Xsphera BIOSCIENCES CT

A Human Tissue Based Platform to Advance Cancer Cell Therapy Development

Solid Tumors Present Challenges for Cancer Cell Therapies

Cancer cell therapy holds the promise to revolutionize the options for patients refractory to conventional treatments including immune checkpoint inhibitors[1].

While CAR-T and other cell therapies have shown remarkable outcomes in hematologic malignancies, their efficacy in solid tumors has proved to be more challenging[2].

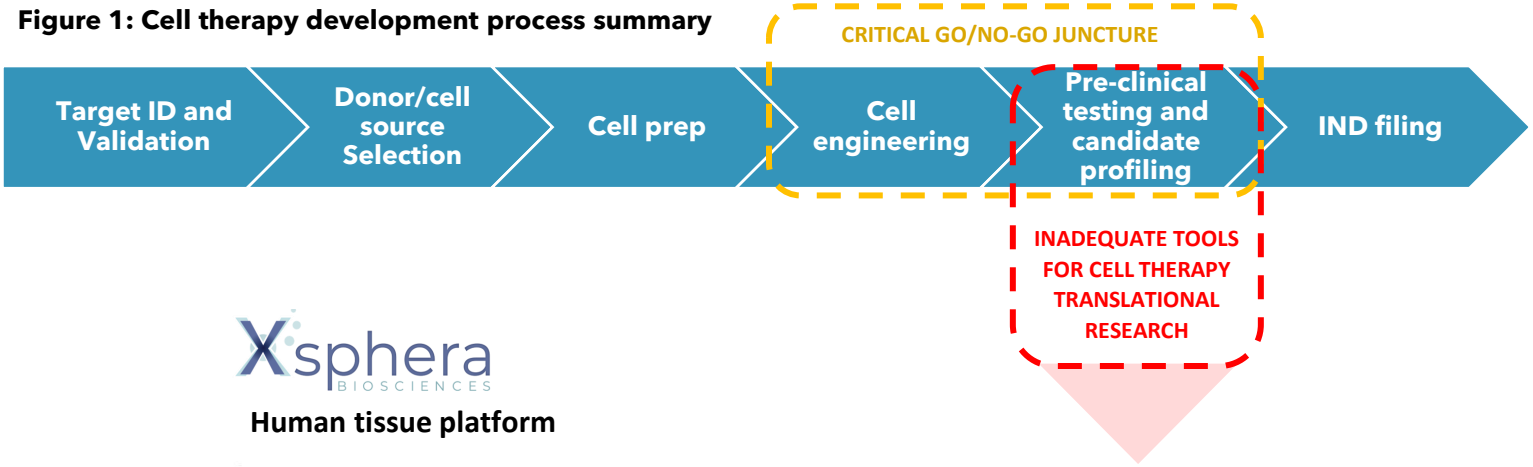
Only 1 cell therapy has been approved for solid tumors and a mere 14% of cell therapies advance from phase II trials, which can be primarily attributed to high toxicity or lack of efficacy[3].

A number of reasons underpin the lack of efficacy seen in the clinic. This is because solid tumors pose unique challenges for cell therapy persistence, migration, and chemotaxis[4].

Addressing key translational model barriers for cell therapy

- ☑ Migration and chemotaxis through heterogeneous solid tumor environments
- ☑ Persistence and exhaustion of cell therapy in diverse immune suppressive environments
- ☑ Representation of human tumor diversity
- ☑ Ability to predict the in-situ patient response

Figure 1: Cell therapy development process summary



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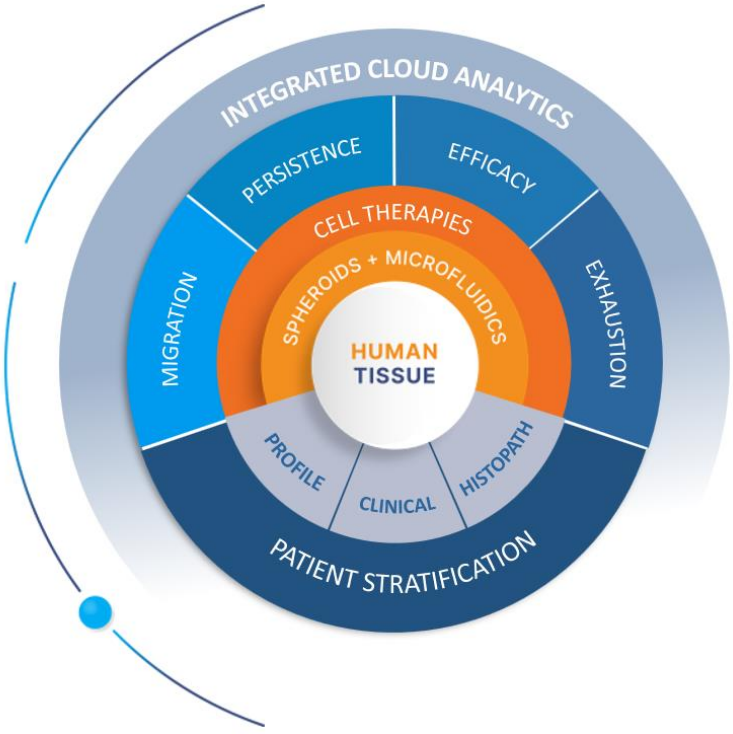
Human tissue platform



