cancers

Supplementary Metrials: Early response prediction framework in CD19-specific CAR-T cell immunotherapy using a quantitative systems pharmacology model

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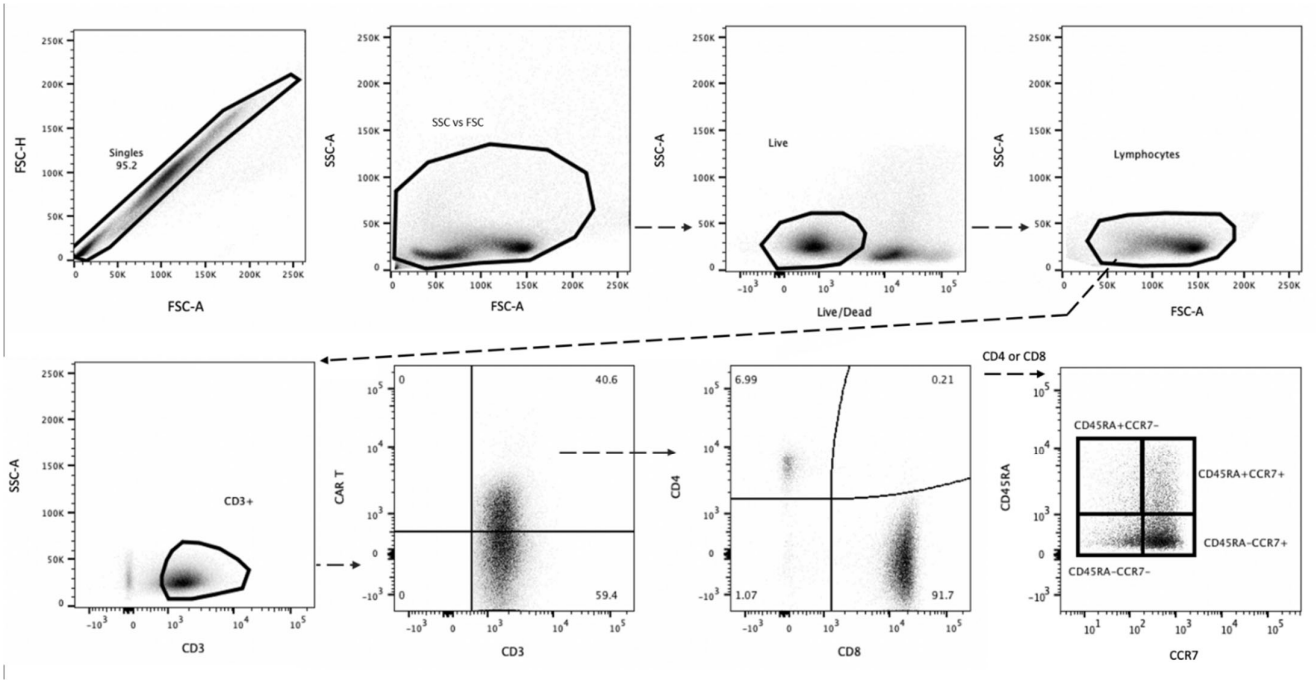

Figure S1. Flow cytometry gating strategy for the isolation of CD4⁺ and CD8⁺ CAR-T cells from peripheral blood samples of patients at days 7, 14 and 28 after infusion and identification of the different phenotypes. *Abbreviations:* SSC: sideward scatter; FSC: forward scatter; CAR T: chimeric antigen receptor T cells.

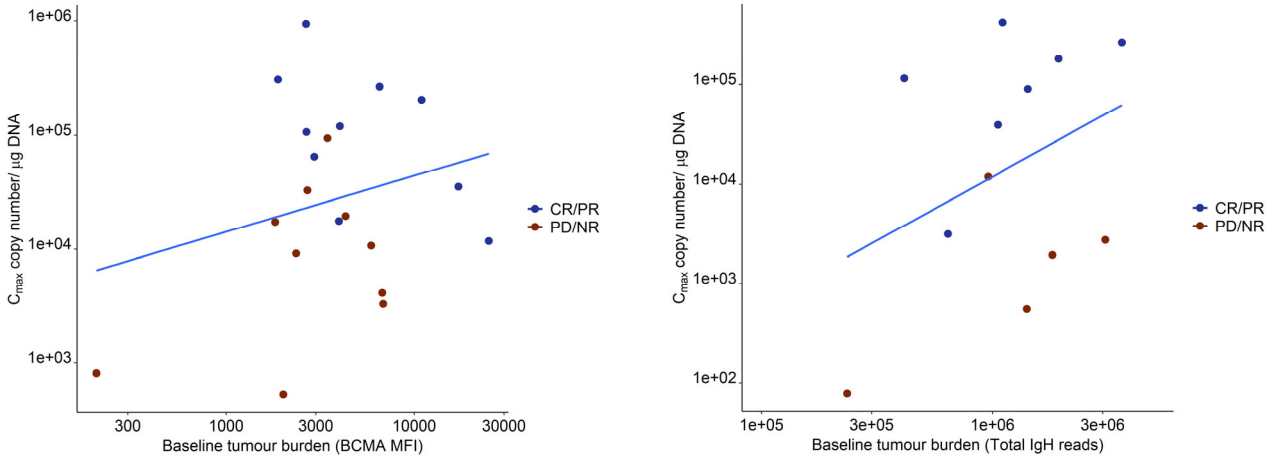

Figure S2. Digitised data on C_{max} and Baseline tumour burden in patients with MM and CLL. **Right:** MM data; **Left**: CLL data; both digitised from the publication by Liu et al. *Abbreviations:* CLL: chronic lymphocytic leukaemia; CR: complete response; MM: multiple myeloma; NR: no response; PR: partial response.

