Establishment of an ex vivo tissue culture platform as a preclinical model to assess the mechanism of action of *Bicycle®* tumor-targeted immune cell agonists in NSCLC

#A28

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ABSTRACT

The translation of drug discovery to bedside for cancer therapy is low with only 5% of anti-cancer compounds getting past phase III clinical trials. Since the tumor microenvironment is complex, there is a need for a relevant preclinical model to bridge the gap between tumor behavior and clinical therapeutic response for novel therapies.

- We have developed a novel class of modular synthetic drugs, termed *Bicycle®* tumor-targeted immune cell agonists (*Bicycle®* TICAs), which are multifunctional molecules comprised of constrained bicyclic peptides. [1]
- To investigate the mechanism of action of *Bicycle®* TICAs in a human model system, here we describe the development and optimization of an ex vivo organotypic histoculture model that uses freshly resected human non-small cell lung cancer (NSCLC) tumor tissue and preserves the tumor microenvironment and heterogeneity.

INTRODUCTION

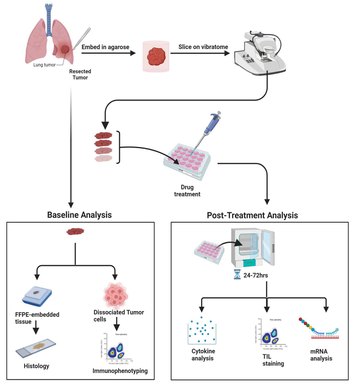


Figure 1: Schematic of the process of the ex vivo human tumor histoculture platform. Freshly resected lung tumor tissue from Brigham and Women's hospital was chopped into smaller pieces. Part of the tissue was removed for baseline analysis and the rest of the tissue was embedded in agarose and sliced on a Vibratome to generate tissue explants. Explants were cultured in 6-well tissue culture plates with or without treatment for 24-72hrs. Post-treatment analysis included cytokine measurements, Tumor Infiltrating Lymphocyte (TIL) phenotyping by flow cytometry, and/or generating Formalin-Fixed Paraffin-Embedded (FFPE) block for NanoString mRNA analysis.

Bicycle® tumor-targeted immune cell agonists deliver immune agonism to the tumor

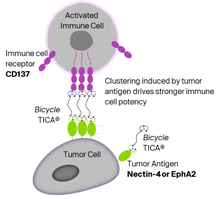
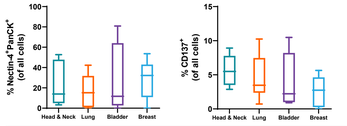


Figure 2: Introduction to the CD137 *Bicycle* TICA® concept and mechanism of action. CD137 is a costimulatory receptor that drives T cell function and survival and is also expressed on NK and myeloid cells. BT7480 is a fully synthetic *Bicycle* TICA™ that delivers CD137 immune agonist activity to Nectin-4 expressing tumors. BT7480 leads to complete tumor regression and reprogramming of TME in syngeneic mouse models including early myeloid cell activation that precedes T cell infiltration and upregulation of cytotoxicity-related genes.[2] We are also developing CD137 *Bicycle* TICAs that are targeted to the EphA2 receptor. Nectin-4 and EphA2 have both been reported to be overexpressed lung cancer[3,4] and our data using immunohistochemistry (IHC) of lung tumor microarrays (TMAs) supports these findings.[5,6]

INTRODUCTION

CD137 and Nectin-4 proteins are highly co-expressed in human lung tumors

A.



B.

Indication	Total Samples (N)	%Nectin-4/CD137+ (of samples with >1% target+ cells)
Head & Neck	5	100
Lung (All)	19	73.7
Lung adenocarcinoma	8	75
Lung squamous cell carcinoma	10	70
Bladder	5	60
Breast	14	57.1

Figure 3: Spatial proteomic profiling of Nectin-4+ and CD137+ cells across 43 human FFPE tumor samples performed using Multiomyx™ technology. (A) Tumor Nectin-4 expression where total Nectin-4+ PanCK+ cells are normalized to total cells and CD137+ immune infiltrate where total CD137+ cells detected are normalized to total cells. (B) Frequency of samples co-expressing Nectin-4 and CD137 at the protein level (>1% positive cells) is shown.

