Exploring the Influence of Bispecific Antibody Mechanisms on *In Vitro* Dose Response: Insights from an Open-Science Quantitative Systems Pharmacology Model in Julia

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Abstract

Objectives: Bispecific antibodies (bsAb) are a large family of molecules engineered to recognize two different epitopes. A bell-shaped concentration vs. response relationship, also called the hook effect (Figure 1), is often observed in *in vitro* cell viability experiments. Previous work found that the hook effect depends on variables that impact trimer (the trimolecular complex formed by the bsAb and the two receptors) formation, including the expression of each receptor and the binding affinity of the bsAb to each receptor [1]. However, other biological mechanisms may also influence the hook effect. The work presented here explores the potential impact of additional mechanisms through the development and analysis of a quantitative systems pharmacology (QSP) model of a bsAb in an *in vitro* setting.

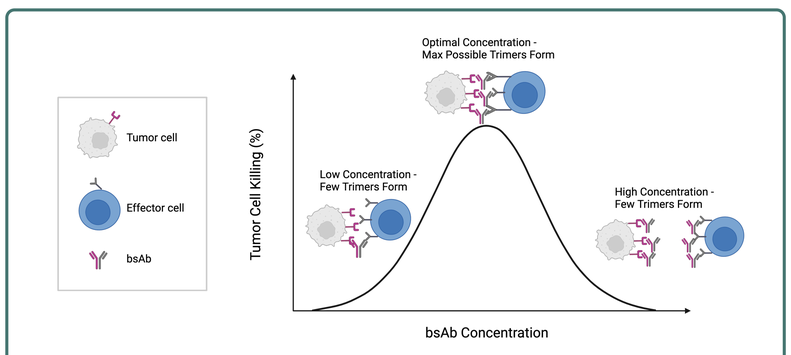

Figure 1. Bispecific antibodies exhibit a bell-shaped concentration-response relationship (also called the hook effect). At low concentrations, few trimers form which results in limited tumor cell killing. As the concentration increases, additional trimers form and eventually reach a maximum. This maximum is expected to lead to maximal tumor cell killing. Beyond this concentration, more dimers form, saturating the individual receptors, and trimer formation decreases. This results in reduced efficacy at high bsAb concentrations.

Methods: An *in vitro* QSP model for a bsAb was developed *de novo*. The model describes the mechanisms of bsAb:receptor binding, trimer formation, receptor turnover, cell growth, and trimer-induced death of the target cell. Hypothetical-but-realistic, values for all parameters were used to explore a wide range of potential model behaviors. Target cell viability was simulated over a range of bsAb concentrations, and the influence of different mechanisms on target cell viability was investigated with sensitivity analyses. Open-source Julia tools were used for model building and analyses.

Results: Analysis of the QSP model found that the internalization rate of the target receptors may be an important, yet often underestimated, factor for understanding bsAb efficacy *in vitro*. Simulations of the model indicated that high rates of receptor turnover can increase model predictions of efficacy, particularly at higher antibody concentrations.

Conclusions: Analyses of the *in vitro* QSP model for bsAbs highlighted the potential impact of receptor internalization on bsAb efficacy. Understanding target cell-specific internalization rates of free and bound receptors could be an important consideration during target selection for novel bsAbs and preclinical development of new bsAbs.

Methods

An *in vitro* QSP model for a bsAb was developed *de novo* using open-source Julia tools.

Model species:

- Number of effector and tumor cells
- Concentrations (nM) of
  - bsAb
  - Unbound effector receptors (Reff) and target receptors (Rtum)
  - Dimers between bsAb and either receptor (Reff:bsAb and Rtum:bsAb)
  - Trimers (Reff:bsAb:Rtum), the immune synapse between the effector receptor, the bsAb, and the target cell receptor

Key assumptions:

- The molecular representation of Reff and Rtum receptors is based on average expression on effector and tumor cells respectively
- Binding:
  - All binding and unbinding reactions follow mass action kinetics
- Receptor dynamics:
  - Receptors are synthesized and internalized/degraded at a constant rate per cell
  - Dimers are internalized at the same rate as unbound receptors (calculated from the internalization half-life of each receptor)
  - Trimers are not internalized
- Cell dynamics:
  - The number of effector cells remains constant over the short time frame of *in vitro* experiments
  - Tumor cells can grow or be killed
  - Tumor cell killing is triggered by the formation of the Reff:bsAb:Rtum trimer
  - Tumor cell killing is proportional to the average number of trimers per tumor cell
  - Tumor cell death leads to a release of the effector cell, including its receptor Reff, from the trimer