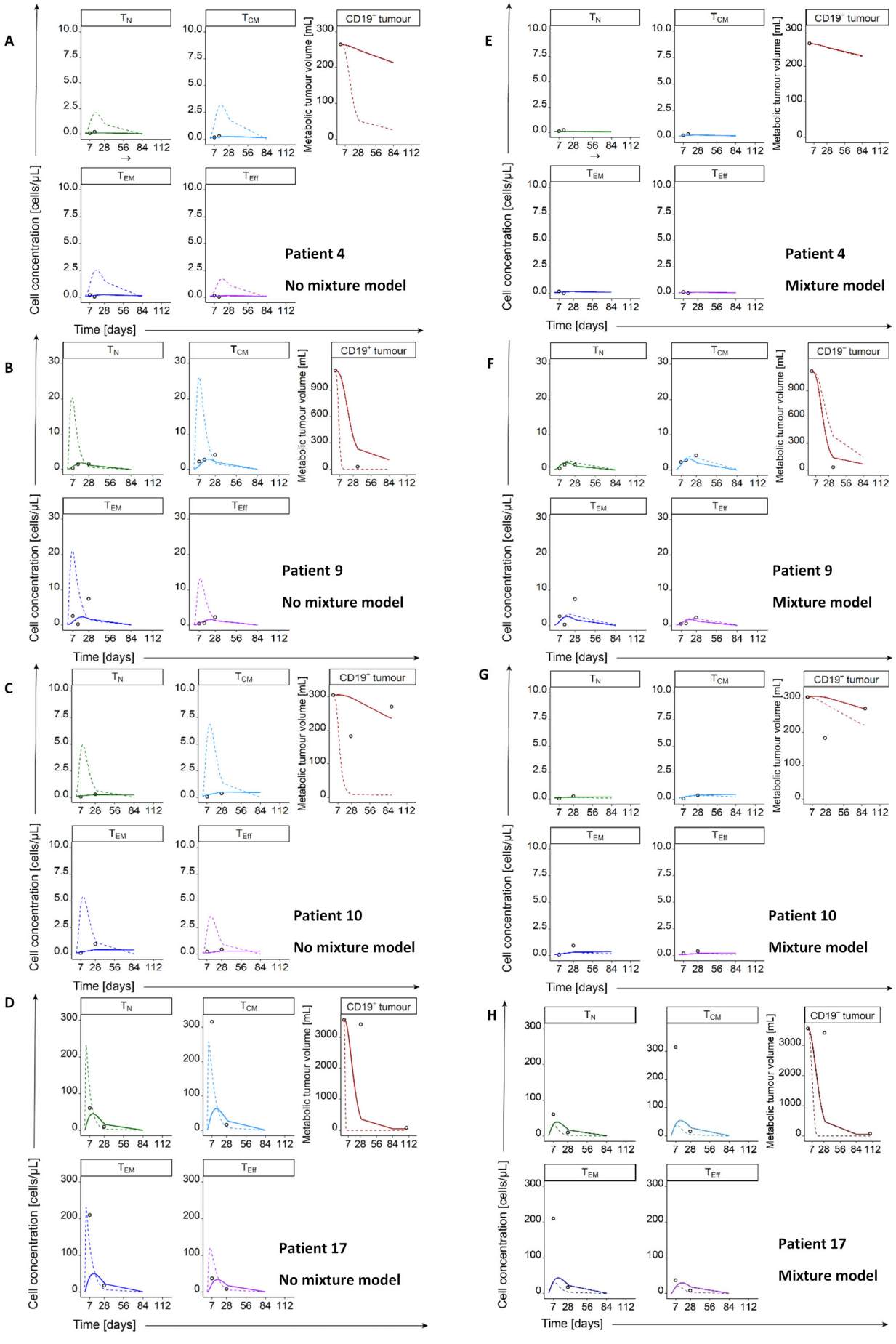
A
B
C
D
E
F
G
H
T_N
T_{CM}
T_{EM}
T_{Eff}
$CD19^+$ tumour
$CD19^-$ tumour
Metabolic tumour volume [mL]
Cell concentration [cells/μL]
Time [days]
Patient 4
No mixture model
Patient 9
No mixture model
Patient 10
No mixture model
Patient 17
No mixture model
Patient 4
Mixture model
Patient 9
Mixture model
Patient 10
Mixture model
Patient 17
Mixture model

Figure S3. Measured concentrations and simulated typical and individual model predictions of different species after CART-cell infusion for patients in the low expansion subpopulation before and after implementation of the mixture model. *Data points*: measured concentrations. *Dashed lines*: simulated typical model predictions. *Solid lines*: individual model predictions. Panels (**A**)-(**D**) show individual plots using the original model and panels (**E**)-(**H**) show individual plots using the mixture model. Abbreviations: T_N: Naïve T cells, T_{CM}: central memory T cells, T_{EM}: effector memory T cells, T_{Eff}: Effector T cells, $CD19^+$ tumour: $CD19^+$ metabolic tumour volume.