Study design to evaluate functionality of T-cells in tumor microenvironment

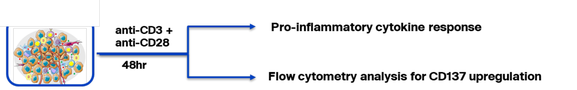


Figure 4: Study design for Figure 9 and 10. NSCLC tissue explants (N= 2 Adenocarcinoma, N=2 Squamous cell carcinoma) were subjected to T cell stimulation using 1ug/ml of soluble anti-CD3 (OKT3) and anti-CD28 (CD28.2) antibody for 48hr. The cytokine response was evaluated post-stimulation. In vitro T cell activation with anti-CD3 along with anti-CD28 co-stimulation led to an increase in CD137 surface expression [7]. CD137 induction as an activation marker was evaluated post-treatment using flow cytometry.

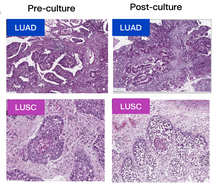
RESULTS

Tumor tissue viability and integrity was preserved up to 72 hours in culture

A.

NSCLC type	Stage (TNM)	Smoking status	Gender/ Age	Prior treatment
Adenocarcinoma	pT1cN1	Y	M/60	Naïve
Adenocarcinoma	pT2N2	Y	M/78	Naïve
Squamous Cell Carcinoma	pT2aN0	Y	F/76	Naïve
Adenocarcinoma	pT2N2	Y	M/78	Naïve
Squamous Cell Carcinoma	pT4N0	Y	F/69	Naïve
Adenocarcinoma	pT2aN0	Y	F/82	Naïve

B.



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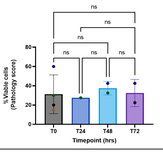

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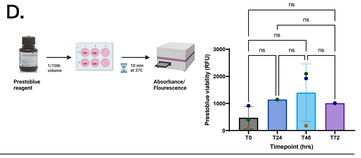
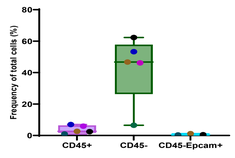


Figure 5: (A) Patient pathological information is shown in the table (N=6). (B) Tumor tissue viability and integrity was measured using H&E staining at baseline and post-culture without treatment. Representative H&E images are shown for lung adenocarcinoma and squamous cell carcinoma. (C) %viable tumor cells (as determined by pathologist) plotted against time showed no significant change in tumor viability and integrity during the culture period up to 72 hours. (D) The finding is also confirmed using an orthogonal assay that is based on the reducing power of cells as a measurement of viability. Each data point represents one donor (average of two biological replicates per donor). Statistical analysis was performed using One way ANOVA.

RESULTS

Baseline tumor and immune cell phenotyping by flow cytometry shows high CD45(-) cells and about 1% CD3+ T cells

A.



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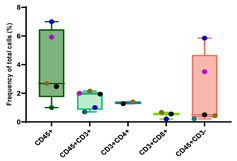
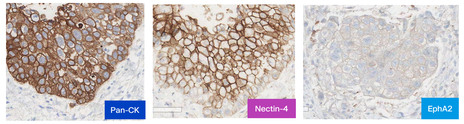


Figure 6: Dissociated tumor cells generated from the NSCLC tumor tissue profiled for tumor (6A) and immune (6B) cell content using flow cytometry (N=5). Data represents % positive cells out of total cells. Each data point represents one donor.

Nectin-4 and EphA2 protein detected at baseline in NSCLC tumors with variable expression profile

A.



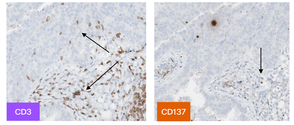
B.

