Supplementary Material

Analytical solution for the compartmental model

When time (t) ≤ time to maximal expansion (T_{max}), the compartmental model in **Figure 2** is described by the equation dE/dt = ρt, which has the analytical solution $E(t) = R_0\exp(\rho t)$, where as described earlier, R0 = Cmax/foldx. This gives the exponential growth equation (Eq 1).

When t > T_{max}, the analysis that links the equations for the compartmental model to the analytical expression are provided below, where the rate at which effector cells decline is (α-k), the rate at which effector cells become memory cells is k, and the rate at which memory cells decline is β.

dE/dt = -(α-k)E – kE = -αE

dM/dt = αE - βM

Rewritten in matrix notation and grouping terms gives:

$$\frac{d}{dt}\begin{pmatrix} E \\ M \end{pmatrix} = \begin{pmatrix} -\alpha & 0 \\ k & -\beta \end{pmatrix}\begin{pmatrix} E \\ M \end{pmatrix}$$

The eigenvalues of the above system are clearly $\lambda_1 = -\alpha$ and $\lambda_2 = -\beta$, and the associated eigenvectors are

$$v_1 = \begin{pmatrix} 1 \\ -k/(\alpha-\beta) \end{pmatrix}, \qquad v_2 = \begin{pmatrix} 0 \\ 1 \end{pmatrix}$$

Eigenvector v_2 is easy to check, and a check of v_1 is shown below.

$$\begin{pmatrix} -\alpha & 0 \\ k & -\beta \end{pmatrix}\begin{pmatrix} 1 \\ -k/(\alpha-\beta) \end{pmatrix} = \begin{pmatrix} -\alpha \\ k+\beta k/(\alpha-\beta) \end{pmatrix} = \begin{pmatrix} -\alpha \\ \alpha k/(\alpha-\beta) \end{pmatrix} = -\alpha\begin{pmatrix} 1 \\ -k/(\alpha-\beta) \end{pmatrix}$$

This results in the solution below, where C_1 and C_2 are 2 constants to be defined further below.

$$\begin{pmatrix} E(t) \\ M(t) \end{pmatrix} = C_1\begin{pmatrix} 1 \\ -k/(\alpha-\beta) \end{pmatrix}e^{-\alpha(t-T_{max})} + C_2\begin{pmatrix} 0 \\ 1 \end{pmatrix}e^{-\beta(t-T_{max})}$$

The measurement variable is:

$$f(t-T_{max}) = E(t-T_{max}) + M(t-T_{max}) = C_1\left(1-\frac{k}{\alpha-\beta}\right)e^{-\alpha t} + C_2 e^{-\beta t}$$

Also, at time T_{max}, it is assumed that all cells are effector cells; thus, $E(T_{max})$ = maximal concentration (C_{max}) (giving $C_1 = C_{max}$) and $M(T_{max}) = 0$ [giving $C_2 = C_1 k/(\alpha - \beta) = C_{max} k/(\alpha - \beta)$]. Note that in the analytical formula, $C_2 = C_{max} \cdot F_B$. Thus, the differential equation model is mapped to the analytical model:

$$C_2 = C_{max} \cdot F_B = C_{max} k/(\alpha - \beta)$$
$$k = F_B \cdot (\alpha - \beta)$$

Semi-mechanistic Model Selection

The semi-mechanistic model presented here is not the only model that gives rise to exponential growth followed by biexponential decay. If memory cells were produced during the expansion phase as well or if cells could transition back and forth from the effector to memory state, then the analytic solution to such a model would have the same functional form. It is also possible that terminal phase is not due to formation of memory cells, but rather the low level of constant proliferation in response to the continual production of CD19 antigen; the kinetics of this process would look similar. Furthermore, DeBoer and Perelson[4] also showed in equations 8-10 of their paper that rather than have expansion stop at Tmax, one could use a "cascade" model of cell division, modeling each generation of expansion complex model and realistic model with successive generations of expansion. The cascade model could be approximated by the simpler model used here; the advantage of the model used here is it is simpler to implement and to present.

