIT assay because

Surrogate material used consists of the tisagenlecleucel formulation medium (b) (4)

(b) (4)

3.2.P.2.6 Compatibility

Compatibility Studies Performed by the Vendor

The (b) (4) bag was tested for biocompatibility. The data were a part of the 510(k) clearance process. The table below reports a summary of the biological testing performed on the (b) (4) bag.

Compatibility Studies Performed by Novartis

Compatibility of the (b) (4) Bag (bag size range tested: 50 m L - 250 mL) and of different infusion sets for DP administration was tested in a study performed a two target dose levels: a low and a high dose corresponding to $1x10^8$ ($1x10^7$ cells/mL) and $3x10^9$ ($1x10^8$ cells/mL) total cells respectively.

Compatibility studies were performed using (b) (4) were used in the study. (b) (4)

(b) (4) .

(b) (4)

2 Pages determined to be not relesable: (b)(4)

(b) (4)

The Applicant's Conclusion

Compatibility of tisagenlecleucel with the infusion bags with different infusion sets intended to be used in the US, EU or Japan was evaluated by viable cell recovery prior to and after the infusion process by different analytical methods (cell count, phenotype, potency) and effective dosing. The results show that infusion bags with infusion sets are compatible with tisagenlecleucel.

Reviewer comment: The applicant conclusion was acceptable.

3.2.P.3 Manufacture

3.2.P.3.1 Manufacturer(s)

Refer to section 3.2.S.2.1 in this BLA review.

3.2.P.3.2 Batch Formula

Table 36 Batch Formula for Tisagenlecleucel

Ingredients	Target dose	Function	Reference to standards
tisagenlecleucel cells[1]	For patients ≤ 50 kg: 0.2 to 5 x 10^6 transduced viable T cells/kg For patient > 50kg: 0.1 to 2.5 x 10^8 transduced viable T cells	Active ingredient	Novartis internal
Excipients	Concentration of excipients stock solution		
Plasma-Lyte A injection (b) (4)	31.25% (V/V)	(b) (4)	(b) (4)
Dextrose[2] and sodium chloride[3] injection	31.25% (V/V)	(b) (4)	(b) (4)
Albumin (human)[4]	20% (V/V)	(b) (4)	(b) (4)
Dextran 40[5] in Dextrose injection	10% (V/V)	(b) (4)	(b) (4)
Cyroserv DMSO[6] (b) (4)	7.5% (V/V)	(b) (4)	Novartis internal

3.2.P.3.3 Description of Manufacturing Process and Process Controls

Refer to section3.2.S.2.2 in this BLA review.

3.2.P.3.4 Controls of Critical Steps and Intermediates

Refer to section 3.2.S.2.4 in this BLA review.

3.2.P.3.5 Process Validation and/or Evaluation

Refer to section 3.2.S.2.5 in this BLA review.

3.2.P.4 Control of Excipients (noncompendial Cryoserv®)

3.2.P.4.1 Specifications

Cryoserv® (≥99.0% Dimethyl Sulfoxide solution) is an excipient that is not included in a major pharmacopoeia as an injectable solution. Therefore, Cryoserv® must be fully qualified prior to use. Cryoserv® is tested by Novartis according to process requirements and meets the requirements for injectable solutions including compendial methods for particulates, sterility and endotoxin.

Reviewer comment: The information in the original submission was inadequate for a full review, therefore the FDA requested more information on 4/24/2017 and was received in amendment 23 received on 5/5/2017. With the provided information, the qualification of Cryoserv is appropriate and adequate for use in the tisagenlecleucel product. Refer to next section for qualification testing for Cryoserv®.

3.2.P.4.2 , 3.2.P.4.3 Analytical Procedures and Validation of Analytical Procedures and 3.2.P.4.4 Justification of Specifications

(b) (4)

(b) (4)

(b) (4)

(b) (4)

3.2.P.4 Control of Excipients(b) (4)

3.2.P.4.1 Specifications

The following excipients of the drug product formulation comply with the quality requirements of the listed compendial monographs:

Plasma-Lyte A (b) (4)

Plasma-Lyte A (b) (4) complies with the quality requirements of (b) (4) . In addition, a visual examination of primary and secondary packaging and a specific identity test is routinely performed by Novartis. The chemical components of Plasma-Lyte A are tested for identity according to (b) (4)

Dextrose and Sodium Chloride Injection

Dextrose and Sodium Chloride Injection, (b) (4) complies with the quality requirements of the (b) (4) monograph as verified on the vendor Certificate of Analysis. In addition, a visual examination of primary and secondary packaging and a specific identity test is routinely performed by Novartis. The chemical components are tested for identity according to (b) (4) .

Dextran 40 in Dextrose Injection

Dextran 40 in Dextrose Injection complies with the quality requirements of the (b) (4) Additionally, extra specifications are performed by Novartis. The chemical components are tested for identity according to (b) (4)

The (b) (4) identity methods for Plasma-Lyte A Injection, Dextrose in Sodium Chloride Injection and Dextran 40 in Dextrose Injection are not validated but verified to demonstrate the specificity

and can be used for the intended purpose, under actual conditions for use and users have the appropriate experience, knowledge, and training to understand and perform the compendial procedures as written.

Vendor's CoAs are provided.

3.2.P.4.5 Excipients of Human or Animal Origin

Albumin (Human)

Albumin (Human) contains Sodium N-Acetyltryptophanate and Sodium caprylate and is a pharmaceutical grade product complying with the quality requirements of the applicable (b) (4) monographs. Additionally, extra specifications are performed by Novartis. as provided in below.

Appearance test by visual examination: Visual examination of the product to verify it is the marketed product. Evaluate the appearance of the secondary packaging (carton). Evaluate the integrity of the primary packaging (container) and container label. **Acceptance criteria**: Results comply with the requirements in the test procedure.

Identity by (b) (4) **analysis:** (b) (4) analysis to detect HSA protein with respect to reference material. **Acceptance criteria**: positive for HSA compared to reference material. Pass on vendor certificate of analysis. The Human Serum Albumin identity test by (b) (4) is validated for system suitability, repeatability and specificity. Vendor's CoA is provided.

3.2.P.4.6 Novel Excipient

There are no novel excipients used in the manufacture of tisagenlecleucel.

3.2.P.5 Control of Drug Product

3.2.P.5.1 and 3.2.P.5.6 Specification(s) and Justification of Specification(s)

Specification(s)

The specifications are set based on the process capability, the results from release and stability testing of clinical batches during development, corresponding clinical data, and statistical evaluation. The original lot release specifications submitted in the original BLA have been updated based on the discussion between the FDA and the applicant and documented in amendment #46 of the BLA, the applicant agreed to the FDA recommendation for narrowing the specifications for percentage of viable T cells from (b) (4) and for lowering the upper limit for CAR transgene (b) (4) (CMC-OTAT IR 21 July 2017). The acceptance criteria for release of IFN-γ in response to CD19-expressing target cells to the value proposed during the BLA filing of (b) (4) IFN-γ/transduced cell remains unchanged at (b) (4) /transduced cell. The applicant provided updated specifications for tisagenlecleucel final product lot release as shown below.

