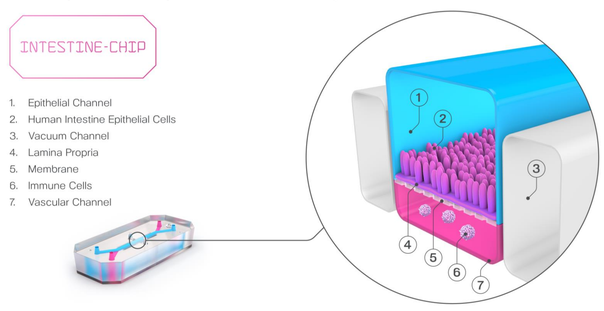
Assessing the Safety Liability of T-Cell Bispecific (TCB) Antibodies Using Organs-on-Chips Technology

Contributors: S. Jordan Kerns[1], Ville J. Kujala[1], Marianne Kanellias[1], John Sauld[1], William Tien-Street [1], Heather S. Grant[1], Katia A. Karalis[1], Geraldine A. Hamilton[1], Lorna Ewart[1], Adrian B. Roth[2], Annie Moisan[2] and Nikolche Gjorevski[2]

Abstract: T-cell bispecific antibodies (TCBs) are a promising class of immunotherapeutic agents that promote tumor cell killing by physical crosslinking of effector T-cells to target expressing cells. While effective in targeting less-immunogenic tumors, TCBs have safety liabilities, which may express at low levels. Preclinical assessment of safety risks is crucial but species differences between human and rodent immune responses necessitate the development of advanced human cell-based models for safety profiling. While tractable, conventional killing assays lack physiological organization and cytoarchitecture and fail to accurately predict efficacy and off-tumor cytotoxicity.

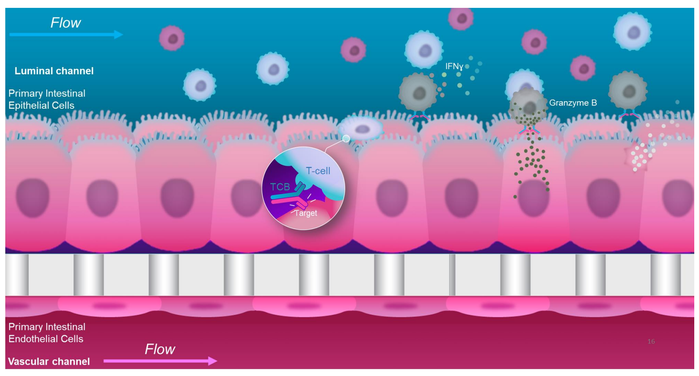
Here, we show the robustness of the Colon Intestine-Chip to capture variability in immune cell activation at physiologically relevant TCB concentrations. With increased confidence in the predictive capabilities of the model, we then confirmed expected regional dependence to gastrointestinal (GI) toxicity of the TCB by showing elevated immune cell activation in the large- versus small-intestine. Further, we demonstrated vascular recruitment and transmigration of circulating immune cells to the intestinal epithelium to more accurately capture the *in vivo* mechanisms of TCB-mediated toxicity. Together, these data show that our models are suitable for safety profiling of novel engineered immunotherapies and provide clinically relevant results.

A.



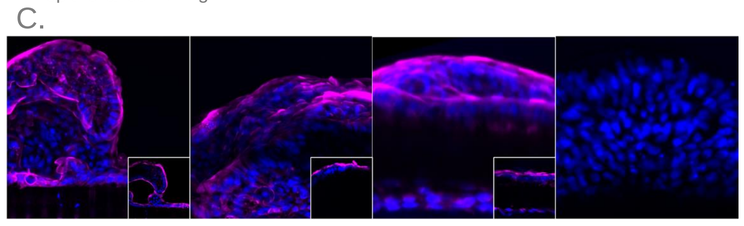
A. Schematic representation of the Intestine-Chip highlighting the different fluidic compartments and corresponding epithelial and endothelial cell types

B.



