Supplementary information

Virtual clinical trials of Anti-PD-1 and Anti-CTLA-4 Immunotherapy in Advanced Hepatocellular Carcinoma Using a Quantitative Systems Pharmacology Model

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S.1 Simulation outcome

Table S1: Simulation results of the virtual patients based on RECIST 1.1 without confirmation of complete and partial responders at subsequent time for different treatment groups: no treatment group; ipilimumab treatment; nivolumab treatment; combination treatment of nivolumab and ipilimumab with dose regimen of arm A in CheckMate 040

	No treatment	Nivolumab	Ipilimumab	Combination (arm A)
	Simulations			
No of patients	1365	1365	1365	1365
Complete Response, No. (%)	0(0)	27(2)	0(0)	23(1.7)
Partial Response, No. (%)	12(0.9)	288(21.1)	49(3.6)	356(26.1)
Stable Disease, No. (%)	733(53.7)	568(41.6)	831(60.8)	609(44.6)
Progressive Disease, No. (%)	620(45.4)	482(35.3)	485(35.5)	373(27.6)
ORR % (95% CI)	0.9(0 to 2.5)	23.1(17.3 to 28.9)	3.6(1 to 7)	27.8(16 to 40)

Table S2: Simulation results of the virtual patients based on RECIST 1.1 (with confirmation of responders) for no treatment group; ipilimumab treatment; combination treatment of nivolumab and ipilimumab with dose regimen of arm B and arm C in CheckMate 040

	No treatment	Ipilimumab	Combination (arm B)	Combination (arm C)
	Simulations			
No of patients	1365	1365	1365	1365
Complete Response, No. (%)	0(0)	0(0)	17(1.3)	17(1.3)
Partial Response, No. (%)	7(0.5)	36(2.6)	309(22.6)	315(23.1)
Stable Disease, No. (%)	738(54.1)	844(61.8)	666(48.8)	671(49.2)
Progressive Disease, No. (%)	620(45.4)	485(35.5)	373(27.3)	362(26.5)
ORR % (95% CI)	0.5(0 to 2)	2.6(1 to 6)	23.9(12 to 37)	24.3(12 to 37)

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Table S3: Summary of the primary endpoint results for monotherapy of pembrolizumab as predicted by our simulations for proposed patients and the phase II clinical trial Keynote – 224

	Keynote-224	Simulations
	Pembrolizumab	
No of patients	104	879
Complete Response, No. (%)	1(1)	15(1.71)
Partial Response, No. (%)	17(16)	131(14.90)
Stable Disease, No. (%)	46(44)	328(37.32)
Progressive Disease, No. (%)	34(33)	405(46.08)
ORR %	17	16.61

To perform the preliminary simulations of Pembrolizumab monotherapy according to the clinical trial Keynote – 224 with a dose regimen of 200 mg every 3 weeks, the pharmacokinetics of the proposed QSP model was calibrated for pembrolizumab. One thousand proposed patients were generated by parameterizing the same 89 parameters as performed in our earlier analysis of Nivolumab. But the proposed cohorts in the two cases are different due to different pharmacokinetics of the two drugs. Out of 1000 proposed patients, 87.9% were successful simulations while 5 % of the cohorts did not reach initial conditions and 7.1% failed due to numerical instabilities. We categorized all the 879 proposed patients according to RECIST 1.1 and found that the overall response rate was 16.61 % with 1.71 %as complete responders and 14.9 % as partial responders. This preliminary analysis is consistent with the phase II clinical trial of Keynote – 224.