This white paper presents the power of microfluidics and human tumor tissue alongside your cell therapy program:

Better Lead Optimization

✓ Engineer candidates to elicit better chemotaxis and migration through the human TME

Better Candidate Selection

✓ Select for candidate properties that reduce exhaustion in human TME and under physiological stress

Better Candidate Profiling

✓ Test candidates in diverse tumor tissues to establish patient stratification

Support your analyses, results and conclusions with AI-enabled, integrated analytics

Box 1 abstract:

Cancer cell therapy has emerged as a promising next-generation modality for the treatment of solid tumors. However, moving assets from pre-clinical development to clinical implementation presents significant challenges, particularly in evaluating efficacy and understanding cell activity within complex tumor environments. This white paper explores the current limitations of cell therapy translational research and how the **Xsphera CT** program can improve and de-risk pre-clinical to clinical stage programs by addressing the gaps and unmet needs, and the critical role of microfluidics in bridging this gap. By providing relevant in-vitro models that mimic the complexities of the tumor microenvironment, microfluidic systems that bring together autologous, 3D human tumor architecture offer insights into cell therapy efficacy, patient heterogeneity, and treatment mechanisms, ultimately facilitating precision medicine approaches in cancer treatment.

The drug development cycle for cell therapy lacks humanized models for candidate selection and profiling

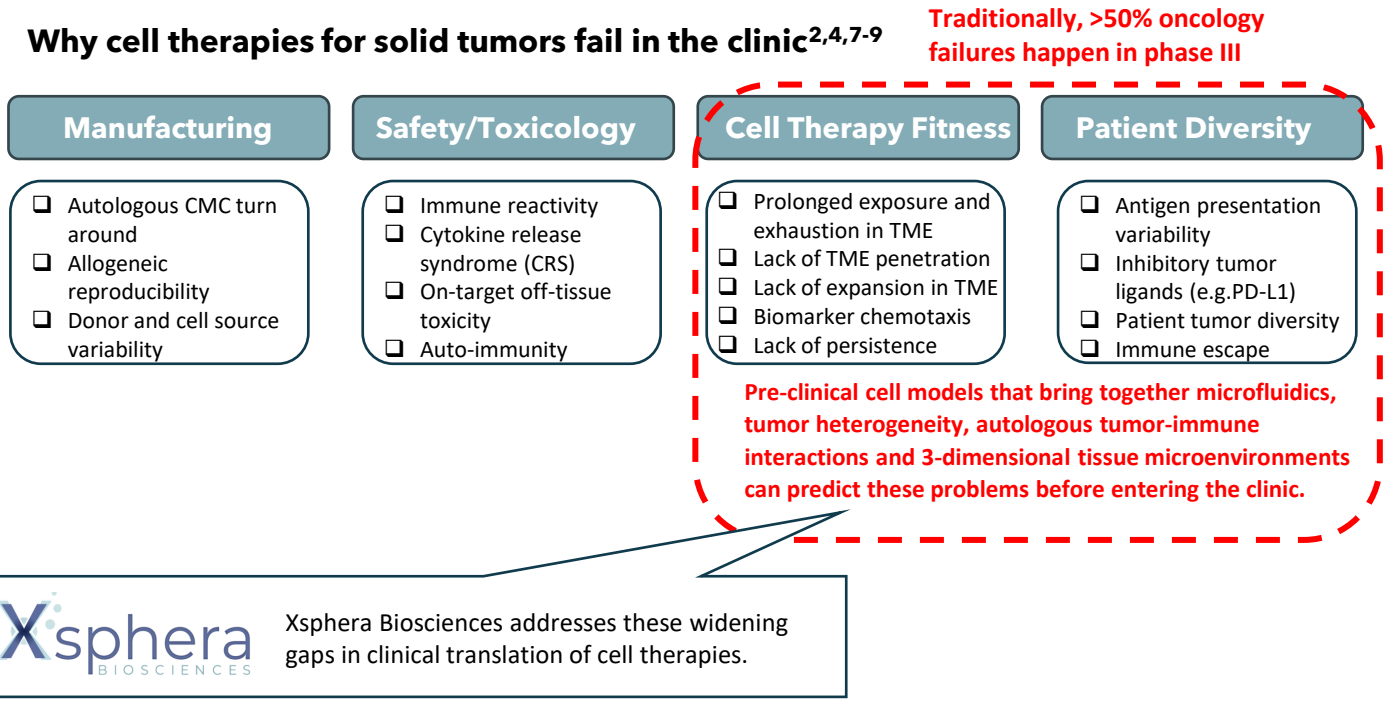
Safety, preliminary efficacy, immunogenicity and patient stratification are key limitations to translational success

90% of all drugs fail progress to approval, >30% fail going into phase II and >50% fail in phase III[5]. While these numbers account for multiple modalities, cell therapy is considered among them. These failures are largely attributed to early pre-clinical decisions that affect safety, toxicity and efficacy[6].

