Final Report-

Pharmacometrics Analysis of Tisagenlecleucel-T (CTL019, BLA 125646)

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Pharmacometrics Analysis Working Group

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EXECUTIVE SUMMARY

CBER/OBE and CDER/OCP received a consult request from CBER/OTAT on April 11, 2017 to conduct a pharmacometric analysis of tisagenlecleucel-T (CTL019, BLA125646) and inform regulatory questions pertaining to CMC and clinical review. Tisagenlecleucel-T is indicated for treatment of pediatric and young adult patients (3 to 25 years of age) with relapsed/refractory B-cell acute lymphoblastic leukemia (ALL). The working group identified nine major regulatory questions considering impact of key product attributes, patient baseline characteristics concomitant therapies and CAR-T kinetics on safety and efficacy outcomes. To address these questions, we conducted univariate/multivariate statistical analysis, and the output of the logistic regression models were explored using visual effect plots. We also used predictive pharmacokinetic (PPK) models to explore the association between CAR-T kinetics and the clinical outcomes. Two documents describing details of univariate/multivariate statistical analysis (Attachment A and B) and pharmacokinetics/pharmacodynamics modelling (Attachment B) are included after this executive summary. Below we summarize the analysis results and conclusion for each identified question.

Q1. Are there correlations between critical product attributes and clinical outcomes of efficacy and safety?

A univariate/multivariate statistical analysis on the key product attributes (bodyweight adjusted/unadjusted cell dose, interferon-gamma (IFN- γ), vector batch and transduction efficiency) did not reveal any significant correlation of these attributes with occurrence of grade 3/4 CRS ($p>0.1$). The visual effect plots show weak positive correlation between the dose of transduced CAR-T cells, and the grade 3/4 CRS. We also found that IFN-γ level was positively correlated with overall remission rate (ORR) at day 28 ($p=0.08$). Some CAR-T cells subpopulation related attributes are significantly associated with ORR at day 28. Please see Attachment A for detailed analysis.

Q2. What is the impact of steroid treatment for CRS on the treatment response and duration of response?

No significant impacts of steroids were found through either a regression analysis on ORR at day 28 or a Kaplan-Meier model analysis on duration of response. Please see Attachment A for detailed analysis. Also, it is important to point out that the design of the B2202 study was not suitable for an unbiased estimate of the impact of steroids because the data for administered and non-administered groups was unbalanced and because of confounding factors (other concomitant therapies, initial tumor burden). We suggest continuous monitoring of patients

who receive tisagenlecleucel-T in future clinical trials to better understand the impact of steroids on ORR.

Q3. What is the impact of CBC lymphoblast counts or levels of baseline blast burden on the efficacy outcome?

We conducted a univariate analysis to evaluate patient related demographic factors and baseline tumor burden (%blast cells, %MRD in blood, %MRD in bone marrow) on ORR at day 28. There is no statistically significant correlation between percent blast cells (%blast cells) and ORR. A visual effect plot identified trend of higher ORR for patients who had lower minimal residual disease (%MRD) in blood or bone marrow and no steroid treatment. Please see Attachment A for detailed analysis.

Q4. Whether prior transplantation makes a difference in the CAR-T cells therapeutic outcome?

Our analysis shows prior transplantation has no discernable association with ORR at day 28. Please see Attachment A for detailed analysis.

Q5. Are any cytokines predictive of CRS?

Multiple classification models (Logistic Regression, Decision Trees, and Random Forest) and several variable selection methods were explored. The results indicate some cytokines (Ferritin, IFNG, IL10, IL12, IL13, IL2, IL4, IL6, IL8 and TNF) are significantly associated with occurrence of grade 3/4 CRS. Depending on the model selection algorithm, different cytokines were used as predictors of CRS. These models provide prediction of grade 3/4 CRS with a certain degree of accuracy and sensitivity. Please see Attachment A for detailed analysis. In future study, modeling cytokine groups with similar functions instead of individual cytokine may be considered in order to improve the accuracy, sensitivity and robustness of model prediction.

Q6. Are any cytokines associated with clinical response?

Some cytokines (C Reactive Protein, Ferritin and IL10) are significantly associated with ORR at day 28. Please see Attachment A for detailed analysis.

Q7. What is the relationship between CAR-T kinetics and cytokine release syndrome (CRS)?

The analysis indicates that a higher CAR-T expansion rate is associated with higher probability of CRS onset. A more rapid declining rate of CAR-T is associated with a higher likelihood of CRS remission in the next time interval. Besides CAR-T changing rate, a greater CAR-T concentration is associated with higher probability of CRS onset. These relationships between CRS status

change and CAR-T kinetics are statistically significant. Please see Attachment B for detailed analysis.

Q8. What is the relationship between CAR-T kinetics and efficacy?

A trend that non-responders had slower CAR-T expansion and longer time to peak concentration was observed (difference is not statistically significant due to limited sample size). In addition, the analysis did not show a statistically significant relationship between T cell persistence (T cell declining rates) and disease relapse. Please see Attachment B for detailed analysis.

Q9. Does the co-medication of tocilizumab or corticosteroid impact the CAR-T cell expansion?

The population PK analysis indicates the impact of the co-medication of tocilizumab and corticosteroid upon CAR-T expansion is mild and not statistically significant. Please see Attachment B for detailed analysis.

In summary, due to small sample size, missing data and cofounding factors associated with the clinical trial data, the analysis results must be interpreted with caution. Most of the results are inconclusive based on the currently available data. However, we showed a possible trend for further investigation, and suggest potential approaches for future study. Our analysis indicates CAR-T kinetics (such as expansion rate) is associated with both treatment response and occurrence of cytokine release syndrome (CRS). Therefore, it may be a potential predictor for both clinical safety and efficacy. In future work, more sophisticated PPK modeling of CAR-T and cytokines may be conducted to identify CRT-T kinetics profiles for a better treatment response and reduced risk of severe CRS.

Attachment A

Modeling the impact of product attributes, concomitant therapies and patient baseline characteristics on safety and efficacy outcomes

Methods: A univariate and multivariate statistical analysis was conducted to understand the impact of key product attributes (bodyweight adjusted/unadjusted cell dose, interferon-gamma (IFN- γ), vector batch and transduction efficiency) on safety or efficacy outcomes. A univariate screening was also conducted to identify the impact of patient baseline characteristics (demographics, diseases burden) and key concomitant therapies (lymphodepletion, steroids, tocilizumab). A logistic regression model for safety outcome estimating the probability of cytokine release syndrome (CRS) with binary response of "yes" for severe CRS (grade 3/4) and "no" for mild CRS (grade 1/2) or no-CRS was fit to the data. The efficacy outcome was modeled as the probability of overall remission rate at day 28 (ORR-28) with binary response, "yes" for ORR-CR/CRi and "no" for unknown/no response. A dose-response model with clinically relevant parameters was specified as:

$$P = \text{Dose}^{\gamma}/(\text{Dose}_{50}{}^{\gamma} + \text{Dose}^{\gamma});$$

Where P is the probability of response (i.e. CRS or ORR), Dose_{50} is the dose corresponding with 50% probability of the response under evaluation, and γ is the steepness of the dose versus response relationship. This mathematical equation is similar to a typical logistic regression model with (coefficient and intercept) but the estimate of Dose_{50} and γ are more easily understood clinically. A multivariate model for predicting CRS was developed using a forward-selected logistic regression model, similar to the approach used in a Novartis publication (Teachey et al. 2016).

We report associations with a p-value less than 0.1 as statistically significant because this is an exploratory analysis based on a small number of patients. The goal of the analysis is to identify some associations that may warrant further exploration.

Q1. Are there correlations between critical product attributes and clinical outcomes of safety and efficacy?

A univariate and multivariate statistical analysis for key product attributes did not reveal significant correlation for predicting severe grade 3/4 CRS ($p>0.1$). A visual effect plot describes how predicted probabilities of an outcome of interest change as we vary the independent variables (Fox, 2003). A visual effect plot for key product attributes versus probability of CRS was displayed in Figure 1 & the Appendix (Fig. A1). There is a trend for increased probability of severe CRS with increasing the transduced CAR T cell dose (Fig. 1). The probability of severe CRS was very weakly correlated with IFN-γ level (a measure of product biological potency). The visual trend for the effect of transduction efficiency (CAR expression by flow cytometry) suggests very weak positive correlation in predicting CRS outcome (Fig. A1). The corresponding effect plot for vector batch in predicting CRS demonstrate essentially comparable results for three different vector batch (b) (4) but the predicted CRS was slightly lower for vector batch (b) (4) Fig. A1).

A similar statistical analysis was conducted for efficacy outcome, ORR at day 28. The transduced CAR T cell dose essentially showed a flat relationship with efficacy outcome following brief increase within narrow dose range (Fig. 2 & A3). We found that IFN-γ level was positively correlated with ORR at day 28 independent of the infused cell dose (Fig. 2). For example, model predicted ORR was 83% and 99% for IFN-γ level of 0.1 and 0.7 pg/transduced cells, respectively(Fig. 2). The comparison of IFN-γ level between respondent versus non-respondent for day 28 response was significant ($p=0.08$).

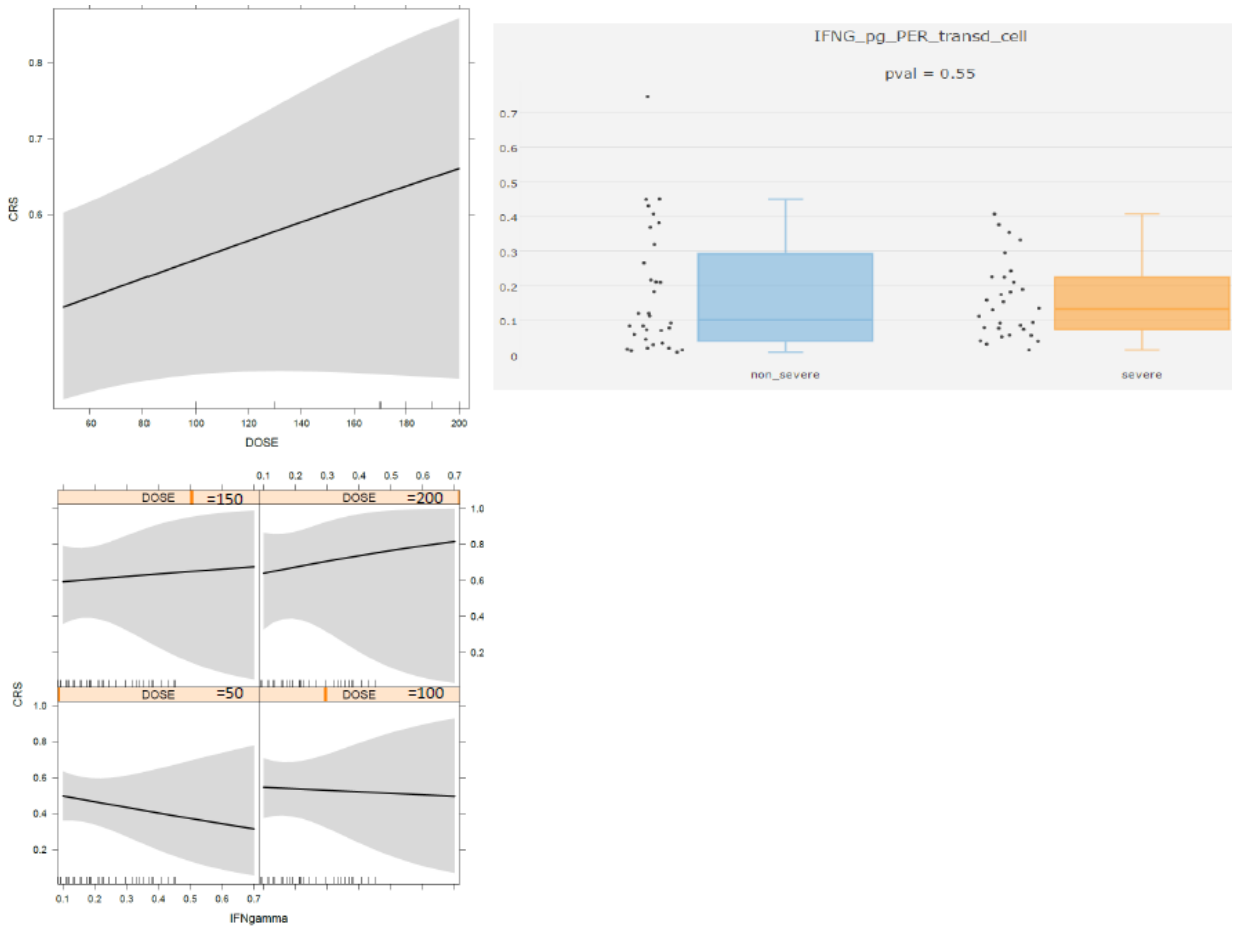

Figure 1. Effect plot for dose (10^6 transduced cells/kg) or IFN-γ level (pg/transduced cells) versus probability of grade 3/4 CRS. The shaded region represents the 95% confidence interval.

