TRANSLATIONAL STATISTICAL ANALYSIS PLAN KT-US-471-0114 (ZUMA-14)

Sponsor:	Kite Pharma, Inc. 2400 Broadway Santa Monica, CA 90404 United States of America
Product Name:	KTE-C19
Protocol:	KT-US-471-0114 A Phase 2 Open-Label, Multicenter Study Evaluating the Safety and Efficacy of Axicabtagene Ciloleucel in combination with Rituximab in Subjects with Refractory Large B-Cell Lymphoma (ZUMA-14)
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LIST OF ABBREVIATIONS

AE	Adverse event
ASCT	Autologous stem cell transplant
AUC	Area under the curve
CAR	Chimeric antigen receptor
CI	Confidence Interval
CR	Complete response / Complete Remission
CRS	Cytokine release syndrome
CSF	Cerebrospinal fluid
DLBCL	Diffuse large B cell lymphoma
CCI	[redacted]
ECOG	Eastern Cooperative Oncology Group
IHC	Immunohistochemistry
IQR	Interquartile range
mITT	Modified intent-to-treat
NE	Neurologic event
NHL	Non-Hodgkin Lymphoma
PA	Primary Analysis
PBMC	Peripheral blood mononuclear cells
PD	Progressive disease
PK	Pharmacokinetic
PR	Partial response
qPCR	quantitative polymerase chain reaction
RCR	Replication competent retrovirus
SAE	Serious adverse event
SD	Stable disease
SOA	Schedule of assessments
TSAP	Translational Statistical Analysis Plan
WBC	White Blood Cell and also called Leukocytes (unit: 10^9/L)

1. INTRODUCTION

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2. OBJECTIVES

2.1. Objectives

- Characterize the presence, expansion, persistence and clearance of anti-CD19 CAR T cells in blood (pharmacokinetic profile, PK) and to characterize the serum cytokine (pharmacodynamic) profile
- Evaluate anti-CD19 CAR T pharmacokinetic/pharmacodynamic data by efficacy and safety outcomes
- Evaluate anti-CD19 CAR T pharmacokinetic data by baseline characteristics
- Characterize Rituximab pharmacokinetic (PK) level
- Assess Rituximab PK data by clinical efficacy
- Characterize the product attributes
- Describe key product attributes and explore association between anti-CD19 CAR T pharmacokinetic, clinical efficacy and safety outcomes
- Explore the association between product characteristics and clinical efficacy and safety outcomes
- Explore the association between Peripheral blood mononuclear cells (PBMC) immune phenotype and clinical efficacy and safety outcomes if data is available.
- Assess B cell aplasia over time
- Explore the association between anti-CD19 CAR T Pharmacokinetics and B cell aplasia
- Explore the association between Rituximab PK and B cell aplasia

2.2. Hypothesis

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3. ENDPOINTS, SUBGROUPS AND COVARIATES

3.1. Biomarker datasets

Table 3-1. Data overview on assay methods and biomarker lists

Data type	Assay method/ Sample type	Assessment time points	Biomarker set
CAR T PK (in combination with Rituximab)	qPCR/PBMC	Leukapheresis, Day 7, 14, 21, 28, 49, 105, and 180	Number of CAR T cells (/µL)
Rituximab PK	Gyrolab immunoassay /serum	Day -5, 21, 49, 77, 105, 133	Serum rituximab level (ng/mL)
Pharmacodynamic data (cytokines)	Cytokine assay/serum (MSD, ELLA, Luminex)	- Leukapheresis, Day -5 0, 1, 3, 5, 7, 14, 21, 28, 49 - Blood draw for chemistry panel - Blood draw for CRP, ferritin	• Refer to appendix 8.5
MRD	NGS-based Minimum Residual Disease assay	Screening, Day 28, 105, 180	• MRD + or – • Frequency of sequence within IgH of IgL population
Product characteristics	Flow Cytometry		• Total Number of T Cells, • Total Number of CAR T Cells, • Total number of Tnaive cells • Transduction Rate (%), • CD4/CD8 Ratio, • % and number of Tnaive/Tcm/Tem/Teff in viable CD3 cells • (% Tnaive + % T central memory) / (% T effector memory + % T effector cells) Ratio, • IFN-gamma level (pg/ml), • Vector Copy Number, • % viability, • CD4 Cells (% and number), • CD8 Cells (% and number), • CCR7+ (Tnaive + Tcm) % and number, • CCR7- (Tem + Teff) %, • Total number of CCR7+ T cells infused