S.2 Biomarker Identification

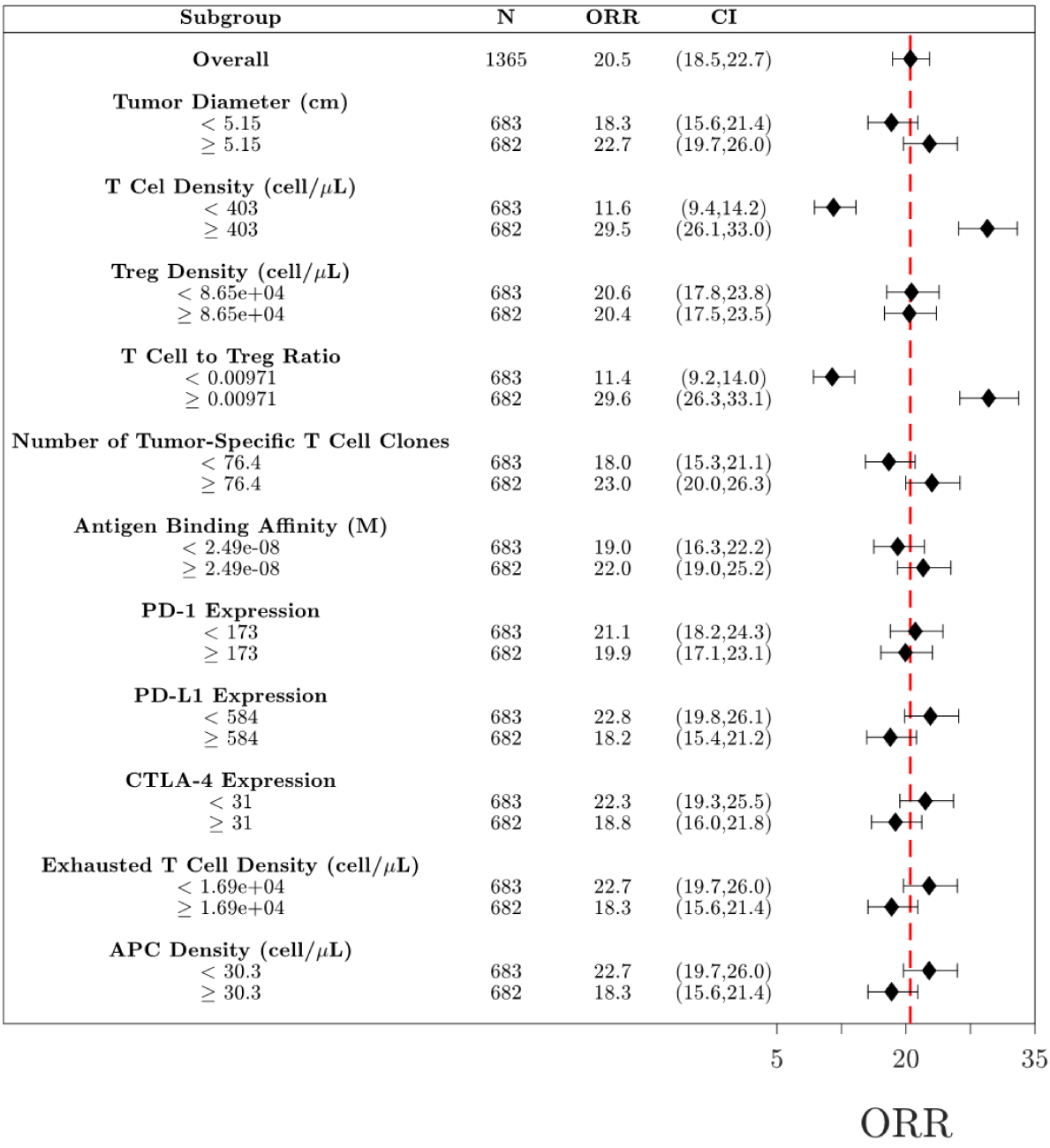

Figure S1: Subgroup analysis of anti-PD-1 (nivolumab) therapy. Virtual patients (N = 1365) are divided into 22 subgroups based on the pretreatment values of selected biomarkers. Objective response rates (ORR) for each group are given along with the 95% confidence interval (CI) estimated by the Agresti-Coull interval. The red dashed line indicated the ORR for the total population.

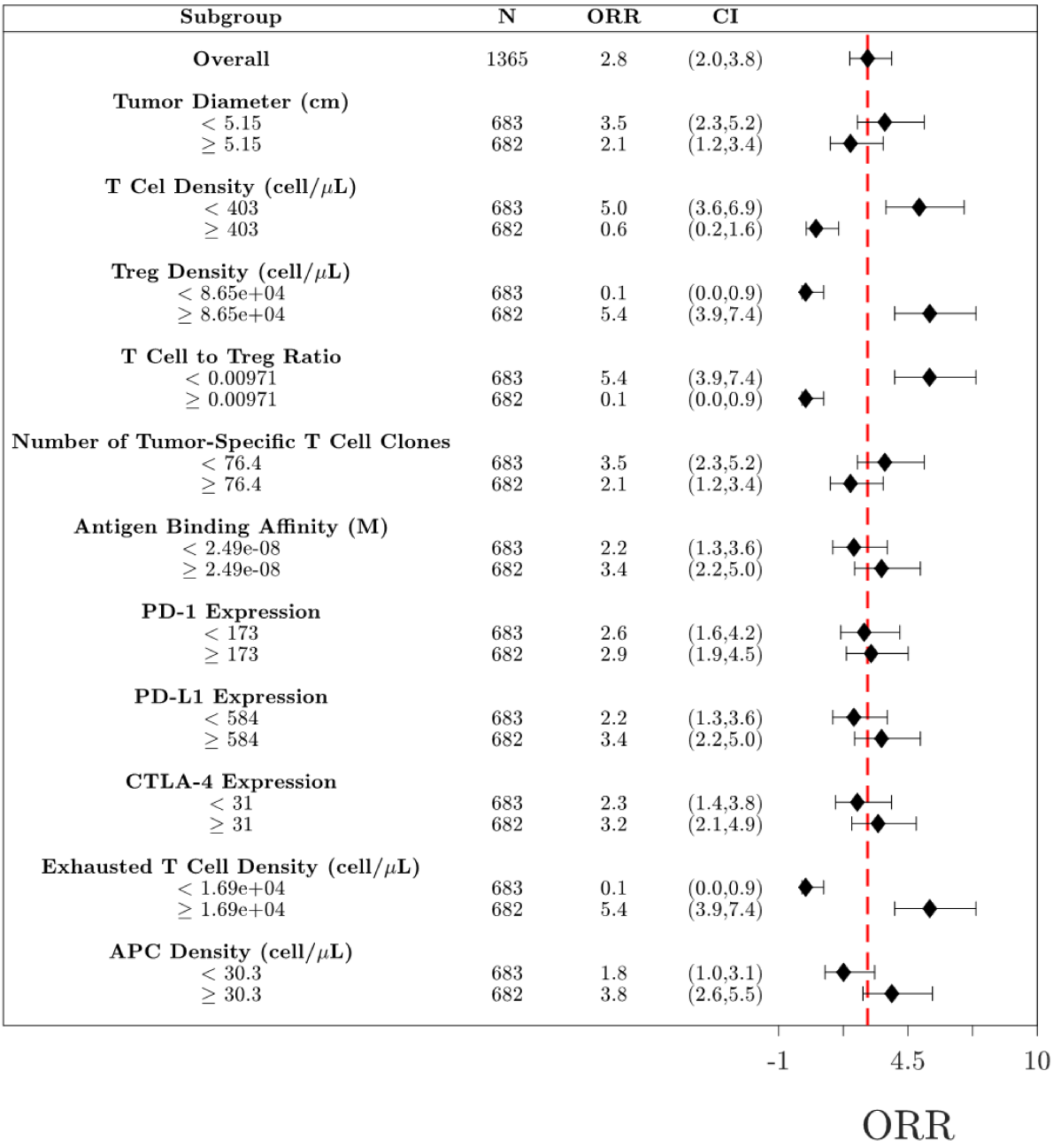

Figure S2: Subgroup analysis of anti-CTLA-4 (ipilimumab) therapy. Virtual patients (N = 1365) are divided into 22 subgroups based on the pretreatment values of selected biomarkers. Objective response rates (ORR) for each group are given along with the 95% confidence interval (CI) estimated by the Agresti-Coull interval. The red dashed line indicated the ORR for the total population.