Lack of translational models negatively impact clinical success of cell therapy

Translating pre-clinical findings into clinical success remains a significant bottleneck for cell therapy. Traditional biological models, including cell lines and animal models, often fail to accurately recapitulate the complexities of human tumors, leading to discrepancies between pre-clinical efficacy and clinical outcomes.

Existing models often overlook critical aspects of the tumor microenvironment, such as spatial organization, nutrient gradients, and intercellular interactions, which play pivotal roles in therapy response and resistance.



Pre-clinical models for cell therapy drug development are emerging

A number of advanced in-vitro models are emerging to enable the study of immuno-oncology drugs such as checkpoint inhibitors[11,12]. 2D in-vitro models remain the go-to platform for testing cell therapy affinity, potency and activity before developers advance candidates to in-vivo models and ultimately patients. However, these tools limit the unique dynamics of cell therapy such as migration through the extracellular matrix (ECM) and persistence in the tumor-immune microenvironment (TIME).

Migration through ECM:
Controlled experiments should evaluate cell therapy target tropism and chemotaxis in candidate selection stages.

Persistence in the TIME:
Key experiments in the lead optimization stage should ensure genetic modifications withstand the pressures of the TIME.

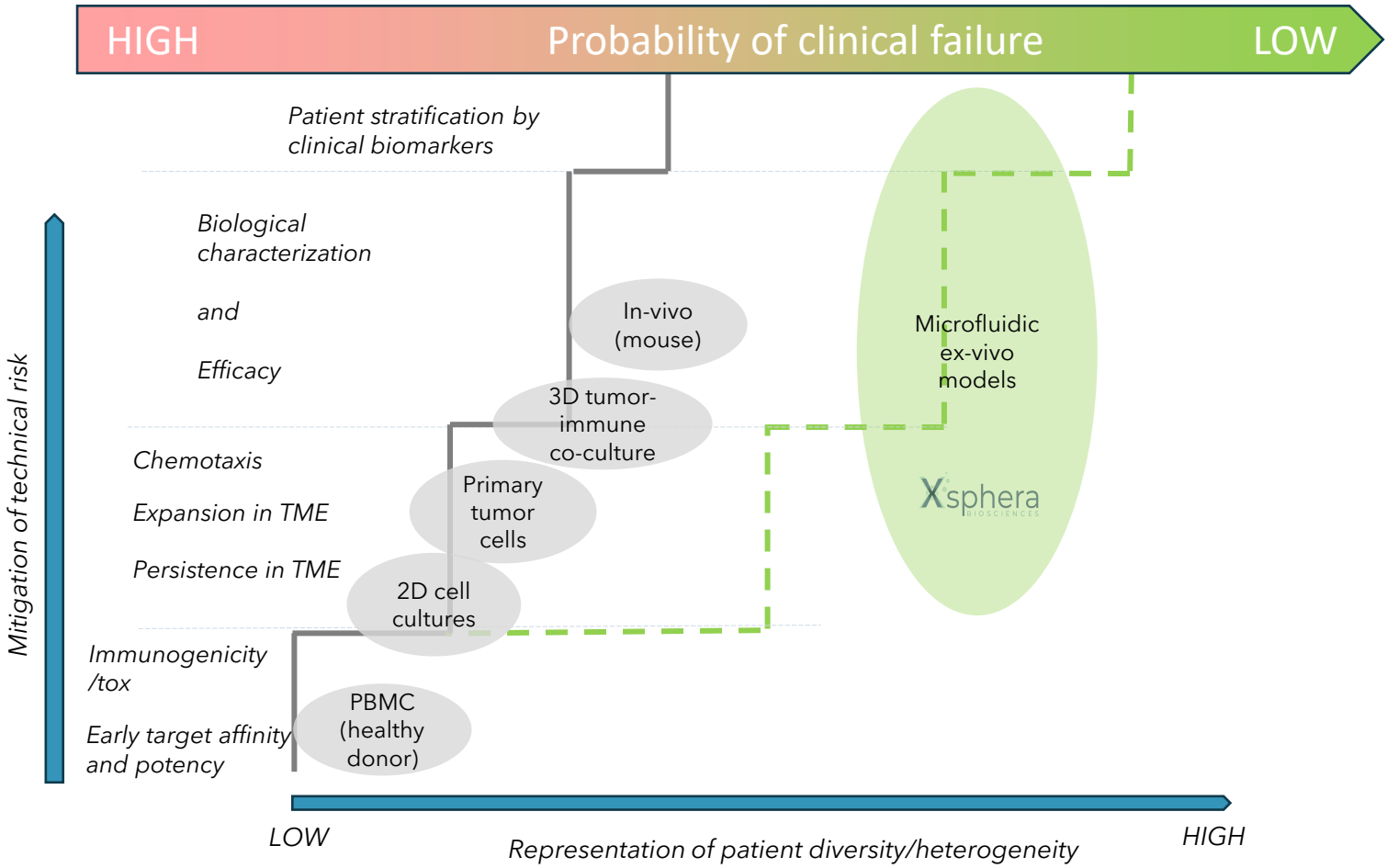
Microfluidics combined with bioengineering are the next generation biotools for cell therapy:

Xsphera CT and our microfluidic platform offers a promising solution to the challenges of pre-clinical development and clinical translation in cancer cell therapy. By leveraging microscale technologies, these platforms enable the precise control of fluid flow, cell behavior, and biochemical gradients within three-dimensional (3D) tissue constructs, recapitulating the complexities of the native TIME.

Why microfluidics is advantageous

- **Plastics provide for real-time imaging and analysis**
- **Migration captured in 3D across diverse patient TIME**
- **Longitudinal fluid sampling to capture protein and cytokine changes.**
- **Micronization of tumor spheroids ensure biological replication.**

Comparing and contrasting biological models for the pre-clinical evaluation of cell therapy



The Xsphera microfluidic system is a next-generation oncology platform

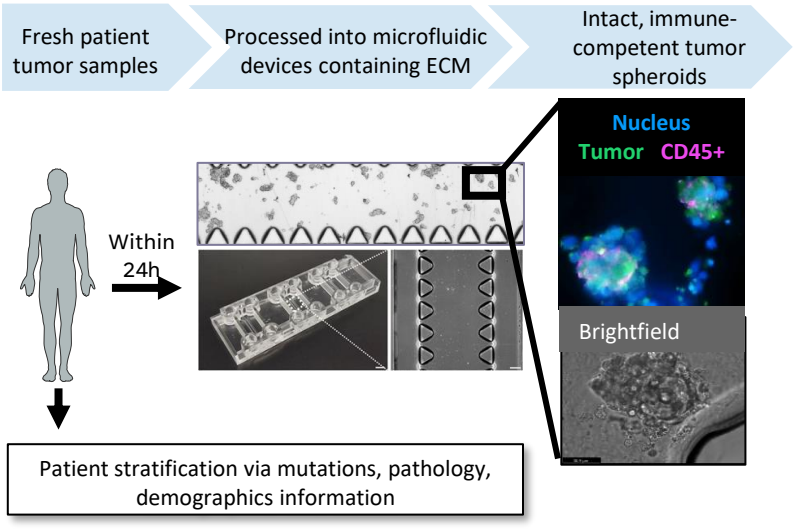
Unlike other microphysiological systems, Xsphera integrates a next-generation microfluidics platform with a proprietary clinical network and cloud data infrastructure for high content data capture and biologically validated analysis and interpretation.

- **Accrual**: Fresh human tumor tissue is sourced from qualified clinical sites across North America, which include clinical data and demographics.
- **Processing**: Tissue is micronized into spheroids using a patent protected, published protocol invented at Dana Farber Cancer Institute.
- **Devices**: Spheroids are embedded in an extracellular matrix and loaded into microfluidics devices with controlled gas permeability and exchange.
- **Actionable Endpoints**: Cytotoxicity, transcriptomics, imaging data, cell migration and phenotype, flow cytometry and proteomics are collected during and after each study.
- **Data**: Leveraging powerful visualization capabilities, AI and machine learning, data are developed on a cloud interface that integrates bioinformatics and analytics
- **Support**: The Xsphera Biosciences team helps to analyze and interpret data employing bioinformatics and 1:1 interactions.

Robust internal and published data provide confidence in the Xsphera Biosciences platform[6-9]

The spheroid generation process is tightly controlled and yields reproducible data and uniform biological components with complete immune architecture[13-17] (See Figure 1):