Data type	Assay method/ Sample type	Assessment time points	Biomarker set
CAR-T cell phenotype (PBMC) if data is available	Flow cytometry		• CAR+ T cells (% and number): - CD4 T naïve/Tcm/Tem/Teff cells (%); PD-1+; TIM3+; LAG3+; CD28+; CD27+, 41BB+. - CD8 T naïve/Tcm/Tem/Teff cells (%); PD-1+; TIM3+; LAG3+; CD28+; CD27+, 41BB+ • CAR- T cells(negative) (% and number): - CD4 T naïve/Tcm/Tem/Teff cells (%); PD-1+; TIM3+; LAG3+; CD28+; CD27+, 41BB+. - CD8 T naïve/Tcm/Tem/Teff cells (%); PD-1+; TIM3+; LAG3+; CD28+; CD27+, 41BB+

CCR7: Chemokine Receptor 7; IFN: Interferon; Tcm: Central Memory T-cell phenotype; Teff: Effector T-cell phenotype; Tem: Effector Memory T-cell phenotype; Tnaïve: Naïve T-cell phenotype

3.2. Endpoints

The following pharmacokinetic endpoints for anti-CD19 CAR T and rituximab and pharmacodynamic endpoints for 33 analytes (Appendix X) will be included for the analysis listed in section 2.1 and 2.2. Detailed definitions can be found in Section 4.

- Fold change from baseline at Day X (cytokine analytes)
- Fold change from Day 0 (pre-dose) at Day X (cytokine analytes)
- Peak (Cmax) (anti-CD19 CAR T and rituximab PK)
- Time to peak (anti-CD19 CAR T and rituximab PK)

Area-Under-Curve (Day 0 to 28 for anti-CD19 CAR T PK; Day -5 to 133 for rituximab PK)

All measurable values at each visit will be used as main endpoints for all biomarkers listed in Table 3-1 as following

- Levels of anti-CD19 CAR T cells in blood samples measured as anti-CD19 CAR+ cells/µL by visit, peak, Day 0-28 AUC, and time to peak (details for the derivations are included in the definition section)
- Levels of rituximab in blood samples measured as ng/mL by visit (pre and post), peak (maximum value between Day -5 to 133), AUC (Day -5 to 133)

- Levels of cytokines in serum by visit, fold change from baseline, Day 0, peak, Day 0-28 AUC, and time to peak
- Product attributes measurements after product manufacturing and prior to dosing.

3.3. Outcomes, Subgroups, and Covariates

1) Objective response: responder (CR/PR) vs. non-responder*; CR vs. PR vs. CR+PR vs. non-responder*
2) Ongoing response: ongoing response vs. relapsed vs non-responder*
3) Best response: complete response (CR) vs. partial response (PR) vs. stable disease (SD) vs. progressive disease (PD); CR vs. PR vs. non-responder*
4) Worst Neurologic event (NE) grade: grade 3 or higher vs. grade 2 or lower
5) Worst NE grade: grade 2 or higher vs. grade 1 or lower
6) Worst cytokine release syndrome (CRS) grade: grade 3 or higher vs. grade 2 or lower
7) Worst CRS grade: grade 2 or higher vs. grade 1 or lower
8) Baseline covariates will be utilized when appropriate:
    a) Refractory subgroup (primary refractory, refractory to 2nd or greater line therapy, refractory after autologous stem cell transplant [ASCT])
    b) Baseline covariates
        i) age (<65 vs. >=65 years)
        ii) race (Asian, White, Other)
        iii) ethnicity (Hispanic or Latino vs. Not Hispanic or Latino)
        iv) gender (Female vs. Male)
    c) Corticosteroid (Yes vs. No)
    d) Tocilizumab use (Yes vs. No)
    e) Baseline tumor burden (SPD in mm^2) quartile/median subgroups
    f) Other baseline covariates if applicable

    *Non-responder is defined as subjects who had neither CR nor PR by the analysis data cutoff.

4. DEFINITIONS

4.1. General

Study Day 0 is defined as the day the subject received the first KTE-C19 infusion. The day prior to study day 0 will be study day −1. Any days after enrollment and prior to study day −1 will be sequential and negative integer-valued.

4.2. Key Measurements of Pharmacokinetics

4.2.1. Anti-CD19 CAR+ T-Cell

The expansion and persistence of anti-CD19 CAR T cells in peripheral blood will be measured by quantitative polymerase chain reaction (qPCR) analysis.