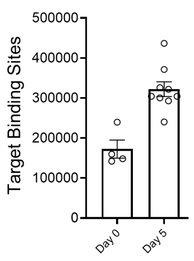
B. Modeling on-target/off-tumor safety with acute exposure of T-cells and TCB to target expressing epithelial cells. TCBs physically link immune cells to the target causing activation and release of their cytotoxic payload and downstream epithelial cell killing

C.



C. Immunofluorescence cross sections of the Colon Intestine-Chip with an inset of a single cross sectional plane showing the cytoarchitectures and correct, apically polarized cancer Target antigen expression relative to a background

D.



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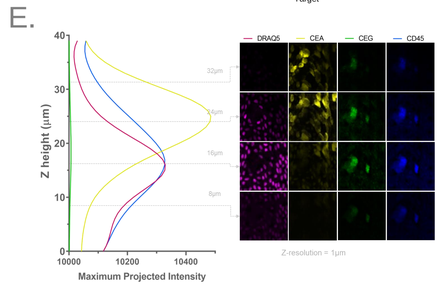
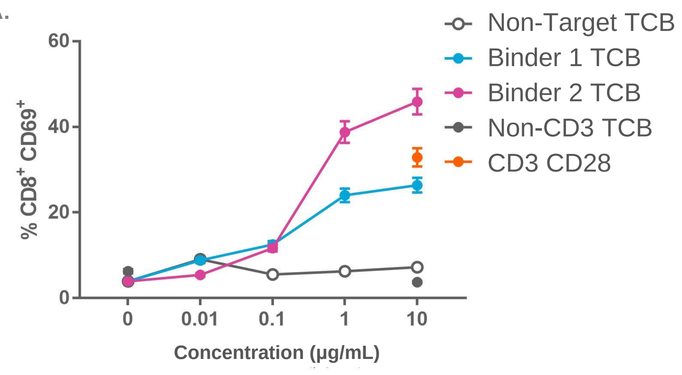


D. Quantification of apical cancer target sites in the Colon-Chip epithelium through flow cytometry pre-seeding, Day 0 and Day 5 of culture

E. Z-plane intensity analysis showing apical polarization of the target. The polarization and surface exposure of the target is important as the TCBs will need these antigens surface exposed in order to link to migrating immune cells

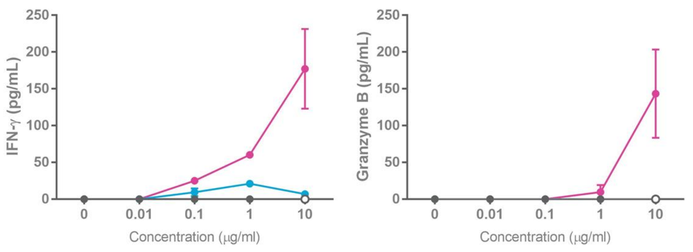
TCB-Mediated Immune Cell Activation

A.



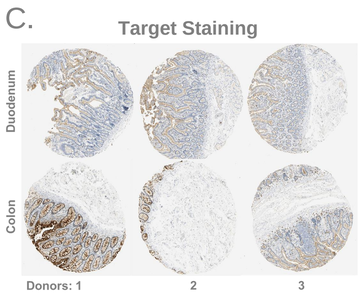
A. TCBs show a target-dependent increase in CD69⁺ immune cell activation above the monospecific non-target and non-CD3 controls as measured by flow-cytometry of immune cells harvested from the Colon Intestine-Chip 72 hours after treatment. The model discriminates between Binder 1 and Binder 2 TCB variants, indicative of the Colon Intestine-Chip being able to discriminate and predict the safety profile of engineered TCB variants

B.



B. Target- and TCB-dependent immune cell activation also corresponds to inflammatory cytokine production (IFN-γ and granzyme-B) indicative of clinically relevant safety signals

C.