Model analysis

- Model was simulated using generalized parameters for a hypothetical bsAb to explore model behavior and thus validate model architecture
- Local sensitivity analysis was used to investigate influential parameters for this hypothetical bsAb
- Model was then parameterized to represent a specific, approved T-cell engaging bsAb, teclistamab (a CD3xBCMA bsAb). Model parameters were optimized to fit published *in vitro* data, validating the model's ability to represent experimental reality

Results: Generalized Model

***In vitro* QSP model**: model structure and hypothetical parameters

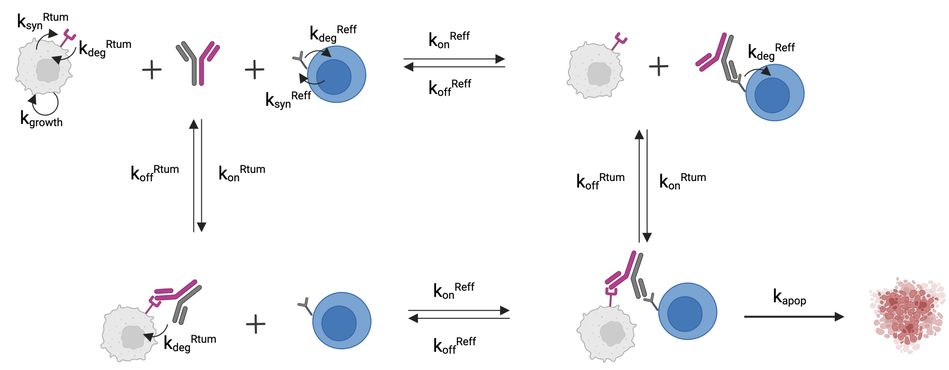

Figure 2. *In vitro* QSP model schematic. The model describes binding dynamics, tumor cell growth and killing, and receptor turnover processes.

Table 1. Parameterization describing a hypothetical (*in silico*) cytotoxicity assay, as typically performed during bsAb preclinical development.

Quantity	Value
E:T ratio	3:1
Incubation time	72 h
k_growth	24 h doubling time
KD_Reff	2 nM
KD_Rtum	0.2 nM
Reff_per_cell	30,000 receptors per effector cell
Rtum_per_cell	10,000 receptors per tumor cell
k_apop	1e-6 1/s * 1/#trimer/#tumor cell
Thalf_Reff	1 h
Thalf_Rtum	1 h

Example simulation

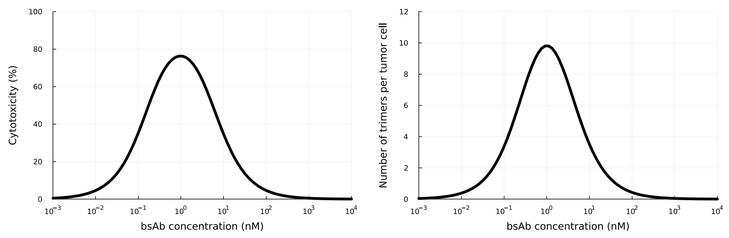

Figure 3. Simulated cytotoxicity (left) and number of trimers per tumor cell (right) across a range of bsAb concentrations. Cytotoxicity is calculated as the percentage of dead tumor cells at the end of the simulation. The example simulation illustrates the connection between the bell-shaped trimer curve and the bell-shaped cytotoxicity curve.

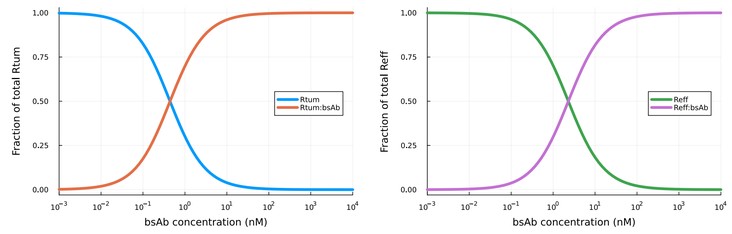

Figure 4. Simulated fractions of total Rtum receptors (left) and total Reff receptors (right). At low concentrations, most receptors are unbound (Rtum and Reff), and at high concentrations most receptors form dimers with the bsAb (Rtum:bsAb and Reff:bsAb).

Local sensitivity analysis: individual parameters are scaled up and down to evaluate their individual impact on the simulated cytotoxicity