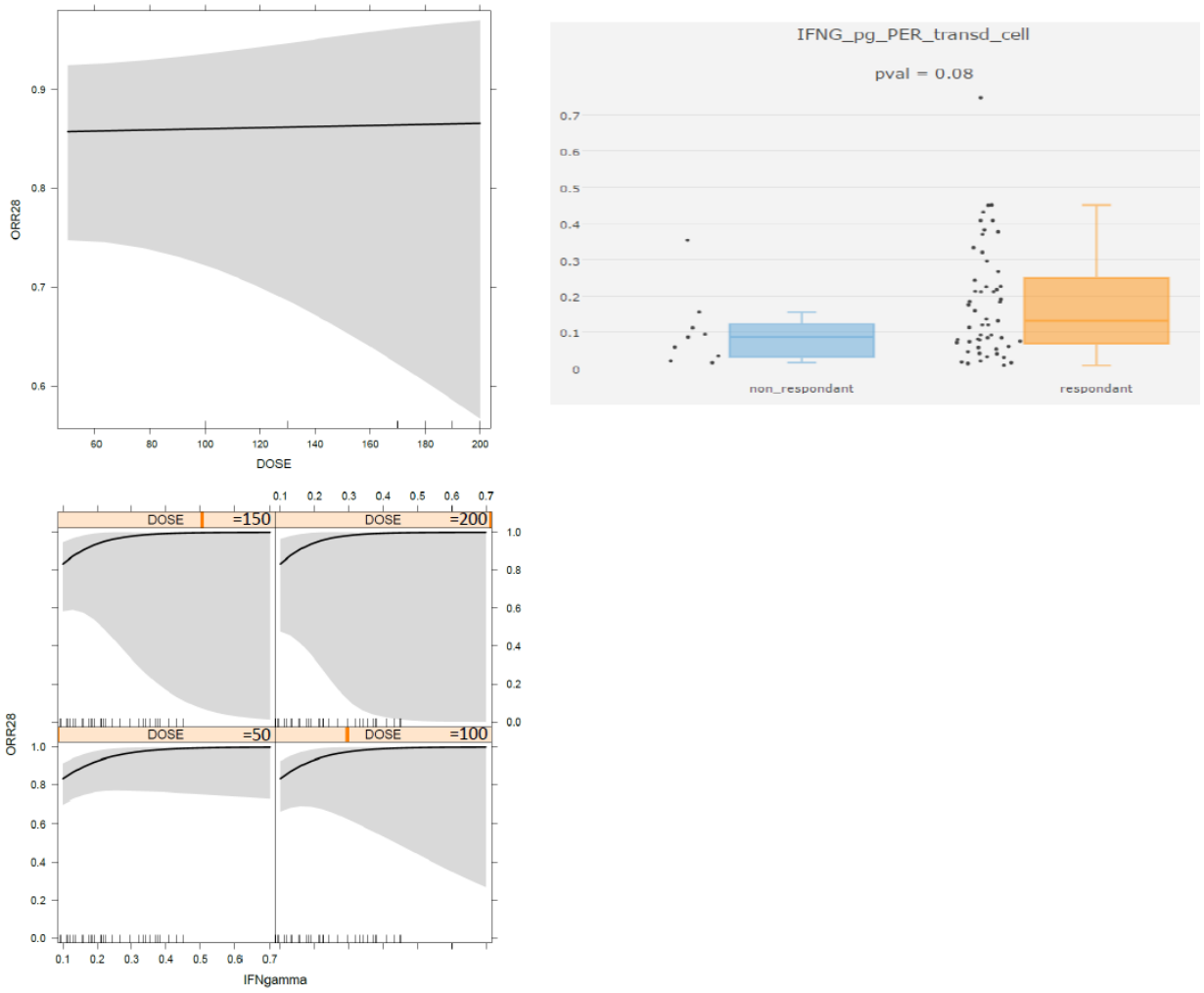

Figure 2. Effect plot for dose (10^6 transduced cells/kg) or IFN-γ level (pg/transduced cells) versus probability of ORR at day 28. The predicted ORR increases with IFN-γ level independent of the infused dose. The shaded region represents the 95% confidence interval.

The following significant associations between CAR T cells subpopulation related attributes and day 28 response rates were observed using a t-test analysis (Table 1). Other product attributes such as transduction efficiency and vector batch did not significantly impact ORR at day 28 (Fig. A2).

Table 1: T cell subpopulation factors significantly associated with day 28 response rates.

VARIABLE	p-value	Direction of Association
% EM CAR – CD4	0.09	+
% Effectors CAR+ CD4	0.02	+
% Naive Tscm CAR- CD8	0.0	+
% Naive Tscm CAR- CD4	0.01	+
% Naive Tscm CAR+ CD8	0.07	+
% Naive Tscm CAR+ CD4	0.0	+

Figure A3 shows the results of the logistic regression model for CRS and ORR using the parameter for predicting 50% probability of severe CRS and 50% probability of achieving CR/CRi. The predicted probability of grade 3/4 CRS modestly increases with increasing dose. The probability of ORR steeply increases within a narrow dose range (<2X10^6 cells/kg) after which it exhibits an almost flat relationship with increasing dose (Fig. A3). The predicted dose that results in 50% probability of CR/CRi was 1X10^3 transduced cells while model predicted dose that results in 50% probability of grade 3/4 CRS was 12.6X10^6 transduced cells/kg (Fig. A3). From a typical dose-response modeling perspective these results suggest wide safety margin (~10000 fold) to achieve 50% probability of efficacy while minimizing the chance of severe CRS. However, it is important to note the wider confidence interval around the effects of dose for both CRS and ORR (Fig. 1 & 2). At present it is difficult to understand whether these high uncertainties are due to the small sample size or inherent variability of the response to the cellular therapy.

The target indication for tisagenlecleucel-T in the current submission is the treatment of pediatric and young adult patients with relapsed/refractory (r/r) B-cell acute lymphoblastic leukemia (ALL) with a recommended intravenous (iv) dose of 0.2 to 5.0×10^6 transduced viable T cells per

kg body weight for patients ≤ 50 kg and 0.1 to 2.5×10^8 transduced viable T cells for patients >50 kg. The dose-response relationship indicates efficacy over this wide dose range and supports the current sponsor dose recommendation. Our model also suggests favorable benefit-risk profile with the lowest dose (<2X10^6 cells/kg) but the number of subjects in this lowest dose range are very limited so no firm conclusion can be drawn at this time.

Conclusion: Our model based analysis identifies a trend for the impact of key CAR T cells product related attributes (dose and IFN-γ level) on CRS and efficacy outcome (ORR at day 28). Our model also suggests favorable benefit-risk profile with the lowest dose (<2X10^6 cells/kg), but the number of subjects in this lowest dose range are very limited to draw firm conclusion at this time. The statistical analysis shows significant impact of CAR T cell product attributes (IFN-γ level, T cell sub-population) on the ORR. The modeling result can be tested and verified with more data.

Q2. What is the impact of steroid treatment for CRS on the treatment response?

About 69% of patients that received steroids for CRS management were responders (CR/CRi) versus 85% who did not receive steroids. However, it is important to note that 16 patients received steroids while 47 did not. Moreover, there was a higher proportion of patients with unknown response who are exposed to steroids (25%) versus unexposed patients (9%).

We conducted a regression analysis to evaluate the impact of steroids on ORR at day 28. We found that there was no statistically significant difference in ORR between patients who were treated with steroids versus untreated patients. The visual steroid effect plot (Fig. 3A) suggests a decrease in ORR with steroids exposure. The logistic regression model predicted mean ORR was 84% for patients who were not exposed to steroids versus 68% for exposed patients (Fig. 3A). It is important to note that patients who are treated with steroids may have also been exposed to other concomitant therapies (e.g. tocilizumab) or have other confounders (e.g. initial tumor

burden, see below for detail). Hence, the design of the B2202 study was not suitable for an unbiased estimate of the impact of steroids on ORR.

A Kaplan-Meier model showed no significant impact of CRS treatment with steroids on duration of response (DOR). Tocilizumab treatment also did not show a significant effect on DOR(Fig. 4).

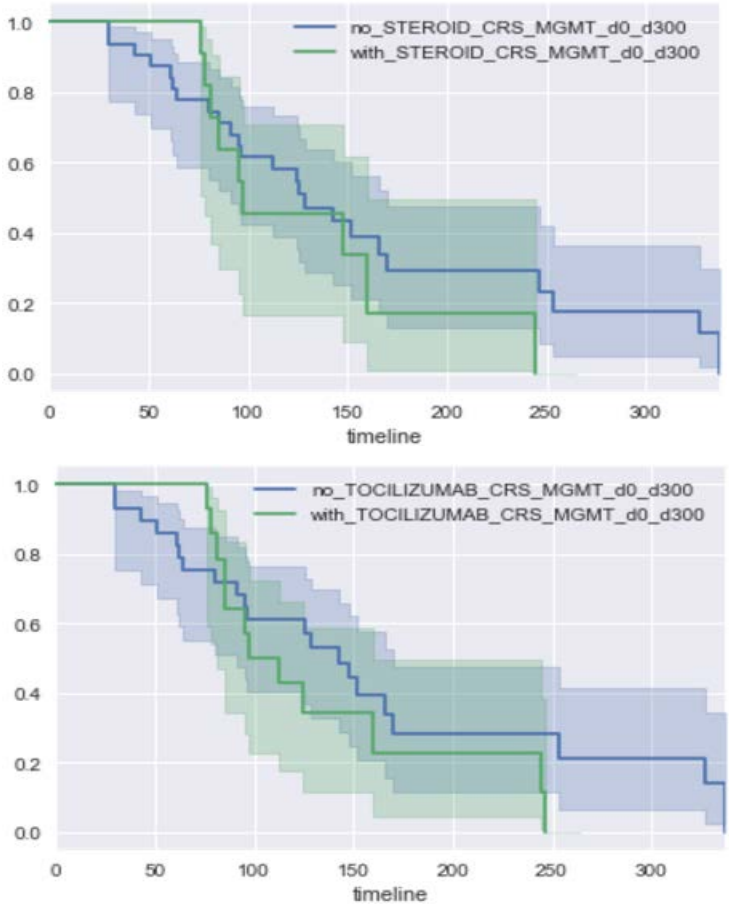

Figure 4. Kaplan-Meier model of the impact of steroids or tocilizumab treatment for management of CRS on duration of response.

Conclusion: We conclude that the current analysis did not show a statistically significant impact of steroids treatment on ORR and DUR. We identify trends toward slightly reduced efficacy for patients who were treated with steroids.

Q3. What is the impact of CBC lymphoblast counts or levels of baseline blast burden on the efficacy outcome?

It should be noted that there are a number of confounding parameters, such as patient baseline characteristics. Therefore, we conducted a separate univariate screening for patient related demographic factors (age, weight, height, sex, race, ethnicity), prior transplantation and baseline tumor burden (%blast cells, %MRD in blood, %MRD in bone marrow). The demographic factors and baseline disease burden factors (% MRD in blood, %MRD in bone marrow, and %blast cells) were studied for their impact on response rates. The following significant associations between demographics/tumor burden and ORR were observed using a t-test analysis.

The visual effect plot for initial disease burden (%MRD blood or bone marrow) on efficacy outcome (ORR 28 days) was displayed (Fig. 3B&C). Our analysis shows that patients with high %MRD (blood count or bone marrow) have a slightly lower predicted probability of ORR (Fig. 3B&C). However, the effect of initial tumor burden (blood or bone marrow) on ORR has a wide confidence interval and was not statistically significant ($p=0.2$). The logistic regression model predicted ORR was 85% and 73% for patients with lowest versus highest disease burden, respectively. Again this effect can be confounded by other factors (e.g. steroids exposure) since patients with high initial tumor burden have a higher probability of severe CRS and most likely were exposed to steroids for management of severe CRS. Hence, we examined whether there was an interaction for the effect of steroid exposure and initial disease burden on ORR 28 days (Fig. A4). We found that that the effect of initial disease burden*steroid exposure was not statistically significant ($p=0.9$). The visual effect plot for steroid*bone marrow residual disease burden demonstrates that patients with no steroid exposure and low disease burden have a higher chance of overall response at day 28 (Fig. A4). For example, the predicted ORR 28 days was 87% for patient with lowest disease burden and no steroid exposure. The corresponding value was 59% for patient with highest disease burden and steroid exposure (Fig. A4).

Conclusion: Based on the current data we identify a trend for higher ORR for patients who have lower %MRD (blood or bone marrow) and no steroid exposure.

Q4. Whether prior transplantation makes a difference in the CAR T cells therapeutic outcome?

The visual effect plot for prior transplantation on efficacy outcome (ORR 28 days) was displayed (Fig. 3D). The effect plot demonstrates an essentially flat relationship between prior transplantation (yes=1 or no=0) versus ORR (Fig. 3D). A further analysis demonstrates no statistically significant difference in tisagenlecleucel-T efficacy outcome (ORR 28 days) in relation with prior transplantation (p=0.9). It was not possible to stratify by transplantation type because data on transplantation type were not available for most of the patients.

Conclusion: We conclude that prior transplantation has no association with the day 28 overall response.

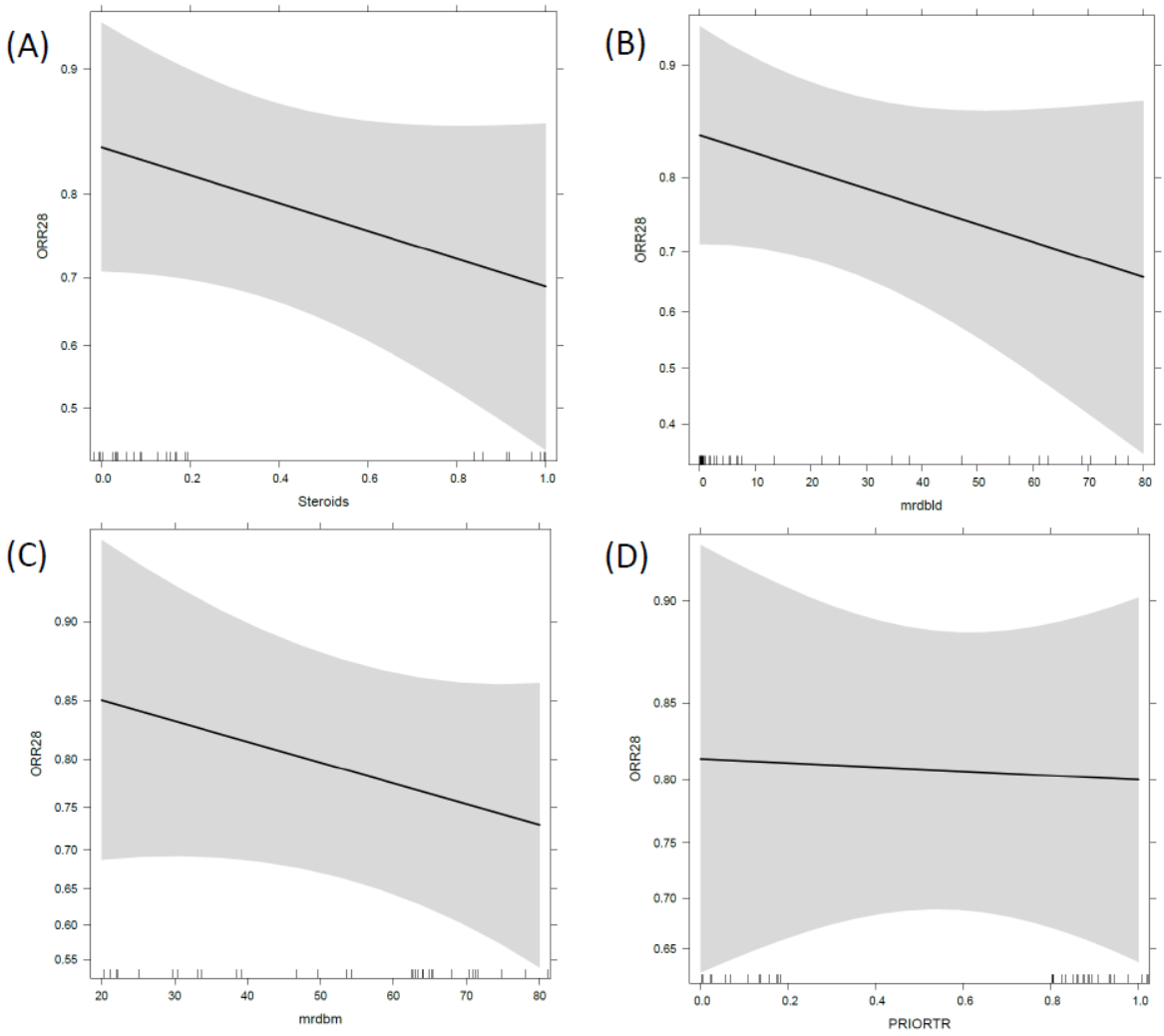

Figure 3. Effect plot for steroids exposure, initial disease burden or prior transplantation versus probability of ORR at day 28. (A) Effect of steroids (1=exposed or 0=unexposed), or (B) residual disease burden in blood or (C) disease burden in bone marrow , and (D) prior transplantation (yes=1 or no=0). The shaded region represents the 95% confidence interval.