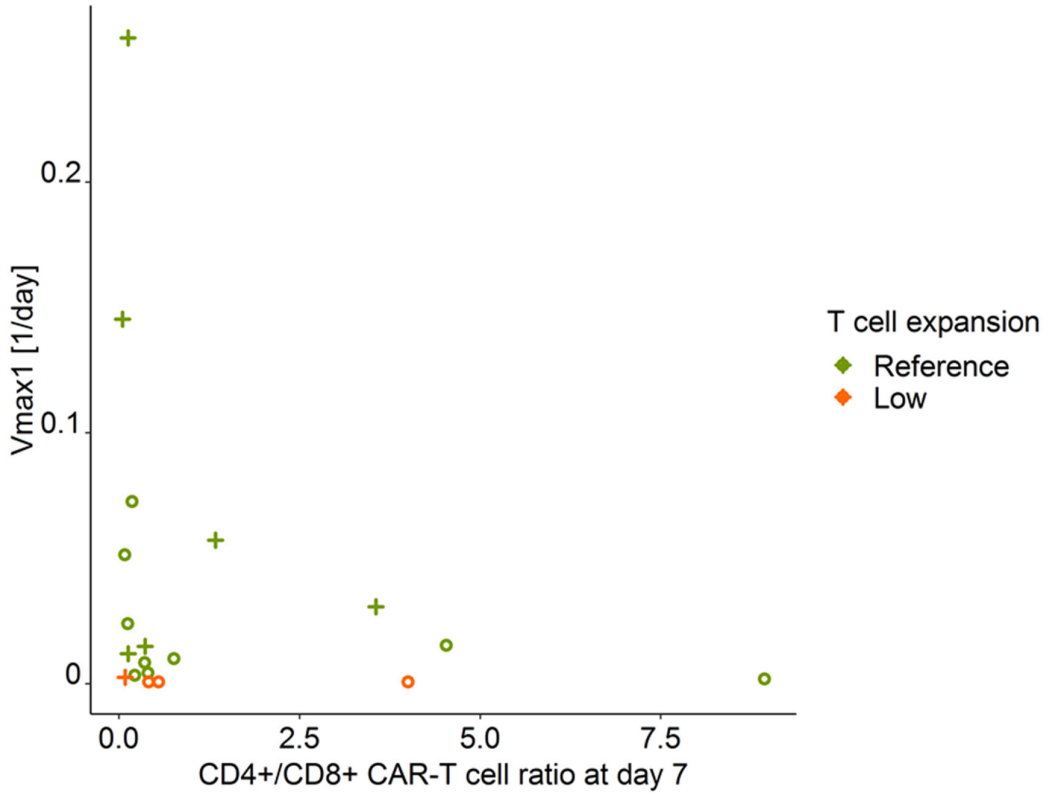

Figure S4. Estimated maximum expansion capacity upon tumor contact parameter $Vmax_1$ versus the CD4/CD8 CAR-T cell ratio at day seven. Circles: no previous autologous stem cell transplantation, plus signs: previous autologous stem cell transplantation.

