

Assessing tobemstomig binding dynamics through integrated experimental and computational modeling approaches to inform therapeutic dosing

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Background

Tobemstomig is a bispecific antibody targeting PD-1 and LAG-3, with a 10-20 fold higher affinity for PD-1 than for LAG-3, allowing a unique avidity-mediated selectivity gain and consequently a preferential binding to tumor infiltrating lymphocytes (TILs) that co-express PD-1 and LAG-3. Maximal blockade of both PD-1 and LAG-3 is required for the intended therapeutic effect.

The key areas of analysis in this work include:

- Investigating the dose-dependent targeting of T cells by tobemstomig, accounting for tumoral cell density and the molecule's binding kinetics;
- Determining the minimal dose required to achieve > 90% targeting of T cells expressing PD-1 and/or LAG-3.

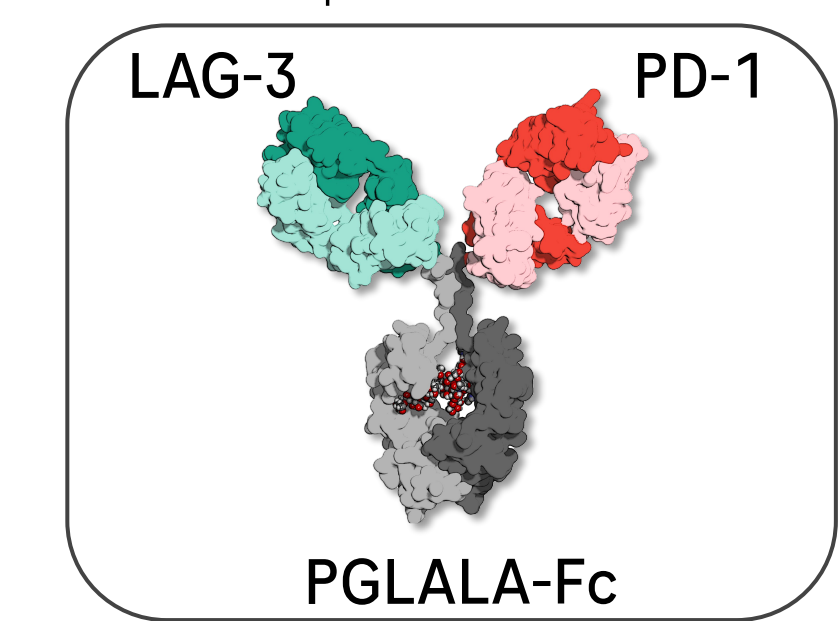


Figure 1: Schematic representation of tobemstomig.

Methods : Tumoral Cell Density of PD-1 and LAG-3

Multiplex immunofluorescence (mIF, Ventana) technology was adopted to evaluate key CD8+ T cell phenotypes, focusing on PD-1 and LAG-3 expression, as summarized in **Table 1**.

Table 1: Phenotypic markers of CD8+ T cells assessed in the analysis.

Description	Abbreviation	Phenotype
Double Positive	dP	PD1+LAG3+
PD-1 single positive	sPD1	PD1+LAG3-
LAG-3 single positive	sLAG3	PD1-LAG3+

The concentration of each T cell phenotype was determined according to the methodology described in [1], taking into account the cellular density of each phenotype and the total tumor burden of each patient.

Samples were analyzed from two different studies, NP41300 and BP42772, across a total of 117 patients. **Figure 2** shows the distribution for the three main indications in the patient population: melanoma, esophageal squamous cell carcinoma (ESCC), and non-small cell lung cancer (NSCLC).