Q5. Are any cytokines predictive of CRS?

The following statistically significant associations were observed between the maximum cytokine levels recorded between 14 days before treatment and 3 days after treatment, and rates of severe CRS.

Table 3: Cytokines significantly associated with the occurrence of severe CRS.

VARIABLE	p-value	Direction of Association
Ferritin	0.04	+
IFNG	0.04	+
IL10	0.01	+
IL12	0.02	+
IL13	0.02	+
IL2	0.0	+
IL4	0.07	+
IL6	0.02	+
IL8	0.02	+
TNF	0.01	+

A multivariate model for predicting severe CRS was developed by considering several classification models (Logistic Regression, Decision Trees, and Random Forest) and several variable selection methods. The best preforming multivariate model for predicting CRS using cytokine levels was a logistic regression model with mutual information variable selection method. This model had 80% accuracy with a 74% positive recall (sensitivity). This model used levels of IL2 and IL6 to predict occurrences of severe CRS.

Table 4: Results of model for predicting occurrences of severe CRS using cytokine levels

Model	Accuracy	Sensitivity	Confusion Matrix		
Logistic Regression	80 %	74 %	True Class -	23	4
			True Class +	7	20
				-	+
				Predicted Class	

Conclusion: Our analysis identifies several cytokines that appear to be predictive of severe CRS. A multivariate model that employ level of IL2 and IL6 for predicting severe CRS were developed. This model had 80% accuracy with a 74% positive recall (sensitivity).

Q6. Are any cytokines associated with response?

The following significant associations were observed between the maximum cytokine levels recorded between 14 days before treatment and 3 days after treatment, and day 28 response rates.

Table 5: Cytokines significantly associated with day 28 response rates.

VARIABLE	p-value	Direction of Association
C Reactive Protein	0.02	+
Ferritin	0.02	+
IL10	0.03	+

Conclusion: Our analysis identifies CRP, Ferritin and IL10 as predictive biomarkers for day 28 response.

References

1. Fox J (2003). Effect Displays in R for Generalized Linear Models, Journal of Statistical Software 15: 1-9.
2. Teachey DT, Lacey SF, Shaw PA, et al. (2016). Identification of Predictive Biomarkers for Cytokine Release Syndrome after Chimeric Antigen Receptor T-cell Therapy for Acute Lymphoblastic Leukemia. Cancer Discov. 6: 664-679.

Appendix: Additional figures

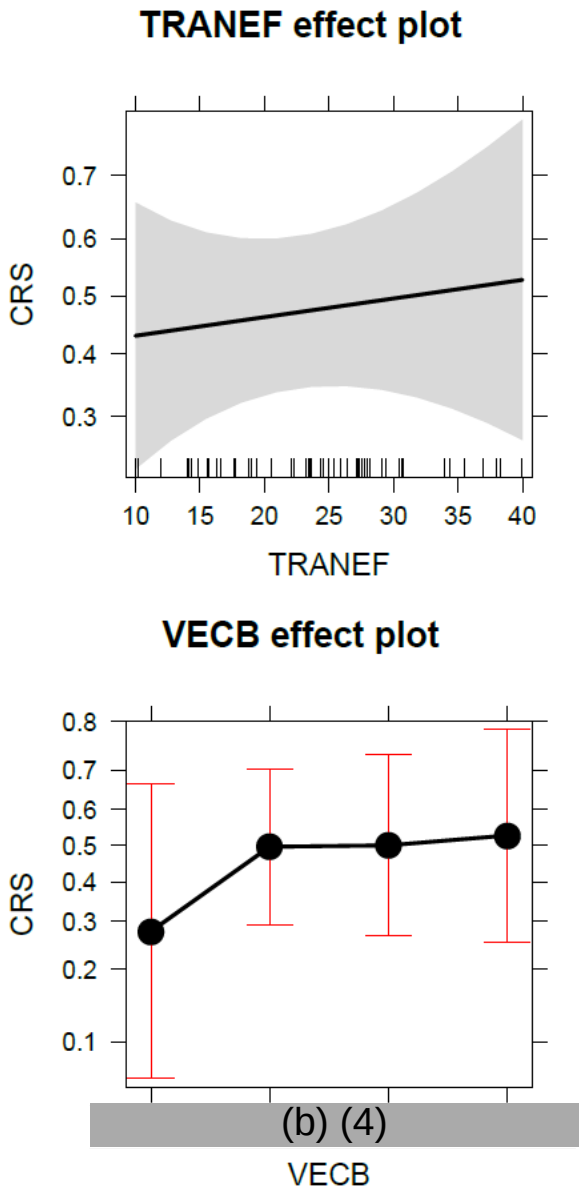

Figure A1. Effect plot for transduction efficiency (TRANEF) or vector batch (VECB) versus probability of grade 3/4 CRS. The shaded region or error bar represents the 95% confidence interval.

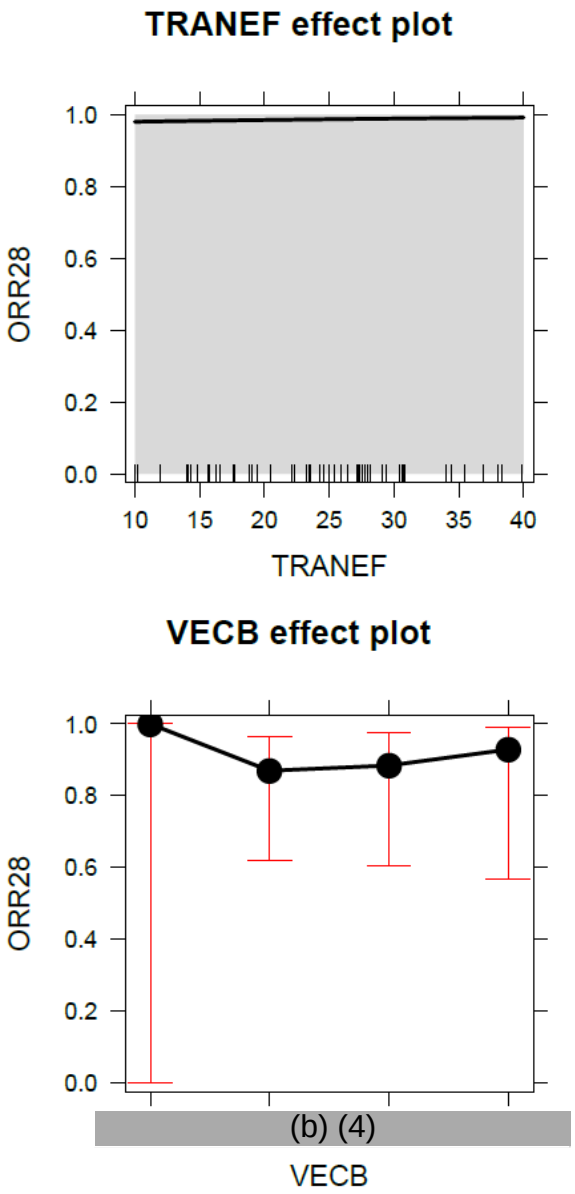

Figure A2. Effect plot for transduction efficiency (TRANEF) or vector batch (VECB) versus probability of ORR at day 28(ORR28). The shaded region or error bar represent the 95% confidence interval.

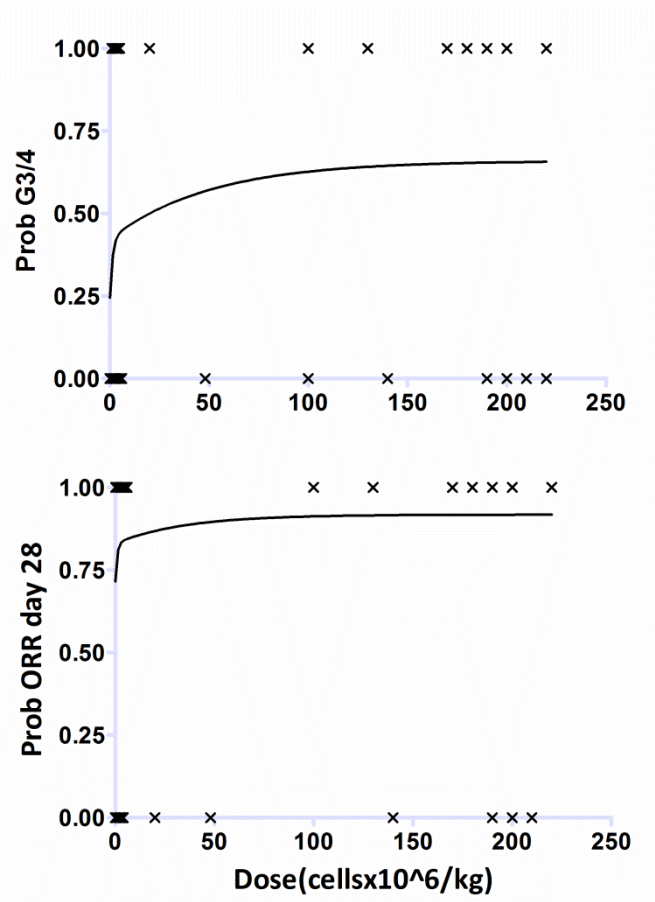

Figure A3. A dose-response relationship for CRS and ORR. The symbol (x) represent observed values and the solid line is logistic regression model prediction.

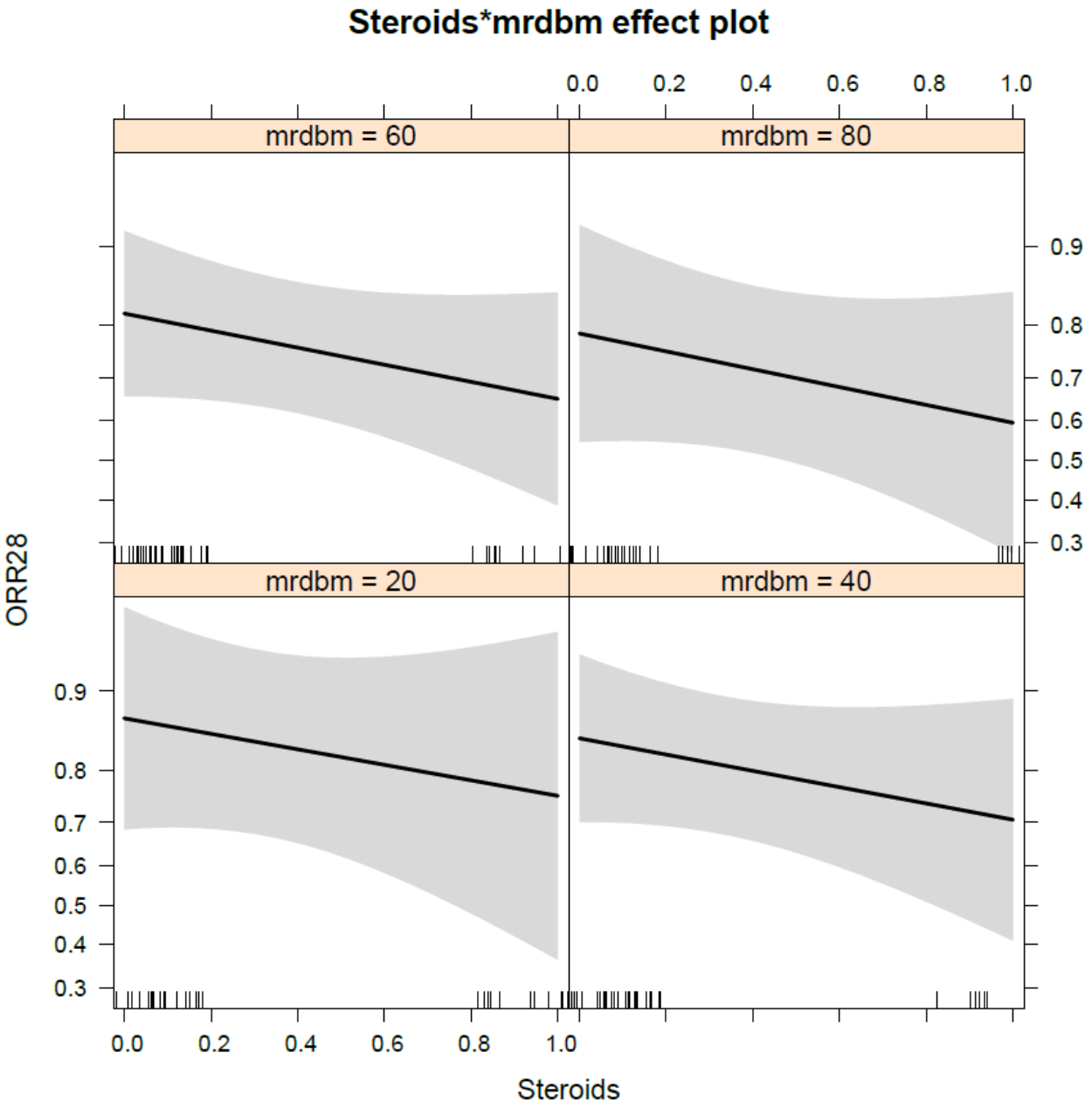

Figure A4. Effect plot for steroids*bone marrow disease burden (mrdbm) versus probability of ORR at day 28. The predicted ORR decreases with steroid exposure at all level of disease burden in bone marrow (range from 20 to 80). The plot demonstrates no significant interaction for steroid and initial disease burden effect. The shaded region represents the 95% confidence interval.