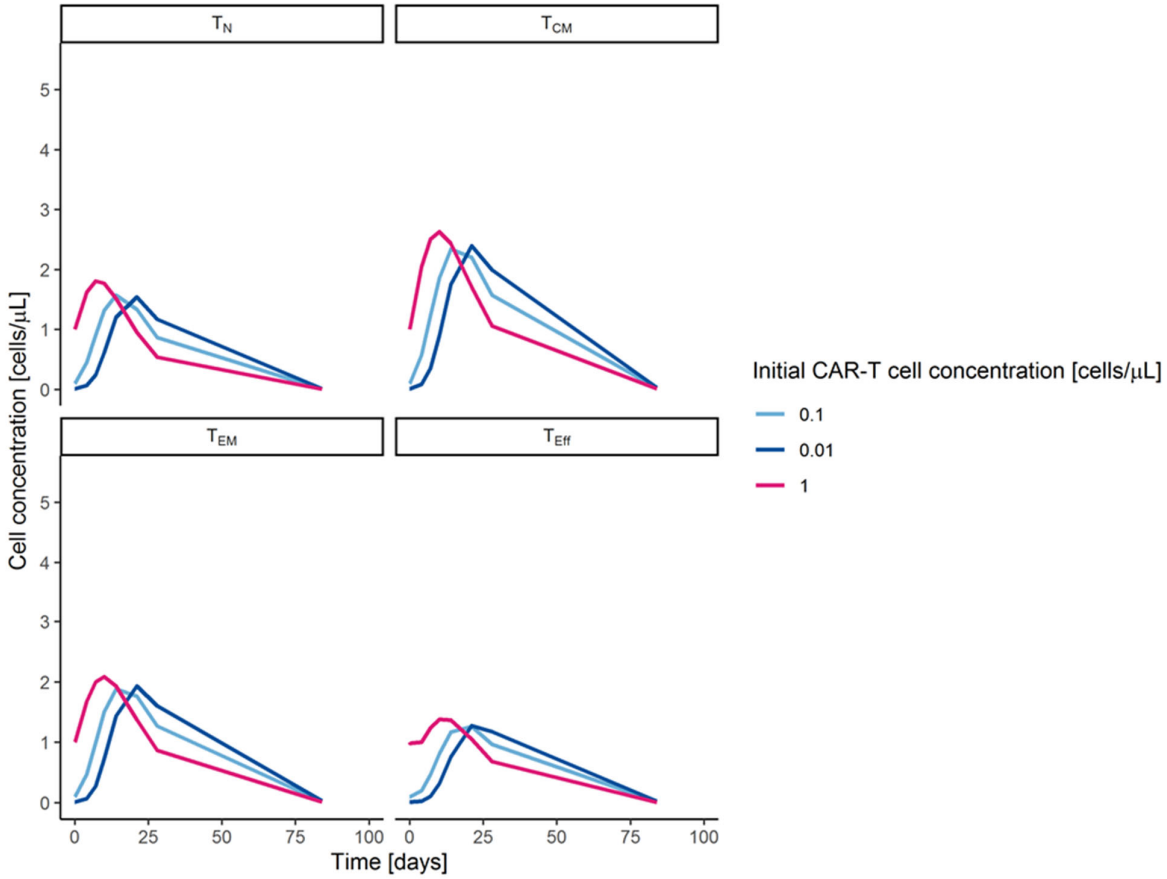

Figure S5. Simulated typical CAR-T cell concentration-time profiles using different initial CAR-T cell concentrations (0.1 cells μL^{-1} as used in our model (light blue) and ten-fold lower (dark blue) or ten-fold higher (purple) and a baseline metabolic tumour volume of 85.7 mL (median baseline metabolic tumour volume in our dataset) (assuming reference covariate values of no previous autologous stem cell transplantation and a CD4/CD8 CAR-T cell ratio of 1).

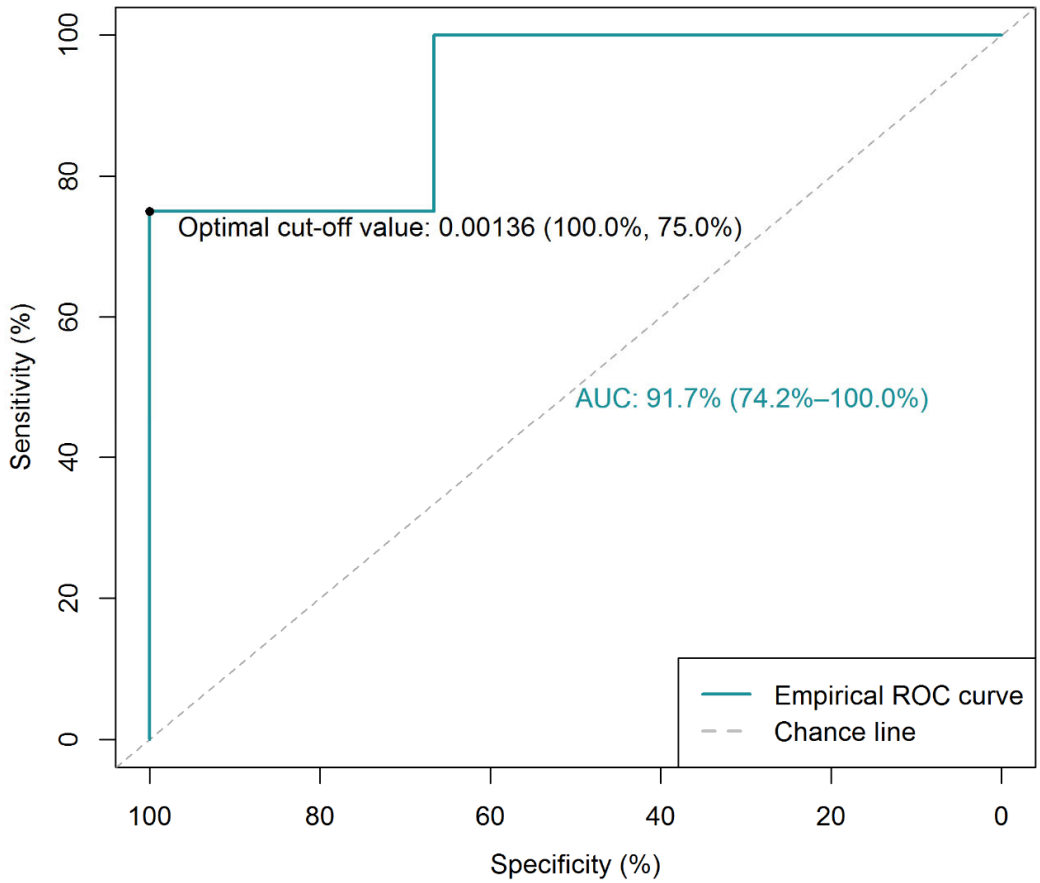

Figure S6. Receiver operating characteristic (ROC) curve for deriving an optimal cut-off value of the clinical composite score (CCS) Maximum CAR-T_N cell concentrations (C_{max})/Baseline metabolic tumour volume [(cells · μL^{-1}) · mL^{-1}] to determine if patients belong to low expansion subpopulation. The optimal cut-off value marks the value with optimal predictive capability (0.0143 [(cells · μL^{-1}) · mL^{-1}]; with 75% sensitivity, 100% specificity and an area under the curve of 91.7% (95% confidence interval: 74.2%-100%).

