Long-term Characterization of T Cell Product Interactions with *in vitro* 3D Tumor Models using the Go-Rex Platform

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Introduction

- *In vitro* assays are valuable tools for pre-clinical development and assessment of function of T cell therapy products.
- To be effective treatments, T cells have to:
  - ❑ Detect and migrate towards chemokine gradients
  - ❑ Navigate and infiltrate the tumor environment
  - ❑ Eradicate tumor cells
  - ❑ Proliferate and persist for extended efficacy
- Current *in vitro* assays used to assess anti-tumor activity of T cells often uncouple important T cell functions from the measured outcome. For example, ^{51}Chromium release or other suspension-based co-culture assays assess T cell cytotoxicity but not their ability to migrate or infiltrate tumors, which is a crucial factor for patient outcome.
- Furthermore, current *in vitro* assays characterize T cell function and cancer cell interactions over short timeframes.
- New tools and longer *in vitro* timeframes are needed to adequately assess critical properties of adoptive cell therapies: T cell migration, tumor infiltration and killing, as well as T cell expansion, and persistence.
- ***The Go-Rex, developed here, is an innovative platform that can assess multiple T cell properties, including killing, over much longer periods of time compared to current in vitro assays***.

Conventional *in vitro* assay devices limit cell growth to short timeframes

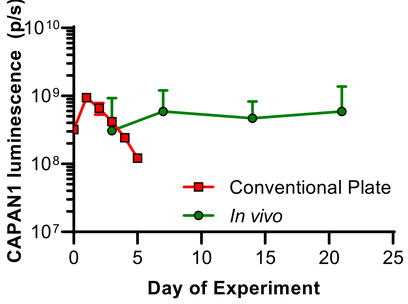

Figure 1. Cancer cell growth in conventional tissue culture devices fails to reach *in vivo* timeframes. CAPAN1 pancreatic cancer cells were engineered to express firefly luciferase to create bioluminescent cells, allowing measurement of luminescence over time representative of cell growth. Luminescence of CAPAN1 cells over time was determined *in vitro* using a conventional tissue culture dish versus an *in vivo* animal model.

The Go-Rex device allows long-term culture without disruption

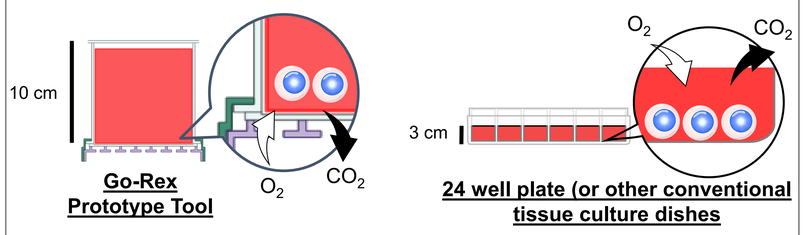

Figure 2. Gas permeable membrane enables long-term culture of cells. The Go-Rex builds upon G-Rex® technology which permits gas exchange at the bottom of the device, allowing a significantly larger media-to-surface area ratio and providing a superior source of nutrients relative to conventional assay devices.

Tumor growth in a Go-Rex provides unprecedented *in vitro* timeframes

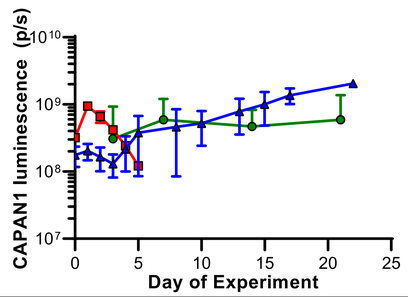

Figure 3. Comparison of experimental timeframes. The Go-Rex device provides an expanded experimental timeframe for *in vitro* tumor assessment similar to *in vivo* experiments.