Attachment B

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1 SUMMARY OF FINDINGS

- A higher CAR-T expansion rate and slower declining rate was associated with greater probability of CRS onset and exacerbation. With the same CAR-T changing rate, a greater CAR-T concentration was associated with higher probability of CRS onset.
- Patients who had no response to the CAR-T treatment tended to show a slower expansion and longer time to the peak CAR-T concentrations.
- There were no evident relationship between T cell persistence and the risk of disease relapse.

1.1 Consulted Questions

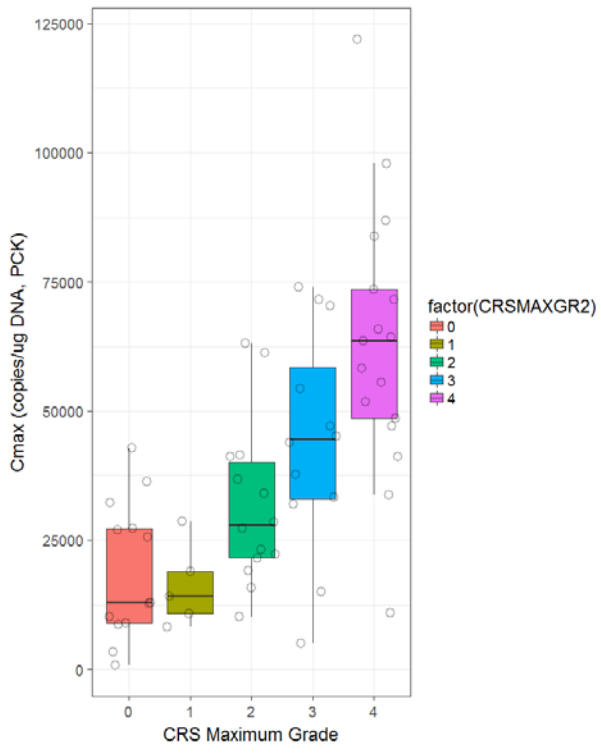
The purpose of this review is to address the following key questions.

1.1.1 What is the relationship between CAR-T kinetics and cytokine release syndrome (CRS)?

A positive relationship between CAR-T concentration and changing rate was identified. Here, a positive rate indicates CAR-T is expanding and a negative rate corresponds to declining CAR-T concentration.

According to the analysis at the subject level, there was a trend that greater maximal concentration of CAR-T was associated with the maximal toxicity grade of CRS (Figure 1). The time to peak concentration (T_{max}) of CAR-T was not associated with the CRS onset and severity.

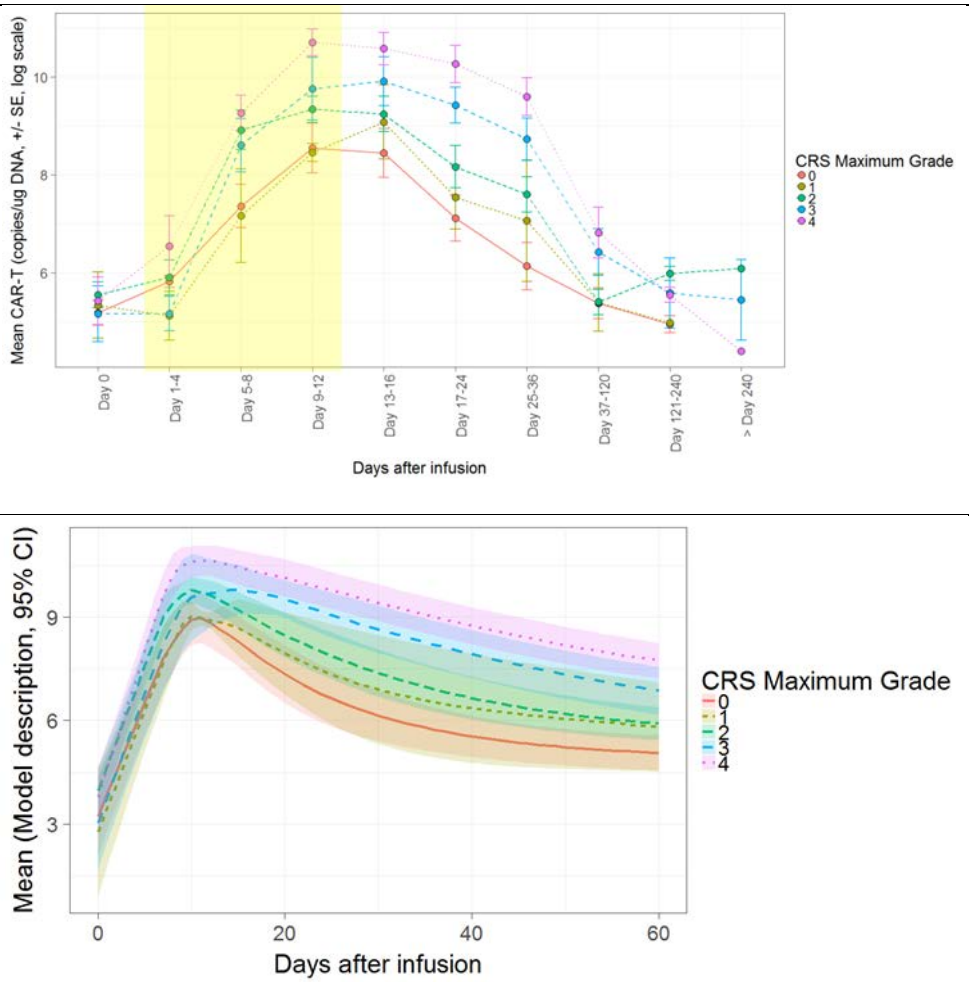
Figure 1: Higher CRS Grade correlates with greater CAR-T Cell maximal concentrations.



Source: FDA reviewer's analysis

The relationship between longitudinal CAR-T exposure and CRS was firstly explored by graphical analysis. The results showed a positive trend between CAR-T exposure and the risk of increased CRS severity. According to both PPK model predicted or observed CAR-T concentration, patients with greater CRS toxicity showed more rapid CAR-T expansion, leading to a greater CAR-T exposure (Figure 2). Considering the biological mechanism of CRS and the positive feedback between CRS and CAR-T expansion, the causality of the CAR-T exposure upon CRS should be interpreted with caution.

Figure 2: Subjects with greater CRS toxicity showed greater CAR-T expansions and higher maximal concentrations



Note: These two plots showed the concentration-time profile of CAR-T grouped by worst CRS grade. The CAR-T kinetics on the top pan was based on the observed data and the one at the bottom was derived from PPK model. Analysis was performed based on data from Study 2202.
Source: FDA reviewer's analysis

To further quantify the longitudinal relationship between CAR-T and CRS, a first order Markov model was developed to explore the time course of CRS and its relationship with longitudinal CAR-T exposure. In the first order Markov model, it was assumed that the probability of moving to the following state (CRS grade) in the next day depends only on the present state of CRS at the current day and not on the previous states.

The following statistically significant relationships between CRS status change and CAR-T kinetics were identified:

- A higher CAR-T expansion rate was associated with higher probability of CRS onset and exacerbation coming up.
- A greater declining rate of CAR-T was associated with higher likelihood of CRS remission in the coming time interval.
- With the same CAR-T changing rate, a greater CAR-T concentration was associated with higher probability of CRS onset.

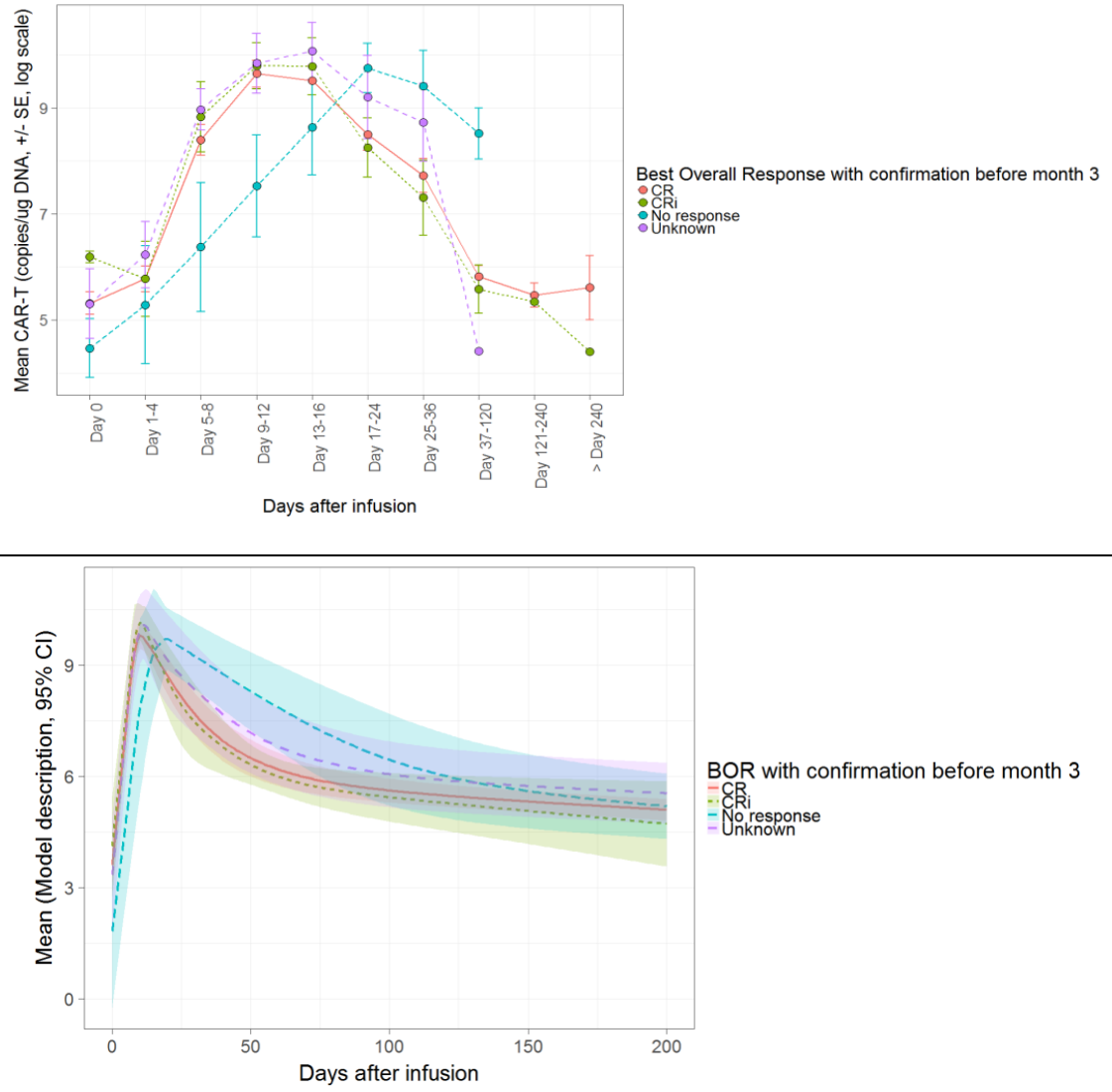
Other factors were screened for covariates under the structure of the Markov model. None of them were statistically significant. This suggested that the CAR-T kinetics in the longitudinal structure could probably carry most of the information for the time course of CRS.

1.1.2 What is the relationship between CAR-T kinetics and efficacy?

CAR-T proliferation v.s. response:

The graphical exploration suggested that patients who had no response to the CAR-T treatment tended to show a slower expansion and longer time to the peak CAR-T concentrations, according to both observed and PPK model predicted CAR-T kinetics (Figure 3).

Figure 3: **Non responders showed slower expansion and longer time to reach the peak CAR-T concentrations**



Note: These two plots showed the concentration-time profile of CAR-T grouped by best overall response. The CAR-T kinetics on the top panel was based on the observed data and the one at the bottom was derived from PPK model. Analysis were performed based on data from Study 2202.
Source: FDA reviewer's analysis

Multivariate logistic regression was performed to screen factors associated with confirmed response rate before month 3. About 200 factors, as measured at baseline or within 28 days after treatment, were screened. As the CAR-T kinetics showed distinct patterns between subjects with no response and with unknown response, two ORR endpoints were chosen: 1) unknown response was treated as non-responder (ORR3); 2) unknown response was censored ($ORR3_{SEN}$).

- For ORR3, two covariates were identified: pre-infusion ferritin level was negatively associated with response probability and average IL13 level was negatively associated with ORR3.
- For $ORR3_{SEN}$, pre-infusion ferritin level and maximal value of $CD19^{+}$ amongst viable WBC (%) in the blood before day 10 were selected: ferritin was negatively associated with response probability and CAR-T at day 3 was positively associated with $ORR3_{SEN}$. There was a trend that longer T_{max} was correlated with no-response as the graphical analysis showed. It could due to the limited sample size in the analysis.

CAR-T persistence v.s. duration of response (DOR)

The relationship between CAR-T persistence and DOR was assessed at subject level, as well as in a longitudinal manner. For subject-level analysis, multivariate Cox proportional hazard model was employed to assess the correlation between CAR-T PK parameters (e.g. declining rate) and hazard. T cell declining rates (rapid and slow) were not associated with duration of response.

The longitudinal Cox model assessed the relationship between time-varying CAR-T concentration/slope and its relationship with the risk of relapse/death. No evident relationship was identified between CAR-T number / declining rate and the risk of relapse. This suggests that disease relapse may not due to insufficient CAR-T concentration at that moment.