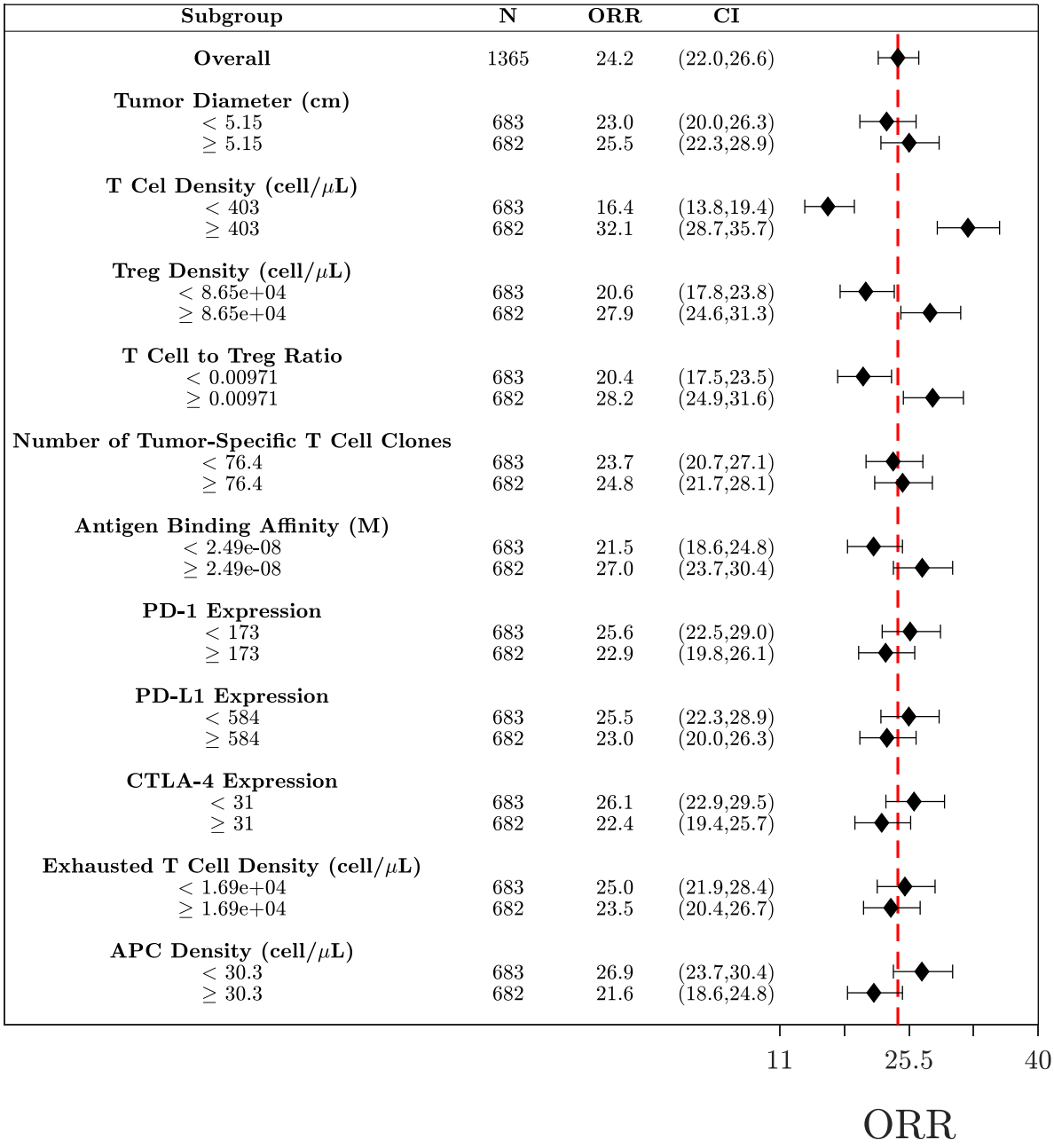

Figure S3: Subgroup analysis of anti-PD-1 (nivolumab) in combination of anti-CTLA-4 (ipilimumab) therapy; with dose regimen of arm B of CheckMate 040. Virtual patients (N=1365) are divided into 22 subgroups based on the pretreatment values of selected biomarkers. Objective response rates (ORR) for each group are given along with the 95% confidence interval (CI) estimated by the Agresti-Coull interval. The red dashed line indicated the ORR for the total population.