Table 37 Revised Analytical Methods and Specifications for Release

Test	Requirements for commercial use	Sample used for testing
Appearance	Colorless to slightly yellow	Formulated product (b) (4)
Identity by CAR q-PCR	Positive	Post-harvest[1]
Percentage of viable T cells	(b) (4)	Final product (b)
Determination of transduction efficiency by CAR-q-PCR	(b) (4)	(b) (4)
Cell viability	(b) (4)	Final product (b)
Determination of	(b) (4)	(b) (4)

residual beads by microscopy		
Percentage of viable CD19+ B cells	(b) (4)	Final product (b)
Total cell count[4]	Report cells/mL	Final product (b)
Number of viable cells (calculated)	(b) (4)	Final product (b) (4)
Dose (calculated)	• 0.2 to 5.0 × 10^6 transduced viable T cells/kg body weight (≤50 kg) • 0.1 to 2.5 ×10^8 transduced viable T cells (> 50 kg)	Final product (after thaw)[2] Calculation formula: *(%CAR expression x Viable cell concentration x Volume per dose)/100 (per patients (≤ 50 kg this number is divided per Kg body weight)*
Determination of CAR expression by flow cytometry	(b) (4)	Final product (b)
Release of IFNγ in response to CD19-expressing target cells	• (b) (4)	Final product (b)
Bacterial Endotoxins	(b) (4)	Final product (b)
Sterility	Negative	Formulated product (b) (4)
Mycoplasma	Negative	(b) (4)
Determination of VSV-G DNA by quantitative PCR (qPCR)	(b) (4)	(b) (4)

[1]Post-harvest samples are taken before the addition of cryopreservation media, to avoid potential interference of (b) (4) present in the final formulation.
[2]Final product (after thawing) sample tests are performed on an aliquot of the final formulation of the cellular product, collected just prior to filling the drug product bag, stored in the vapor phase of liquid nitrogen (≤ -120 C), and thawed at the time of analysis.
[3] Testing for residual beads is performed using a sample collected (b) (4) prior to dose formulation, to allow for accurate measurement of residual beads present per (b) (4) , without being impacted by the range of concentrations of cells per mL at which final dose may be formulated.
[4] No specification is set for Total cell count since it is not a critical quality attribute; result from Total cell count is used to calculate Number of viable cells, essential for the Dose calculation.
[5] Pre-harvest samples are taken from the cell culture supernatant at the end of cell culture just prior to harvest processing steps and final formulation, to provide maximal opportunity to detect any contaminating mycoplasma present in the manufacturing process.

Justification of Specifications

A total of (b) (4) batches were manufactured under study CCTL019B2202 (b) (4) batches manufactured for clinical development that were used for the analysis to justify how the commercial specifications have been set. All (b) (4) batches were manufactured by Novartis Morris Plains for use in clinical study CCTL019B2202.

The release specifications were based on product batches shown to be safe during clinical use. Of the batches excluded from the specification setting analysis, (b) (4) batches were not released for clinical use due to out-of-specification testing results. In addition, (b) (4) manufactured batches meeting product release specifications but not infused (due to changes in patient health status) were also excluded from the analysis due to lack of supportive clinical outcome data.

To model tisagenlecleucel release data, two different distributional fits were required. The first, a Beta distribution, was employed in cases where the data were bounded (*e.g.* viability with limits of 0% and 100% and could describe non-normal distributions). The second, an exponential distribution, was employed in cases where the data grew or decayed exponentially.

In order to choose the best model that would fit the data, a best visual fit of the data was performed as well as Diagnostic Plots as a measure of the goodness-of-fit (here represented by the log-likelihood of the data). The lower the log-likelihood is, the better the curve fit and, as a result, the better the model.

Once a model was chosen that sufficiently explained the variability in the pALL data set, the upper (at the (b) (4) percentile) and/or lower limits (at the (b) (4) percentile) of the data were determined and reported.

In instances where the specification was supported by the estimated limits of the statistical analysis, this was mentioned. In the instances where the chosen specification was not supported by the estimated limits of the statistical analysis, justification of the specification source was provided.

Appearance

Appearance testing is performed on the final formulated product sample collected prior to filling drug product bag (prior to cryopreservation).

- Color

Appearance testing for final product color provides an evaluation of consistency of final formulation. Final product color has been identified as a potential indicator of significant erythrocyte content in the drug product, leading to final product color ranging from (b) (4) . Visual evaluation of drug product formulation is performed in comparison to the color reference solutions as described in (b) (4) .

The acceptance criteria to release are set to “Colorless to slightly yellow”.
Evaluation of Novartis manufactured product showed consistent color across all but one of the batches evaluated. (b) (4) batches tested met the current specification of “colorless to slightly yellow.” One batch (b) (4) did not meet the current specification, with color evaluated as (b) (4) This out of specification batch was found to exhibit persistence of patient erythrocytes during manufacture of the batch. Because autologous erythrocytes are not considered a safety risk, the batch was released under exception, and clinical efficacy was observed at the patient’s (b) (4) evaluation. Subsequent manufacturing process optimizations have been implemented that are expected to prevent further occurrence of erythrocyte persistence.

- Opacity

Appearance testing of drug product opacity provides user evaluation of final formulation consistency. The product contains a cell suspension and is consequently expected to show cell density dependent degree of opacity. The appearance test for opacity is deemed unnecessary considering that this attribute is only able to confirm the presence of a cellular suspension which is tightly controlled by the manufacturing process. Absence of the cellular suspension identified by inspection for opacity would be identified by a number of other assays used for product release testing (and would result in OOS test results), including measurement of cell viability, % T cells, total viable cell count, determination of CAR expression by flow cytometry, and others. Because the appearance assessment for opacity does not measure a quality attribute that is not already captured by other release assays, the assessment for opacity is deemed unnecessary and will be omitted from the commercial specifications.

Review comment: The applicant’s reason to omit opacity as an appearance test is reasonable. However, lots with cell clumps and other foreign matters should not be released based on visual inspection. An IR was sent to the applicant on June 26, 2017 requesting the inclusion of cell clump visual inspection to the lot release testing. The applicant responded on June 30, 2017 indicating that the in-process visual inspection of any foreign matter after formulation but before cryopreservation will be performed. The applicant also indicated that they will continue inspecting each filled bag of each CT019 batch for visible particles and cell clumps. In the highly unlikely event where cell particles or clumps are observed in a filled bag, the operator will photograph the observation, contact management, and a deviation report will be initiated. The concerned filled bag disposition will be determined upon

completion of the investigation. This response is acceptable if the instruction for visual inspection of cell clumps will be included in the batch record, SOPs or work procedures. FDA also proposed to add cell clump inspection in the section of preparation for infusion of the Highlights of Prescribing Information. This information was sent to the applicant on 7/14/2017. In amendment #52 received on 8/11/2017, the applicant accepted the inclusion of the visual examination for cell clumps.

Identity

Identity testing is performed using a sample collected (b) (4) of the final cell culture during manufacture, just prior to dose formulation. This (b) (4) sample is considered representative of the final cellular product, but avoids potential interference of (b) (4) present in the final formulation with the qPCR reaction. The harvested cells are pelleted and cryopreserved until DNA extraction and analysis.

Identity is confirmed via testing for the presence of the CAR19 transgene by a quantitative PCR method. The same q-PCR method is used for determining average transgene copy number per cell.

The acceptance criterion for release is set to "Positive". All (b) (4) batches evaluated were positive for transgene presence.