CAR-T kinetics v.s. event free survival (EFS):

The longitudinal Cox model was employed where the whole time course of CAR-T concentration and changing rate (slope) were included in the analysis. A trend that greater hazard (risk of disease progression, relapse and death) associated with lower CAR-T number and rapider declining was suggested. In this analysis, the average concentration of CAR-T over 3 weeks prior each time interval provided the best data description. This relationship was not statistically significant. More data would be needed to show conclusive results due to the limited sample size in the current analysis.

CAR-T kinetics v.s. overall survival (OS):

The relationship between CAR-T kinetics and overall survival was explored using time-varying Cox proportional hazards model. There is a trend that faster CAR-T expansion is associated with higher risk of death. However, these results should be interpreted with caution because the time to censoring or event did not exclude the therapy shift or HSCT, which may confound this analysis.

In summary, a trend that non-responders had slower CAR-T expansion and longer time to peak concentration was observed. On the other hand, there were no evident relationship between T cell persistence and disease relapse.

1.1.3 Dose the co-medication of tocilizumab or corticosteroid impact the CAR-T cell expansion?

No. The impact of the co-medication of tocilizumab and corticosteroid was evaluated by the population PK analysis. The model assessed whether the CAR-T cell expansion rate changed following tocilizumab or corticosteroid administration. The impact of the co-medication of tocilizumab and corticosteroid upon CAR-T expansion is mild and not statistically significant. It should be highlighted that patients who ever received tocilizumab or corticosteroid showed greater AUC as compared with the ones who did not receive them. This may not be evidence that concomitant medication affects the CAR-T cell expansion, because patients who received these drugs tended to have more severe CRS, which leads to greater CAR-T cell expansion.

2 RESULTS OF SPONSOR'S ANALYSIS

2.1 Population PK Analysis

- Investigate whether there are differences in tisagenlecleucel-T transgene peak levels between patients that do and do not receive tocilizumab or corticosteroids.
- Investigate where there are changes in the rate of tisagenlecleucel-T transgene expansion after tocilizumab or corticosteroids are given.
- Investigate the effects of other intrinsic and extrinsic factors on tisagenlecleucel-T.

2.1.1 Data

This analysis includes data from two studies: [CCTL019B2205J] and [CCTL019B2202]. As mentioned above, the Study B2101J cellular kinetic data was not pooled for this analysis. Because nonlinear mixed effect modeling methods are designed to work with sparse data and because the purpose was to characterize the cellular kinetics, all patients with cellular kinetic data were included in this analysis, regardless of whether the patients had available primary.

Table 1: Summary of Studies included in Population PK Analysis

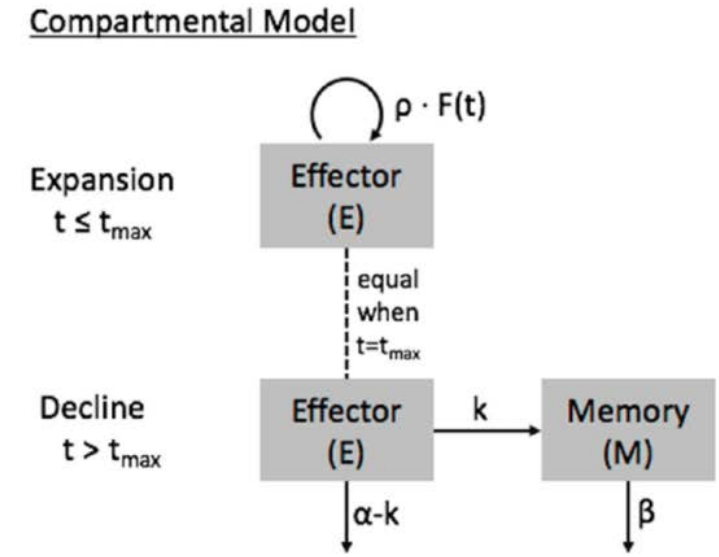
Study	Study	Allowable dose range	(Sample Size, n) Cellular Kinetic sample times, D1 = infusion
B2202	Phase II, single arm, multicenter trial to determine the efficacy and safety of tisagenlecleucel-T in pediatric and young adult patients with relapsed and refractory B-cell acute lymphoblastic leukemia	For patients ≤ 50 kg: 0.2 to 5.0 x 10^6 transduced viable T cells per kg body weight For patients >50 kg: 0.1 to 2.5 x 10^8 transduced viable T cells	n=61 D1-10min post dose, D4, D7, D11, D14, D21, D28, M3, M6, M9, M12, M18, M24, M30, M36, M42, M48, M54, M60
B2205J	Phase II, single arm, multicenter trial to determine the efficacy and safety of tisagenlecleucel-T in pediatric and young adult patients with relapsed and refractory B-cell acute lymphoblastic leukemia	For patients ≤ 50 kg: 0.2 to 5.0 x 10^6 transduced viable T cells per kg body weight For patients >50 kg: 0.1 to 2.5 x 10^8 transduced viable T cells	n=29 D1-10min post dose, D4, D7, D11, D14, D21, D28, M3, M6, M9, M12, M18, M24, M30, M36, M42, M48, M54, M60

Source: Applicant's population PK report

2.1.2 Model structure

The cellular kinetic profile showed that the tisagenlecleucel-T cells undergo an exponential expansion at rate ρ until time tmax, followed by a biexponential decline at rates α (initial slope) and β (terminal slope). The structural model that describes this profile was based on a published model that was used to describe the murine immune response to an infection by *Listeria monocynogenes* or *Lymphocytic choriomeningitis virus*, where similar profiles were observed.

Figure 4: Structure of the population PK model



Definitions

ρ = log(foldx)/tmax

k = FB·(α-β)

Equations

$$\frac{dE}{dt} = \rho \cdot F(t) \cdot E$$

$$\frac{dE}{dt} = -\alpha \cdot E$$

$$\frac{dM}{dt} = k \cdot E - \beta \cdot M$$

Initial Conditions

at t = 0: $E(0) = C_{max}/foldx$

at $t = t_{max}$: $E(t_{max}) = C_{max}$

$M(t_{max}) = 0$

Comedication Effect: F(t)

$$F(t) = f_{toci}(t) \cdot f_{ster}(t)$$

$$f_{toci}(t) = \begin{cases} 1 & t \le t_{toci} \\ F_{toci} & t > t_{toci} \end{cases}$$

$$f_{ster}(t) = \begin{cases} 1 & t \le t_{ster} \\ F_{ster} & t > t_{ster} \end{cases}$$

2.1.3 Results

The final model parameters are provided in Table X. The α and β half-lives was computed by using equation ln(2)/rate and it was found that t1/2-α = 4.3 days and t1/2-β=220 days. The t1/2-β estimate however should be interpreted with caution as the median follow-up time was only 90 days.

Table 2: Parameter estimates of the final population PK model

Type	Parameter	Estimate	RSE %	Eta Shrinkage	Units
Fixed Effect	foldx	3900	30	-	-
Fixed Effect	tmax	9.3	4.2	-	days
Fixed Effect	Cmax	24000	20	-	DNA cop./ug
Fixed Effect	Ftoci	1.2	7.5	-	-
Fixed Effect	Fster	1	9	-	-
Fixed Effect	alpha	0.16	11	-	1/day
Fixed Effect	FB	0.0079	15	-	-
Fixed Effect	beta	0.0032	23	-	1/day
Random Effect	foldx	2.4	9.5	0.39	-
Random Effect	tmax	0.38	7.9	0.14	-
Random Effect	Cmax	0.65	10	0.29	-
Random Effect	alpha	0.91	8.8	0.27	-
Random Effect	FB	0.8	15	0.53	-
Random Effect	beta	0.86	23	0.82	-
Residual Error	a	0.56	3.3	-	-
log Cmax Covariate Effect	Female (vs Male)	0.25	72	-	-
log Cmax Covariate Effect	Asian (vs Cauc.)	0.13	250	-	-
log Cmax Covariate Effect	Race Other/Unknown (vs Cauc.)	0.33	76	-	-
log Cmax Covariate Effect	Downs Syndrome	0.25	130	-	-
log Cmax Covariate Effect	Received HSCT	0.29	62	-	-
log Cmax Covariate Effect	No Fludarabine Received	-0.63	69	-	-
log Cmax Covariate Effect	Study B2205J vs B2202	-0.11	190	-	-
log Cmax Covariate Effect	Transduction Efficiency	0.22	72	-	-
log Cmax Covariate Effect	Dose normalized by body weight	0.093	140	-	-
log Cmax Covariate Effect	Received Tocilizumab	0.44	59	-	-
log Cmax Covariate Effect	Received Corticosteroids	-0.36	75	-	-

RSE denotes the relative standard error of the parameter. Eta shrinkage for each parameter is calculated by the formula: (1-var(η))/ω^2.

Source: Applicant's population PK report

Patients that received tocilizumab had a 2-fold higher Cmax. This is thought to be because patients with greater peak transgene levels are more likely to develop Grade 3 and 4 cytokine release syndrome and therefore are more likely to require tocilizumab therapy. An impact of tocilizumab therapy on the rate of expansion was not detected. None of the other covariates explored (including corticosteroid dosing) were confirmed to have an effect. While it has been observed elsewhere that baseline tumor burden also correlates with an increased expansion, this was not assessed here because in B2202 and B2205J, biopsies were not collected after lymphodepletion and before tisagenlecleucel-T dosing.
Care should be taken when interpreting the lack of effect of corticosteroids. The CRS treatment algorithm specified that corticosteroids only be given when the first dose of tocilizumab did not lead to an improvement in CRS. Furthermore, corticosteroid doses were less than 2 mg/kg/day. Thus the effect of giving corticosteroids at larger doses, before tocilizumab, or without tocilizumab was not assessed.
No relationship between dose and Cmax was detected. While a dose-exposure relationship is generally expected for most drugs, the lack of a relationship here may be due to the capacity of tisagenlecleucel-T to proliferate.

Reviewer's comments:

- *Sponsor's population PK model is reasonable.*
- *The reviewer agrees with sponsor's assessment that there was no evidence that tocilizumab or corticosteroids slowed the rate of expansion. However, as highlighted by the applicant, the effect of giving corticosteroids at larger doses, before tocilizumab, or without tocilizumab was not assessed due to the study design.*

3 RESULTS OF REVIEWER'S ANALYSIS

3.1 Introduction

The reviewer initiated an independent analysis to investigate the consulted questions by the review team, which mainly focused on the relationship between CAR-T kinetics and safety or efficacy endpoints.

3.2 Objectives

Analysis objectives are:

- Develop a longitudinal CRS model and assess the relationship with CAR-T kinetics
- Develop survival models for DOR, EFS and OS, and assess the relationship with time course of CAR-T.
- Evaluate other factors at subject level which may be associated with CRS, ORR, EFS, DOR and OS.

3.3 Software

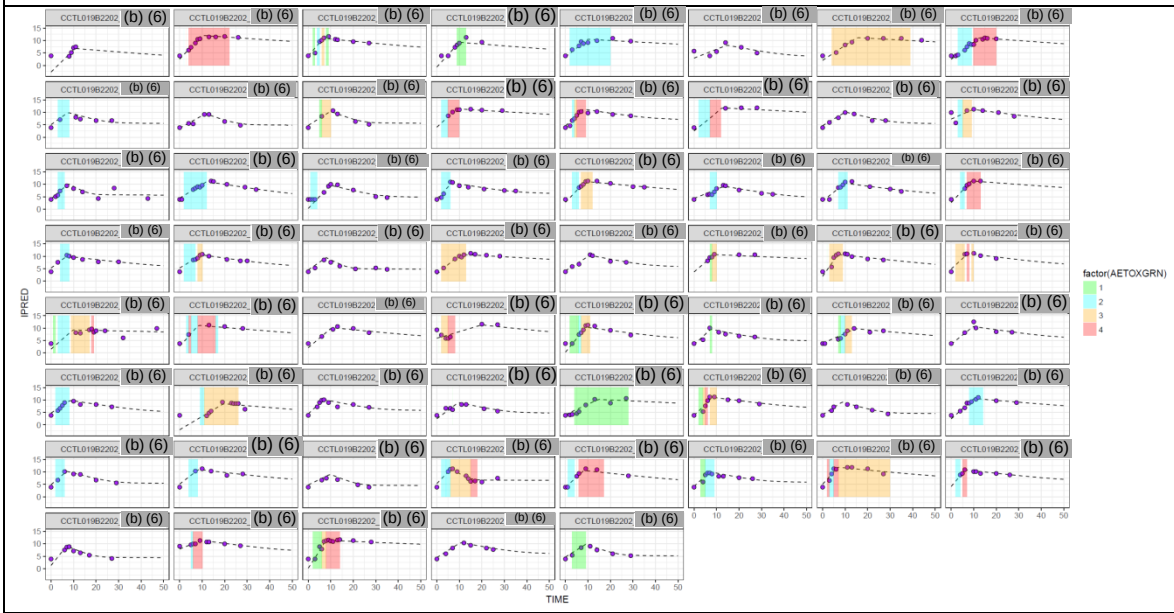
(b) (4) were used for developing the models. (b) (4) was used for data handling, visualization, and post-processing.

