Chimeric antigen receptor-T cell efficacy can be evaluated on an Organ-Chip model system

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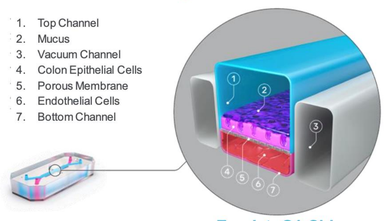
OBJECTIVE: The need for human-centric model systems that can test the efficacy of chimeric antigen receptor (CAR) therapies is expanding rapidly, as these hold great promise for cancer treatment. We recently developed a system for inflammatory immune cell recruitment on the human Colon Intestine-Chip as a model for inflammatory bowel disease (IBD). The goal of the current study was to develop a novel system for measuring the recruitment and killing capacity of CAR-T cells in an Organ-Chip system. Our findings herein suggest that the human-centric Organ-Chip model can evaluate the efficacy of CAR-T cell therapies, and in particular, provide a system that integrates both the recruitment and killing aspects of CAR-T function.

Modeling the Intestine with Organ-on-a-Chip Technology

Colon Intestine-Chip

1. Top Channel
2. Mucus
3. Vacuum Channel
4. Colon Epithelial Cells
5. Porous Membrane
6. Endothelial Cells
7. Bottom Channel



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Key Functionality

- Multi-lineage epithelial differentiation
- Microvilli, polarization, mucin secretion
- Tight junctions & low permeability
- Human-relevant gene expression

Human cells: Tissue-specific biopsy-derived organoids & primary microvascular endothelium
Mechanical forces: Tunable media flow rates and cyclic stretch

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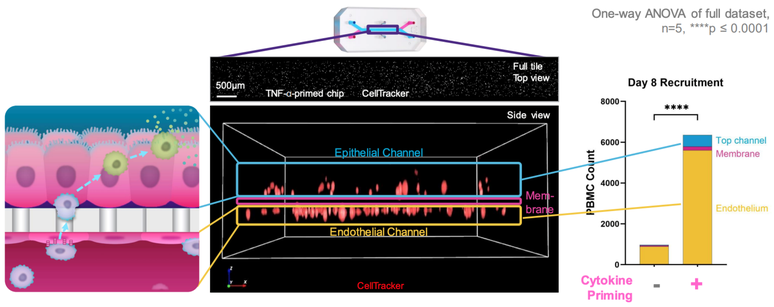
Apostolou A, et.al, Cell Mol Gastroenterol Hepatol. 2021;12(5):1719-41

PBMC Recruitment, Attachment, and Migration On-Chip



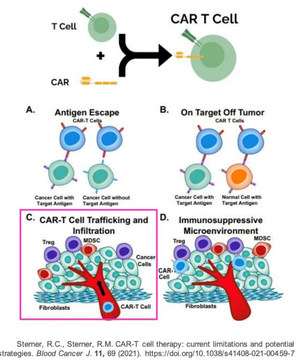
One-way ANOVA of full dataset, n=5, ****p ≤ 0.0001

Robust, *inflammation-specific* recruitment & migration of PBMCs on-chip

Living Drugs Derived From Human Immune Cells | CAR-T

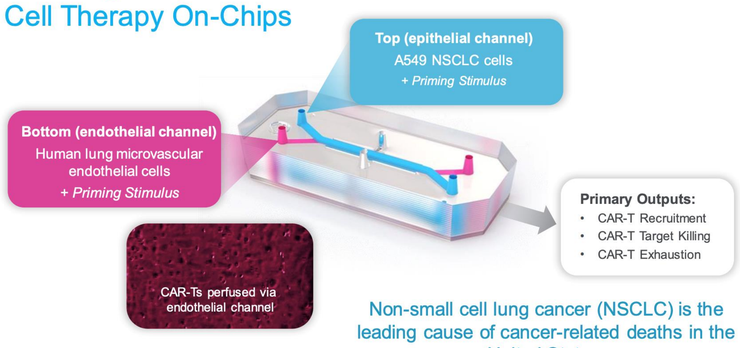
CAR-T Challenges & Opportunities

- T cells can be engineered to express **cancer-specific** Chimeric Antigen Receptors (CAR)
- First CAR-T therapy against B cell malignancies approved in 2017, with 5 others FDA approved for **blood cancers** since
- **Solid tumors** account for ~**90%** of all adult human cancers, yet CAR-T therapy against them has been **largely unsuccessful**
- **Immune cell trafficking** & immunosuppressive factors within the TME are key factors for this discrepancy
- Current *in vitro* models fail to capture challenges of CAR-T recruitment & infiltration