A 6 chamber Go-Rex "maze" demonstrates T cell migration

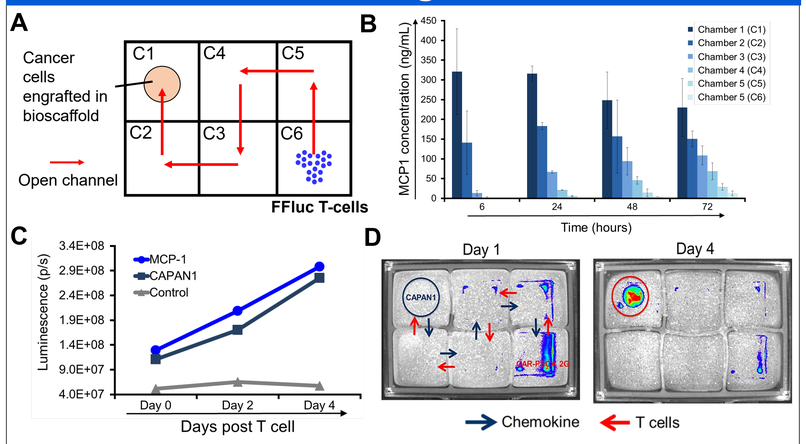

Figure 4. T cells can sense and migrate towards chemokine gradients. A) Prototype chemokine "maze" device where bioluminescent T cells are placed distant from the tumor **B)** A chemokine gradient can be established over 72 hours. **C)** A buildup of firefly-luciferase (FFluc)-modified T cells (luminescence) is detected in chamber 1 (C1 from panel A) over time using a chemokine (MCP-1) or conditioned media from CAPAN1 cells. **D)** Bioluminescent imaging illustrating T cell migration from chamber C6 to chamber C1, which contains CAPAN1 cells engrafted in a bioscaffold to form a 3D tumor.

Single-chamber Go-Rex Killing Assay

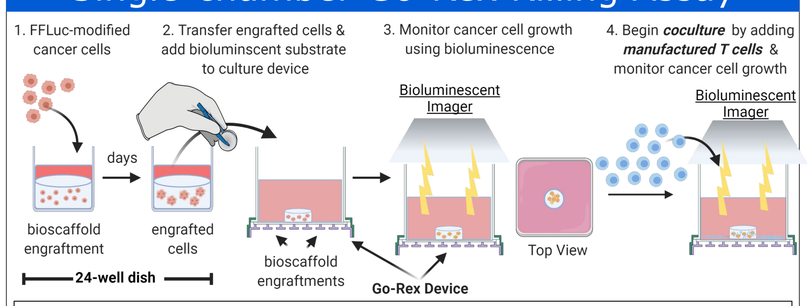

Figure 5. Firefly-luciferase (FFluc)-modified target cells are cultured in a bioscaffold to support 3D growth before transfer to a Go-Rex device. Manufactured T cells are then added, and bioluminescence of 3D tumors are monitored to determine T cell anti-tumor activity.

The Go-Rex distinguishes 1st and 2nd generation CAR-T cells, unlike traditional *in vitro* assays

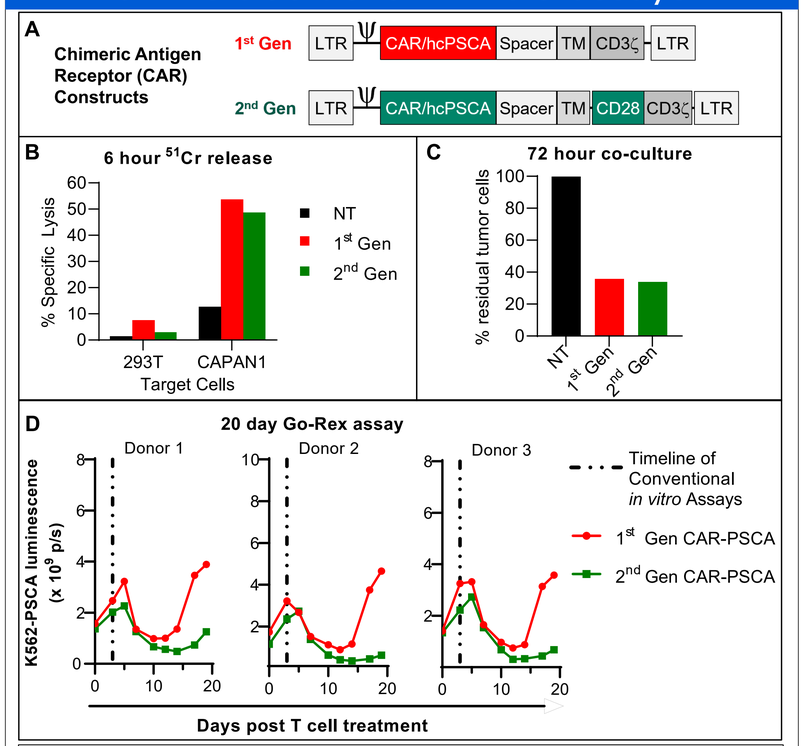

Figure 6. A) 1st and 2nd generation chimeric antigen receptors (CARs). **B)** Traditional short-term chromium (^{51}Cr) release and **C)** flow cytometry assays do not distinguish between 1st & 2nd generation CARs. NT = non-targeting **D)** Long-term Go-Rex co-culture assays comparing 1st and 2nd generation CAR-Ts targeting PSCA-expressing K562 tumors clearly demonstrate their differences in anti-tumor activity already proven in the clinic. Figures B & C adapted from Anurathapan *et al.* Kinetics of tumor destruction by chimeric antigen receptor-modified T cells. *Mol Ther.* 2014;22(3):623-633.