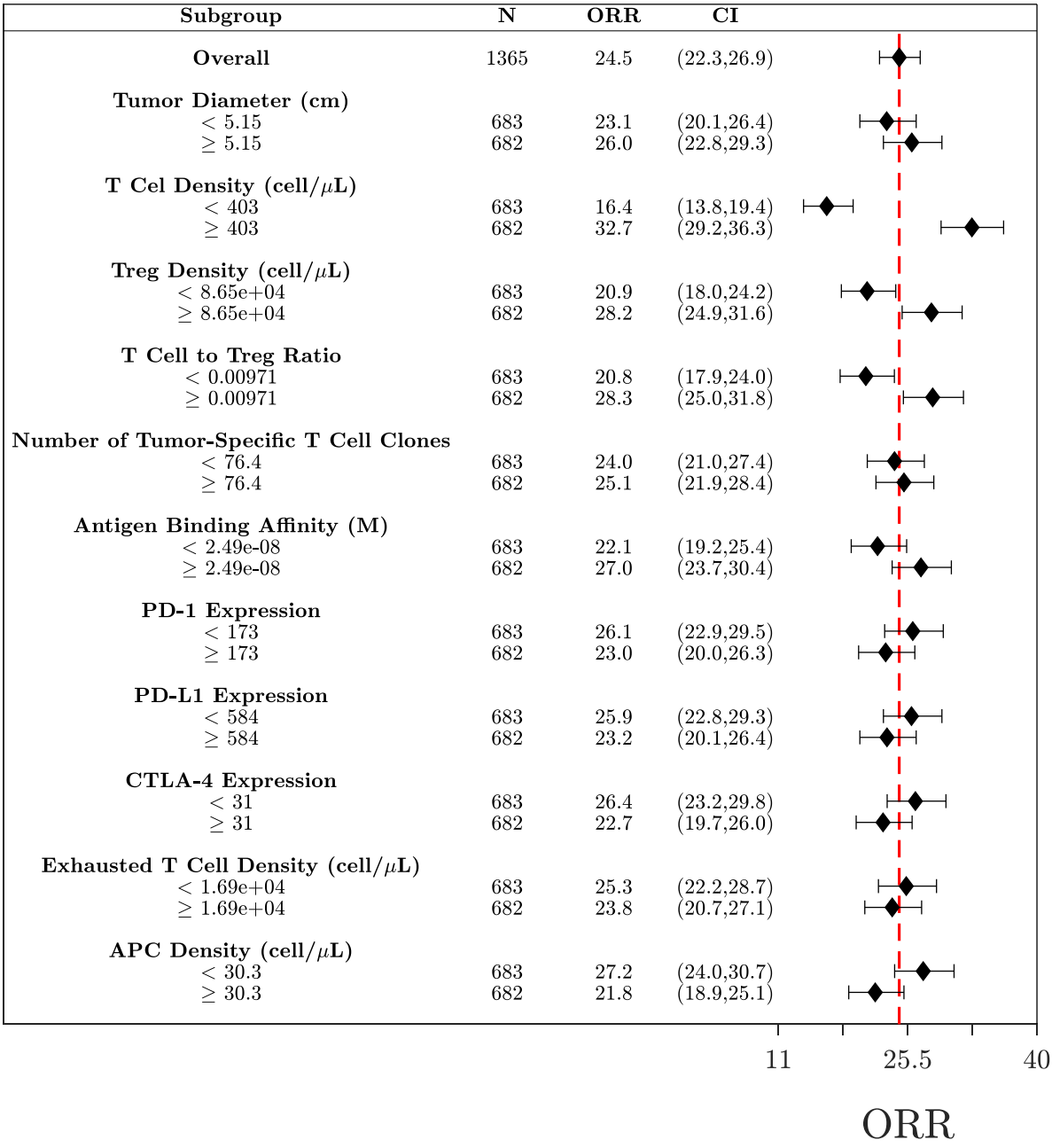

Figure S4: Subgroup analysis of anti-PD-1 (nivolumab) in combination of anti-CTLA-4 (ipilimumab) therapy; with dose regimen of arm C of CheckMate 040. Virtual patients (N = 1365) are divided into 22 subgroups based on the pretreatment values of selected biomarkers. Objective response rates (ORR) for each group are given along with the 95% confidence interval (CI) estimated by the Agresti-Coull interval. The red dashed line indicated the ORR for the total population.