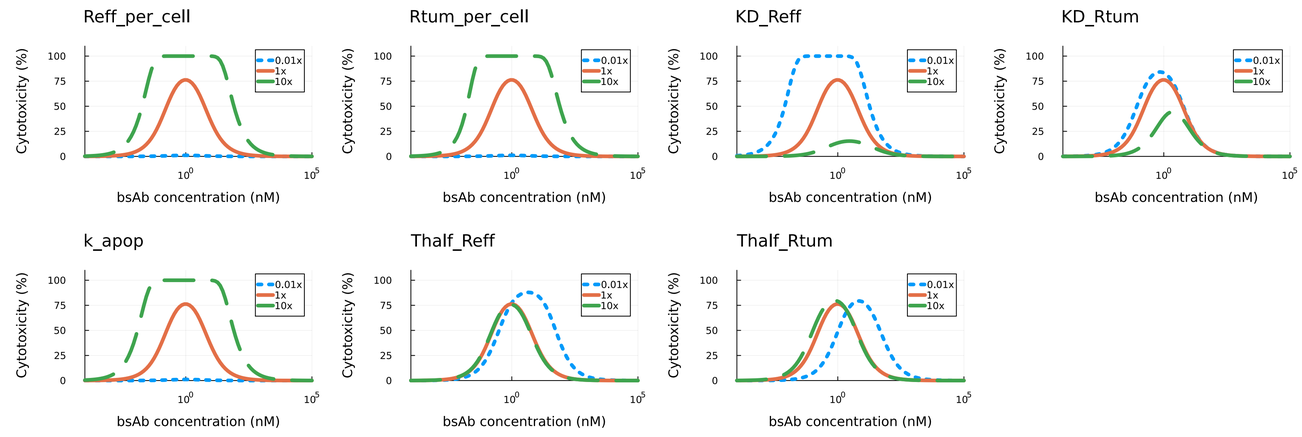

Figure 5. Simulated cytotoxicity levels from local sensitivity analysis. The solid red lines are the example scenario, the dotted blue lines are model output from scaling individual parameters by 0.01, and the dashed green lines are from scaling individual parameters by 10.

- Changes to receptor expression levels (Reff_per_cell and Rtum_per_cell) and the trimer-induced apoptosis rate (k_apop) were all highly influential on the simulation output, confirming expectations and thus supporting model validation
- Changes to the bsAb dissociation constants (KD_Reff and KD_Rtum) were also impactful although the changes to the dissociation constant of the weaker-binding target receptor (in this case KD_Reff) were more influential
- Reducing either of the receptor internalization half-lives (Thalf_Reff and Rhalf_Rtum) increased the concentration needed to reach maximum cytotoxicity, an outcome that highlights the importance of understanding this aspect of trimer formation in the context of optimal dose selection as a novel bsAb progresses towards the clinic

Results: Application of the *in vitro* QSP model to recapitulate teclistamab cytotoxicity

The QSP *in vitro* model was reparameterized to describe teclistamab (a CD3xBCMA bsAb that has been approved for relapsed/refractory multiple myeloma), and two parameters were fit to published *in vitro* data.

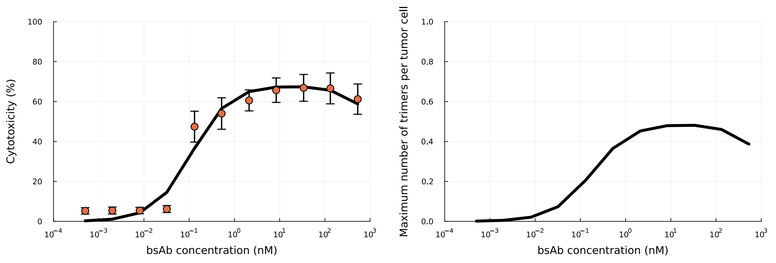

Figure 6. Cytotoxicity (left) and projected maximum number of trimers per tumor cell (right) across teclistamab concentrations. Orange points are the observed data (extracted from [2] for the H929 cell line), and the black lines are model output. Optimizing the killing rate (k_apop) and the CD3 half-life (Thalf_Reff) was sufficient to describe the data well.

Table 2. Parameterization for *in vitro* QSP bsAb model of teclistamab to support *in silico* recapitulation of published experimental results.

Quantity	Value	Source
E:T ratio	5:1	[2]
Incubation time	48 h	[2]
k_growth	50 h doubling time	[3]
KD_Reff	28.03 nM	[4]
KD_Rtum	0.18 nM	[4]
Reff_per_cell	30,000 receptors per effector cell	[5]
Rtum_per_cell	13,173 receptors per tumor cell	[2]
k_apop	1.7e-5 1/s * 1/#trimer/#tumor cell	Optimized
Thalf_Reff	0.1 h	Optimized
Thalf_Rtum	24 h	[6]

Conclusions

- Analyses of the *in vitro* QSP model for bsAbs highlighted the potential impact of receptor internalization on bsAb efficacy
- Understanding the internalization rates of receptors was shown to be an important consideration during target selection as well as the interpretation of preclinical development outcomes of new bsAbs
- The *in vitro* QSP model for bsAbs may be extended to test additional hypotheses (such as the effect of effector cell proliferation, or the impact of the choice of binding affinities)
- Validated *in vitro* QSP models are often used as the basis for a tumor compartment in an *in vivo* PKPD QSP model [7]

References

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