Baseline value for the number of anti-CD19 CAR T in blood (cells/μL) is defined as 0 since KTE-C19 is infused on Day 0.

Scheduled blood draw for anti-CD19 CAR T cell
This TSAP will focus on the anti-CD19 CAR T cell data collected as per planned assessment. The schedule of assessments and the analytic visit windows are defined in Appendix Section 8.1.

Number of anti-CD19 CAR T (cells/ uL blood) is defined as:

(Absolute Monocytes + Absolute Lymphocytes) *%CAR^+ PBMC

Note: unit conversions for absolute monocytes and lymphocytes will need to be considered when calculating the number of anti-CD19 CAR T cells (cells/uL blood) using the formula above.

Peak of anti-CD19 CAR T cell (cells/ uL blood) is defined as the maximum absolute number of anti-CD19 CAR T cells in serum attained after Day 0.

Time-to-Peak of anti-CD19 CAR T cell (days) is defined as "Peak Date – KTE-C19 Dosing Date + 1".

Day 0 -28 AUC of level of anti-CD19 CAR T cell (cells/ uL ▪ days) is defined as the area under the curve in a plot of levels of anti-CD19 CAR T cells against scheduled visits from Day 0 to Day 28. This AUC measures the total levels of anti-CD19 CAR T cells overtime. Given the anti-CD19 CAR T cell is measured at certain discrete time points, the trapezoidal rule will be used to estimate the AUCs.

4.2.2. Rituximab Pharmacokinetics

The expansion and persistence of rituximab in peripheral blood will be measured by Gyrolab immunoassay analysis.

Scheduled blood draw for rituximab
This TSAP will focus on the rituximab data collected as per planned assessment before and after each infusion of Rituximab. The schedule of assessments and the analytic visit windows are defined in Appendix Section 8.2.

Peak of rituximab in blood (ng/mL) is defined as the maximum level of rituximab (ng/mL) in Blood from Baseline (Day -5 pre dose) to Day 133 (post dose).

Time to peak is defined as the number of days from Day 0 (KTE-C19 infusion date) to the date when the rituximab in Blood firstly reached the peak.

AUC of rituximab in blood (ng/mL▪ days) is defined as the area under the curve in a plot of levels of rituximab in Blood (ng/mL) against scheduled visits from Baseline (Day -5 to Day 133).

4.3. Key Measurements of Pharmacodynamics: Serum Cytokines, Chemokines and other Blood Biomarkers

Scheduled blood draw for cytokines:

This TSAP will focus on the cytokine data collected from baseline to Day 28. The Schedule of Assessment and analytic visit window is defined in Appendix Section 8.3.

Baseline of cytokines is defined as the last value measured prior to conditioning chemotherapy.

Fold change from baseline at Day X is defined as

$$\frac{\text{Cytokine level at Day X}}{\text{Cytokine level at Baseline}}$$

Peak of cytokine post baseline is defined as the maximum level of cytokine in serum attained after baseline up to Day 28.

Time to peak of cytokine post KTE-C19 infusion is defined as "Peak Date – KTE-C19 Dosing Date + 1".

AUC of cytokine levels from baseline to Day 28: is defined as the area under the curve in a plot of levels of cytokine against scheduled visits from baseline to Day 28. This AUC measures the total levels of cytokine between baseline and day 28. Given the cytokine is measured at certain discrete time points, the trapezoidal rule will be used to estimate the AUCs.

4.4. Key Measurements of Product Characteristics

- All product characteristics as defined in Table 3-1 will be summarized individually and also for the correlative analysis with anti-CD19 CAR T levels in blood, serum analyte levels, and clinical outcome endpoints.
- Two additional co-variates will be derived for exploration:

— **CD4/CD8 Ratio** is defined as: $\frac{\text{CD4+ Cells (\%)}}{\text{CD8+ Cells (\%)}}$

— **CCR7+ in (%) or (#)** is defined as: Naive (%) or (#) + Central Memory (%) or (#), respectively

5. ANALYSIS SETS

- Modified intent-to-treat (mITT) analysis set consists of all subjects enrolled and treated with the target dose of axicabtagene ciloleucel at 2 x 10^6 CAR T cells/kg (1.0 x 10^6 to 2.4 x 10^6 anti CD19 CAR T cells/kg) and at least one dose of Rituximab after axicabtagene ciloleucel infusion. Subjects are considered to have received the target dose if they receive at least 1 x 10^6 anti-CD19 CAR T cells/kg. This analysis set will be used for all efficacy analyses and Rituximab PK analyses.