Stain	% Avg Positive cells (±SD)	Weak Percent (H-Score ≤30)	Moderate Percent (H-Score 30-100)	Strong Percent (H-score >100)
Pan-CK	33 (±13)	20	60	20
Nectin-4	34 (±14)	20	60	20
EphA2	43 (± 23)	40	60	-

Figure 7: Tumor content and target antigen expression evaluated using single stain immunohistochemistry assay (IHC) (N=5). Representative images of PanCK, Nectin-4 and EphA2 shown (7A). Both Nectin-4 and EphA2 detected in the tumor cells with broad range of intensity for Nectin-4 and weak to moderate intensity for EphA2 across samples tested (7B)

NSCLC tumors show T-cell infiltration at baseline via IHC and a small population of cells expressing CD137

A.



B.

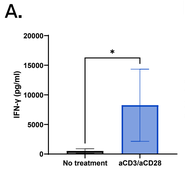
Stain	%Avg positive cells (±SD)	% Avg weak positive cells (±SD)	% Avg moderate positive cells (±SD)	% Avg strong positive cells (±SD)
CD3	14 (±9)	4 (± 2.6)	3 (± 4)	8 (± 7.8)
CD137	0.7 (± 0.8)	0	0	0.7 (± 0.8)

Figure 8: Baseline T cell content and CD137 expression was evaluated using a single stain IHC assay (N=5). (A) Representative images for CD3 and CD137 shown. CD3+ T cells were observed mostly in the stromal compartment and some in the tumor area as shown with arrows (B) On average, about 14% CD3+ T cells were detected in the NSCLC tissues tested by IHC and less than 1% of immune cells expressed CD137.

RESULTS

Treatment of tumor explants with anti-CD3/CD28 led to a T cell mediated cytokine response

A.



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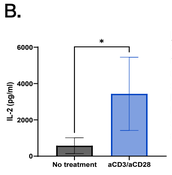
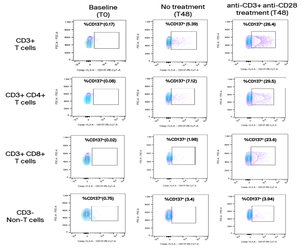


Figure 9: Supernatants from anti-CD3 and anti-CD28 treated tumor explant cultures were tested for presence of pro-inflammatory cytokines using a Luminex assay. A robust T cell response was observed with IFNg (9A) and IL2 (9B) cytokine levels compared to an untreated control (N=4). Each data point represents one donor (average of two biological replicates per donor). Statistical analysis was performed using an unpaired t-test.

Surface CD137 expression induced on CD3+ T cells post T cell stimulation

A.



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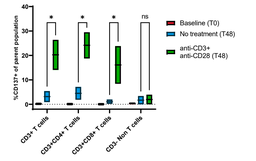

Figure 10: Tissue explants treated with anti-CD3 and anti-CD28 antibodies (1ug/ml) for 48hrs were dissociated into single cell suspension and tested for CD137 expression on T cell or non-T cell populations using flow cytometry. CD137 expression was induced after 48hrs in culture without treatment but a significant increase in expression was observed with anti-CD3 and anti-CD28 treatment. Representative flow cytometry histograms for one donor shown (10A) along with a graph showing induction post-treatment (10B) (N=2). Statistical significance was calculated using an unpaired t-test.

CONCLUSIONS

We demonstrate a rapid and reproducible ex vivo histoculture platform that is potentially poised to evaluate functional proof-of-concept of *Bicycle* TICA™ molecules in a human model system. Our preliminary data shows NSCLC tumor explants can maintain tissue integrity and tumor cell viability up to 72 hours in culture. A heterogenous protein expression profile for Nectin-4 and EphA2 was observed in the samples tested. The tumor explants were infiltrated with viable immune cells. We were also able to elucidate a T cell-specific cytokine response within 48 hours that suggests our histoculture platform contains live and functional tumor infiltrating lymphocytes (TILs).

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8. Images created with BioRender.com (2022)

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