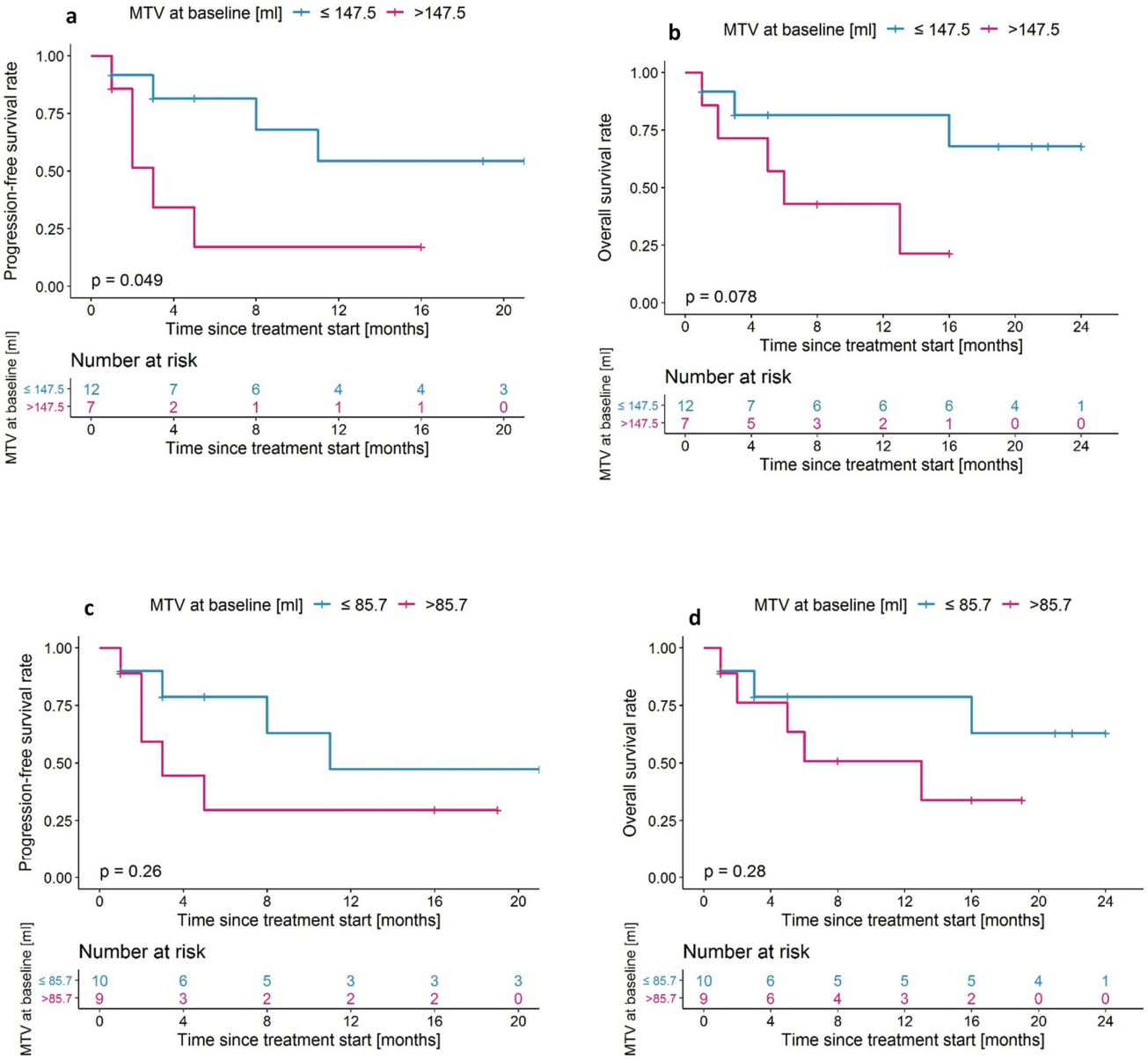

Figure S7. Kaplan-Meier plots for and progression-free survival and overall survival in patients with high and low metabolic tumor volume in mL at baseline. In plots (**a**) and (**b**), the cut-off value of 147.5 mL has been used while in plots (**c**) and (**d**), the median baseline metabolic tumour volume in our dataset has been used as cut-off value; log-rank tests. *Abbreviations:* MTV: metabolic tumour volume.