Sterner, R.C., Sterner, R.M. CAR-T cell therapy: current limitations and potential strategies. *Blood Cancer J.* **11**, 69 (2021). https://doi.org/10.1038/s41408-021-00459-7

Modeling Adoptive Cell Therapy On-Chips



Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths in the United States.

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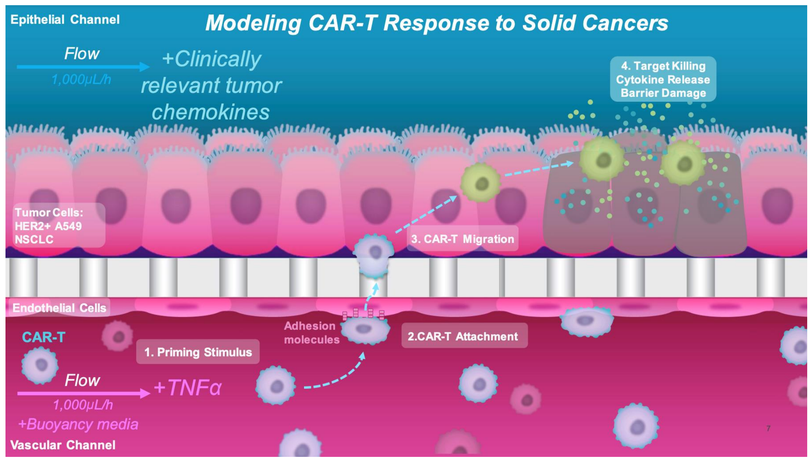
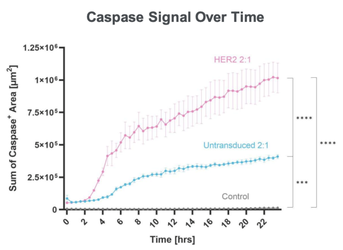


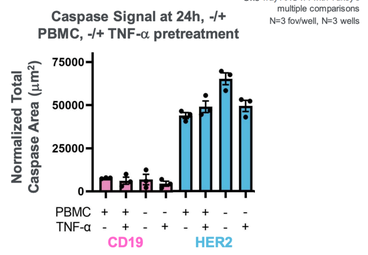
A B

Validation of CAR-T Killing on A549 Plates

One-way ANOVA with Tukey's multiple comparisons N=3 fov/well, N=3 wells

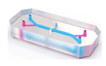


Specific CAR T cytotoxicity demonstrated with Caspase signal over 24h

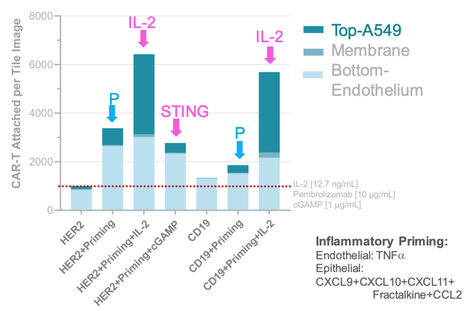


HER2 showed specific Caspase killing vs. CD19 CAR-T TNFα treatment or PBMC addition did not affect killing

CAR-T Recruitment & Migration On-Chip



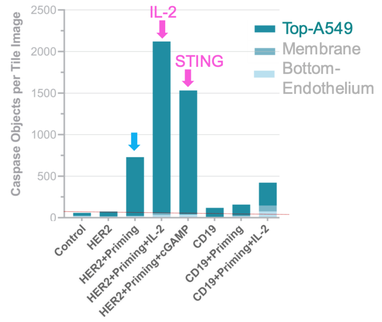
- HER2 & CD19 show enhanced recruitment following ***inflammatory priming (P)*** chips
- IL-2 family cytokines are shown to improve CAR-T robustness
- Pretreatment with ***IL-2*** enhances CAR-T migratory *'fitness' on-chip*