Figure 1: Xsphera microfluidic devices



DISCOVER

Identify better starting material

SELECT

Select better candidates that perform in human tumors

COMBINE

Determine effective drug combinations in the same patient

STRATIFY

Stratify patient populations before entering clinical trial

Xsphera Bio microfluidics system

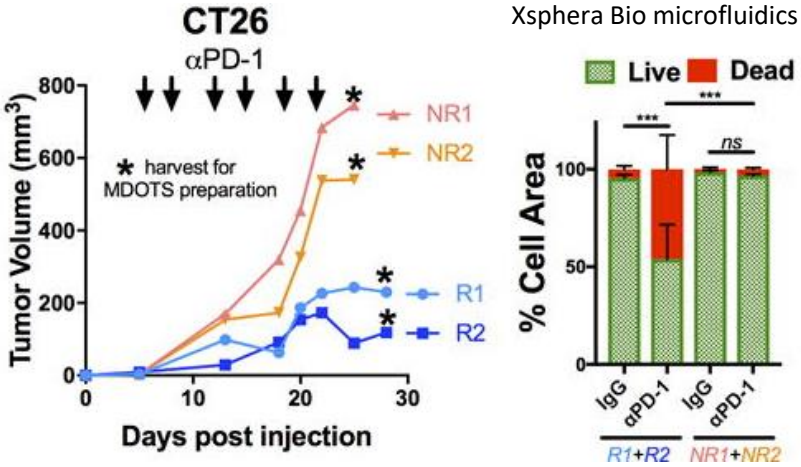


Figure 2: Correlation between in-vivo and ex-vivo response to immunotherapy

CT26 tumor volumes for responder (R1+R2) and non-responder (NR1+NR2) Balb/c mice were also tested in the Xsphera microfluidics platform and live/dead area was assessed at termination (Day 6).

Jenkins RW et al. *Ex Vivo* Profiling of PD-1 Blockade Using Organotypic Tumor Spheroids. **Cancer Discov.** 2018 Feb;8(2):196-215. doi: 10.1158/2159-8290.CD-17-0833.

XspheraCT is a **purpose-built cell therapy program** to improve candidate selection and profiling by leveraging cell therapy specific activity in a patient derived microfluidic platform with high content data generation and validated response criteria.

Xsphera CT brings together information on cell therapy activity with tumor and patient heterogeneity

Migration of CAR-T, autologous and allogeneic cell therapies through the tissue ECM, into the tumor microenvironment and into cancer cells is uniquely enabled by microfluidics (**Figure 3**). This feature of cell therapy is a critical metric of activity and potency, which Xsphera CT combines with cytotoxicity and cell killing, pharmacodynamics and transcriptomics to elucidate patient heterogeneity.

Peer-reviewed Xsphera Bio cell therapy data supports platform confidence:

Validated in peer-reviewed journals[16], Xsphera CT can capture the migration of cell therapy using real-time imaging techniques, interconnect these evidence with anti-tumor effect, and accurately re-capitulate the killing effect of clinically-developed umbilical cord blood derived natural killer cells (aNK) (**Figure 4**).

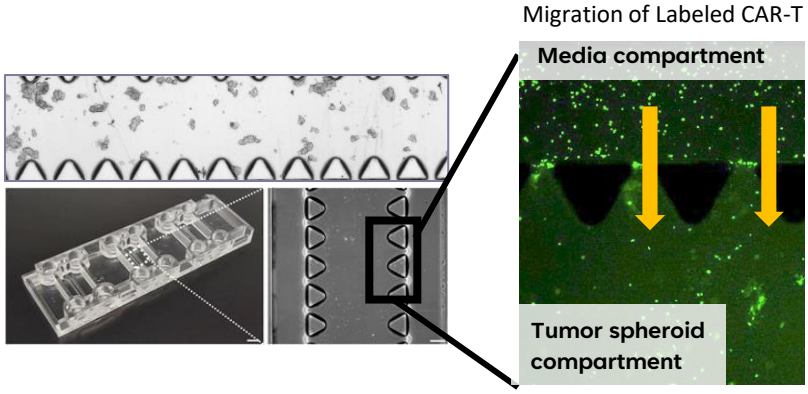


Figure 3: Candidate selection enabled with active chemotaxis in heterogeneous tumors:

Microfluidic devices enable the active migration of cell therapy through microscopic pores and tumor ECM representative of the patient tumor microenvironment, which can be studied in diverse tumor samples with varying stromal content.