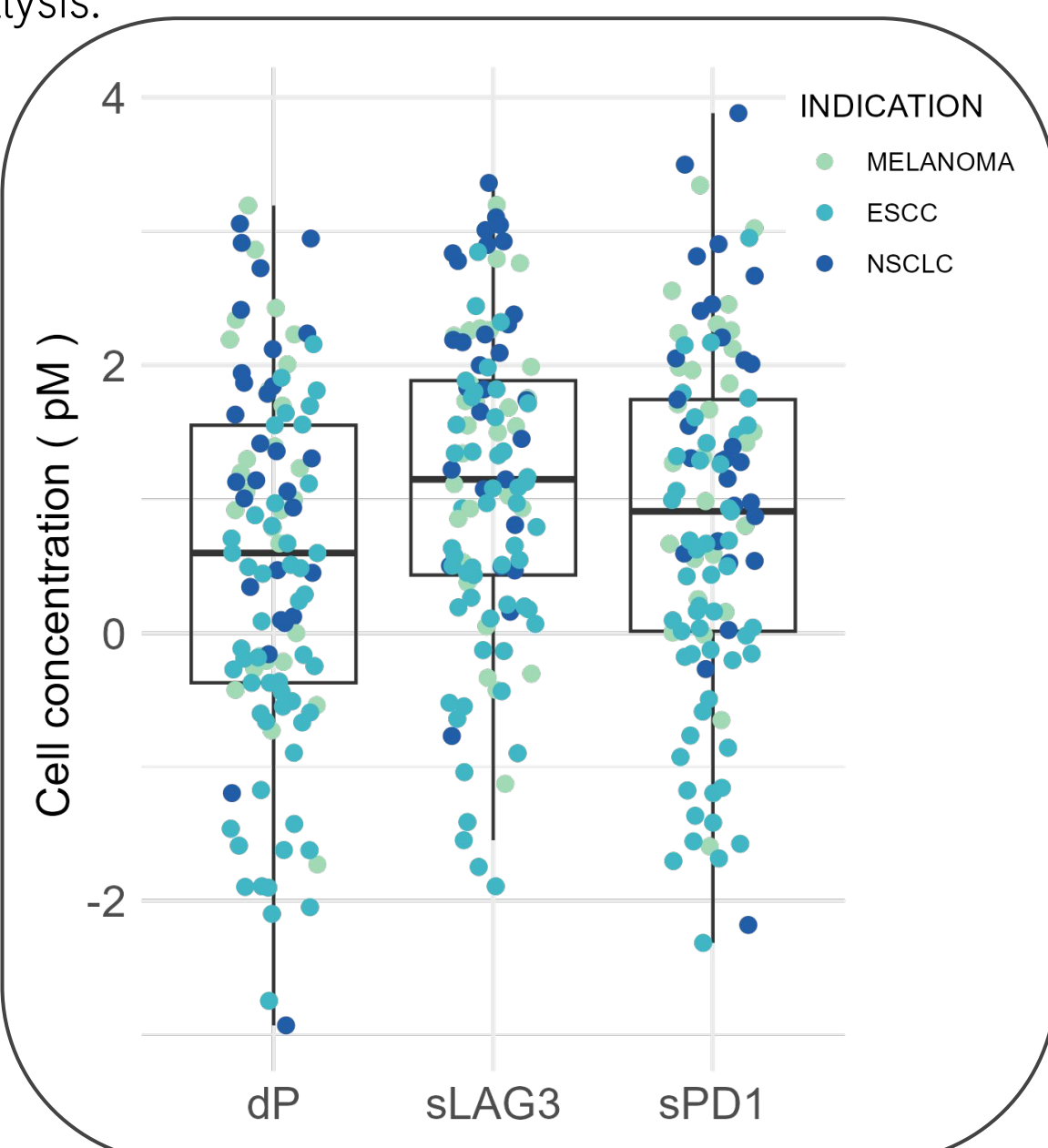


Figure 2: Estimated cell concentration, colour-coded by the three main indications in the patient population. Cell concentration is in log-scale.

Methods : Minimal PBPK model

A minimal physiologically-based pharmacokinetics (mPBPK) model [2] was developed, incorporating PK and mIF data, accounting for patient variability in PK parameters - clearance (CL) and central volume (V1) - and tumoral cell density of PD-1 and LAG-3 expressing cells - **Fig. 2**. **Figure 3** is a schematic representation of the implemented mPBPK model, describing both systemic and intratumoral concentrations of tobemstomig and incorporating its binding to PD-1 and LAG-3 receptors on TILs in the tumor interstitial compartment.

Preclinical binding assay data were used to estimate the binding kinetic parameter (k_D), which varies according to both the T cell phenotype and the receptor type (PD-1 or LAG-3), as represented in **Figure 3**.

A range of tobemstomig doses (10^{-3} - 10^4 mg) was simulated over 10 cycles, with administration every 3 weeks. Additionally, simulations assessed variations in intratumoral concentrations of tobemstomig, reflecting different levels of tumor vascularization, represented by parameter *vasc*. The scenarios explored tumoral interstitial concentrations at 5%, 20%, and 50% relative to plasma levels.