Percentage of Viable T Cells

Evaluation of T cells by flow cytometry is performed on an aliquot of the final formulation of the cellular product, collected just prior to filling the drug product bag. The aliquot is cryopreserved along with the drug product bag, stored in the vapor phase of liquid nitrogen (≤ -120°C), and thawed at the time of analysis, providing a test sample that will accurately reflect the percentage of T cells present in the formulated drug product following cryopreservation and thaw.

Flow cytometry is the industry standard method for evaluation of T cells, which are most commonly measured via the surface expression markers CD45 and CD3. T cell purity of tisagenlecleucel is tested by flow cytometry for % viable CD3+CD45+ cells, of the total viable WBCs. As the CTL019 product is comprised of T cells, this assay is providing information on the cellular purity of the intended cell type in the product.

The acceptance criterion for release is set to (b) (4) viable T cells". The %T cells data was modeled with a Beta distribution fit (b) (4) . The Beta distribution fit was the model that most comprehensively covered the available data of the statistical models available. Based on the (b) (4) percentile for the data, the lower control limit was estimated to be (b) (4) The lowest value detected was (b) (4) which resulted in clinical efficacy based on (b) (4) response. Based on manufacturing history and statistical evaluation, it is proposed to set the commercial acceptance limit for percentage of viable T cells to (b) (4)

Transduction Efficiency by CAR qPCR

Transduction efficiency is measured by qPCR. The test sample is collected from (b) (4) of the final cell culture period during manufacture, just prior to dose formulation. This (b) (4) sample is considered representative of the final cellular product, but avoids potential interference of DMSO present in the final formulation with the qPCR reaction. The results are calculated as the average number of transgene copies/cell. The acceptance criterion for release is set to (b) (4) transgene copies/cell".

The pALL CAR copies by qPCR data were modeled with a Beta distribution fit ((b) (4) curve). This model most closely models the data but it cannot account for all of the variability seen in the pALL dataset. Based on the (b) (4) percentile for the data, the lower control limit is estimated to be (b) (4) . The lowest pALL value for release product observed was (b) (4) . In addition, based on the (b) (4) percentile for the data, the upper control limit is estimated to be (b) (4) , while the maximum pALL value for release product was (b) (4) . An acceptance range for acceptance was set to be (b) (4)

Cell Viability

Cell viability testing is performed on an aliquot of the final formulation of the cellular product that is cryopreserved and thawed prior to testing. Viability of the product is essential for activity. Demonstration of drug product cellular viability serves as an additional measure of purity providing information on the presence of dead cells in the product, and non-viable cells are considered an impurity.

Cell viability was measured by (b) (4) exclusion or by (b) (4) using the (b) (4) during manufacture for clinical development. Cell viability will be measured using (b) (4) for commercial manufacture. The acceptance criterion for release is set to (b) (4) viable cells".

The pALL cell viability data was modeled with a Beta distribution fit ((b) (4)). Based on the (b) (4) percentile for the data, the lower control limit is estimated to be (b) (4) The lowest pALL value observed was (b) (4). A lower limit for acceptance was set to be (b) (4)

Determination of Residual Beads by Microscopy

Testing for residual beads is performed using a sample collected (b) (4) of the final cell culture period during manufacture, immediately following (b) (4) to dose formulation. This (b) (4) sample is considered representative of the final cellular product, and allows for accurate measurement of residual beads present per (b) (4) without being impacted by the range of concentrations of cells per mL at which final dose may be formulated.

CD3/CD28-coated Dynabeads™ used during manufacture. The most sensitive and reliable method for evaluating presence of beads within drug product involves evaluation of an aliquot of drug product by microscopy. Residual beads are visually observed and counted per (b) (4). The acceptance criterion for release is set to (b) (4)

The pALL residual bead count data was most closely modeled with an exponential fit/decay. The model sufficiently explains the data set variability. Based on the (b) (4) percentile for the model, the upper control limit was estimated to be (b) (4). The highest pALL residual bead count data was (b) (4).

Statistical evaluation of data from release testing, and preclinical toxicology assessment, were taken into consideration for the setting of specification. In an intravenous safety study in rats at a dose of (b) (4), no beads were detected in any tissues at day (b) (4) post –mortem and no toxicity observed. Worst case scenario for pediatric patients (b) (4) and for adult patients (b) (4) were applied and resulted in a maximal bead dose of (b) (4) respectively. Based on the statistical evaluation of Novartis manufactured product data, the acceptance criterion was tightened to (b) cells to ensure the consistency of the manufacturing process. This limit is well below the NOAEL in animals and therefore a sufficient safety margin is established.

Percentage of Viable CD19+ B cells

Test of B cells is performed on an aliquot of the final formulation of the cellular product, collected just prior to filling the drug product bag. The aliquot is cryopreserved along with the drug product bag, stored in the vapor phase of liquid nitrogen (≤ -120°C), and thawed at the time of analysis, providing a test sample that will accurately reflect the percentage of B cells present in the formulated drug product following cryopreservation and thaw.

Measurement by flow cytometry is the industry standard assay for evaluation of B cells. The B cells are identified by their expression of CD45 and CD19. The acceptance criterion for release is set to (b) (4).

B cell impurities are tested by flow cytometry for % viable CD19+CD45+ cells, of the total viable WBCs. Within the sensitivity of the assay, no CD19+ B cells were observed for any of the (b) (4) batches tested.

Number of Viable Cells

Total viable cells in the formulated dose is calculated based on bag fill volume and cell count and viability measurements obtained using an aliquot of the final product after thawing of the cryopreserved sample. Because dose is formulated based on number of transduced T cells, the total number of cells (transduced and non-transduced) present within a dose can vary significantly depending on the relative percentage of transduced T cells present in the drug product. Thus, evaluation of total viable cells present in drug product represents an important component to evaluating product identity. Total viable cells are measured by automated count using the (b) (4) . The acceptance criterion for release is set to (b) (4) total viable cells; report cells/mL".

The pALL cell viability data was modeled with an exponential distribution fit/decay. The model sufficiently explains the data set variability. Based on the (b) (4) percentile for the model, the upper control limit was estimated to be (b) (4) cells. The highest pALL viable cell count was (b) (4) cells. The acceptance criterion was set using the data from release testing and considering historical safety data. Maximum total viable cell number was originally based on historical safety data from Penn.
Based on the statistical evaluation of Novartis manufactured product data, Novartis decided to maintain the specification for Number of Viable Cells at (b) (4) total viable cells.

Dose

Based on the clinical development release criteria for earlier pALL trials at University of Pennsylvania, lack of correlation to clinical outcomes and lowest dose 0.17×10^6 cells/kg that resulted in clinical response, the dose specification was set to a range of 0.2 to 5.0×10^6 transduced viable T cells/kg (≤50 kg patients) and 0.1 to 2.5×10^8 transduced viable T cells (>50 kg patients).

Determination Of CAR Expression By Flow Cytometry (Potency)

Evaluation of CAR expression by flow cytometry is performed on an aliquot of the final formulation of the cellular product that is cryopreserved and thawed prior to analysis. Determination of CAR expression by flow cytometry demonstrates discrete expression of the CAR protein on the surface of cells within final product. Additionally it provides the percentage of cells in final product expressing the CAR protein, which is essential for determining dose. The detection of CAR protein by flow cytometry is performed using an anti-idiotypic antibody, conjugated to (b) (4) recognizing the tisagenlecleucel CAR receptor. The acceptance criterion for release is set to (b) (4) positive".