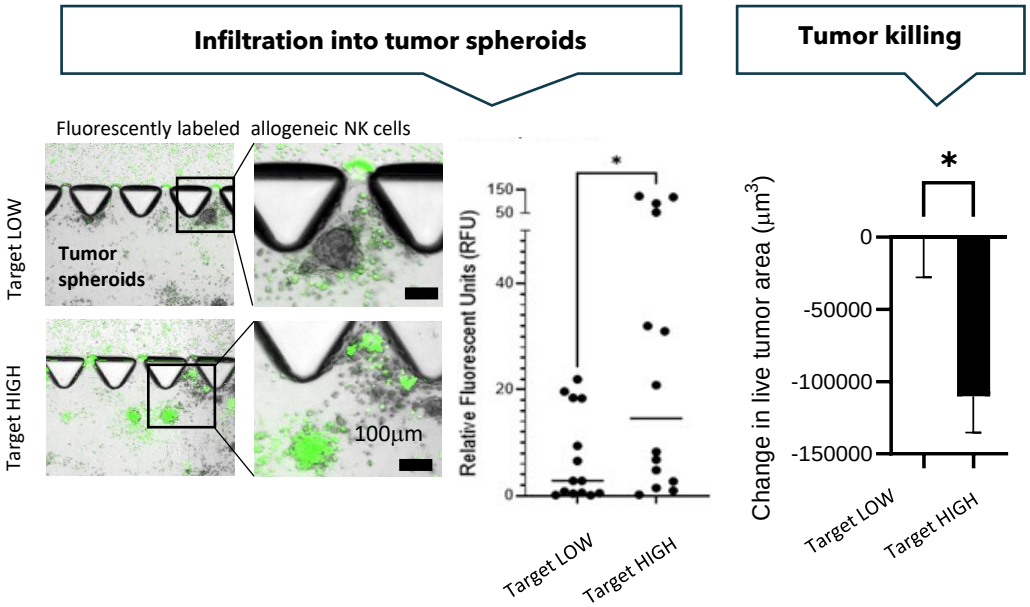


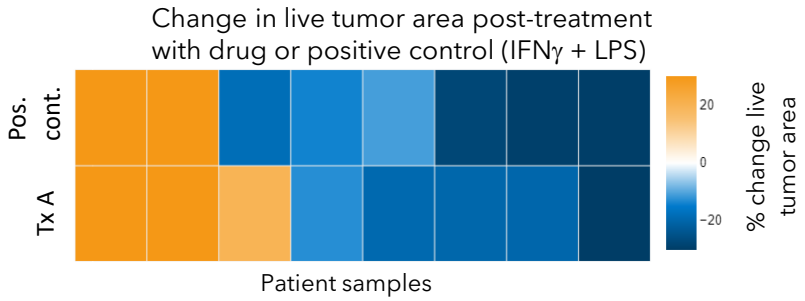
Figure 4: Allogeneic NK cell therapy chemotaxis and killing associates with tumor target expression

Xsphera CT captures penetration of cell therapy, infiltration into tumor spheroids and killing of tumor spheroids when the target on cancer cells is HIGH vs. LOW.

Saha et al. Boosting Natural Killer Cell Therapies in GBM Using Supramolecular Cationic Inhibitors of Heat Shock Protein 90. **Front Mol Biosci**. 2021 Dec 1;8:754443

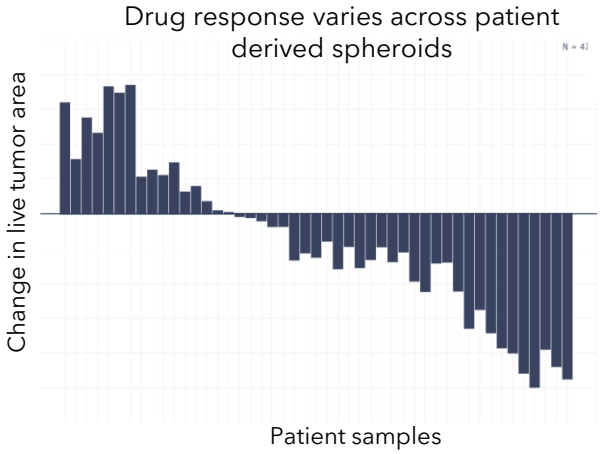
Positive control experimental designs associate mechanisms of action and antitumor activity

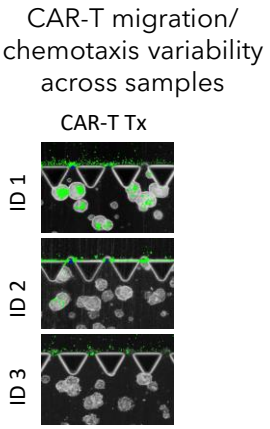
Goal: Adding a positive control alongside the experimental therapeutic correlates the magnitude of drug mechanism with response, and identifies patient sensitivity to pathway activation/down-regulation.