S.3 Random Forest

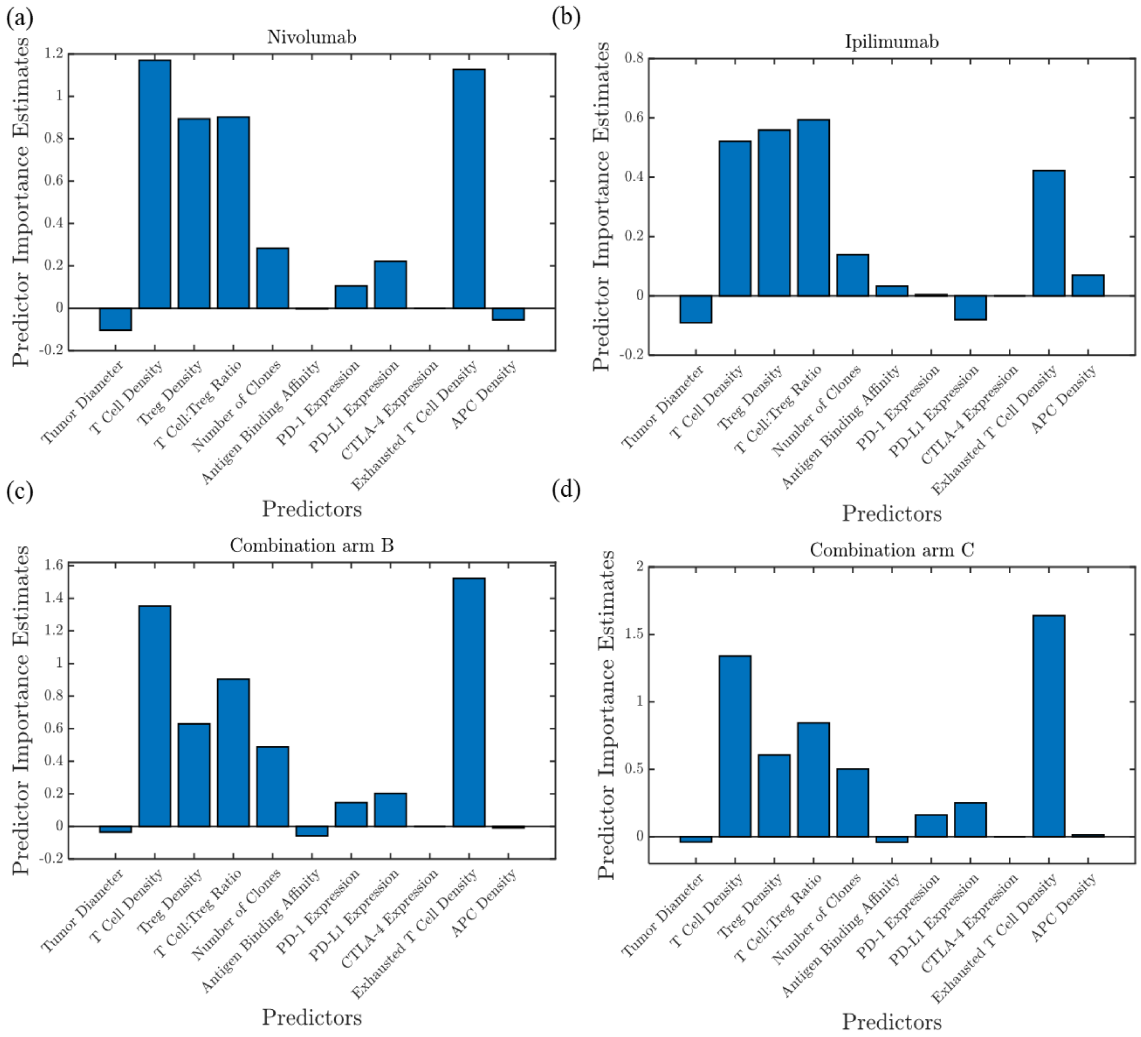

Figure S5: Predictor importance for different treatment therapy estimated by the mean increase in out-of-bag error caused by permuting the observations of each predictor.

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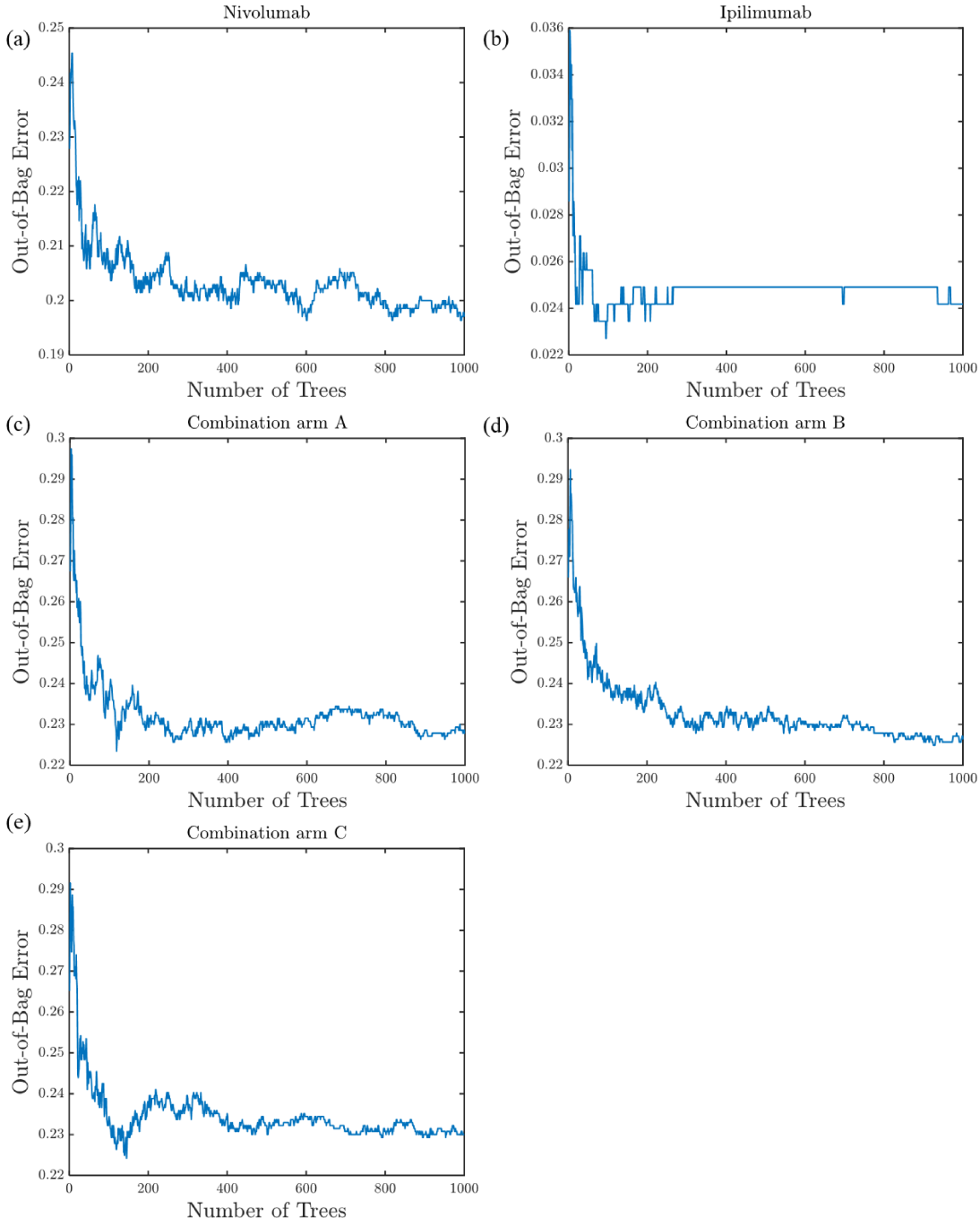

Figure S6: Convergence of the out-of-bag error calculated as the number of trees in the random forest is increased for different treatment therapy.