The pALL CAR expression data were modeled with a Beta distribution fit. Based on the (b) (4) percentile for the data, the lower control limit is estimated to be (b) (4). The lowest pALL value observed for the (b) (4) batches was (b) (4). A lower limit for acceptance was set to be (b) (4) for release.

The acceptance criterion was set based on statistical evaluation of data from release testing and evaluation against clinical outcome. To date, no correlation between the percentage of CAR expressing cells and clinical efficacy has been observed. Transduced cells expand *in vivo*, resulting in an immune response sufficient to allow clearing of the tumor cells, irrespective of the infused percentage of transduced cells. The absolute minimal percentage of transduced cells required to achieve clinical response remains to be defined. Additionally, dose formulation is based on total number of transduced viable T cells; thus the total number of transduced T cells is controlled by the dose. Although the statistical evaluation of batch release testing supports a lower limit of (b) (4), it is noted that recent process improvements during (b) (4) cell enrichment resulted in more consistent transduction efficiencies; thus, specification for Determination of CAR Expression by Flow Cytometry is set as (b) (4) positive.

Reviewer comment: An FMO control was introduced for suitability control into this test method. Refer to section 3.2.P. 5.2 of this BLA review for details.

IFN-γ Release in Response to CD19-Expressing Target Cells

The IFNγ release assay is performed using an aliquot of the final formulation of the cellular product that is cryopreserved and thawed prior to analysis as described previously. The IFNγ release assay detects the responsiveness of tisagenlecleucel cells to CD19-specific stimulation by measuring release of the cytokine IFNγ in cell culture supernatant. In order to control for the CD19-specific response, a negative control consisting of mesothelin (Meso)-expressing cells, which are negative for CD19, was used for co-culture with the tisagenlecleucel cells. The levels of IFNγ in supernatant from the cultures with tisagenlecleucel and Meso-expressing cells should be relatively low (="background"). In comparison, the levels for the CD19-specific stimulation of the tisagenlecleucel cells should be higher than the negative control.

The acceptance criterion for release is set to (b) (4) induction (of OD value in (b) (4) on supernatant from cells stimulated with CD19-expressing cells over background); and (b) (4) IFNγ per transduced cell".

The pALL release of IFNγ pg per transduced cell was modeled with a Beta distribution fit. Based on the (b) (4) percentile for the data, the lower control limit is estimated to be (b) (4) cell. The lowest pALL value observed for this product was (b) (4) transduced cell. In addition, the upper control limit is estimated to be (b) (4) transduced cell, while the maximum pALL value for release product was (b) (4) transduced cell.

Specification was set based on statistical evaluation of data from release testing and evaluation against clinical outcome. No clear correlation between IFNγ release and clinical efficacy has been observed. Because of the wide range of IFNγ assay measurements observed for batches resulting in clinical efficacy, the proposed dual specification is intended to demonstrate both sufficient induction of antigen (CD19)-specific IFNγ release, as well as a minimum absolute amount of IFNγ released per transduced cell in response to antigen stimulation. Specification for

IFNγ release is set as (b) (4) IFNγ per transduced cell. Although there was no relationship of IFNγ release in response to CD19 expressing target cells to the main safety measure CRS grade, the upper limit is set to ensure consistency of manufacturing.

Bacterial Endotoxins

Endotoxin testing is performed using an aliquot of the final formulation of the cellular product, collected just prior to filling the drug product bag. The aliquot is cryopreserved along with the drug product bag, stored in the vapor phase of liquid nitrogen (≤ -120°C), and thawed at the time of analysis, providing a test sample that accurately reflects the formulated drug product following cryopreservation and thaw.

Endotoxin is detected per (b) (4) using the (b) (4) . The principle of the method is based on the (b) (4) by the reaction of endotoxins with the (b) (4) reagent. Proposed specification for release is set to (b) (4) .

The endotoxin limit concentration is based on a maximum human dose as defined in the scope of the analytical acceptance criteria and the maximum acceptable level, which ensures that a patient would not receive more than (b) (4) of body mass and hour as required by (b) (4) is met. The proposed limit is considering a maximum dose volume of (b) (4) and minimum body weight of (b) (4) for pediatric ALL. Within MP manufactured batches, endotoxin was detected in only (b) (4) batches, (b) (4) , reported at (b) (4) , respectively. All other batches tested <LOQ.

Sterility

Sterility testing is performed using an aliquot of the final formulation of the cellular product, collected just prior to filling the drug product bag. The sterility test is initiated immediately following collection of this sample (without cryopreservation and thaw) to maximize the potential detection of any microbial contaminants, as sensitivity might be diminished post-cryopreservation and thaw.

Sterility testing is performed on samples from the final formulation bulk. After sampling for sterility, the product is only transferred to the infusion bag by making use of a closed system therefore no contamination can take place during fill and finish after sterility sampling. The (b) (4) is used for sterility test. The assay allows a valid read out in a shorter timeframe than the classical sterility test, while maintaining the sensitivity. The acceptance criterion for release is set to "Negative". The sterility test was also reviewed by DBSQC and found acceptable. Refer to the review memorandum from Ms. Simleen Kaur from DBSQC for details.

Mycoplasma

Mycoplasma testing is performed using a sample of the cell culture (b) (4) taken at (b) (4) processing steps and final formulation. It is considered the most appropriate sampling point to maximize the chance of detection.

(b) (4) Mycoplasma detection assay was selected to be the assay in order to test product for mycoplasma contamination in the tisagenlecleucel manufacturing process. The (b) (4) was adapted using the (b) (4). Proposed specification for release is set to "Negative".

This assay was reviewed by Ms. Simleen Kaur of DBSCQ.

Review comment: During the PLI at the Novartis Morris Plains NJ facility, two issues were noted with the validation of the (b) (4) *assay used to test for mycoplasma contamination in the cell culture. 1) The mycoplasma test was not performed on the proper sample. Generally, mycoplasma testing is performed on the cells and supernatant to increase assay sensitivity, Novartis performed the test on* (b) (4) *sample only. We discussed this issue with Novartis. Novartis provided a discussion about the risks and literature references related to how most mycoplasma species will be in the* (b) (4) *following the longer term cell culture that is used to make tisagenlecleucel. Novartis also discussed how given the biology of mycoplasma, they felt that the cells, which for some lots can be limited, should be used to make the final product. OTAT CMC reviewers (Xiaobin Victor Lu and Denise Gavin) discussed this issue with DBSQC reviewer (Simleen Kaur). FDA agreed that the risk was low and that the sample may consist of* (b) (4) *only, when procured late in the manufacturing process for tisagenlecleucel. Novartis agreed to provide all supporting data, literature and the risk assessment to the BLA. Literature was provided to FDA inspectors. Review of the supporting documents, we agreed that the test is suitable for tisagenlecleucel mycoplasma testing.*

2) The assay was not validated in the proper matrix, i.e. the assay was validated by the vendor and verified by Novartis, but the comparability between the (b) (4) *method* (b) (4) *and the* (b) (4) *was not performed. When the vendor validated the test it was not in the CAR-T cell matrix, but media. We discussed these issues with the firm staff who outlined the validation process at the vendor and on-site using their product (matrix effect not observed). They described how they looked at over* (b) (4) *of mycoplasma, and showed data demonstrating that the method is* (b) (4) *more sensitive than the* (b) (4) *method. After further discussion with CBER/DBSCQ reviewer (Ms. Simleen Kaur) and the Novartis SMEs, the FDA agreed that the validation was sufficient. Refer to Refer to the review memorandum from Ms. Simleen Kaur from DBSQC for details.*

Presence of VSV-G DNA

VSV-G testing is performed using a sample collected (b) (4) of the final cell culture period during manufacture, just prior to dose formulation. This (b) (4) sample is considered representative of the final cellular product, but avoids potential interference of DMSO present in the final formulation with the qPCR reaction.