- Safety analysis set consists of all subjects treated with any dose of axicabtagene ciloleucel. This analysis set will used to explore the association between biomarker data with safety outcomes.

Generally, this TSAP will utilize the same efficacy analysis strategy described in the SAP, such as analysis sets and related outcome definitions.

6. STATISTICAL ANALYSIS

6.1. General Methods

The following methods will be applied to the data analysis when applicable. All p-values generated will be descriptive.

- **Summary statistics** will summarize data in frequency (N, %) and quartile range (Minimum, 1st quartile (Q1), Median, 3rd quartile (Q3), Maximum) in overall and by appropriate subgroups and covariates (Section 3.3).

- **Simple linear Regression (SLR)** will be conducted to explore relationships between biomarkers with continuous values {Gelman 2006}. The estimated slope and its 95% Confidence Interval (CI) with the unadjusted p-value will be reported.

- **Non-parametric Wilcoxon rank sum tests**
  Non-parametric Wilcoxon rank sum tests {Siegel 1956, Wilcoxon 1945} will be utilized to explore the associations between PK/ Pharmacodynamic , product characteristics, and outcomes. Unadjusted p-values will be reported. The multiplicity adjustment (Holm-Bonferroni step-down method, {Holm 1979, Hommel 1988} may be implemented when further characterization of potential association are identified and adjusted p-values will be reported. Median fold change will be utilized to describe the differences in the outcome.

- **Non-parametric Kruskal-Wallis test**
  Non-parametric Kruskal-Wallis test {Kruskal 1952} will be conducted for three or more-group comparison followed by pairwise comparisons using Dunn's test with Holm's adjustment method {Dunn 1964} implemented in the 'dunn.test' package for R.

- **Clopper Pearson Method** will be used to calculate exact 95% confidence intervals for Objective Response Rate (ORR) and complete response rate {Brown 2002}.

6.2. Primary Analysis

6.2.1. Summarize anti-CD19 CAR T cell pharmacokinetic expansion, rituximab PK, and serum cytokine (Pharmacodynamic) profile

- mITT will be used for Rituximab PK profile and Safety Analysis set will be used for anti-CD19 CAR T cell profile.

- The Median Line plot over time with interquartile range (IQR) will be produced for anti-CD19 CAR T and rituximab.

- Anti-CD19 CAR T Cell profile in blood (PK) and AUC over time will be summarized using summary statistics described in Section 6.1 in overall and by baseline covariates specified in Section 3.3.

- Similarly, pharmacodynamics profile as measured by serum analyte levels overtime will be summarized using summary statistics described in Section 6.1 in overall

  — Pre-selected key serum analytes as listed in Table 3-1 will be presented in the Clinical Pharmacology report.

6.2.2. Explore association between anti-CD19 CAR T, rituximab pharmacokinetic, serum cytokine with safety and clinical outcomes

- Safety analysis set will be used for exploration between PK, and Pharmacodynamic with safety outcomes and mITT analysis set will be used to explore the association between PK, and Pharmacodynamic with efficacy outcomes.
- Clinical response outcomes:

  — Responder vs Non-responder

  — CR vs PR vs Non-responders

  — Ongoing Response vs Relapsed vs Non-responders
- Safety outcomes:

  — Neurologic event grade 3 or higher vs grade 2 or lower

  — Neurologic event grade 2 or higher vs grade 1 or lower

  — CRS grade 3 or higher vs grade 2 or lower

  — CRS grade 2 or higher vs grade 1 or lower
- Statistical tests are listed in Table 6-1.

  — Wilcoxon rank sum test will be used to compare the PK/serum analyte profiles among the subgroups. See Table 6-1 for details.

For comparisons among CR vs. PR vs. SD+PD, the Kruskal-Wallis test will be conducted. Further pairwise comparisons among these 3 groups, following a significant Kruskal-Wallis test, will be using Dunn's test with Holm's adjustment method.