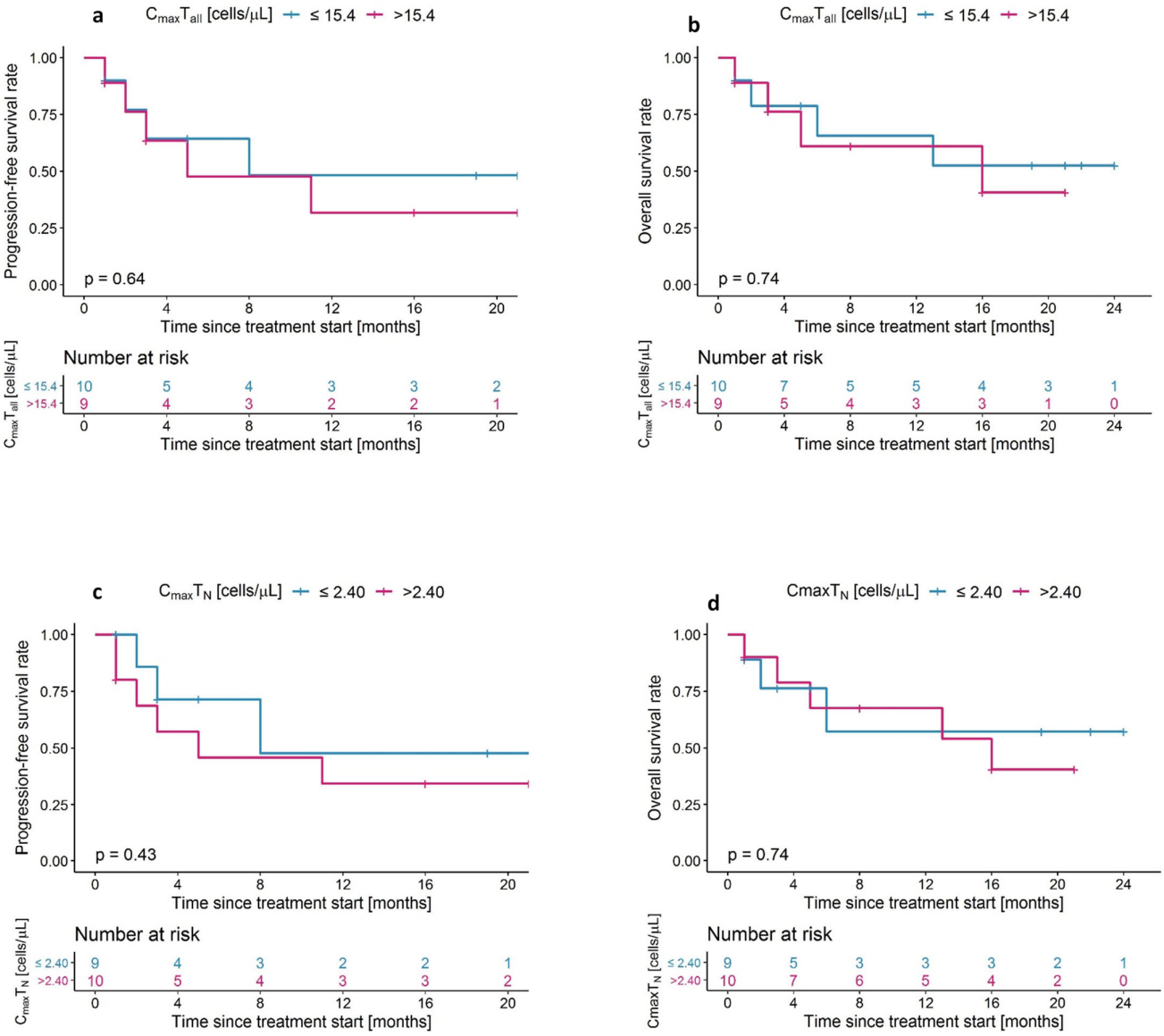

Figure S8. Kaplan-Meier plots for progression-free survival and overall survival in patients with high (above median) and low (lower or equal to median) maximum CAR-T cell concentrations. In plots (**a**) and (**n**), all CAR-T cells are assessed, while in plots (**c**) and (**d**), only naïve T cells are assessed, as the maximum concentration of naïve T cells was highest correlated with our model parameter V_{max1}; log-rank tests. *Abbreviations:* C_{max}: maximum concentration; T_{all}: the sum of all measured CAR-T cell phenotypes; T_N: naïve CAR-T cells.