To evaluate the likelihood of RCL in final product, a (b) (4) qPCR assay for residual plasmid is used. The assay specifically detects the glycoprotein of the Vesicular Stomatitis Virus (VSV-G) sequence in genomic DNA using primers and a probe that hybridize to VSV-G sequences. The acceptance criterion for release is set to (b) (4)

Detection of VSV-G DNA is used as marker for RCL, therefore the acceptance criterion is no detection of RCL. The limit is based on the LOQ of the qPCR assay and remains unchanged from the Penn limit. RCL testing using amplification in indicator cells is performed as part of the LV vector release testing.

3.2.P.5.2 and 3.2.P.5.3 Analytical Procedures and Validation of Analytical Procedures

Summary for 3.2.P.5.2 Analytical Procedures:

Each of the assays used for lot release testing were described generally. The FDA requested detailed SOPs for each assay on 02/24/2017 and they were entered into the BLA eCTD section 1.11.1 in amendment 6. The review of analytical procedures includes all of these documents, including information provided only in the SOPs. The proposed analytical procedures included 3 assays with little or no experience during the clinical trial; cell concentration and viability by (b) (4), Mycoplasma by qPCR, and Phenotyping of White Blood Cell (WBC) Subsets and CAR expression by flow cytometry. Comparability data was requested on 3/23/2017 with a response received in amendment 14. *Reviewer comment: All analytical procedures are acceptable.*

Summary for 3.2.P.5.3 Validation of Analytical Procedures:

Reviewer comment: Overall the predetermined acceptance criteria were met and the validations are adequate. However, the scope of the validations was not rigorous and generally did not assess different lots of reagents or different characteristics of the final product (e.g., various transduction efficiencies). All validation reports were acceptable. All validation tables in this review were generated by the FDA from the assay validation reports.

The analytical procedures and validation of the analytical procedures for testing sterility, mycoplasma, and endotoxin were reviewed by Simleen Kaur in DBSQC.

Appearance:
Procedure: Performed on final formulation of DP, just prior to filling the bag. According to SOP AM10041C. In brief, (b) (4) of DP sample is dispensed into a (b) (4) and compared to (b) (4) reference solutions according to (b) (4) using a (b) (4) for the (b) (4) source. Specification allows range of color, colorless to slightly yellow, corresponding to references (b) (4) Color reference solutions: (b) (4), catalog #87576 (b) (4) ampules of solutions (b) (4)
Validation: None. The applicant states that it follows (b) (4)

Reviewer comment: SOP still includes turbidity assessment. However this analysis was removed from testing of the final product because it was uninformative. The product is always turbid due to the inherent nature of a cell suspension product. This is acceptable.

Determination of Transduction Efficiency by CAR Quantitative PCR (qPCR):
Procedure:

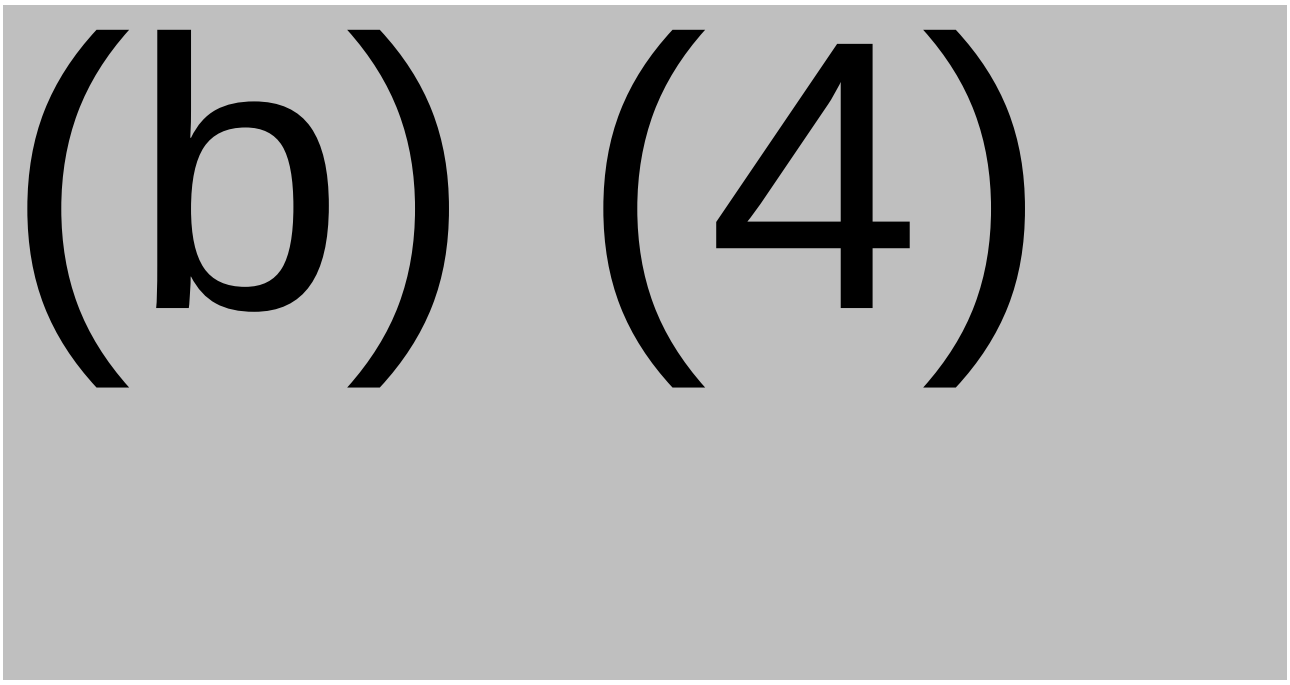
- (b) (4)