CAR-T Target Cytotoxicity On-Chip - *Caspase*

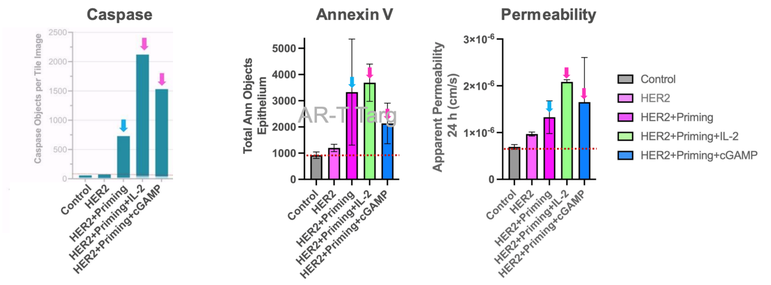
- Pretreatment with IL-2 enhances HER2 mediated target killing
- cGAS-STING pathway supports anti-tumor immunity
- STING agonists are in clinical development as IO therapeutics

Wu, PNAS 2022 119 (49) e2214278119



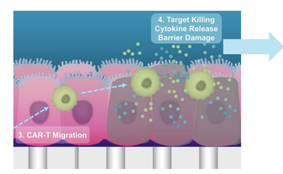
HER2 CAR-T shows robust & selective target killing vs CD19 CAR-T.
IL-2 & STING agonist increase killing – via complementary mechanisms

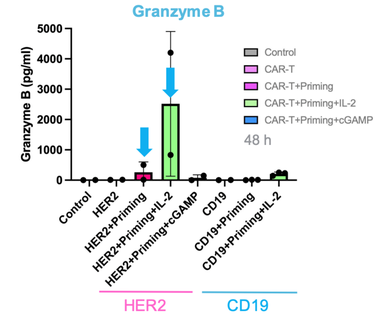
CAR-T Target Cytotoxicity On-Chip – ***Annexin V & Permeability***



CAR-T Cytotoxicity On-Chip – ***Effluent Analysis***

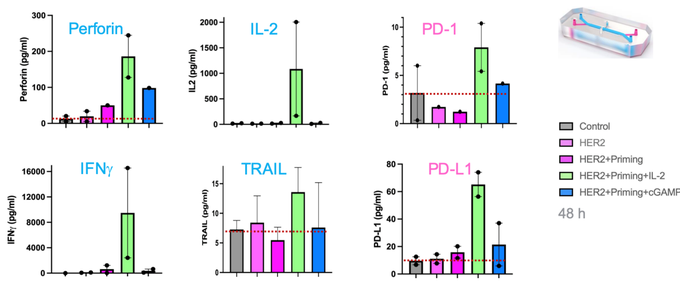
Effluent Analysis:
- Media sampled from outlets every 24 h
- Analyzed by multiplexed MSD





Antigen- & time-dependent CAR-T activity can be assessed through effluent

CAR-T Target Cytotoxicity & Exhaustion On-Chip - Effluent



Antigen- & time-dependent CAR-T activity can be assessed through effluent

CAR-T Cell Recruitment Summary and Next Steps

Model & Application Characterization to Date
- Show CAR-T recruitment & modulation
- Demonstrated specific cytotoxicity
- Efficacy of IO co-therapies
- Assessment of CAR-T exhaustion
- Validated workflow for meaningful CAR-T evaluation

Next Steps
- Assess efficacy & specificity for diverse CAR-T Targets
- Screen novel CAR designs and IO co-Therapies

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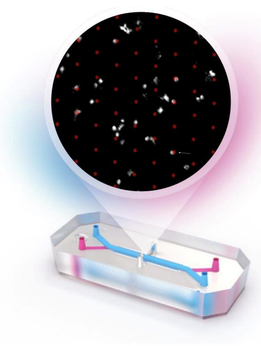

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