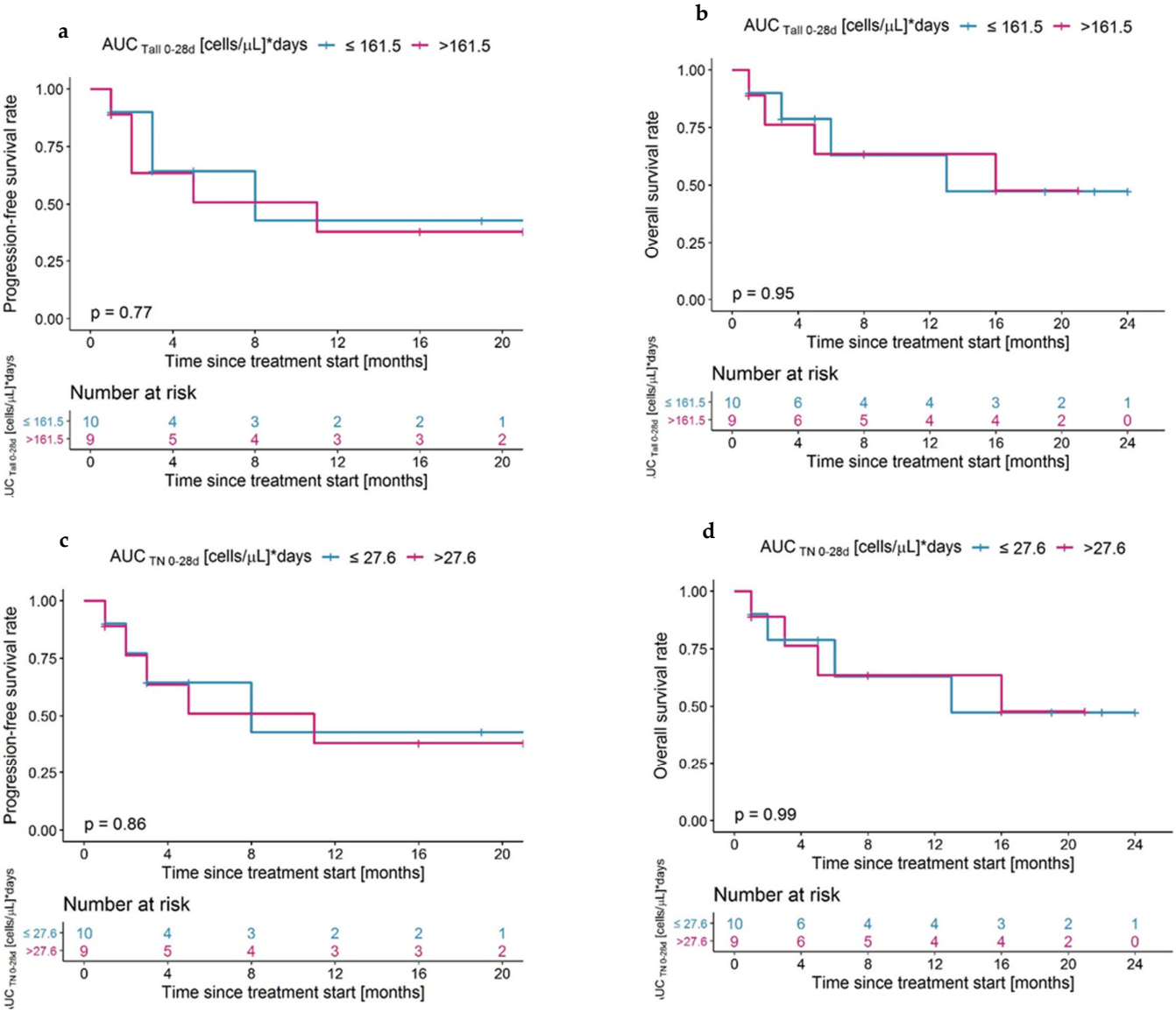

Figure S9. Kaplan-Meier plots for progression-free survival and overall survival in patients with high (above median) and low (lower or equal to median) $AUC_{0\text{-}28d}$ CAR-T cell concentrations. In plots (**a**) and (**b**), all CAR-T cells are assessed, while in plots (**c**) and (**d**), only naïve T cells are assessed, as the maximum concentration of naïve T cells was highest correlated with our model parameter V_{max1}; log-rank tests. *Abbreviations:* $AUC_{0\text{-}28d}$: area under the concentration-time curve from day 0 to day 28; T_{all}: the sum of all measured CAR-T cell phenotypes; T_N: naïve CAR-T cells.

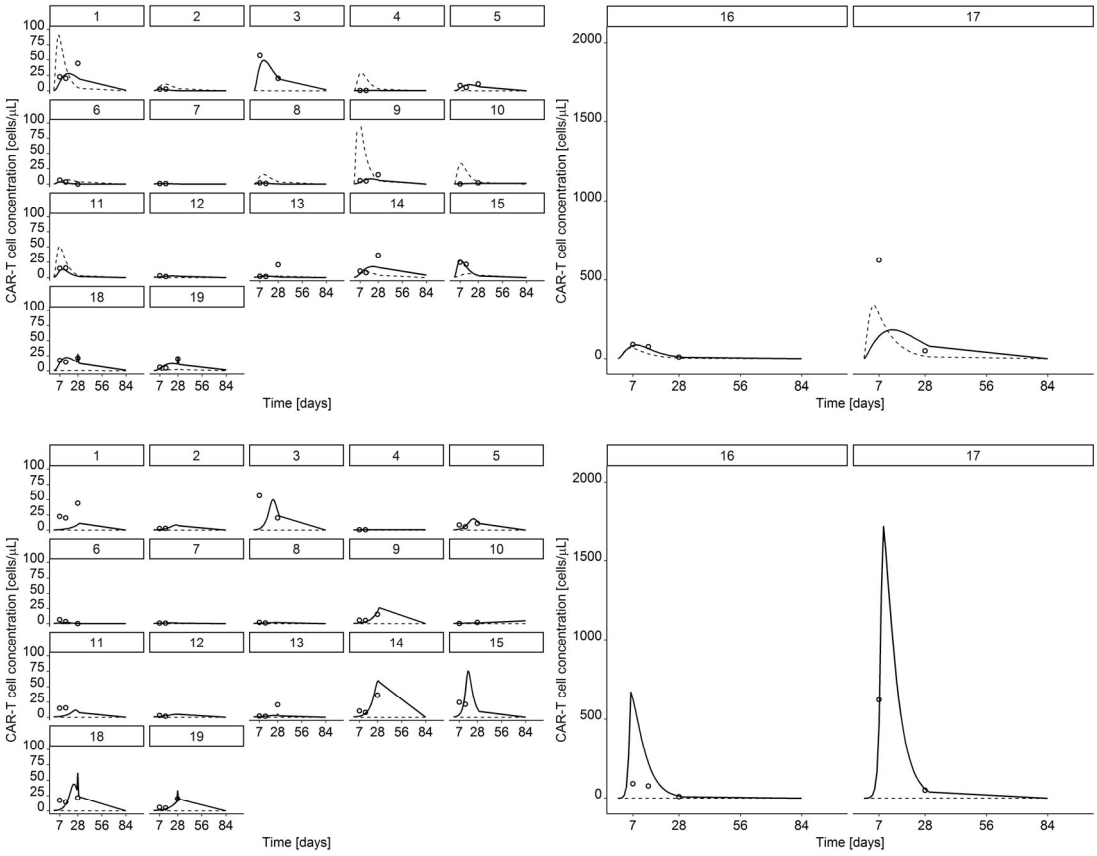

Figure S10. Measured CAR-T cell concentrations (circles) and individual (solid lines) and typical (dashed lines) model predictions using the base model (without covariates or mixture model) and using either the respective CAR-T cell population (**upper plots**) or the $CD19^+$ metabolic tumour volume (**lower plots**) in the denominator of the expansion term describing CAR-T cell expansion in response to tumour contact. The base model using the respective CAR-T cell population in the denominator of the expansion term (**upper plots**) was used for the rest of the work.