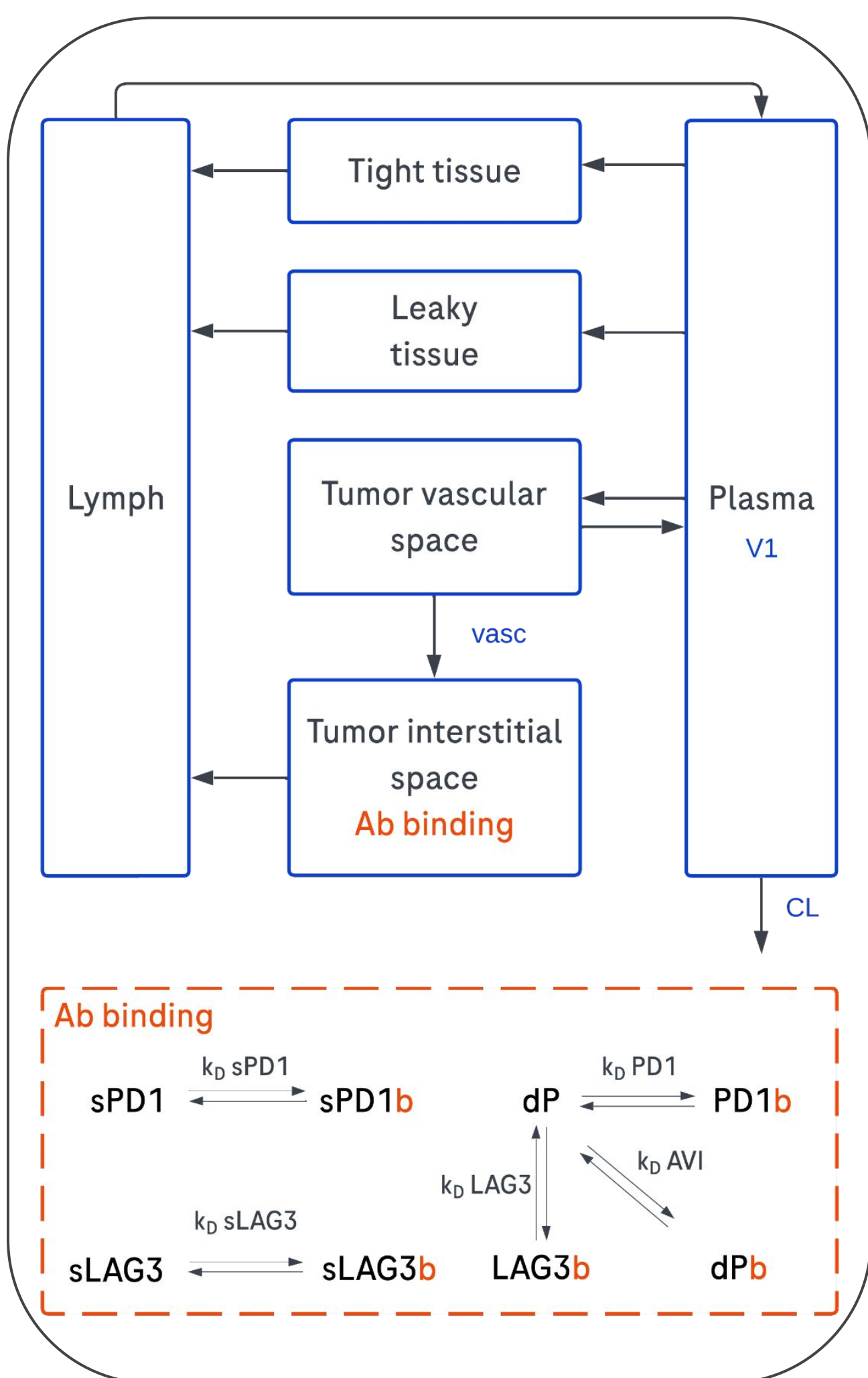


Figure 3: Schematic representation of the mPBPK model and the binding model. The 'b' notation indicates the bound state of each T-cell phenotype.

Results - Simulated receptor occupancy

The main variable analyzed was the simulated receptor occupancy (RO) calculated with the following expression

$$RO = \frac{CELL_{b,t}}{CELL_{t0}} \times 100 (\%) \quad (1)$$

in which $CELL_{b,t}$ represents the concentration of bound cells of a certain T cell phenotype at time point t , while $CELL_{t0}$ denotes the initial concentration of the respective T cell phenotype.

Figure 4 illustrates the main outcomes of the simulation, highlighting three key factors impacting RO

1. T cell phenotype
2. Level of tumor vascularization
3. Individual patient characteristics

- The dose required to achieve a given RO increases from dP to sLAG3, reflecting tobemstomig's higher affinity for double positive cells compared to single positive cells;
- For a given dose range, tumors with low vascularization show a lower range of RO, indicating limited drug delivery. In well-vascularized tumors scenario, a higher range of RO is simulated, reflecting enhanced drug exposure, which underscores the value of assessing intratumoral PK;
- The interpatient variability observed in RO for a given dose and vascularization level is primarily attributed to the previously estimated CL parameter. Higher CL rate leads to reduced drug concentration in plasma and, consequently, lower intratumoral exposure and RO.