Table 6-1. Non-parametric Comparisons

Outcome	Subgroups	Method	Key Measurements	
			CAR T Cell Expansion in Blood	Cytokine Levels in Serum
Worst Neurologic event	Grade 3 or higher vs grade 2 or lower	Wilcoxon rank sum test	AUC Peak	AUC Peak
Worst Neurologic event	Grade 2 or higher vs grade 1 or lower	Wilcoxon rank sum test		
Worst CRS	Grade 3 or higher vs grade 2 or lower	Wilcoxon rank sum test		
Worst CRS	Grade 2 or higher vs grade 1 or lower	Wilcoxon rank sum test		
Response	Responder vs Non-responder	Wilcoxon rank sum test		
Best Response	Complete Response vs Partial Response vs. Non-responders	Kruskal-Wallis test		
Durable Responders	Ongoing Response vs Relapsed vs Non-responders	Kruskal-Wallis test		

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8. APPENDIX

8.1. Definition of Analytic Visit Windows for CAR T Cells in Blood

Table 8-1. Analytic Visit Windows for CAR T Cells (in combination with Rituximab) in Blood

Analytic Visit	Target Day	Visit Window
		Cohort1
Baseline	Leukapheresis	Leukapheresis
Day 7	7	[4, 10]
Day 14	14	[11, 17]
Day 21	21	[18, 24]
Day 28	28	[25, 38]
Day 49	49	[39, 77]
Day 105	105	[78, 142]
Day 180	180	[143, 225]
Month 9	270	[226, 315]
Month 12	360	[316, 405]
Month 15	450	[406, 495]
Month 18	540	[496, 630]
Month 24	720	[631, 900]

* We will continuously make assessments for the PK samples at later time points according to the protocol.

8.2. Definition of Analytic Visit Windows for Rituximab in Blood

Table 8-2. Analytic Visit Windows for Rituximab in Blood

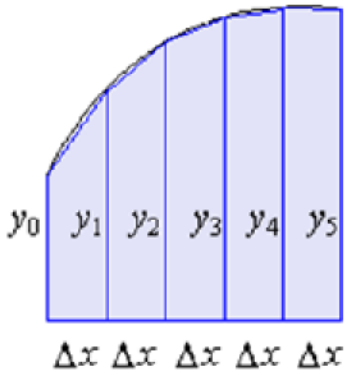
Analytic Visit	Target Day	Visit Window
		Cohort1
Baseline	-5	<=-4
Day 21	21	[18, 35]
Day 49	49	[36, 63]
Day 77	77	[64, 91]
Day 105	105	[92, 119]
Day 133	133	[120,)

8.3. Definition of Analytic Visit Windows for Cytokines in Serum

Table 8-3. Analytic Visit Windows for Cytokines in Serum

Analytic Visit	Target Day	Visit Window
		Cohort1
Baseline	-5	<=-5
Day 0	0	0
Day 1	1	[1, 2]
Day 3	3	[3, 4]
Day 5	5	[5, 6]
Day 7	7	[7, 10]
Day 14	14	[11, 17]
Day 21	21	[18, 24]
Day 28	28	[25, 38]
Day 49	49	[39, 105]

8.4. Using Trapezoidal Rule to Approximate the Area under the Curve (AUC)



$$\text{AUC} \approx \frac{1}{2}(y_0 + y_1) \cdot \Delta x + \frac{1}{2}(y_1 + y_2) \cdot \Delta x + \frac{1}{2}(y_2 + y_3) \cdot \Delta x + \cdots$$

8.5. Serum Cytokine Analyze

1	CRP (mg/L)
2	CXCL10 (pg/mL)
3	Ferritin (ng/mL)
4	Granzyme B (pg/mL)
5	ICAM-1 (ng/mL)
6	IFN-gamma (pg/mL)
7	IL-1 RA (pg/mL)
8	IL-10 (pg/mL)
9	IL-15 (pg/mL)
10	IL-2 (pg/mL)
11	IL-2 R alpha (ng/mL)
12	IL-6 (pg/mL)
13	IL-7 (pg/mL)
14	IL-8 (pg/mL)
15	TNF alpha (pg/mL)
16	VCAM-1 (ng/mL)
17	GM-CSF (pg/mL)
18	ICAM-1 (pg/mL)
19	IL-12 P40 (pg/mL)
20	IL-17 (pg/mL)
21	IL-2 R alpha (pg/mL)
22	IL-4 (pg/mL)
23	IL-23 (pg/mL)
24	IL-5 (pg/mL
25	MDC (pg/mL)
26	MCP-1 (pg/mL)
27	MCP-4 (pg/mL)
28	MIP-1 alpha (pg/mL)
29	SAA (pg/mL)
30	MIP-1 beta (pg/mL)
31	VCAM-1 (ng/mL)
32	VCAM-1 (pg/mL)
33	VEGF (pg/mL)