Derivation of AUC to approximate C_{max}

In the scenario where no comedications are given, the area under the curve (AUC) from time 0 to time "T_{end}" is given by the equation below, where $R = C_{max}/fold_x$, $A = (1 - F_B) \cdot C_{max}$, and $B = F_B \cdot C_{max}$. The final equation below shows that in this model formulation, AUC is proportional to C_{max}; thus, covariates that affect C_{max} also impact the AUC. In the derivation below, we switch from an equality to an approximation because

with the parameters fit here, exp(ρ·Tmax) = foldx = 3900 >> 1, such that [exp(ρ ·Tmax)-1]/exp(ρ ·Tmax) ≈ 1

$$
\begin{aligned}
AUC_{0-T_{\text{end}}} &= \int_0^{T_{\max}} R\exp(\rho t)dt + \int_{T_{\max}}^{T_{\text{end}}} A\exp(-\alpha(t-T_{\max})) + B\exp(-\beta(t-T_{\text{end}}))dt \\
&= \left[\frac{R}{\rho}\exp(\rho t)\right]_0^{T_{\max}} + \left[-\frac{A}{\alpha}\exp(-\alpha(t-T_{\max})) - \frac{B}{\beta}\exp(-\beta(t-T_{\max}))\right]_{T_{\max}}^{T_{\text{end}}} \\
&= \left[\frac{R}{\rho}\left(\exp(\rho\cdot T_{\max})-1\right)\right] + \left[\frac{A}{\alpha}\left(1-\exp(-\alpha(T_{\text{end}}-T_{\max}))\right) + \frac{B}{\beta}\left(1-\exp(-\beta(T_{\text{end}}-T_{\max}))\right)\right]
\end{aligned}
$$

substituting in the equations for R, A, and B gives

$$
\begin{aligned}
&= \left[\frac{C_{\max}}{\rho}\frac{\left(\exp(\rho\cdot T_{\max})-1\right)}{\exp(\rho\cdot T_{\max})}\right] \\
&\quad + \left[\frac{(1-F_B)\cdot C_{\max}}{\alpha}\left(1-\exp(-\alpha(T_{\text{end}}-T_{\max}))\right) + \frac{F_B\cdot C_{\max}}{\beta}\left(1-\exp(-\beta(T_{\text{end}}-T_{\max}))\right)\right] \\
&= C_{\max}\cdot\left[\frac{1}{\rho}\frac{\left(\exp(\rho\cdot T_{\max})-1\right)}{\exp(\rho\cdot T_{\max})}\right. \\
&\quad \left. + \frac{(1-F_B)}{\alpha}\left(1-\exp(-\alpha(T_{\text{end}}-T_{\max}))\right) + \frac{F_B}{\beta}\left(1-\exp(-\beta(T_{\text{end}}-T_{\max}))\right)\right] \\
&\approx C_{\max}\left[\frac{1}{\rho} + \frac{(1-F_B)}{\alpha}\left(1-\exp(-\alpha(T_{\text{end}}-T_{\max}))\right) + \frac{F_B}{\beta}\left(1-\exp(-\beta(T_{\text{end}}-T_{\max}))\right)\right] \\
&\text{as } T_{\text{end}}\to\infty
\end{aligned}
$$

$$
AUC_{0-\infty} \approx C_{\max}\left(\frac{1}{\rho} + \frac{1-F_B}{\alpha} + \frac{F_B}{\beta}\right)
$$

Covariate Analysis – Further Details

Sex, race, and weight were included because they were demographic parameters that are typically included in a model-based analysis of exposure. Weight-adjusted dose, too, is a factor that for standard drugs correlates with exposure. Down's Syndrome was included because ALL is more prevalent in Down's syndrome patients. Prior lymphodepleting therapy and prior stem cell transplant were included because it's possible that both could have affected the CAR-T cell ability to expand. Per protocol patients received fixed, prespecified weight or surface area-adjusted lymphodepleting (LD) fludarabine and cyclophosphamide chemotherapy dosing. A small percent (listed in ELIANA publication) of patients received non-FC LD chemotherapy or non LD chemotherapy if blood counts were very low. In this subset, 85 patients received fludarabine and cyclophosphamide, 2 received only cyclophosphamide, and 3 received neither.

For practical reasons T cell numbers were not included as a covariate in this analysis. While the inclusion of T cell numbers as a covariate could alter Cmax, it is unlikely that it would impact the primary conclusions of the effect of tocilizumab and corticosteroids on the rate of expansion. However, this question warrants study in future analyses. If it is possible to collect data on tumor burden or CD19 antigen density, this data should be included in assessing the impact on Cmax and if the follow-up is long enough, then β as well. The impact of missing this information in this study is that we may have larger portion of unexplained variability in the exposure.