The Go-Rex allows *in vitro* characterization of CAR-T co-stimulatory domains

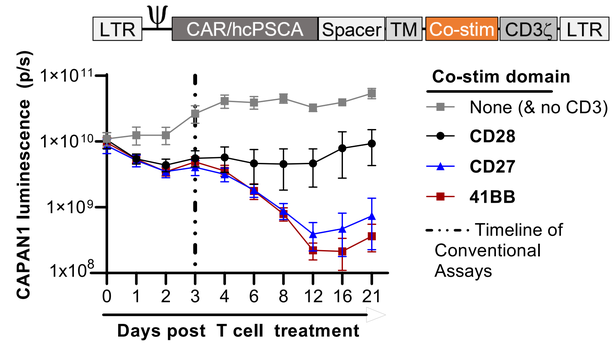

Figure 7. The anti-tumor activity of PSCA-targeting CAR constructs with different co-stimulatory domains was evaluated against bioluminescent PSCA-expressing CAPAN1 tumor cells.

The Go-Rex demonstrates *in vitro* efficacy of mTAA-specific T cells manufactured by Marker Therapeutics

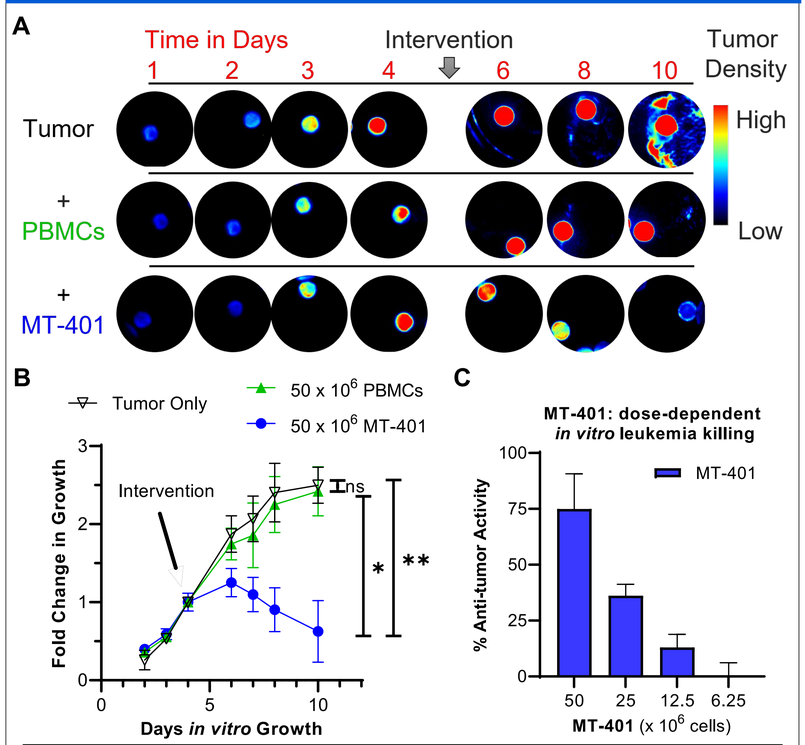

Figure 8. A) Firefly-luciferase-modified leukemic cells (THP-1) were engrafted in a 3D bioscaffold, and bioluminescent images were acquired. These 3D tumors were treated ("intervention") with either PBMC starting material or MT-401 product. MT-401 is a non-engineered multi-tumor associated antigen (mTAA)-specific T cell therapy from Marker Therapeutics that is currently being explored in the clinic for the treatment of AML. **B)** Quantitation of images in A) show a significant decrease in *in vitro* tumor growth when treated with MT-401. **C)** Performing the Go-Rex killing assay at different doses of MT-401 shows a clear dose-dependent reduction in *in vitro* tumor growth.

Conclusions

- **The Go-Rex permits assessment of multiple T cell properties**:
  - ✓ **Migration**: establishment of chemokine gradients allows assessment of bioluminescent T cell migration
  - ✓ **Infiltration**: use of bioscaffolds can assess infiltration
  - ✓ **Killing**: Eradication of bioluminescent target cells
  - ✓ **Proliferation**/**persistence**: endpoint measurements can assess T cell growth and clonality in the presence of tumor models over long periods.
- The Go-Rex provides a critical bridge between *in vitro* and *in vivo* experimental validation of cell therapy products.

Acknowledgements

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