2 Pages determined to be not relesable: (b)(4)

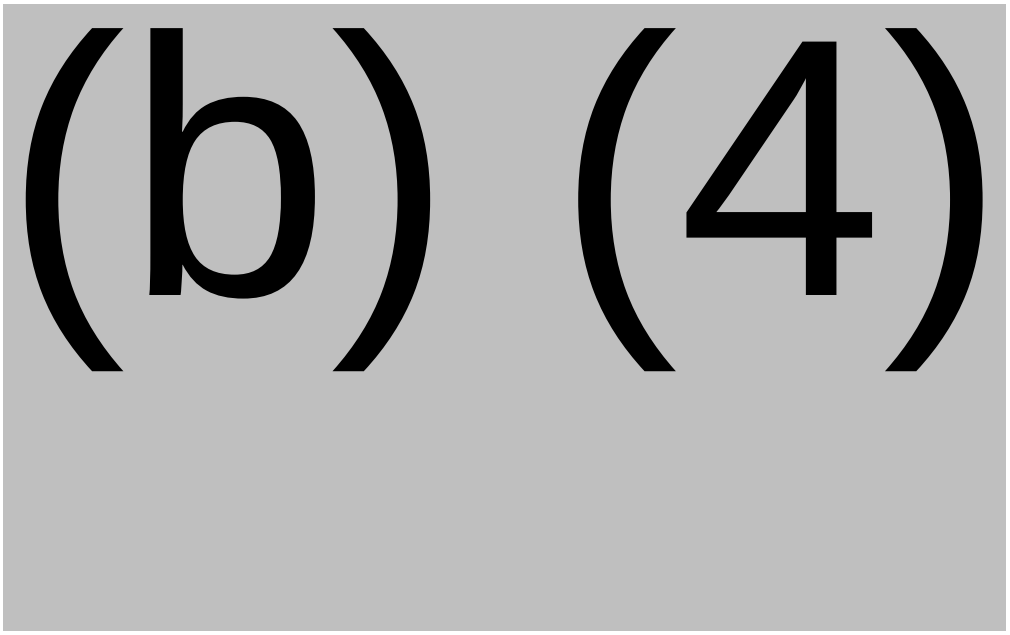
should be minimized. On the other hand, it if the (b) (4) *differ in the quality of DNA that is isolated, this could impact the qPCR assay. The validation is acceptable.*

Phenotyping of White Blood Cell (WBC) Subsets and CAR expression by Flow Cytometry
This assay is critical for lot release as it is the dose determining assay. This assay is not the same as what was used during the clinical study. The terminal stains for % CAR+ and % T-cells are the same between the two procedures, however the staining of higher-order stains (e.g., live-dead, CD45) differ between the two assays. Additionally, this assay is used to test for residual B-cells in the tisagenlecleucel product. Residual B-cells have added risk as evidenced by the SAE reported at UPenn where a reoccurring leukemia resulted from expansion of a transduced B-cell. There is an extended review of the procedures as it is critical for dose determination.

(b) (4)



20 Pages determined to be not relesable: (b)(4)



3.2.P.5.5 Characterization of Impurities

Product-related impurities, process-related impurities and contaminants, are discussed in section [3.2.S.3.2 Cells] of this BLA review.

3.2.P.6 Reference Standards or Materials

Due to wide variability of autologous cells, invasive procedure to collect the cells and limited quantity, no reference material is available at present.

3.2.P.7 Container Closure System

Primary Packaging

(b) (4) freezing bags supplied by (b) (4) is used as a container for tisagenlecleucel storage and infusion. The vendor is qualified according to the Novartis vendor qualification process. Bags of two sizes are used: (b) (4) for volumes of 10 – 30 mL of cell suspension and (b) (4) for volumes of 30 – 50 mL.

The containers are made from (b) (4) and have two hermetically sealed spike ports and one (b) (4) inlet tube sealed on the bag on one end and separated on the other end with a Y-connector into (b) (4) tubes, each ended with a female luer-lock cap. The fluid path is sterile and non-pyrogenic. The container closure system is commonly used for packaging and storage of commercial pharmaceutical products in the vapor phase of liquid nitrogen, meets FDA regulation and is certified according to (b) (4)
Freezing Bags are 510K cleared with the number (b) (4), sterilized bags as supplied by (b) (4) are used within the stated expiry date and not (b) (4) by Novartis.

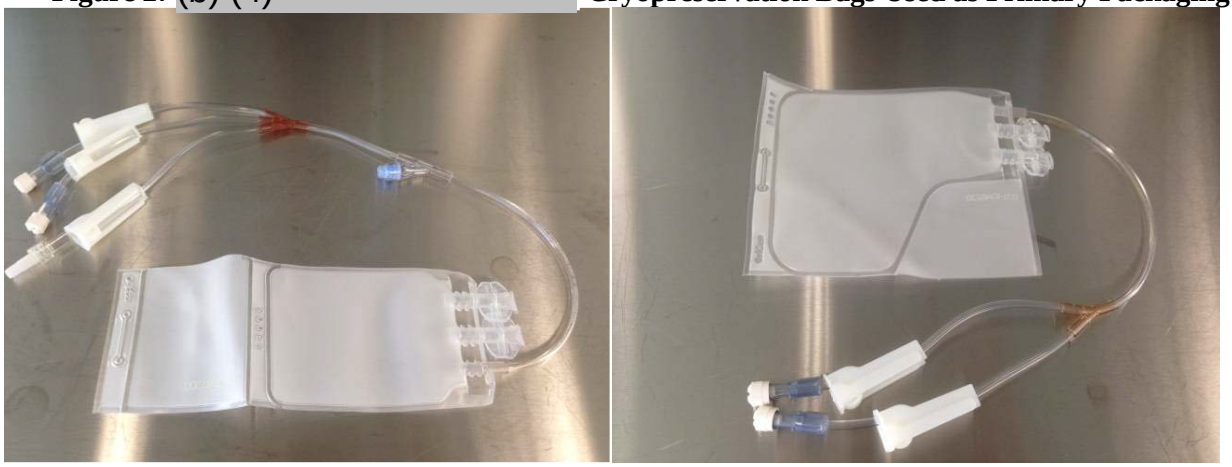
Specifications, Test Procedures and Batch Analysis Data

Each component of the container closure system is purchased against a specification and representative Certificate of Analysis (CoA). The specification and the CoA define attributes that may affect material quality or usability. Each delivery is accepted, and visually inspected by Novartis on the

Certificate of Analysis/Conformity provided by the container closure manufacturer. Compliance and supplier specification are verified against the supplier CoA.

The figure below (Figure 27) shows a representative example of an (b) (4) bags used for primary packaging.

Figure 27 (b) (4) **Cryopreservation Bags Used as Primary Packaging**



A B

In the USA, the (b) (4) Freezing Bags are 510K cleared with the number (b) (4) . In Europe, the (b) (4) Freezing Bags are available as CE marked medical devices (CE number (b) (4) and in Canada are approved with the approval reference number (b) (4)

Specifications, Test Procedures and Batch Analysis Data

Certificate of Analysis (CoA) is provided. The specification and the CoA define attributes that may affect material quality or usability. Each delivery is accepted, and visually inspected by Novartis Quality Unit. The Certificate of Analysis/Conformity provided by the container closure manufacturer. Compliance and supplier specification are verified. tisagenlecleucel primary container is controlled by Novartis according to tests and predefined specifications as part of the Novartis raw material qualification plan.

3.2.P.8 Stability

3.2.P.8.1 Stability Summary and Conclusion

Stability study analysis, summaries, and conclusions are presented to support:

- 9 months shelf life for tisagenlecleucel stored at the long term storage condition (≤ -120°C in vapor phase liquid nitrogen, VPLN2)
- 30 minutes in-use period for tisagenlecleucel at room temperature
- Equivalency between tisagenlecleucel packaged in (b) (4) freezing bags

Novartis proposes the following shelf life and storage conditions under which the product is considered to be stable when packaged and stored as described:

Table 59 tisagenlecleucel Proposed Storage Conditions and Shelf Life for Long-Term Storage Condition

Primary packaging	Storage requirements	Shelf life
(b) (4)	Vapor phase liquid nitrogen (VPLN2: ≤ -120 °C)	9 months

Table 60 tisagenlecleucel Proposed In-Use Period

Primary packaging	Storage requirements	In-use Period	Labeling Recommendation
(b) (4)	Room temperature (RT, 20-25ºC)	30 minutes	The time from when the product is thawed to completion of the infusion should not exceed 30 minutes at room temperature (RT, 20-25ºC) to maintain maximum product viability, including any interruption during the infusion.