The relationship between C_{maxi} (C_{max} for patient i), the random effect η_i with variance ω^2, and the covariate j is given below. Here, x_{ij} denotes the covariate j value for patient i, z_{ij} is a transformation of x_{ij}, and θ_j denotes the size of the covariate effect.

(4) $$C_{maxi} = C_{max} \cdot \exp(\eta_i) \cdot \Pi_j z_{ij}$$

For continuous covariates (eg, dose),

(5) $$z_{ij} = (x_{ij}/x_{median,j})^{\theta_{ij}}$$

For dichotomous covariates, x_i is assigned either 0 or 1 (eg, $x_{ij} = 0$ for no tocilizumab; $x_{ij} = 1$ for tocilizumab). Then z_{ij} is given by

(6) $$z_{ij} = (\theta_j)^{x_{ij}}$$

For categorical covariates with $n > 2$ categories (eg, race) $n - 1$ dichotomous variables (y_{ijk}) are created for each patient i, with k going from 1 to $n - 1$. Then z_{ij} is given by

(7) $$z_{ij} = \Pi_k (\theta_{jk})^{y_{ijk}}$$

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Model MLXTRAN file

```
INPUT:
parameter = {foldx, Tmax, Cmax, Ftoci, Fster, alpha, FB, beta}
regressor = {TOCI1T, STERSTT}

EQUATION:

; Figure out whether tocilizumab or steroids come first
; assign first comedication to t1,F1 and second to t2,F2
;
; If patient does not receive TOCILIZUMAB, then TOCI1T=99999 in dataset
; If patient does not receive STEROIDS, then STERSTT=99999 in dataset
        if TOCI1T <= STERSTT
                t1 = min(TOCI1T,Tmax)
                F1 = Ftoci
                t2 = min(STERSTT,Tmax)
                F2 = Fster
        else
                t1 = min(STERSTT,Tmax)
                F1 = Fster
                t2 = min(TOCI1T,Tmax)
                F2 = Ftoci
        end

; Compute constants for solving system
; Note that: foldx = exp(rho*tau)
        rho = log(foldx)/Tmax
        R0 = Cmax/exp(rho*Tmax)
        R1 = R0*exp(      rho* t1      )
        R2 = R1*exp(F1   *rho*(t2  -t1))
        CmaxAdjust = R2*exp(F1*F2*rho*(Tmax -t2))

        AA = (1-FB)*CmaxAdjust
        BB = FB*CmaxAdjust

; Analytical solution for system
        if t<0
                ylin = R0
        elseif t < t1
                ylin = R0*exp(rho*t)
        elseif t < t2
                ylin = R1*exp(F1*rho*(t-t1))
        elseif t < tmax
                ylin = R2*exp(F1*F2*rho*(t-t2))
        else
                ylin = AA*exp(-alpha*(t-Tmax)) + BB*exp(-beta*(t-Tmax))
        end

        logy = log(ylin)

OUTPUT:
output = {logy}
```

Figure S1 Correlation between direct and model-based estimates of maximal concentration (C_{max}) and area under the curve at day 28 (AUC_{d28}). The data-driven estimates of C_{max} and AUC_{d28} were calculated in patients who had ≥ 21 days of follow-up data. C_{max} was the maximum quantitative polymerase chain reaction value in the first 28 days, and AUC_{d28} was calculated using the trapezoidal rule. The model-based estimate for C_{max} was derived directly from the *post hoc* estimate of the C_{max} parameter. The model-based AUC_{d28} was calculated using the *post hoc* estimates of all the cellular kinetics and the formula above for $AUC_{0\text{-}Tend}$ (where T_{end} = 28). Significant correlation between the noncompartmental analysis (NCA) and model-based estimates was observed. T_{end}, time of final measurement of transgene copy number. The blue line and shaded area correspond to the linear regression with 90% confidence intervals. The dotted line is the identity line.