3.4 Results and Discussions

3.4.1 Longitudinal exposure-CRS Model

The longitudinal exposure-CRS analyses were based on data from Study 2202. The CRS were treated as ordered categorical (grades 0, 1/2, 3/4), and an extension of the proportional odds model was used to describe the probability and severity of CRS over time. (**Figure 5**)

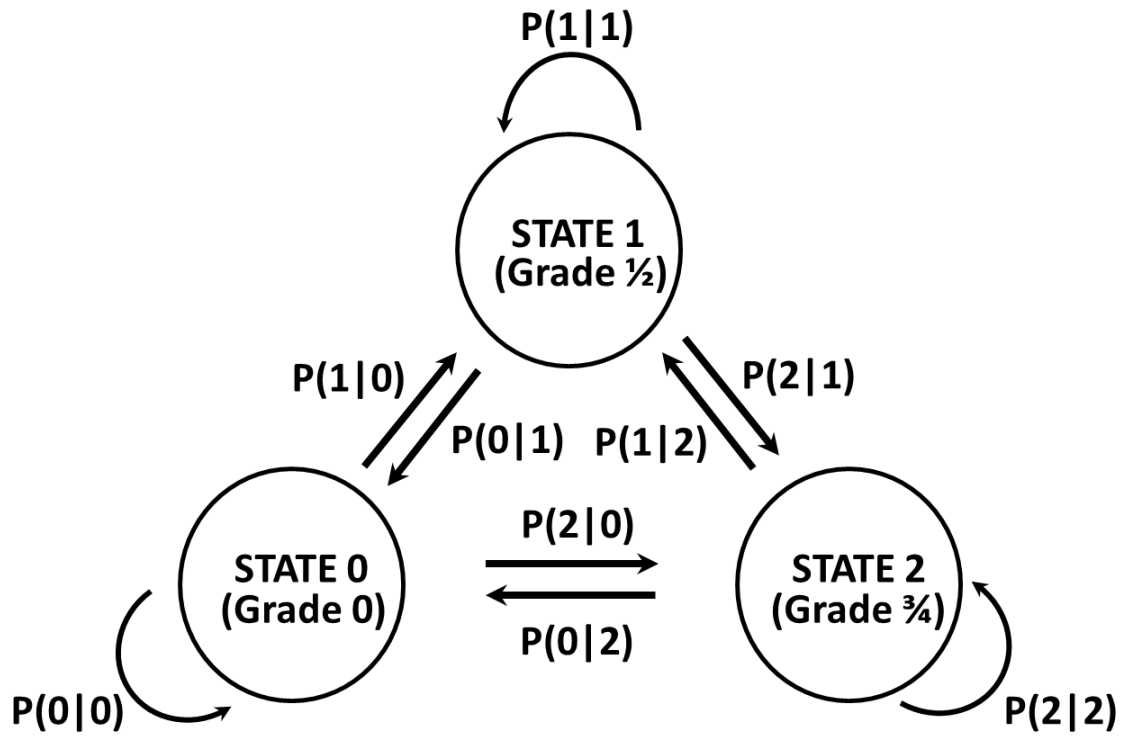
Figure 5: Data structure for CAR-T kinetics vs. longitudinal CRS



Note: The round dots represent the observed CAR-T concentration; the dashed lines are model predicted CAR-T kinetics; the color areas are the time intervals where CRS occurred, different CRS grades are represented by distinct colors: green, 1st grade CRS;
blue, 2nd CRS; orange, 3rd CRS; red, 4th CRS.
Source: FDA reviewer's analysis

The extension included a first-order Markov model to condition the probability of transition between different severities based on the preceding one. This accounts for the likely association between the severity of the adverse effects between one time point and another. Logit transformations were used to constrain the estimated probabilities to values between 0 and 1, and the function describing the probability of transition from state $s^{(n)}$ to grade $s^{(m)}$ for the i^{th} patient at the j^{th} time interval was given the structure shown in Figure 6.

Figure 6: Model structure for CAR-T kinetics vs. longitudinal CRS



Note: each circle represents distinct states: no CRS, Grade ½ CRS, Grade ¾ CRS. The arrow is the transition from the current state to the next state. P(X|Y) is the transition probability to state X given the current state is Y.
Source: FDA reviewer's analysis

The logit model was of the form:

$$Logit\left(P_{(ijs^{(m)}|s^{(n)})}\right) = log\left(\frac{P_{(ijs^{(m)}|s^{(n)})}}{1 - P_{(ijs^{(m)}|s^{(n)})}}\right) = f_{s^{(m)}|s^{(n)}} + \eta_i$$

$$f_{s^{(m)}|s^{(n)}} = B_{s^{(m)}|s^{(n)}} + \beta_{1|s^{(n)}} \cdot \log(CART) + \beta_{2|s^{(n)}} \cdot SLP_{\log(CART)}$$

where $P_{(ijs^{(m)}|s^{(n)})}$ is the transition probability from $s^{(n)}$ to $s^{(m)}$. $B_{s^{(m)}|s^{(n)}}$ is a baseline logit from state $s^{(n)}$ to $s^{(m)}$, $\beta_{1|s^{(n)}} \cdot \log(CART)$ is the effect of log-transformed CAR-T concentration modeled as being linear; $\beta_{2|s^{(n)}} \cdot SLP_{\log(CART)}$ the effect of CAR-T changing rate at logarithm scale. η is the subject-specific random effect. The parameter estimates, precision of the estimate, and 95% confidence interval for sponsor's model are shown in the table:

Table 3: Parameter Estimates (95% CI) for CRS Markov Model

Parameter	Estimate	Relative SE	95% CI
$B_{1\|0}$	-6.97	5.6%	-8.872 - -7.108
$B_{2\|0}$	-1.54	17.2%	-3.972 - -1.968
$B_{1\|1}$	3.49	31.8%	0.787 - 3.393
$B_{2\|1}$	-9.59	17%	-11.66 - -5.82
$B_{1\|2}$	1.79	10.9%	1.621 - 2.499
$B_{2\|2}$	-0.181	10.9%	1.621 - 2.499
$\beta_{slp\|0}$	5.95	7%	6.908 - 9.112
$\beta_{slp\|1}$	2.58	20.6%	3.224 - 7.576
$\beta_{slp\|2}$	1.7	47.8%	0.097 - 2.963
$\beta_{CAR\text{-}T\|0}$	0.705	37.5%	0.086 - 0.56
$\beta_{CAR\text{-}T\|1}$	0 FIX	N/A	N/A
$\beta_{CAR\text{-}T\|2}$	0 FIX	N/A	N/A

3.4.2 Longitudinal exposure-TTE (time to event) Analysis

The FDA reviewer developed Cox proportional hazards models to evaluate the relationship between CAR-T exposure and multiple time to event efficacy endpoints, including event free survival (EFS), overall survival (OS), and duration of response (DOR). Various CAR-T time-varying exposure measures were evaluated as shown in the following Table 4.

Table 4: Time-vary CAR-T exposure metrics

Exposure Metrics	Definition
Cavg1D	Average CAR-T concentration over prior one day at each day
Cavg1W	Average CAR-T concentration over prior one week at each day
Cavg10D	Average CAR-T concentration over prior ten days at each day
Cavg2W	Average CAR-T concentration over prior two weeks at each day
Cavg3W	Average CAR-T concentration over prior three weeks at each day
Cavg4W	Average CAR-T concentration over prior four weeks at each day
Cavg6W	Average CAR-T concentration over prior six weeks at each day
CavgT	Average CAR-T concentration from the first exposure to each day

The Cox model was specified as:

$$h\left(t, X'_{ex}(t)\right) = h_o(t) \cdot exp\left(\beta_{ex2} \cdot X'_{ex}(t)\right)$$

where $X_{ex}(t)$ is the CAR-T exposure measure which may vary with t, β_{ex2} represents the slope of CAR-T concentration or changing rate. The selection of the time-varying CAR-T exposure metrics as shown in was based on Akaike information criterion (AIC) and biological plausibility. Both linear and log-linear models were estimated. The model parameter estimates for these models are illustrated in Table 5.

Table 5: Parameter Estimates for Exposure - TTE Model

Endpoint	Parameter	Scale	Estimate	SE	P-value
DOR	β_{cavg2w}	Linear	-0.0012	0.002	0.55
	$B_{slp(CAR\text{-}T)}$		0.0289	0.085	0.73
	β_{cavg1w}	Logarithm	-0.21	0.394	0.59
	$\beta_{slp(CAR\text{-}T)}$		2.00	6.19	0.75
EFS	β_{cavg4w}	Linear	-0.0015	0.0012	0.24
	$\beta_{slp(CAR\text{-}T)}$		-0.0721	0.0545	0.19
	β_{cavg1w}	Logarithm	0.321	0.31	0.30
	$\beta_{slp(CAR\text{-}T)}$		-0.173	2.86	0.55
OS	β_{cavg6w}	Linear	0.00009	0.000035	0.013
	$\beta_{slp(CAR\text{-}T)}$		6.86	3.47	0.048
	β_{cavg1w}	Logarithm	0.256	0.296	0.387
	$\beta_{slp(CAR\text{-}T)}$		4.625	2.706	0.087

3.4.3 Regression analysis at subject level for CRS, ORR, DOR and EFS

Approximately two hundred factors were screened for the regression analysis. Due to the relevantly large number of covariates, univariate analyses were performed to minimize the impact of missing values. The factors selected served as the candidates for the subsequent multivariate analysis. For CRS, subjects were tagged as the most severe toxicity grade. An ordinal logistic regression model was selected to take adverse reaction severity into consideration. ORR was treated as dichotomous and analyzed using logistic model. Time to event endpoints like EFS or DOR were analyzed using Cox proportional hazard model.

In the univariate analysis, the significance level was selected at 0.05 and no overall type I error control was performed at this stage. It is important to note that this is an exploratory analysis based on a small number of patients. The goal of the analysis is to identify some associations that may warrant further exploration. The factors screened were classified into the following categories:

a. Product characteristics [batch, T cell subpopulation (CD8/CD4, SCM/CM/EM, CART+/-, etc.)].
b. Dose
c. Baseline patient characteristics and biomarker profile (pre-infusion)

d. CAR-T pharmacokinetics
e. Metrics of cytokines and other biomarkers post treatment

The detail of the factor screening can be found in the Appendix A.

Based on the covariates selected in the univariate analysis, multivariate analysis was performed to reduce the redundancy of the covariates selected. Stepwise selection was performed out of the candidate covariates as chosen in the univariate analysis. The selection was based on AIC and biological plausibility. The AIC measures the tradeoff between the accuracy of the predicted outcomes and the number of independent variables included in the model. The model parameter estimates for these models are shown in Table 6.

Table 6: Parameter Estimates for Regression Analysis

Endpoint	Covariate	Estimate	SE	P-value
ORR3	Ferritin (µg/L), pre-infusion, maximal value per subject	-0.00045	0.00016	0.005
	IL13(pg/mL), Day 0-28, average value per subject	-0.1329	0.0609	0.03
$ORR3_{SEN}$	Ferritin (µg/L), pre-infusion, maximal value per subject	-0.00047	0.00026	0.06
	CD19+ amongst viable WBC (%) - Blood, Day 10, maximal value per subject	-0.0358	0.0202	0.07
DOR	T cell exponential expansion rate, PPK	-4.37	2.595	0.09
	Tmax (days), PPK	0.301	0.181	0.09
EFS	CD19+ amongst viable WBC (%) - blood, day 0-28, average value per subject	0.0573	0.0225	0.01
	Ferritin (µg/L), Day 0-3, maximal value per subject	0.0007	0.00033	0.03
	T cell exponential expansion rate, PPK	-3.626	1.8716	0.05
	Ferritin (µg/L), pre-infusion, average value per subject	-0.00067	0.00038	0.07

FDA reviewer's comments:
As sample size was very limited and no overall alpha was adjusted, this analysis may have low power and high probability of type I error. Thus, these results from regression analysis should be interpreted with caution.

4 APPENDIX

4.1 Appendix A: Factors Screened for Efficacy and Safety Endpoints

Variable Name	Description	Maximal CRS Grade (0/12/34)	ORR3	ORR3SEN	DOR	EFS
AGE	Age					
SEX	Sex					
MNFSBWT	Subject Weight for Manufacturing	X				
WGTBASE	Weight at Baseline	X				
PSBASEN	Performance Status at Baseline					
PRHSCTFL	Prior HSCT (Y/N)					
PRHSCTN	Number of Prior HSCT Performed					
PRLINTHY	Number of Previous Lines of Therapies					
NUMPRCR	Number of Previous Complete Remissions					
MLLFL	MLL rearrangement at Baseline Flag					
CPXKARBL	Baseline Complex Karyotypes(>=5 abnorm.)					
HRKMUTFL	High risk mutations at Baseline Flag					
LDTPGR1	LD Chemotherapy Type Group					
RESPENF	Response status at study entry					X
FRTPGMN	Timing of First Relapse (mon)					
BLEXMDFL	Baseline extramedullary disease presence					
BLTUMBRD	Baseline bone marrow tumor burden	X				
DOWNSFL	Down's syndrome					
CNSLEUFL	CNS leukemia prior to enrollment					
BLMRDBM	Baseline MRD in bone marrow by flow cytometry (%)					
BLMRDPB	Baseline MRD in peripheral blood by flow cytometry (%)					
BLHEMLBP	Baseline Morphologic lymphoblast count in peripheral blood (%)					

Variable Name	Description	Maximal CRS Grade (0/12/34)	ORR3	ORR3SEN	DOR	EFS
TCTLDOS	Total CTL019 Cell Dose (10E8 cells)					
TCTLDSKG	Total CTL019 Cell Dose (10E6 cells/kg)					
AUC0TMAX.NCA	AUC0-Tmax (copies/ug*days), NCA, observed				X	
CLAST.NCA	CLAST Clast (copies/ug), NCA, observed				X	
CMAX.NCA	Max Conc (copies/ug), NCA, observed				X	
TLAST.NCA	Tlast (days), NCA, observed				X	
TMAX.NCA	Time of CMAX (days), NCA, observed				X	
AUC28D.NCA	AUC0-28d (copies/ug* days), NCA, observed				X	
AUCTM84D.NCA	AUC0-84d (copies/ug* days), NCA, observed					X
ALPHA.NM	CAR-T rapid declining rate (1/day), PPK	X		X		
BETA.NM	CAR-T slow declining rate (1/day), PPK					
TMAX.NM	Tlast (days), PPK			X	X	X
CMAX.NM	Max Conc (copies/ug), PPK	X				
FB.NM	the fraction of cells contributing to Cmax that exhibit a gradual decline at rate β (memory cell), PPK					
RHO.NM	T cell exponential expansion rate, PPK				X	X
CART.DAY3	CAR-t conc. (copies/ug) at day 3 post infusion, PPK			X		
AUC.DAY0_3	AUC0-3d (copies/ug* days), PPK					X
AUC.DAY0_10	AUC0-10d (copies/ug* days), PPK					
AUC.DAY0_28	AUC0-28d (copies/ug* days), PPK					
AUC.DAY0_90	AUC0-90d (copies/ug* days), PPK					
AUC.DAY0_180	AUC0-180d (copies/ug* days), PPK					
AUC.DAY0_Tmax	AUC0-Tmax (copies/ug* days), PPK					
CAVG.DAY0_Tmax	Cavg0-Tmax (copies/ug* days), PPK					X