Table S1. Patient characteristics.

Pat. ID	Disease Type	Sex [M/F]	Age group [years]	C_{max} naïve CAR-T cells [cells·µL^{-1}]	Baseline metabolic tumour volume [mL]	Clinical composite score $_{TN}$ * [(cells · µL^{-1}) · mL^{-1}]	Low expansion subpopulation [yes/no]	Previous ASCT [yes/no]	CD4/CD8 ratio of CAR-T cells at day seven	Progressive disease [yes/no]	PFS [months]	OS [months]	Dead [yes/no] (and reason) or lost to follow-up†
1	DLBCL	F	51-60	44.1	894	0.00542	No	No	8.94	Yes	2	8	Lost to follow-up
2	DLBCL	M	21-30	3.04	102	0.00863	No	No	0.357	No	1	1	Lost to follow-up
3	PMBCL	M	51-60	56.8	13.0	0.723	No	Yes	0.126	No	21	21	No
4	TFL	F	21-30	0.64	264	0.000870	Yes	No	4.01	No	1	1	Yes (septic shock)
5	DLBCL	F	51-60	11.0	14.2	0.169	No	Yes	1.34	No	21	21	No
6	TFL	M	31-40	6.43	70.5	0.0174	No	Yes	0.364	Yes	8	24	No
7	DLBCL	F	61-70	1.02	13.1	0.0236	No	Yes	3.56	No	22	22	No
8	PMBCL	M	71-80	2.04	142	0.00230	No	No	0.405	No	19	19	No
9	PMBCL	M	51-60	15.3	1118	0.00136	Yes	No	0.413	Yes	2	2	Yes (PD)
10	DLBCL	M	51-60	1.98	305	0.000884	Yes	No	0.549	Yes	3	6	Yes (PD)
11	DLBCL	M	61-70	15.4	479	0.00751	No	No	0.223	Yes	1	13	Yes (PD)
12	DLBCL	F	61-70	2.82	2.54	0.299	No	Yes	0.0512	No	5	5	Lost to follow-up
13	DLBCL	M	51-60	16.8	34.2	0.014	No	No	4.53	No	3	3	Lost to follow-up
14	PMBCL	M	61-70	36.2	85.7	0.0705	No	Yes	0.130	No	1	1	Lost to follow-up
15	DLBCL	M	61-70	24.7	64.1	0.164	No	No	0.0845	Yes	3	3	Yes (PD)
16	PMBCL	F	61-70	90.8	663	0.0388	No	No	0.764	Yes	5	5	Yes (PD)
17	DLBCL	M	51-60	623	3555	0.017	Yes	Yes	0.0866	No	16	16	No
18	PMBCL	M	51-60	17.3	20.0	0.224	No	No	0.182	Yes	1	1	Yes (PD)
19	DLBCL	M	51-60	15.8	32.5	0.124	No	No	0.126	Yes	11	16	Yes (PD)

Abbreviations: ASCT: autologous stem cell transplantation; C_{max}: maximum concentration; DLBCL: Diffuse large B cell lymphoma; F: female; M: male; OS: overall survival; PD: progressive disease; PFS: progression-free survival; Pat: patient; PMBCL: primary mediastinal B-cell lymphoma; TFL: transformed follicular lymphoma.

*Clinical composite score$_{TN}$: Maximum naïve CAR-T cell concentrations/Baseline metabolic tumour volume

†Patients who were lost to follow-up were from overseas or other US states and did not return to MD Anderson Cancer Center for further monitoring.

Table S2. Observed and predicted CAR-T cell kinetic parameters in the reference population and the low expansion sub-population.

Pharmacokinetic parameter	Reference population (*n* = 15)	Low expansion subpopulation (*n* = 4)
Observed CAR-T cell kinetic parameters in the reference population and the low-expansion subpopulation		
Maximum CAR-T cell concentration (C_{max}) [cells·µL^{-1}]	Median: 15.8 Range: 1.02–90.8	Median: 8.64 Range: 0.64–623
Area under the concentration-time curve from day 0 to day 28 ($AUC_{0\text{-}28d}$) [(cells·µL^{-1}) · day^{-1}]	Median: 162 Range: 6.48–1195	Median: 102 Range: 4.34–7068
Time at maximum CAR-T cell concentration (T_{max}) [day]	Median: 14 Range: 7–28	Median: 17.5 Range: 7–28
C_{max}/Baseline metabolic tumour volume [(cells·µL^{-1})·mL^{-1}]	Median: 0.386 Range: 0.0143–4.38	Median: 0.0101 Range: 0.00242–0.175
Model-predicted CAR-T cell kinetic parameters in the reference population and the low-expansion subpopulation		
Maximum CAR-T cell concentration (C_{max}) [cells·µL^{-1}]	Median: 13.8 Range: 1.05 –79.9	Median: 5.231 Range: 0.619–166
Area under the concentration-time curve from day 0 to day 28 ($AUC_{0\text{-}28d}$) [(cells·µL^{-1}) · day^{-1}]	Median: 179 Range: 11.3–1271	Median: 94.0 Range: 6.41–2765
Time at maximum CAR-T cell concentration (T_{max}) [day]	Median: 12 Range: 7–28	Median: 23.5 Range: 14–30
C_{max}/Baseline metabolic tumour volume [(cells·µL^{-1})·mL^{-1}]	Median: 0.121 Range: 0.0136–3.59	Median: 0.00594 Range: 0.00234–0.0467