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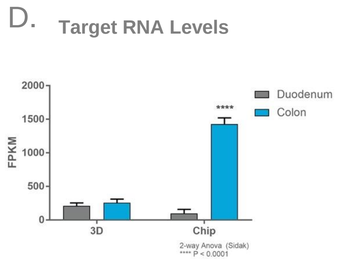
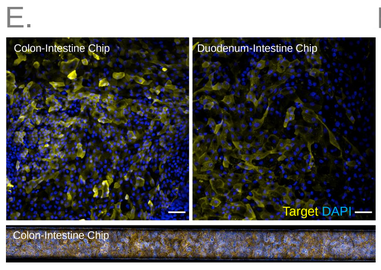


C. Histological sections of human colon and duodenum sections stained for target expression from 3 different donors. The relative intensity of target staining reveals regionally dependent expression of the target. These differences in target expression could help explain the poor predictivity of other preclinical models.

D. RNA-seq transcript levels of the target gene in healthy duodenum and colon 3D organoids in comparison to these enterocytes reconstituted on the Intestine-Chip showing that the Colon Intestine-Chip epithelium recapitulates the elevated target expression observed clinically.

E.



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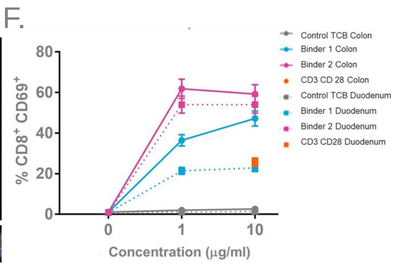
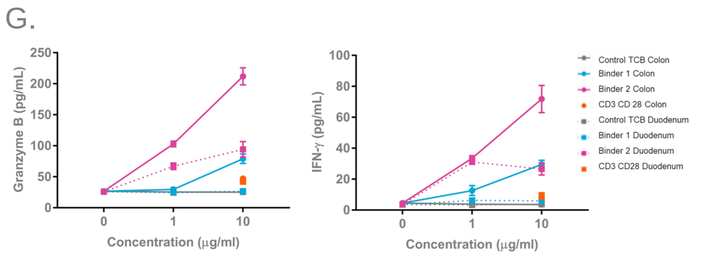


E. Representative immunofluorescent micrograph of target expression in the epithelial compartment of the Colon Intestine-Chip and Duodenum Intestine-Chip

F. Flow-cytometry analysis of CD69⁺ CD8⁺ T-cells at 72 hours post-treatment demonstrates the ability of the Colon- and Duodenum-Chips to discriminate between the low (Binder 1) and high-affinity (Binder 2) TCB treatments as well as the regional differences in the tissue expression levels of the target (n=3; mean ± SEM)

G.

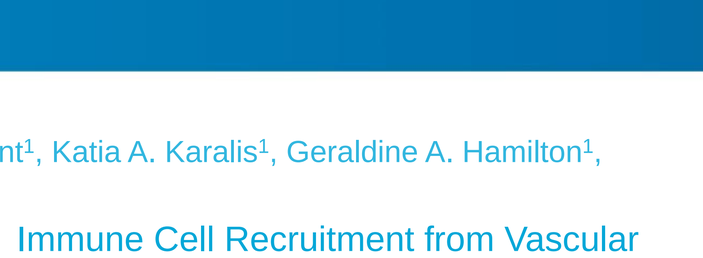


G. Cytokine analysis of supernatant collected from epithelial channels after 72 hours of treatment highlights the ability of the Colon and Duodenum Intestine-Chips to discriminate between the low (Binder 1) and high-affinity (Binder 2) TCB treatments as well as the regional differences in the tissue expression levels of the target (n=3; mean±SEM)

Immune Cell Recruitment from Vascular to Epithelial Compartment

A.