Variable Name	Description	Maximal CRS Grade (0/12/34)	ORR3	ORR3SEN	DOR	EFS
SCN.C3LYMBM.AVG	T cells amongst mono-nuclear cells (Lymphocytes and monocytes with the exclusion of granulocytes) (%) - Bone Marrow, screen, average value per subject					
DAY28.CD19ILBM.MAX	CD19+ Intensity Level among B-ALL cells (PE Molecules/cell) - Bone Marrow, screen, maximal value per subject					X
SCN.CD19ILBM.AVG	CD19+ Intensity Level among B-ALL cells (PE Molecules/cell) - Bone Marrow, screen, average value per subject		X	X		
SCN.CD19ILNM.MAX	CD19+ Intensity Level among normal B cells (PE Molecules/cell) - Bone Marrow, screen, maximal value per subject					
SCN.CD19ILNM.AVG	CD19+ Intensity Level among normal B cells (PE Molecules/cell) - Bone Marrow, screen, average value per subject					
PRE.IF.IFNG.MAX	IFN-γ (pg/mL), pre-infusion, maximal value per subject					
PRE.IF.IFNG.AVG	IFN-γ (pg/mL), pre-infusion, average value per subject					
PRE.IF.IL10.MAX	IL10 (pg/mL), pre-infusion, maximal value per subject					
PRE.IF.IL10.AVG	IL10 (pg/mL), pre-infusion, average value per subject			X		
PRE.IF.IL12P70.MAX	IL12p70 (pg/mL), pre-infusion, maximal value per subject					
PRE.IF.IL12P70.AVG	IL12p70 (pg/mL), pre-infusion, average value per subject					
PRE.IF.IL13.MAX	IL13 (pg/mL), pre-infusion, maximal value per subject					
PRE.IF.IL13.AVG	IL13(pg/mL), pre-infusion, average value per subject					

Variable Name	Description	Maximal CRS Grade (0/12/34)	ORR3	ORR3SEN	DOR	EFS
PRE.IF.IL1B.AVG	IL1β (pg/mL), pre-infusion, average value per subject					
PRE.IF.IL2.MAX	IL2 (pg/mL), pre-infusion, maximal value per subject					
PRE.IF.IL2.AVG	IL2 (pg/mL), pre-infusion, average value per subject					
PRE.IF.IL4.MAX	IL4 (pg/mL), pre-infusion, maximal value per subject					
PRE.IF.IL4.AVG	IL4 (pg/mL), pre-infusion, average value per subject					
PRE.IF.IL6.MAX	IL6 (pg/mL), pre-infusion, maximal value per subject					
PRE.IF.IL6.AVG	IL6 (pg/mL), pre-infusion, average value per subject	X		X		
PRE.IF.IL8.MAX	IL8 (pg/mL), pre-infusion, maximal value per subject	X				
PRE.IF.IL8.AVG	IL8 (pg/mL), pre-infusion, average value per subject	X				
PRE.IF.TNFA.MAX	TNFα (pg/mL), pre-infusion, maximal value per subject					
PRE.IF.TNFA.AVG	TNFα (pg/mL), pre-infusion, average value per subject					
PRE.IF.C3LYMBD.MAX	T cells amongst mono-nuclear cells (Lymphocytes and monocytes with the exclusion of granulocytes) (%) - Bone Marrow, pre-infusion, maximal value per subject		X			X
PRE.IF.C3LYMBD.AVG	T cells amongst mono-nuclear cells (Lymphocytes and monocytes with the exclusion of granulocytes) (%) - Bone Marrow, pre-infusion, average value per subject		X			X
PRE.IF.CRP.MAX	CRP (mg/L), pre-infusion, maximal value per subject					
PRE.IF.CRP.AVG	CRP (mg/L), pre-infusion, average value per subject					
PRE.IF.FERRITIN.MAX	Ferritin (µg/L), pre-infusion, maximal value per subject	X	X	X		X
PRE.IF.FERRITIN.AVG	Ferritin (µg/L), pre-infusion, average value per subject	X	X	X		X

Variable Name	Description	Maximal CRS Grade (0/12/34)	ORR3	ORR3SEN	DOR	EFS
DAY3.CRP.AVG	CRP (mg/L), Day 3, average value per subject					
DAY3.FERRITIN.MAX	Ferritin (µg/L), Day 3, maximal value per subject	X	X	X		X
DAY3.FERRITIN.AVG	Ferritin (µg/L), Day 3, average value per subject	X	X	X		X
DAY3.C3LYMBD.MAX	T cells amongst mono-nuclear cells (Lymphocytes and monocytes with the exclusion of granulocytes) (%) - Bone Marrow, Day 3, maximal value per subject					
DAY3.C3LYMBD.AVG	T cells amongst mono-nuclear cells (Lymphocytes and monocytes with the exclusion of granulocytes) (%) - Bone Marrow, Day 3, average value per subject					
DAY3.IFNG.MAX	IFN-γ (pg/mL), Day 3, maximal value per subject					
DAY3.IFNG.AVG	IFN-γ (pg/mL), Day 3, average value per subject					
DAY3.IL10.MAX	IL10 (pg/mL), Day 3, maximal value per subject	X		X		
DAY3.IL10.AVG	IL10 (pg/mL), Day 3, average value per subject	X				
DAY3.IL12P70.MAX	IL12p70 (pg/mL), Day 3, maximal value per subject					
DAY3.IL12P70.AVG	IL12p70 (pg/mL), Day 3, average value per subject					
DAY3.IL13.MAX	IL13 (pg/mL), Day 3, maximal value per subject					
DAY3.IL13.AVG	IL13(pg/mL), Day 3, average value per subject					
DAY3.IL1B.MAX	IL1β (pg/mL), Day 3, maximal value per subject					
DAY3.IL1B.AVG	IL1β (pg/mL), Day 3, average value per subject					
DAY3.IL2.MAX	IL2 (pg/mL), Day 3, maximal value per subject	X				
DAY3.IL2.AVG	IL2 (pg/mL), Day 3, average value per subject	X				
DAY3.IL4.MAX	IL4 (pg/mL), Day 3, maximal value per subject					
DAY3.IL4.AVG	IL4 (pg/mL), Day 3, average value per subject					
DAY3.IL6.MAX	IL6 (pg/mL), Day 3, maximal value per subject	X				
DAY3.IL6.AVG	IL6 (pg/mL), Day 3, average value per subject	X				

Variable Name	Description	Maximal CRS Grade (0/12/34)	ORR3	ORR3SEN	DOR	EFS
DAY3.IL8.AVG	IL8 (pg/mL), Day 3, average value per subject	X				
DAY3.TNFA.MAX	TNFα (pg/mL), Day 3, maximal value per subject	X				
DAY3.TNFA.AVG	TNFα (pg/mL), Day 3, average value per subject	X				
DAY10.C3LYMBD.MAX	T cells amongst mono-nuclear cells (Lymphocytes and monocytes with the exclusion of granulocytes) (%) - Bone Marrow, Day 10, maximal value per subject					
DAY10.C3LYMBD.AVG	T cells amongst mono-nuclear cells (Lymphocytes and monocytes with the exclusion of granulocytes) (%) - Bone Marrow, Day 10, average value per subject					
DAY10.CD19ILBD.MAX	CD19+ Intensity Level Among B-All Cells (PE Molecules/Cell) - Blood, Day 10, maximal value per subject					X
DAY10.CD19ILBD.AVG	CD19+ Intensity Level Among B-All Cells (PE Molecules/Cell) - Blood, Day 10, average value per subject					X
DAY10.CD19PWBC.MAX	CD19+ Amongst Viable WBC (%) - Blood, Day 10, maximal value per subject			X		X
DAY10.CD19PWBC.AVG	CD19+ Amongst Viable WBC (%) - Blood, Day 10, average value per subject			X		X
DAY10.CRP.MAX	CRP (mg/L), Day 10, maximal value per subject					
DAY10.CRP.AVG	CRP (mg/L), Day 10, average value per subject					
DAY10.FERRITIN.MAX	Ferritin (µg/L), Day 10, maximal value per subject					
DAY10.FERRITIN.AVG	Ferritin (µg/L), Day 10, average value per subject					
DAY10.IFNG.MAX	IFN-γ (pg/mL), Day 10, maximal value per subject					
DAY10.IFNG.AVG	IFN-γ (pg/mL), Day 10, average value per subject					
DAY10.IL10.MAX	IL10 (pg/mL), Day 10, maximal value per subject					

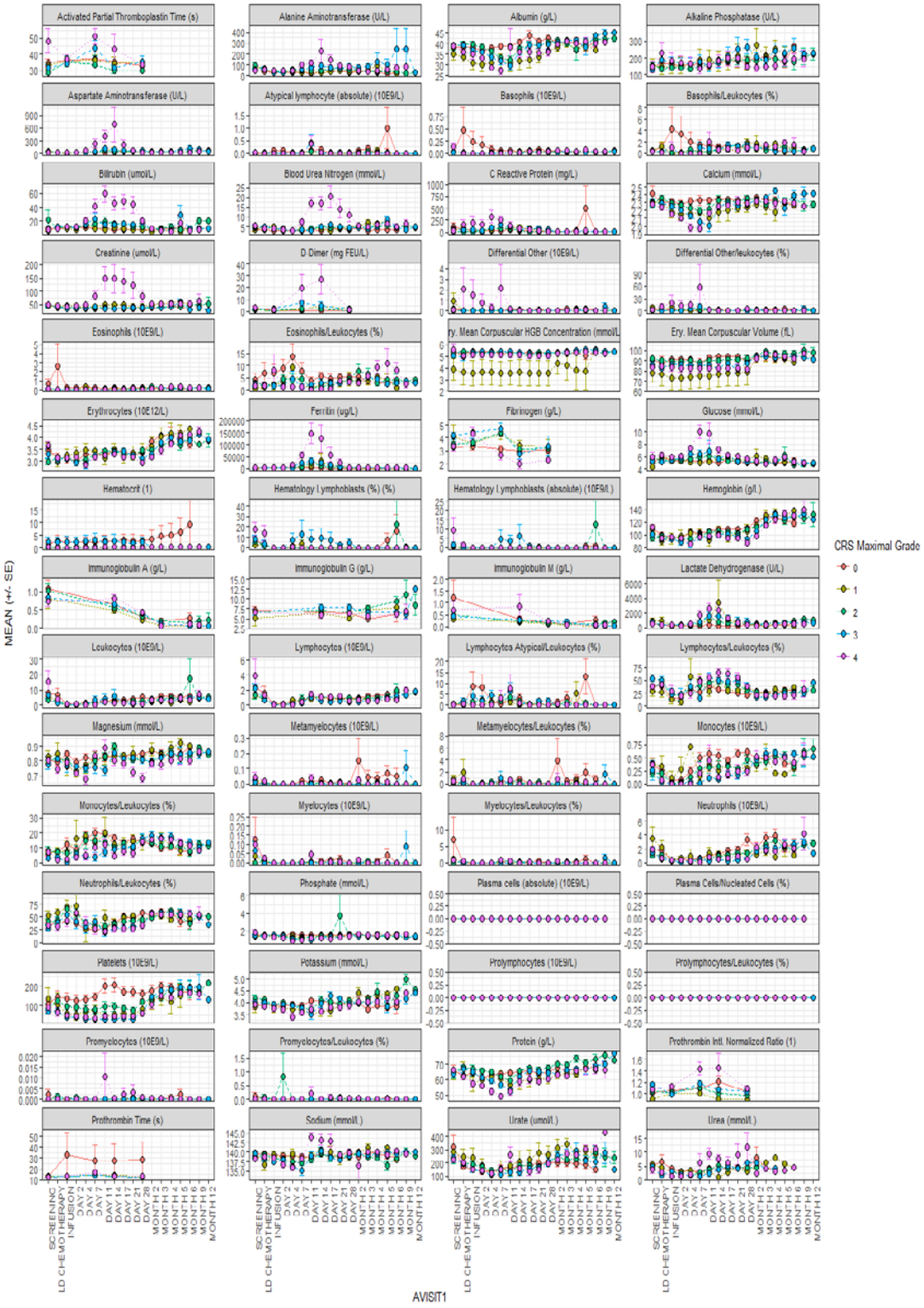
Variable Name	Description	Maximal CRS Grade (0/12/34)	ORR3	ORR3SEN	DOR	EFS
DAY10.IL12P70.MAX	IL12p70 (pg/mL), Day 10, maximal value per subject					
DAY10.IL12P70.AVG	IL12p70 (pg/mL), Day 10, average value per subject					
DAY10.IL13.MAX	IL13 (pg/mL), Day 10, maximal value per subject					
DAY10.IL13.AVG	IL13(pg/mL), Day 10, average value per subject					
DAY10.IL1B.MAX	IL1β (pg/mL), Day 10, maximal value per subject					
DAY10.IL1B.AVG	IL1β (pg/mL), Day 10, average value per subject					
DAY10.IL2.MAX	IL2 (pg/mL), Day 10, maximal value per subject					
DAY10.IL2.AVG	IL2 (pg/mL), Day 10, average value per subject					
DAY10.IL4.MAX	IL4 (pg/mL), Day 10, maximal value per subject					
DAY10.IL4.AVG	IL4 (pg/mL), Day 10, average value per subject					
DAY10.IL6.MAX	IL6 (pg/mL), Day 10, maximal value per subject					
DAY10.IL6.AVG	IL6 (pg/mL), Day 10, average value per subject					
DAY10.IL8.MAX	IL8 (pg/mL), Day 10, maximal value per subject					
DAY10.IL8.AVG	IL8 (pg/mL), Day 10, average value per subject					
DAY10.TNFA.MAX	TNFα (pg/mL), Day 10, maximal value per subject					
DAY10.TNFA.AVG	TNFα (pg/mL), Day 10, average value per subject					
DAY28.C3LYMBD.MAX	T cells amongst mono-nuclear cells (Lymphocytes and monocytes with the exclusion of granulocytes) (%) - Bone Marrow, Day 28, maximal value per subject					
DAY28.C3LYMBD.AVG	T cells amongst mono-nuclear cells (Lymphocytes and monocytes with the exclusion of granulocytes) (%) - Bone Marrow, Day 28, average value per subject					