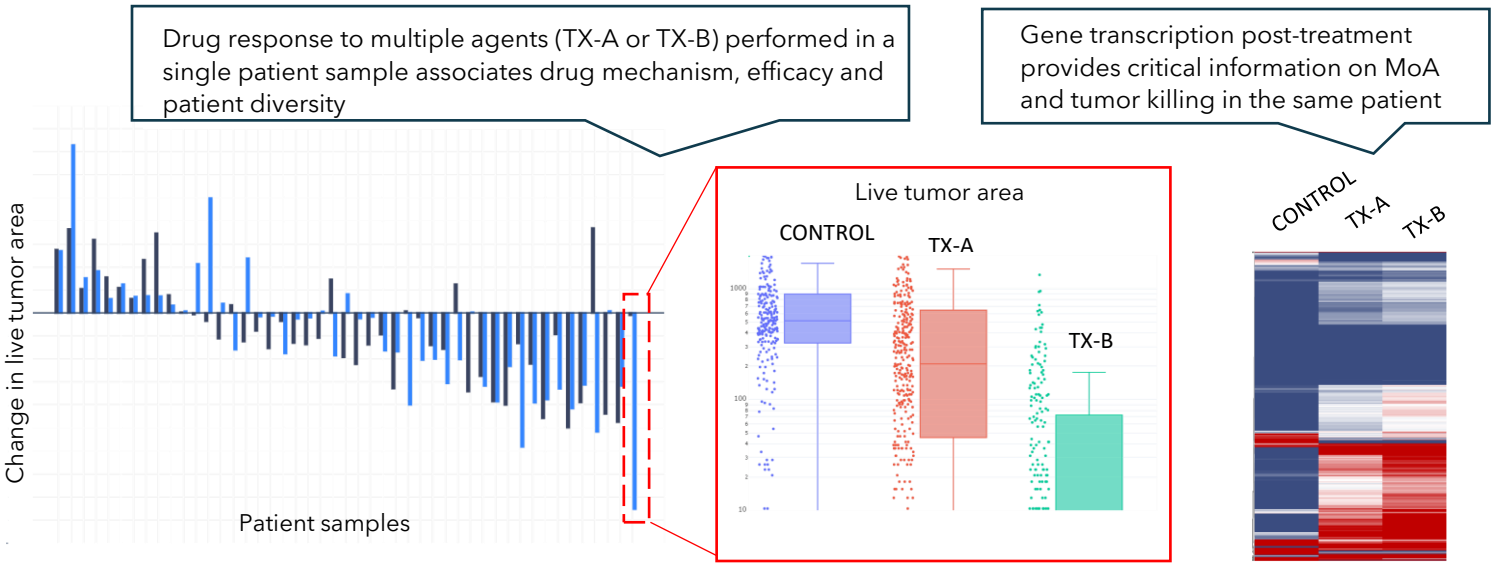


Drug efficacy and cell therapy activity across patients

Goal: Evaluating individual drugs across a diverse patient population provides unique, clinically-relevant and actionable information on drug effect and patient heterogeneity.







Data analytics and interpretation

Patient stratification	❑ Baseline mutation status ❑ Immunohistochemistry (IHC), H&E and pathology ❑ Patient demographic and clinical history ❑ Baseline tumor-immune flow cytometry ❑ Spatial proteomics and transcriptomics profiling
Mechanisms of cell therapy activity and action	❑ Fluorescent microscopy and live cell tracking, migration and chemotaxis ❑ Cytokine/chemokine protein expression ❑ Post treatment gene transcription
Therapeutic efficacy	❑ Cytotoxicity ❑ Live and dead tumor area ❑ Cell morphology

Representative case study:

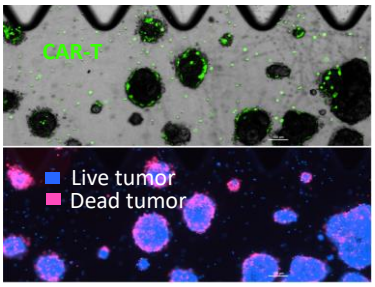
Goal	Candidate Selection: To determine the activity and efficacy of two CAR constructs in T-cell therapy within multiple patient samples
Desired outcome	To triage multiple cell therapy candidates expressing different CAR constructs for further selection and profiling towards IND.
Study design	Tumor type: Non-small cell lung cancer patient samples ➢ Fluorescent imaging to detect migration towards target ➢ CAR-T cytokine expression ➢ Cytotoxicity and antitumor activity

Identify effective CAR engineered therapies among multiple candidates

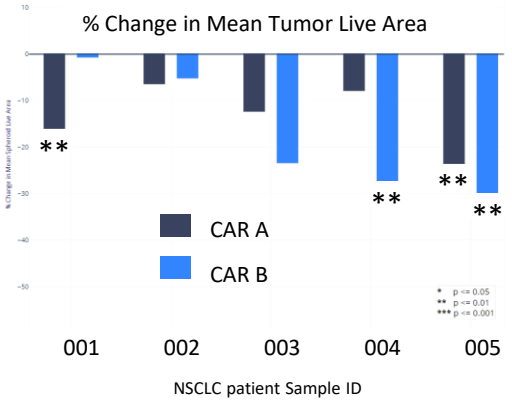
300+ tumor spheroids are assessed in every experiment, which is performed multiple times across unique patient samples. At Candidate selction, CAR can be compared for all endpoints. Analysis of tumor spheroid live area is captured after 3-6 days of culture, ex-vivo to assess effect on killing efficiency. Data are interpreted by an algorithm that detects tumor killing via high resolution fluorescent microscopy.

Data informatics is generated and delivered on the cloud based on a spheroid by-spheroid basis to conclude on population-wide efficacy and identify more efficacious CAR-T.