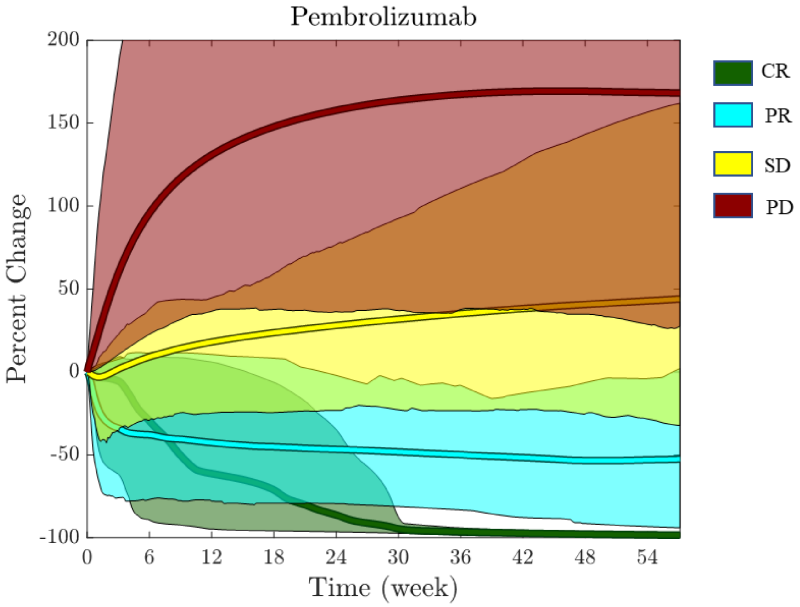

Figure S7: Mean percentage change in tumor diameter for proposed patients as a function of time for monotherapy of pembrolizumab as predicted by the QSP model. Shaded regions correspond to the 95% confidence interval of the simulations in the corresponding RECIST category.

S.4 Model equations

Dependent variables in the model

Dependent Variables	Description
C	Number of cancer cells in the tumor
H_{PD1}	Hill function representing the fraction of PD1-PDL1/L2 interactions
V_T	Tumor compartment volume
D_{T}	Number of naïve antigen presenting cells in the tumor
D_{LN}	Number of naïve antigen presenting cells in the lymph node compartment
$\widehat{D_{\mathrm{T}}}$	Number of mature antigen presenting cells in the tumor
$\widehat{D_{\mathrm{LN}}}$	Number of mature antigen presenting cells in the lymph node compartment
$[c]$	Concentration of maturation cytokines
$[P]_T$	Extracellular antigen concentration in the tumor
$[P]_e$	APC endosomal antigen concentration
$[p]$	APC endosomal epitope concentration
$[M]_e$	Free APC endosomal MHC concentration
$[M]_s$	Free APC surface MHC concentration
$[Mp]_e$	APC endosomal epitope-MHC complex concentration
$[Mp]_s$	APC surface epitope-MHC complex concentration

$\mathcal{N}_C$	Number of naïve T cells in the central compartment
$\mathcal{N}_P$	Number of naïve T cells in the peripheral compartment
$\mathcal{N}_{LN}$	Number of naïve T cells in the lymph node compartment
H_p	Hill function representing the strength of antigen-TCR binding
TCR_{active}	Concentration of active T cell receptors in the synapse
C_{tot}	Total TCR-antigen-MHC complex concentration
$\mathcal{P}$	Number of proliferating cells in the lymph node compartment
N	Division destiny
N_{IL2}	Number of divisions due to the presence of IL2
$[IL2]$	IL2 concentration in the lymph node compartment
T_C	Number of activated T cells in the central compartment
T_P	Number of activated T cells in the peripheral compartment
T	Number of activated T cells in the tumor compartment
T_{LN}	Number of activated T cells in the lymph node compartment
$[A]_C$	Antibody concentration in the central compartment
$[A]_P$	Antibody concentration in the peripheral compartment
$[A]_T$	Antibody concentration in the tumor compartment
$[A]_{LN}$	Antibody concentration in the lymph node compartment

Tumor Growth Dynamics:

$$\frac{\mathrm{dC}}{\mathrm{dt}} = \mathrm{k_{growth}C}\left(1 - \frac{\mathrm{C}}{\mathrm{C_{max}}}\right) - \left(\mathrm{k_{death}} + \mathrm{k_{Tcell}}\frac{\mathrm{T}}{\mathrm{T} + T_{reg} + \mathrm{C}}(1 - \mathrm{H_{PD1}})\right)\mathrm{C}$$

$$\mathrm{H_{PD1}} = \frac{\mathrm{X^n}}{\mathrm{X^n} + \mathrm{PD1_{50}^n}}$$

$$\mathrm{V_T} = \mathrm{V_{cancer}C} + \mathrm{V_{Tcell}}(\mathrm{T} + \mathrm{T_{reg}})$$

Parameters for tumor growth dynamics:

Parameter	Description
k_{growth}	Maximal rate of cancer cell growth
C_{max}	Cancer cell carrying capacity
k_{death}	Cancer cell death rate
k_{Tcell}	Maximal T cell-mediated cancer cell death rate
$PD1_{50}$	Concentration of PD1-PDL1/L2 complex for half-maximal T cell-mediated killing
n	Hill coefficient for T cell-mediated killing
V_{cancer}	Average volume of a cancer cell
V_{Tcell}	Average volume of a T cell

Antigen Presenting Cell Dynamics:

$$\frac{dD_T}{dt} = k_D\left(\rho_T^D V_T - D_T\right) - k_{mat}\frac{[c]}{[c]+[c]_{50}}D_T$$

$$\frac{dD_{LN}}{dt} = k_D\left(\rho_{LN}^D V_{LN} - D_{LN}\right)$$

$$\frac{d\widehat{D_T}}{dt} = k_{mat}\frac{[c]}{[c]+[c]_{50}}D_T - \left(k_{\widehat{D}} + k_{mig}\right)\widehat{D_T}$$

$$\frac{d\widehat{D_{LN}}}{dt} = k_{mig}\widehat{D_T} - k_{\widehat{D}}\widehat{D_{LN}}$$

$$\frac{d}{dt}(V_T[c]) = k_c([c]_0 - [c])V_T + k_{Tcell}\frac{TC}{T+C}(1 - H_{PD1})x_c$$