Variable Name	Description	Maximal CRS Grade (0/12/34)	ORR3	ORR3SEN	DOR	EFS
DAY28.CD19PWBC.MAX	CD19+ Amongst Viable WBC (%) - Blood, Day 28, maximal value per subject				X	X
DAY28.CD19PWBC.AVG	CD19+ Amongst Viable WBC (%) - Blood, Day 28, average value per subject			X		X
DAY28.CRP.MAX	CRP (mg/L), Day 28, maximal value per subject					
DAY28.CRP.AVG	CRP (mg/L), Day 28, average value per subject				X	
DAY28.FERRITIN.MAX	Ferritin (µg/L), Day 28, maximal value per subject				X	
DAY28.FERRITIN.AVG	Ferritin (µg/L), Day 28, average value per subject				X	
DAY28.IFNG.MAX	IFN-γ (pg/mL), Day 28, maximal value per subject					
DAY28.IFNG.AVG	IFN-γ (pg/mL), Day 28, average value per subject					
DAY28.IL10.MAX	IL10 (pg/mL), Day 28, maximal value per subject					
DAY28.IL10.AVG	IL10 (pg/mL), Day 28, average value per subject					
DAY28.IL12P70.MAX	IL12p70 (pg/mL), Day 28, maximal value per subject					
DAY28.IL12P70.AVG	IL12p70 (pg/mL), Day 28, average value per subject					
DAY28.IL13.MAX	IL13 (pg/mL), Day 28, maximal value per subject					
DAY28.IL13.AVG	IL13(pg/mL), Day 28, average value per subject		X			
DAY28.IL1B.MAX	IL1β (pg/mL), Day 28, maximal value per subject					
DAY28.IL1B.AVG	IL1β (pg/mL), Day 28, average value per subject					
DAY28.IL2.MAX	IL2 (pg/mL), Day 28, maximal value per subject					
DAY28.IL2.AVG	IL2 (pg/mL), Day 28, average value per subject					
DAY28.IL4.MAX	IL4 (pg/mL), Day 28, maximal value per subject		X			
DAY28.IL4.AVG	IL4 (pg/mL), Day 28, average value per subject		X			
DAY28.IL6.AVG	IL6 (pg/mL), Day 28, average value per subject					

Variable Name	Description	Maximal CRS Grade (0/12/34)	ORR3	ORR3SEN	DOR	EFS
DAY28.IL8.AVG	IL8 (pg/mL), Day 28, average value per subject					
DAY28.TNFA.MAX	TNFα (pg/mL), Day 28, maximal value per subject					
DAY28.TNFA.AVG	TNFα (pg/mL), Day 28, average value per subject					
DAY28.C3LYMBM.MAX	T Cells Amongst Mono-Nuclear Cells (Lymphocytes And Monocytes With the Exclusion of Granulocytes) (%) - Bone Marrow, Day 28, maximal value per subject					
DAY28.C3LYMBM.AVG	T Cells Amongst Mono-Nuclear Cells (Lymphocytes And Monocytes With the Exclusion of Granulocytes) (%) - Bone Marrow, Day 28, average value per subject					
DAY28.IGSIG.MAX	Signal (MFI) of Anti-CTL019 Antibodies, Day 28, maximal value per subject					
DAY28.IGSIG.AVG	Signal (MFI) of Anti-CTL019 Antibodies, Day 28, average value per subject					
BATCH	Vector Batch					
TCYTO.CAR.NEGATIVE	Tcyto.CAR.negative (%)					
THELPER.CAR.NEGATIVE	Thelper.CAR.negative (%)					
CAR.NEGATIVE.CD4.VS.CD8	CAR.negative.CD4.vs.CD8 (%)					
TCYTO.CAR.POSITIVE	Tcyto.CAR.positive (%)					
THELPER.CAR.POSITIVE	Thelper.CAR.positive (%)					
CAR.POSITIVE.CD4.VS.CD8	CAR.positive.CD4.vs.CD8 (%)					

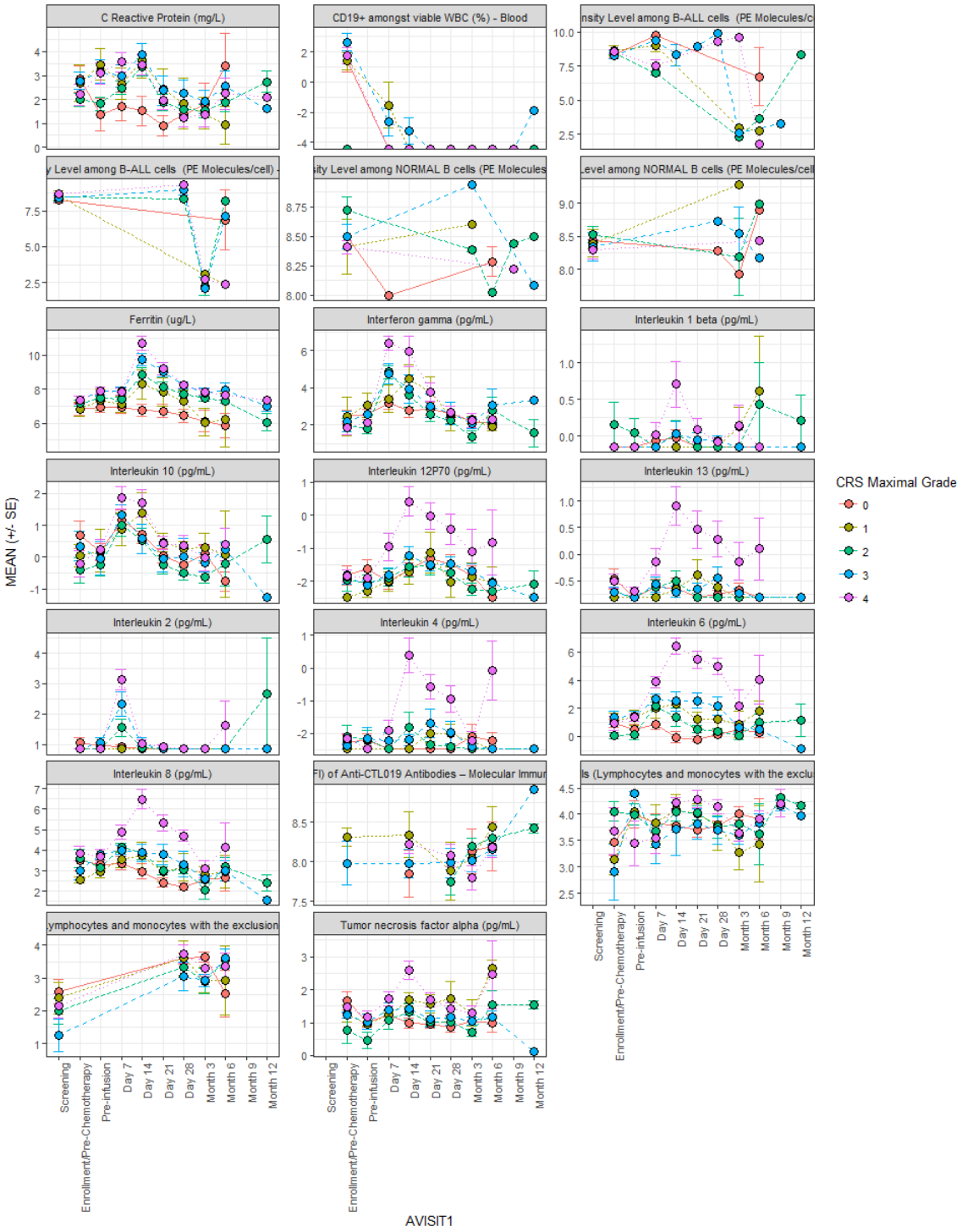
Variable Name	Description	Maximal CRS Grade (0/12/34)	ORR3	ORR3SEN	DOR	EFS
CM.CAR.NEGATIVE.THELPER	CM.CAR.negative.Thelper (%)					
CM.CAR.POSITIVE.TCYTO	CM.CAR.positive.Tcyto (%)					
CM.CAR.POSITIVE.THELPER	CM.CAR.positive.Thelper (%)					
EM.CAR.NEGATIVE.TCYTO	EM.CAR.negative.Tcyto (%)					
EM.CAR.NEGATIVE.THELPER	EM.CAR.negative.Thelper (%)					X
EM.CAR.POSITIVE.TCYTO	EM.CAR.positive.Tcyto (%)					
EM.CAR.POSITIVE.THELPER	EM.CAR.positive.Thelper (%)					
EFFECTORS.CAR.NEGATIVE.TCYTO	Effectors.CAR.negative.Tcyto (%)					
EFFECTORS.CAR.NEGATIVE.THELPER	Effectors.CAR.negative.Thelper (%)					
EFFECTORS.CAR.POSITIVE.TCYTO	Effectors.CAR.positive.Tcyto (%)					
EFFECTORS.CAR.POSITIVE.THELPER	Effectors.CAR.positive.Thelper (%)					
NAIVE_TSCM.CAR.NEGATIVE.TCYTO	Naive_Tscm.CAR.negative.Tcyto (%)				X	X
NAIVE_TSCM.CAR.NEGATIVE.THELPER	Naive_Tscm.CAR.negative.Thelper (%)				X	X
NAIVE_TSCM.CAR.POSITIVE.TCYTO	Naive_Tscm.CAR.positive.Tcyto (%)					
NAIVE_TSCM.CAR.POSITIVE.THELPER	Naive_Tscm.CAR.positive.Thelper (%)					X

4.2 Appendix B: Longitudinal plot of biomarkers vs. Maximal CRS grade (Part 1)



The round dots represent the mean value of the biomarker over time; the error bar is the standard deviation of the mean value. The dot color corresponds to the maximal CRS grade

Appendix B: Longitudinal plot of biomarkers vs. Maximal CRS grade (Part 2)

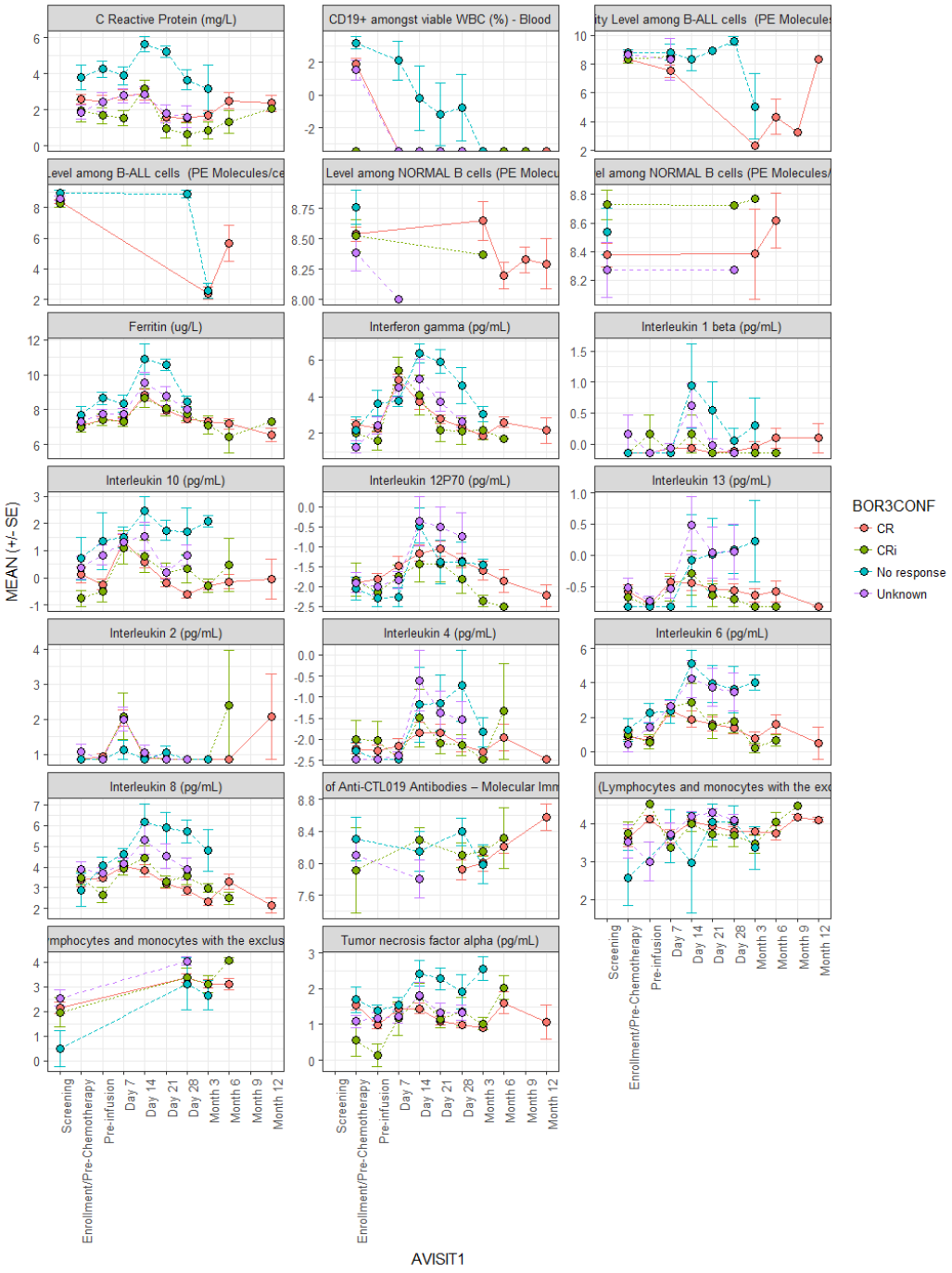


The round dots represent the mean value of the biomarker over time; the error bar is the standard deviation of the mean value. The dot color corresponds to the maximal CRS grade

4.3 Appendix C: Longitudinal plot of biomarkers vs. Best overall response with confirmation at month 3 (Part 1)

The round dots represent the mean value of the biomarker over time; the error bar is the standard deviation of the mean value. The dot color corresponds to best overall response by month 3.

Appendix C: Longitudinal plot of biomarkers vs. Best overall response with confirmation at month 3 (Part 2)



The round dots represent the mean value of the biomarker over time; the error bar is the standard deviation of the mean value. The dot color corresponds to best overall response by month 3.