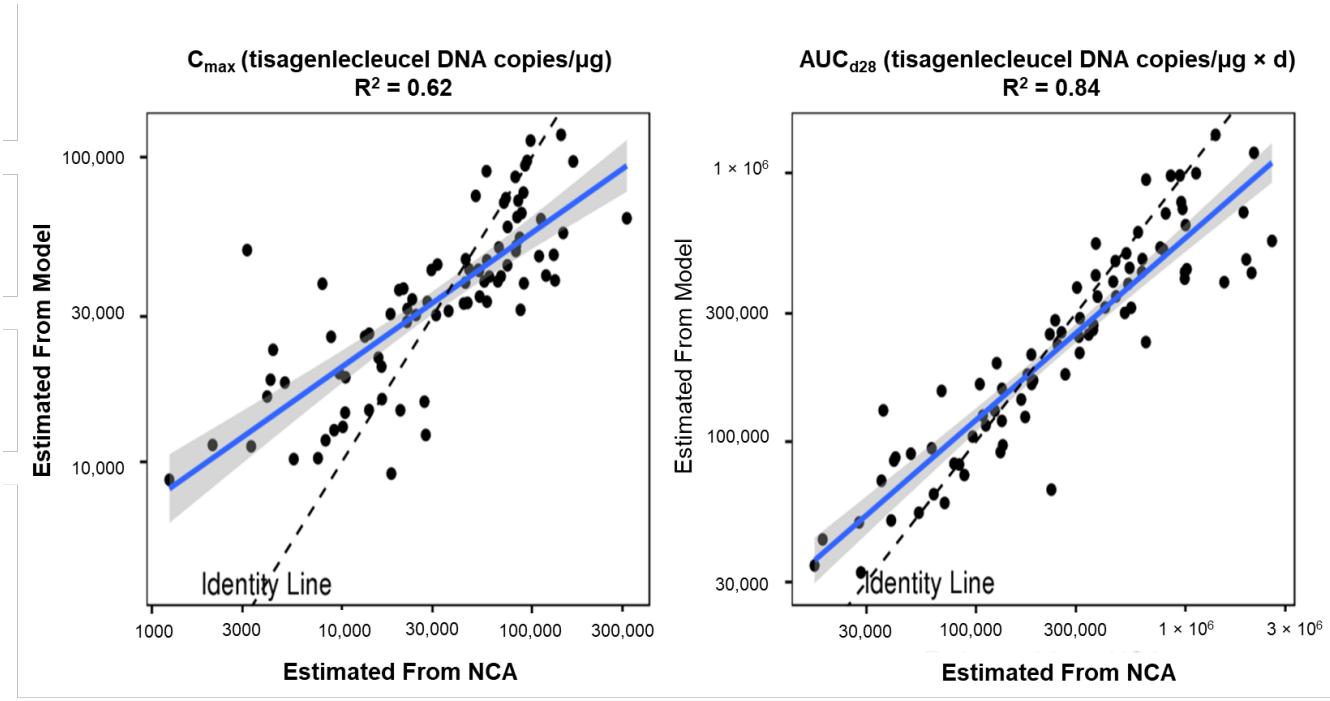