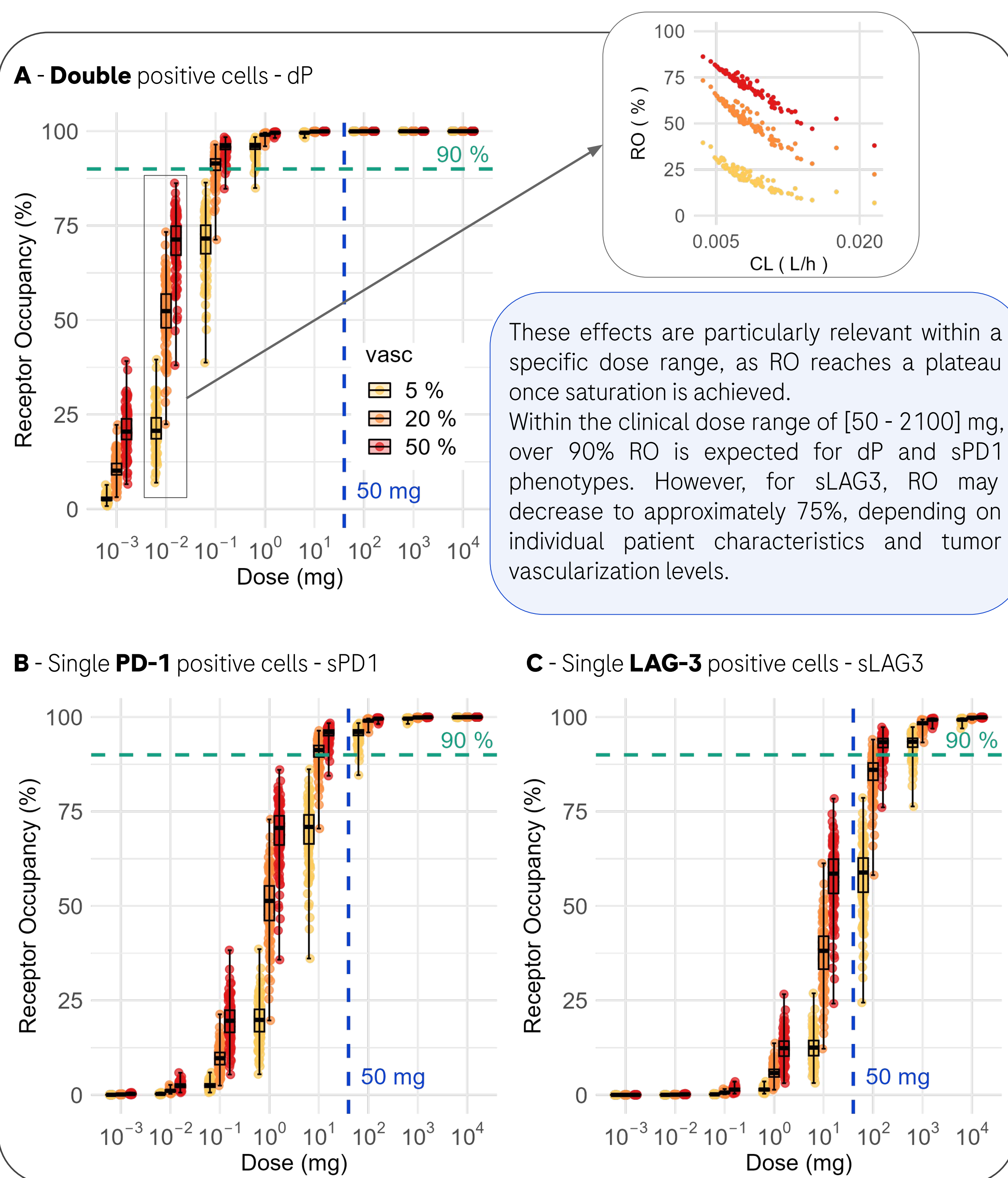


Figure 4: Simulated RO at different doses, with each dot representing an individual patient and colors indicating the distinct levels of tumor vascularization simulated. Results are shown at Ctrough after 10 cycles of tobemstomig, administered every 3 weeks.

Conclusions

- Simulations provided insights into RO and drug distribution, driven by T-cell phenotype, tumor vascularization, and individual patient characteristics;
- In the clinical dose range of 50-2100 mg, >90% RO is expected for dP and sPD1, while sLAG3 may drop to 75%, depending on clearance and vascularization;
- In the absence of direct measurements of tumoral RO in the clinic, measuring tumor PK could be an effective approach to estimate drug exposure particularly in light of the significant influence of tumor vascularization on RO.

References

1. Sender et al. Proc Natl Acad Sci U S A. 2023 Oct 31;120(44):e2308511120
2. Li et al. Clin Pharmacol Ther. 2021 Jul;110(1):200-209