Parameters for antigen presenting cell dynamics:

Parameter	Description
k_D	Death rate of naïve APCs
$k_{\widehat{D}}$	Death rate of mature APCs
k_{mat}	Maximal APC maturation rate
k_{mig}	APC migration rate
ρ_T^D	Steady-state APC density in the tumor
ρ_{LN}^D	Steady-state APC density in the lymph node compartment
V_{LN}	Volume of the lymph node compartment
$[c]_0$	Steady-state maturation cytokines concentration
$[c]_{50}$	Concentration of cytokines for half-maximal maturation of APCs
k_c	Maturation cytokines turnover rate
x_c	Concentration of maturation cytokines released from cancer cell death

Antigen-Related Equations:

These equations describe the release of antigenic proteins from cancer cells and their subsequent update, degradation into smaller peptide fragments (epitopes) and their presentation on the surface of APCs.

$$\frac{d}{dt}(V_T[P]_T) = n_{clones}[P]_0 V_T\left(k_{death} + k_{Tcell}\frac{T}{T+C}(1 - H_{PD1})\right)C - \left(k_{up} + k_{deg}\right)[P]_T V_T$$

$$\frac{d[P]_e}{dt} = k_{up}[P]_T - k_{deg}^P[P]_e$$

$$V_e\frac{d[p]}{dt} = k_{deg}^P[P]_e V_e + k_{off}[Mp]_e A_e - \left(k_{on}[M]_e A_e + k_{deg}^p V_e\right)[p]$$

$$A_e\frac{d[M]_e}{dt} = k_{off}[Mp]_e A_e + k_{in}[M]_s A_s - (k_{on}[p] + k_{out})[M]_e A_e$$

$$A_s\frac{d[M]_s}{dt} = k_{off}[Mp]_s A_s + k_{out}[M]_e A_e - k_{in}[M]_s A_s$$

$$A_e\frac{d[Mp]_e}{dt} = k_{on}[p][M]_e A_e + k_{in}[Mp]_s A_s - (k_{off} + k_{out})[Mp]_e A_e$$

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$$A_s \frac{d[Mp]_s}{dt} = k_{out}[Mp]_e A_e - (k_{off} + k_{in})[Mp]_s A_s$$

Parameters for antigen-related equations:

Parameter	Description
n_{clones}	Number of T cell clones
$[P]_0$	Concentration of antigen released by apoptotic cancer cells
k_{up}	Antigen uptake rate by APCs
k_{deg}	Antigen extra cellular degradation rate
k_{deg}^P	Antigen intracellular degradation rate
V_e	APC endosomal volume
A_e	APC endosomal surface area
A_s	T cell-APC synapse surface area
k_{on}	Epitope-MHC association rate
k_{off}	Epitope-MHC dissociation rate
k_{in}	MHC internalization rate
k_{out}	MHC externalization rate

T Cell Dynamics:

Naïve T Cells:

These equations model the production of naïve T cells in the central compartment and their transport to the lymph node and peripheral compartments.

$$\frac{d\mathcal{N}_C}{dt} = \sigma + k_{prolif}\frac{\mathcal{N}_C}{K_m + \mathcal{N}_C} + \sum_{i=P,LN}\left(q_i^{out}\mathcal{N}_i - q_i^{in}\mathcal{N}_C\right) - k_{death}^{\mathcal{N}}\mathcal{N}_C$$

$$\frac{d\mathcal{N}_P}{dt} = k_{prolif}\frac{\mathcal{N}_P}{K_m + \mathcal{N}_P} + q_P^{in}\mathcal{N}_C - q_P^{out}\mathcal{N}_P - k_{death}^{\mathcal{N}}\mathcal{N}_P$$

$$\frac{d\mathcal{N}_{LN}}{dt} = q_{LN}^{in}\mathcal{N}_C - q_{LN}^{out}\mathcal{N}_{LN} - k_{act}\frac{n_{sites}\widehat{D_T}}{n_{sites}\widehat{D_T} + T_{total}}\frac{[Mp]_s}{[Mp]_s + K_{m,p}}H_p\mathcal{N}_{LN}$$

T Cell Activation:

Activation of naïve T cells by mature APC is based on the interaction of TCRs and antigen-MHC complex. This interaction is modeled with kinetic proofreading model with limited signaling. In this model free antigen-MHC complex binds to free TCRs on naïve T cells to form TCR-antigen-MHC complex which undergo 'm' biochemical modifications to reach signaling – competent TCR state. Since this model assumes signaling through individual TCRs is limited, following which the signaling signaling -competent TCR is rendered to non-signaling state [1].

$$H_p = \frac{TCR_{active}}{TCR_{active} + K_{p,50}}$$

$$TCR_{active} = \left(\frac{k_{off}^{TCR}}{k_{off}^{TCR} + \phi}\right)\left(\frac{k_p}{k_p + k_{off}^{TCR}}\right)^m C_{tot}$$

$$C_{tot} = \frac{1}{2}\left(Mp_s + TCR_{tot} + K_D - \sqrt{(Mp_s + TCR_{tot} + K_D)^2 - 4Mp_s TCR_{tot}}\right)$$

T Cell Proliferation and the Dynamics of IL2:

$$\frac{d\mathcal{P}}{dt} = n_{clones} k_{act} \frac{n_{sites}\widehat{D_{LN}}}{n_{sites}\widehat{D_{LN}} + T_{total}} \frac{[Mp]_s}{[Mp]_s + K_{m,p}} H_p \mathcal{N}_{LN} - k_{pro}\mathcal{P}$$

$$N = N_{TCR} + N_{costim} + N_{IL2}$$

$$N_{IL2} = \Delta N \frac{[IL2]}{[IL2] + [IL2]_{50}}$$

$$V_{LN}\frac{d[IL2]}{dt} = k_{sec}\mathcal{P} - k_{deg}^{IL2}[IL2]V_{LN} - k_{cons}\left(\left(T_{LN} + T_{LN}^{Treg}\right)\frac{[IL2]}{[IL2]_{50}+[IL2]} + T_{LN}^{Treg}\frac{[IL2]}{[IL2]_{50}^{Treg}+[IL2]}\right)$$