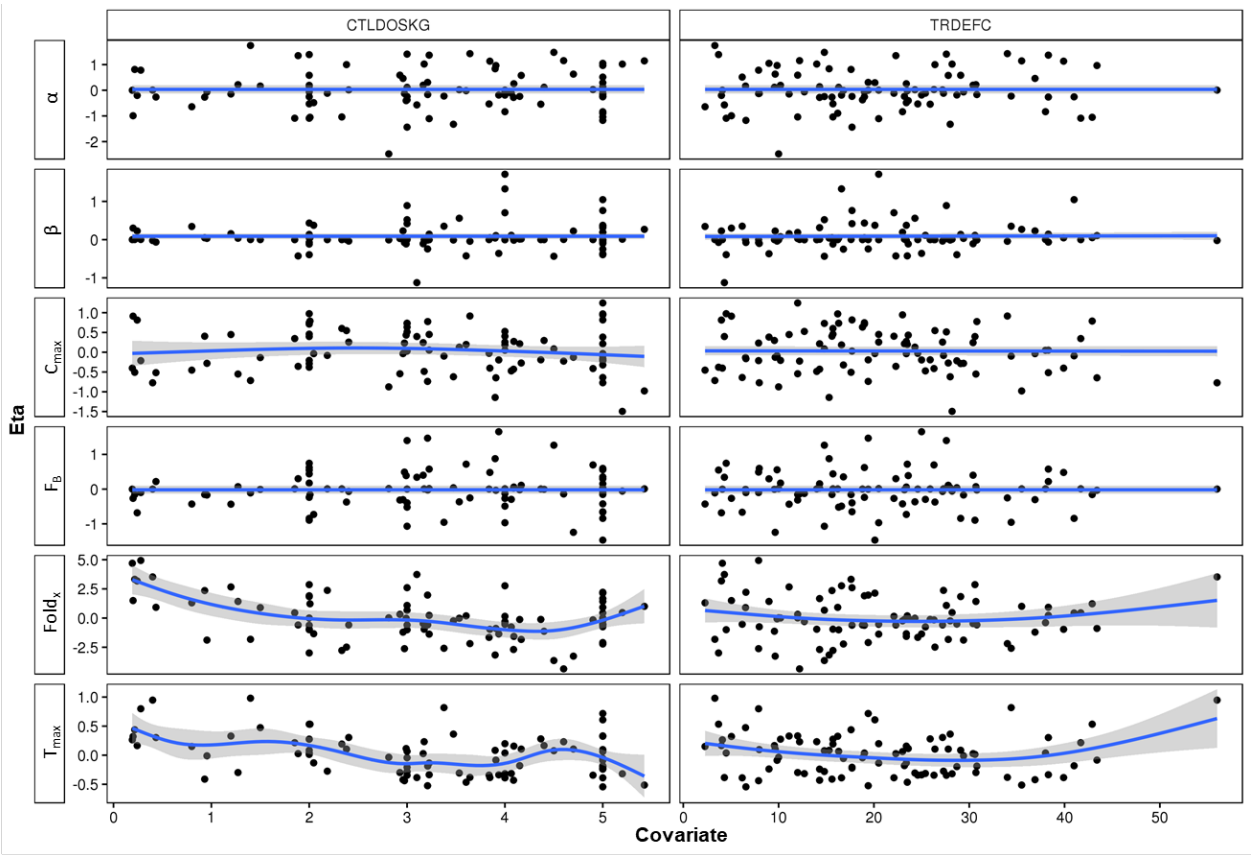


