Background
Conditionally activated bispecific T cell engagers (TCEs) have the potential to provide a larger therapeutic window by reducing off-site on-target toxicity. Conditionally Bispecific Redirected Activation (COBRAs) are novel TCEs designed to be activated preferentially in the tumor microenvironment (TME).\textsuperscript{1,2} The conditionality of the COBRA prodrug is mediated upon binding of the high affinity target binding domain to the tumor antigen and by cleavage of the prodrug by matrix metalloproteases such as MMP9/2. The cleavage of the prodrug results in the release of the inactive CD3e VH/VL domains leading to the formation of the active dimer, responsible for tumor killing. In order to understand the dependence of treatment effect on drug and patient properties for this novel modality, we developed a mathematical model of COBRAs’s mechanism of action.

Methods
A mechanistic model was developed to describe the preclinical pharmacokinetics, target engagement and immune synapse formation in the TME, and tumor volume change due to resulting T-cell dependent tumor cell-mediated cytotoxicity (TDCC). Unknown parameters were optimized using \textit{in vitro} TDCC data and \textit{in vivo} efficacy data. Sensitivity analyses were conducted to investigate the influence of molecule- and TME-specific characteristics on synapse formation and treatment effect.

Results
The model recapitulates the experimental data \textit{in vitro} and \textit{in vivo}. We used the model to identify parameters that govern the synapse formation and treatment outcome, such as COBRA affinity to TAA and CD3 (figure 1A), and the active protease concentration in the TME (figure 1B). The model also suggests that the drug-induced antigen internalization along with the abundance of target cells and effector T cells can impact the predicted treatment effect of COBRA and that COBRA concentration-synapse formation relationship does not exhibit the bell-shaped TCE concentration-synapse profile due to its conditionality on homodimerization once cleaved.

Conclusions
The model framework was used to integrate preclinical datasets and project the short-term treatment response in human. This work demonstrates the impact of conditionality of the COBRA design on its dose-response relationship. Importantly, factors such as target abundances and protease activity in the TME can strongly influence synapse formation and the resulting anti-tumor effect, and therefore may serve as baseline biomarkers for patient stratification.

REFERENCES

Ethics Approval
The animal study protocol received ethics approval by In-Vivo Technologies, Inc Institutional Animal Care and Use Committee IVT-17-002-Y6.