Applicant's Conclusions

Study DSR5135 provides evidence that the tisagenlecleucel final product is stable at the long term storage condition (≤ -120°C, VPLN2) for all parameters evaluated for at least 9 months. The study has determined tisagenlecleucel final product shelf life of 9 months at the long term storage (≤ -120°C, VPLN2).

Study DSR5135_1 provides evidence that the long term storage (≤ -120°C, $VPLN_2$) is an appropriate storage condition for tisagenlecleucel final product manufactured with pediatric ALL patient leukapheresis material. The data from the clinical batches confirm the 9 month shelf life at ≤ -120°C ($VPLN_2$). Comparison of the final time point data, t≥ 6m, from the clinical batches with healthy donor final product batches, the data indicate that there is no change in final product stability at ≤ -120°C ($VPLN_2$).

Study DSR51354 provides evidence that tisagenlecleucel final product packaged into (b) (4) (b) (4) freezing bags (b) (4) were equivalent for (b) (4) containers types when stored at the long term storage condition (≤ -120°C, $VPLN_2$). Furthermore, for (b) (4) freezing bags (b) (4) over time there are no significant differences for at least (b) (4) at the long term storage condition (≤ -120°C, $VPLN_2$).

Collectively, the available stability data support the 9 months expiry date from the time of cryopreservation of the formulated final product.

Study DSR5135_3 provides support for the intended post-thaw in-use period at the infusion centers. For post-thawed tisagenlecleucel final product, the proposed in-use time period at (b) (4) at 20 - 25°C is 30 minutes.

Reviewer comment: based on the stability data provided in section 3.2.P.8.3 Stability Data in this review, the applicant's conclusions are acceptable.

3.2.P.8.2 Post-Approval Stability Commitment

Novartis does not intend to perform annual stability studies post approval but will evaluate process changes for the potential impact on stability. Changes with the potential to impact stability will be assessed.

Reviewer comment: The applicant's plan is acceptable.

3.2.P.8.3 Stability Data

4 studies were performed to support the proposed shelf life at the long term storage condition at ≤ -120°C ($VPLN_2$) and the in-use period after thaw of the cryopreserved product. The predefined acceptance criteria are shown in Table 61.

Table 61 Stability Tests and Acceptance Criteria

Test method	**Criteria** [1]
Appearance	
Appearance by visual inspection	Colorless to slightly yellow, slightly opaque to opaque cell suspension
Purity	
Percentage of viable T cells	(b) (4)
Cell viability	(b) (4)
Quantity	
Total cell count (concentration)	Report cells/mL
Number of viable cells	Report total viable cells
Potency	
Determination of CAR expression by Flow cytometry	(b) (4)
Release of IFN-γ in response to CD19 expressing target cells	(b) (4) Report pg/transduced cell
Safety	
Sterility by (b) (4) culture (rapid assay)	Negative
Other test	
Cytotoxicity [2]	For characterization only

[1] In addition to the listed criteria, the study was evaluated for t≥ 6m final time point data to be within 3x intermediate precision from t= 0 data
[2] Cytotoxicity is an additional potency assay used for characterization.

1. <u>DSR5135: Stability Study At The Long Term Storage Condition, Using Final Product Batches Manufactured From Healthy Donor Leukapheresis Material.</u>

(b) (healthy donor batches (b) (4) were analyzed for long term storage at ≤ -120°C ($VPLN_2$) with the test interval of at t= 0, 6 months for (b) (4) bag and at t= 1.5, 3, 4.5, 6, 9 months (b) (4) . Appearance test was performed at t=0 and 9 months. Sterility test was performed at t=0, 6 months (b) (4) and 9 months (b) (4) . Percentage of viable T cells, Cell viability, Total cell count Report cells/mL, CAR expression by flow cytometry, and Release of IFN-γ in response to CD19-expressing target cells were tested for all intervals.

Summary results: All tests were within the lot release specification. No downward trend was noted.

2. <u>DSR5135_1: Stability Study At The Long Term Storage Condition Using Final Product Batches Manufactured From Patient Leukapheresis Material.</u>

(b) (patient batches (b) (4) were analyzed for long term storage at ≤ -120°C ($VPLN_2$) with the test interval of at t= 0, 8, (b) (4) . Percentage of viable T cells,

Cell viability, total cell count (cells/mL), CAR expression by flow cytometry, release of IFN-γ in response to CD19-expressing target cells and cytotoxicity tests were performed for all intervals.

Summary results: All tests were within the lot release specification. No downward trend was noted.

3. DSR5135_3: In-Use Stability Study, Using Final Product Batches Manufactured From Healthy Donor Leukapheresis Material.

3 full scale batches, manufactured from healthy donor leukapheresis material using the (b) (4) pathway, were evaluated. The final product batches were formulated, packaged into the (b) (4) bags, cryopreserved and stored at ≤ -120°C (VPLN2). The bags were thawed and the final product was sampled per time point directly from the (b) (4) bags stored at the different temperature conditions post-thaw (b) (4) .

Summary results: Tests were within the lot release specification within 1 hour of thawing at either (b) (4) . No downward trend was noted. However, beyond (b) (4) , test results start to drop. At (b) (4), the test sample started to show significant drops in cell viability within 1 hour.

4. DSR5135_4: Stability Study To Determine Equivalency Between Tisagenlecleucel Packaged In (b) (4) And Final Product Bags, Using Final Product Batches Manufactured From Healthy Donor Leukapheresis Material.

Final formulated tisagenlecleucel samples are packaged into cryovials and cryopreserved along with the tisagenlecleucel final product bags for product release testing: cell viability, percentage of viable T cells, percentage of viable B cells, total cell count, number of viable cells and dose, CAR expression, release of IFN-γ and endotoxin.

Study was conducted to support equivalency between final product packaged in (b) (4) cryovials and (b) (4) freezing bags under long term storage conditions (VPLN2; ≤ -120°C) over a (b) (4) time period.

(b) full scale batches manufactured from (b) (4) healthy donor leukapheresis material using the (b) (4) pathway were used for this study. The final product batches were formulated, packaged into (b) (4) bags and (b) (4) , cryopreserved and stored at ≤ -120°C ($VPLN_2$).

Summary results: Tests were within the lot release specification at all intervals tested up to (b) (4) . No downward trend was noted. All stability indicating parameters were close between the samples from (b) (4) bags and (b) (4) when cryopreserved and stored at ≤ -120°C ($VPLN_2$).

Summary of the Overall Stability Results

- Percentage of viable cells derived from patient cells was stable for 9 months in (b) (4) for 9 months in (b) (4) when stored at ≤ -120ºC.
- Percentage of viable cells derived from healthy donor was stable for 12 months in (b) (4) and in (b) (4) when stored at ≤ -120ºC.
- Viable cell concentration and recovery derived from healthy donor were stable for 6 months in Cryo-Bags and for 9 months in (b) (4) when stored at ≤ -120ºC.
- There were no significant change trends in viable cell concentration and recovery derived from healthy donor were stable for 6 months in Cryo-Bags and for 9 months in (b) (4) when stored at ≤

-120ºC.There were no significant change trends in percentage of transduction in viable cells derived from healthy donor were stable for 6 months in Cryo-Bags and for 9 months in (b) (4) when stored at ≤ -120ºC.