Figure S2 Exploratory plots for covariate analysis. **(a)** Continuous covariates plotted against *post hoc* random-effects estimates (Eta). CTLDOSKG is defined as the dose of tisagenlecleucel per kg. TRDEFC is defined as the transduction efficiency. **(b)** Categorical covariates plotted against *post hoc* random-effects estimates. Downs denotes Down syndrome, LDFLUD denotes that the patient received fludarabine lymphodepleting therapy, Ster denotes that the patient received corticosteroids, Stud denotes the study (ELIANA, B2202; ENSIGN, B2205J), and Toci denotes that the patient received tocilizumab. C_{max}, maximal concentration; F_B, fraction of transgene copies present during the decline at the gradual rate β, starting from T_{max}; $fold_x$, fold expansion; HSCT, hematopoietic stem cell transplant; T_{max}, time to maximal expansion. The blue line and shaded area shows the loess smoothed line; no obvious trends are observed.

(a)



(b)

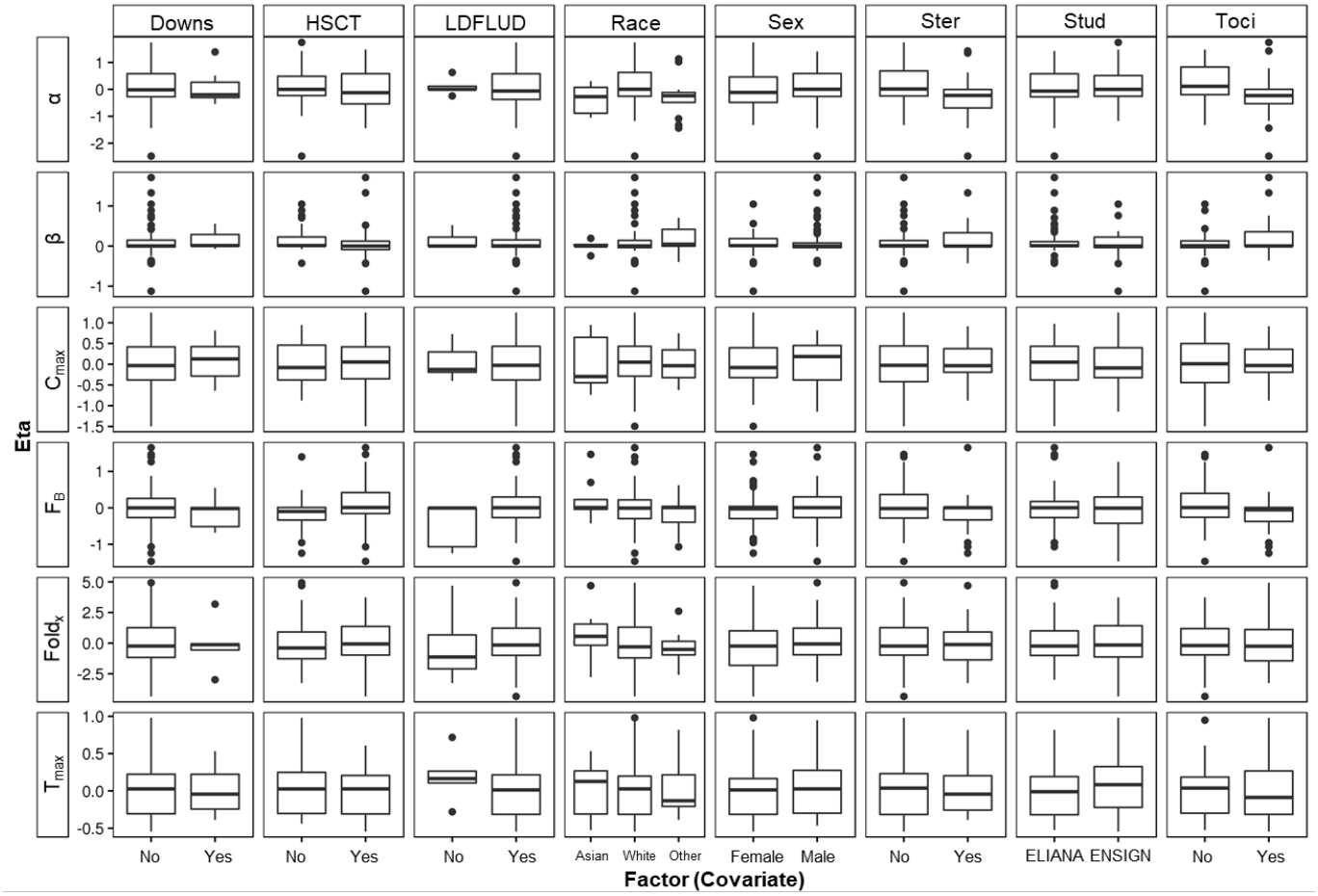
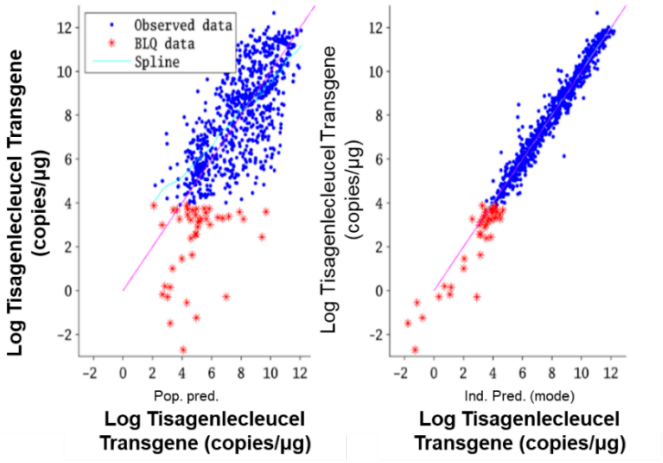

Downs
HSCT
LDFLUD
Race
Sex
Ster
Stud
Toci
α
β
C_{max}
F_B
$Fold_x$
T_{max}
Eta
No
Yes
Asian
White
Other
Female
Male
ELIANA
ENSIGN
Factor (Covariate)

Figure S3 Predictions vs observations. **(a)** Blue dots are quantifiable values, and red asterisks signify simulated residuals below the limit of quantification (BLQ). **(b)** Blue dots are quantifiable values, and red asterisks signify simulated residuals BLQ. The green solid lines in the pdf plot demonstrate the true residual distribution, and the black lines that nearly overlie the green lines denote the theoretical distribution. IWRES, individual weighted residual; NPDE, normalized distribution prediction errors; pdf, probability distribution function.

(a)



(b)