Variability of CAR-T migration and Spheroid Infiltration



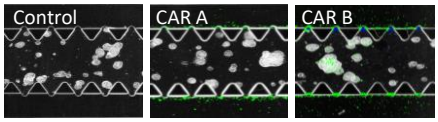
Diverse efficacy with different CAR tested within single patient samples and across multiple patients



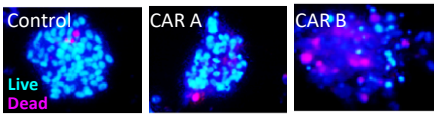
Patient-level interrogation to validate mechanisms of action: Patient ID 004

Individual patient samples are analyzed for cell therapy activity including migration towards the target, killing, transcriptomics and protein cytokine changes after treatment.

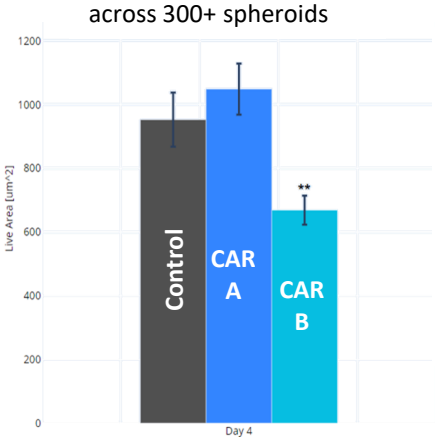
Elevated CAR B Migration and activity



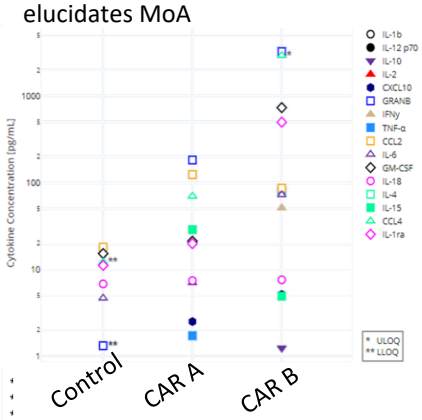
Elevated CAR B Tumor killing



Mean live tumor area across 300+ spheroids



Pharmacodynamic cytokine profile elucidates MoA



DECISION TREE

How should my Xsphera CT study be designed?

Have you identified your cell therapy target or clinical biomarker

NO →

We can provide primary patient samples, bulk RNA seq, single cell RNA seq, spatial proteomics and transcriptomics profiling to identify new biomarkers and clinical targets for cell therapy.

YES ↓

Have you selected your optimized donor material?

NO →

Test article(s)	Number of patient samples	Xsphera CT Experiment
Single cell therapy engineered from donor 1, 2, 3…	Minimum 5 patient samples per donor-derived cell therapy. Recommend multiple patient indications	Cell migration through the ECM Tumor killing and post-treatment gene transcription, cytokine expression

YES ↓

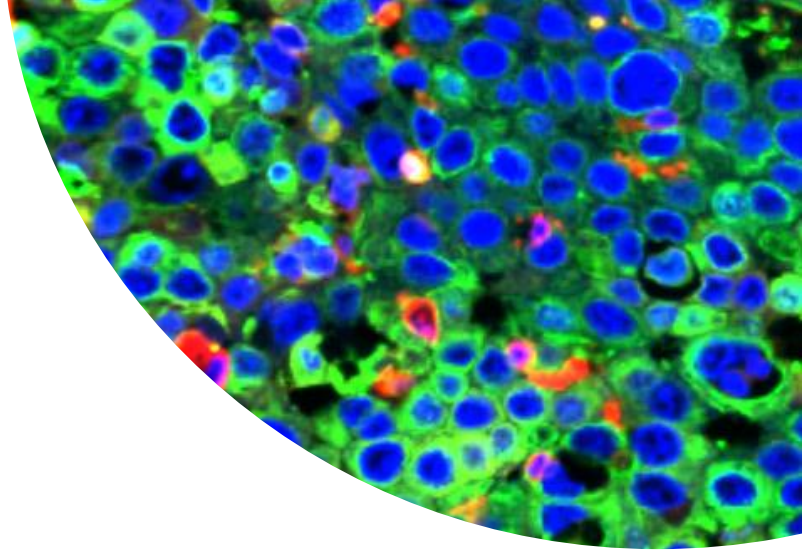
Have you identified your lead candidate and CAR construct for clinical development?

NO →

Test article(s)	Number of patient samples	Xsphera CT Experiment
Multiple cell therapy candidates engineered with different CAR or engineered properties from the same donor material	Recommended 10 patient samples per candidate to ensure target heterogeneity Select a single tumor indication	Dose finding Positive control design Cell migration through ECM Tumor killing and post-treatment gene transcription

YES ↓

Test article(s)	Number of patient samples	Xsphera CT Experiment
Single cell therapy candidate	Recommended 30 patient samples per indication to achieve likelihood of statistically significant responses. Multiple tumor indications selected from patients with target pos./neg., suggested minimum of 3 indications	Dose finding Baseline patient stratification (transcriptomics and mutation analysis) Cell migration through ECM Tumor killing and post-treatment gene transcription



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