B. C. D.



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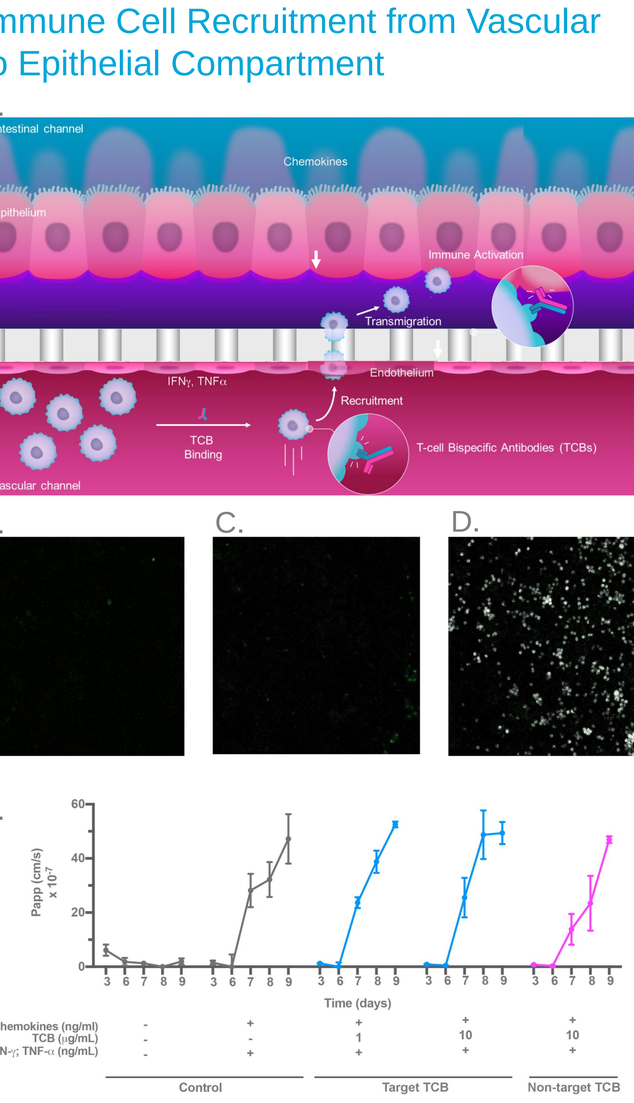

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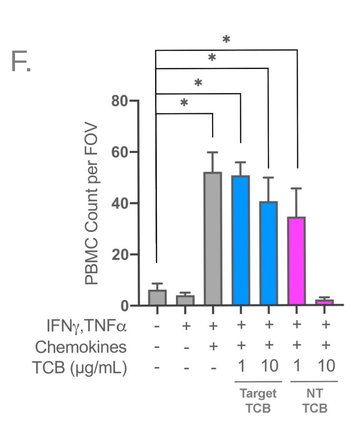

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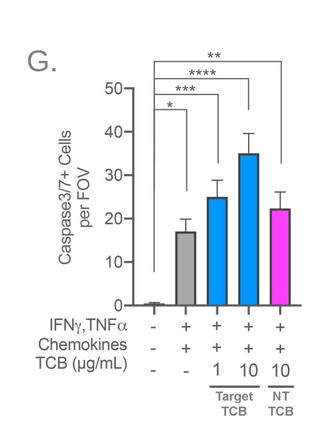

Figure 2: Vascular recruitment of immune cells.

A. Schematic outlining vascular recruitment concept
B. PBMC infiltration to epithelium in untreated Colon Intestine-Chip.
C. PBMC infiltration to epithelium in Colon Intestine-Chip treated with inflammatory cytokines only.
D. PBMC infiltration to epithelium in Colon Intestine-Chip treated with inflammatory cytokines, chemokines, and 1 ug/mL Target TCB.
E. Inflammatory cytokine exposure leads to increased apparent permeability (P_{app}) in chip.
F. Apically dosed chemokines induce PBMC migration towards epithelium. Number of cells are quantified in the epithelial compartment only. NT: Non-target.
G. Epithelial cell apoptosis is increased in response to vascular dosing of PBMCs treated with TCBs. NT: Non-target.

Qualifying the Intestine-Chip for Testing the Safety Liability of Immunotherapies

Results

- Confirmed the subcellular polarization and expression of the target on the Colon and Duodenum Intestine-Chips
- Demonstrated the predictive capability the Intestine-Chip for accurate assessment of on-target/off-target safety risk with acute exposure of immune cells and TCBs via direct administration to the target expressing epithelium
- Recapitulated expected regional differences in target expression and the impact of TCB binding affinity on the modulation of immune cell activation
- Development of new methods for the recruitment of immune cells from the vascular channel lead to target-mediated epithelial apoptosis when treated with TCBs

Summary/Conclusions

- The human Intestine-Chips better recapitulates the *in vivo* context and can be used to assess and predict the safety liabilities of TCBs.

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