Activated T Cell Transport

$$\frac{dT_C}{dt} = q_P^{out}T_P + q_{LN}^{out}T_{LN} - \left(q_P^{in} + q_T^{in}V_T + k_{death}^T\right)T_C$$

$$\frac{dT_P}{dt} = q_P^{in}T_C - q_P^{out}T_P - k_{death}^T T_P$$

$$\frac{dT}{dt} = q_T^{in}V_T T_C - \left(k_{death}^T + k_{Treg}\frac{T_{reg}}{C + T + T_{reg}} + k_C\frac{C}{C + T + T_{reg}}\right)T$$

$$\frac{dT_{LN}}{dt} = 2^N k_{pro}\mathcal{P} - q_{LN}^{out}T_{LN} - k_{death}^T T_{LN}$$

Regulatory T Cells:

Regulatory T cells are modelled using the same equations as those for the cytotoxic T cells with minor differences outlined below. First, the activation of Tregs is modelled by presentation of self-reactive peptides on immature APCs. The activation term is modified as follows:

$$k_{act}\frac{n_{sites}\widehat{D_{LN}}}{n_{sites}\widehat{D_{LN}} + T_{total}}\frac{[Mp]_s}{[Mp]_s + K_{m,p}}H_p \rightarrow k_{act}\frac{n_{sites}D_{LN}}{n_{sites}D_{LN} + T_{total}}\frac{[Mp]_s}{[Mp]_s + K_{m,p}}H_p$$

The second difference is that the exhaustion terms from Tregs and cancer cells become zero. In the equations

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in this document, Tregs in the central, peripheral and lymph node compartments are denoted as T_i^{Treg}, where the subscript, i, refers to the central (C), peripheral (P) and lymph node (LN) compartments. Tregs in the tumor are denoted T_{reg}.

Parameters for T cells dynamics

Parameter	Description
σ	Rate of naïve T cell release from the thymus
k_{prolif}	Maximum rate of naïve T cell proliferation
K_m	Number of naïve T cells for half-maximal rate of proliferation
k_{death}^{N}	Rate of naïve T cell death
k_{act}	Rate of T cell activation
n_{sites}	Number of MHC-TCR binding sites on APCs
$K_{m,p}$	Concentration of antigen-MHC complexes for half-maximal activation
$k_{\mathrm{off}}^{\mathrm{TCR}}$	TCR to antigen-MHC dissociation rate
k_D	TCR to antigen-MHC binding affinity
ϕ	Modification rate to the non-signaling state (kinetic proofreading)
k_p	TCR modification rate
m	Number of intermediate states in the kinetic proofreading model
$\mathrm{TCR}_{\mathrm{tot}}$	Total amount of TCR on naïve T cells
k_{pro}	Rate of activated T cell proliferation
N_{TCR}	Number of divisions due to TCR binding
N_{costim}	Number of division due to costimulatory signals
ΔN	Maximum number of division due to IL2
$[IL2]_{50}$	IL2 concentration for half-maximal consumption by all T cells
k_{sec}	Rate of IL2 secretion
$k_{\mathrm{deg}}^{\mathrm{IL2}}$	Rate of IL2 degradation
k_{cons}	Rate of IL2 consumption by T cells
$[IL2]_{50}^{\mathrm{Treg}}$	IL2 concentration for half-maximal consumption by Tregs
$k_{\mathrm{death}}^{\mathrm{T}}$	Rate of activated T cell death
k_{Treg}	Rate of T cell exhaustion by Tregs
k_{C}	Rate of T cell exhaustion by cancer cells

Physiologically Based Pharmacokinetics:

The following are used to model the pharmacokinetics of nivolumab and ipilimumab. For brevity only one set of equations are presented here with the variable, A, representing either antibody.

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$$V_C \frac{d[A]_C}{dt} = \sum_{i=P,T,LN} Q_i \left(\frac{[A]_i}{\gamma_i} - \frac{[A]_C}{\gamma_C} \right) + Q_{LD} \frac{[A]_{LN}}{\gamma_{LN}} - k_{cl}[A]_C V_C$$

$$V_P \frac{d[A]_P}{dt} = Q_P \left(\frac{[A]_C}{\gamma_C} - \frac{[A]_P}{\gamma_P} \right)$$

$$\frac{d}{dt}(V_T[A]_T) = Q_T \left(\frac{[A]_C}{\gamma_C} - \frac{[A]_T}{\gamma_T} \right) - Q_{LD} \frac{[A]_T}{\gamma_T}$$

$$V_{LN} \frac{d[A]_{LN}}{dt} = Q_{LN} \left(\frac{[A]_C}{\gamma_C} - \frac{[A]_{LN}}{\gamma_{LN}} \right) + Q_{LD} \frac{[A]_T}{\gamma_T} - Q_{LD} \frac{[A]_{LN}}{\gamma_{LN}}$$

Parameters for Pharmacokinetics

Parameter	Description
V_C	Volume of the central compartment
V_P	Volume of the peripheral compartment
V_{LN}	Volume of the lymph node compartment
Q_i	Rate of transport between the central compartment and compartment $i = C, P, T$
Q_{LD}	Rate of lymphatic drainage
γ_i	Ratio of the volume of distribution to the compartment volume
k_{cl}	Elimination rate of the antibody from the central compartment

References:

1. Lever M, Maini PK, van der Merwe PA, Dushek O. Phenotypic models of T cell activation. Nat Rev Immunol. Nature Publishing Group; 2014;14:619–29.