- There were no significant change trends in INF-γ in viable cells derived from healthy donor were stable for 6 months in Cryo-Bags and for 9 months in (b) (4) when stored at ≤ -120ºC.
- There were no significant change trends in percentage of transduction in viable cells derived from healthy donor were stable for 9 months when stored at ≤ -120ºC.
- There was no significant change trend in INF-γ in viable cells derived from patient cells for 9 months when stored at ≤ -120ºC
- The data analysis provides support for the intended post-thaw in-use period at the infusion centers. For post thawed tisagenlecleucel final product, the proposed in-use time period is 30 minutes at 20 - 25°C (b) (4) temperature is not recommended for the in-use period.

Reviewer comment: The stability data and conclusions are acceptable.

3.2.A Appendices Table of Contents

3.2.A.1 Facilities and Equipment

Refer to DMPQ review memorandum.

3.2.A.2 Adventitious Agents Safety Evaluation

The potential risks derived from adventitious agents and microbial contamination of tisagenlecleucel addressed through the following plan.

- Control of raw materials and the tisagenlecleucel vector
  - Control of animal and human derived raw materials entering the manufacturing process through certificates of origin and suitability. For human derived materials such as human serum, viral inactivation steps in the manufacturing process of these materials are performed.
  - Production of tisagenlecleucel vector using (b) (4) cells, which are not known to express endogenous viruses
  - Additional controls through filtration of raw materials (media) performed prior to use in manufacturing.
  - Control of the tisagenlecleucel vector through testing for adventitious viral agents
  - Testing for relevant human viruses as part of the patient eligibility assessment
- Process and environmental controls
  - Control of the tisagenlecleucel drug product manufacturing process (antibiotics free) through use of closed systems. Where there are open steps, the process is performed under environmentally controlled conditions
  - Environmental controls
  - Cleaning and decontamination of work surfaces and equipment
  - Aseptic verification, simulating all process steps and interventions is conducted to verify that the process is capable of maintaining sterility;
  - Control of the tisagenlecleucel
- Microbial contaminants testing as part of drug product release testing:
  - Bacterial endotoxin
  - Sterility
  - Mycoplasma

- Raw materials of human or biological origin used for product manufacture as well as the tisagenlecleucel LV and patient donor cells are qualified and in compliance with current guidelines for TSE transmission.
- Raw materials of human or biological origin used for product manufacture as well as the tisagenlecleucel LV are tested for the presence of adventitious viral agents according to ICH Q5A and for bovine viruses according with the 9CFR53 dispositions. Materials of recombinant origin are produced with animal-free reagent processes.
- tisagenlecleucel LV is designed as a SIN, replication-defective 3rd-generation, VSV-G pseudotyped lentiviral vector that includes a number of key additional safety features including codon-optimized Gag/Pol such that the likelihood of RCL generation either during vector production or in the host target cells is minimized. This is confirmed by the negative results consistently obtained the CLT019 LV batches produced to date, and in tisagenlecleucel batches and in plasma sample of treated patients.
- Raw materials produced through hybridoma technology have been tested for the presence of VLP as well as for the presence of adventitious Murine leukemia virus-like retroviruses and its production process has been validated for the viral clearance ability. Removal /inactivation factors validated for MLV are deemed to provide effective safety margins so that the introduction of VLP or infectious particles in the tisagenlecleucel manufacture is unlikely.
- Extensive viral testing on (b) (4) cell banks, CTL019 LV batches and the careful selection of the raw materials used are considered sufficient to ensure the viral safety of the product.

The applicant concluded that the infusion of the tisagenlecleucel product presents an overall low risk of patient infection from adventitious agents from contaminated reagents in the process or from endogenous viruses.

Reviewer comment: The applicant's conclusion is acceptable.

3.2.A.3 Novel Excipients

There are no novel excipients used in tisagenlecleucel manufacturing and formulation.

3.2.R Regional Information (U.S.A.)

3.2.R.1 Executed Batch Records

(b) (4)

- (b) (4)

Reviewer comment: the version of the batch record that was submitted as a clean batch record is the current FRM-7061151, version 1. This is not the same batch record form that was used for either executed batch record supplied. The clean batch record has a QA approval date of 12/16/2016. It is not clear how many batches have been manufactured under this new system. This was followed up during the inspection of the Morris Plains manufacturing site by Drs. Gavin and Lu. FDA noted that there are two versions of batch records (clinical and commercial) although the manufacturing process is identical. The commercial batch record contains more time limits and tighter CPPs for certain unit operations and fewer sampling points for exploratory product characterization tests. The batch records are also distinguished by color. The commercial batch records are acceptable.

Batch Records Clean:

- FRM-7061151, Version 1.0
- Batch records are used in conjunction with WP-7004671: Commercial (b) (4) Work Procedure for Cell and Gene Therapies (eCTD 1.11.1 submitted in amendment 6: 03/14/2017)
    - WP-7004671 is a new document and Novartis has listed a QA approval date of 03/01/2017

Reviewer comment: The alignment of the batch record and the work procedure is not clear. The WP and batch records follow the same general flow; however the line items do not align. The batch records have headings in grey that say "task XX - refer to related work procedure WP-7004671" that reference heading names, but do not direct the operator to where in the WP to find the task (no page or heading numbers). This may be due to the fact that the WP was not yet approved at the time of batch record approval. This was followed up during the inspection of the Morris Plains manufacturing site by Drs. Gavin and Lu. During the PLI, FDA observed that an operator and a verifier followed the SOP and the Work Procedure on the computer monitor during the unit operation. No issues were identified.

All steps have performed by and verified by sign off

- Clear instructions for error signs on instruments
- Historical ranges of time for some steps is stated
- Documentation of work station pre- & post cleaning
- Color coding is used
    - Red: historical norms, approx. times, temp ranges, operator monitoring, optional steps depending on situation
    - Blue: Perform, check,
    - Brown: record/verify information in LIMS is usually denoted in brown, some calculations, reagent/equipment choice based on calculation
- Indication of which equipment to use based on product (e.g., pg 42 70 mL CS kit for $<10^9$ TNC, 225mL CS5 kit for $\geq 10^9$ TNC)
- Page 48 item 53: Record the Hematocrit % from the Leukapheresis: NOTE: if ≥30% stop and call area management

Reviewer comment: This in-process control was likely added a result of OOS appearance due to "pink color" during IND. This will increase control of the process and likelihood that the product will meet lot release specifications.

In response to 483 observations raised during inspection of the tisagenlecleucel manufacturing facility in Morris Plains, NJ, a revised unexecuted batch record was submitted in amendment 32. The unexecuted batch record, FRM-7061152 v3, references WP-7004671.

3.2.R.2 Method Validation Package

According to 21 CFR 610.2(a), Novartis may be required to provide samples of tisagenlecleucel batches for the CBER lot release program. However, the FDA has determined that tisagenlecleucel will not be evaluated for batch release. The method validation reports have been submitted and batch samples will be submitted only upon request.

3.2.R.3 Comparability Protocols

No formal product comparability protocols have been submitted. Manufacturing changes at the Novartis Gene Therapy Manufacturing Facility in Morris Plains New Jersey will be addressed through BLA supplements.