Microinjection of Nectin-4/CD137 tumor-targeted immune cell agonist (TICA™) activates the local tumor microenvironment

POSTER# 1724

Johanna Lahdenranta[1], Stephen J. Blakemore[1], Kristen Hurov[1], Marc O. Grenley[2], Punit Upadhyaya[1], Carly Campbell[1], Elizabeth Repash[1], Eric Haines[1], Philip E. Brandish[1], Richard A. Klinghoffer[2] and Nicholas Keen[1]

[1]Bicycle Therapeutics, Lexington, MA, [2]Presage Biosciences Inc, Seattle, WA

ABSTRACT

- After disappointing first clinical experiences with agonistic anti-CD137 (4-1BB) antibodies, a new generation of both systemic and targeted CD137 agonists is entering clinical development (1-3). These strategies rely on biologic agents with suboptimal properties for CD137 agonism due to their relatively large sizes and long circulating half-lives. These properties may limit their tissue penetration and cause sustained agonism resulting in overstimulation and activation-induced cell death of lymphocytes due to continuous exposure.
- Fully synthetic constrained bicyclic peptides (*Bicycles*®) with antibody-like affinities and target selectivity are uniquely suited to circumvent the above barriers to optimal targeted CD137 agonistic therapeutics. BT7480 and BCY11864 are tumor-targeted immune cell agonists (TICAs™) designed to deliver a highly potent CD137 agonist to Nectin-4 overexpressing tumor tissue with a flexible dosing schedule maximizing anti-tumor activity while circumventing the need for continuous systemic exposure (4, 5).
- The Comparative In Vivo Oncology (CIVO) platform has been developed to enable in situ investigation of multiple microdosed drugs simultaneously in human tumors (6) with safety and feasibility of this platform recently demonstrated in patients with soft tissue sarcomas (7). CIVO injects drug microdoses directly into accessible tumor tissue as trackable columns where tissue can be analyzed after resection for the effect of the drug treatment in the tumor.
- Both BT7480 and BCY11864 demonstrate extremely potent Nectin-4-dependent CD137 agonism in primary human PBMC/tumor cell co-culture assays. *In vivo*, BT7480 induces significant modulation of the tumor immune microenvironment leading to significant increase in the cytotoxic cell population. (See our poster #1728 for more details.)
- Here we report on an evaluation of the feasibility of using the CIVO platform to demonstrate the mechanism of action of our tumor target-dependent CD137 agonist TICAs.

INTRODUCTION

Activating CD137 through receptor cross-linking

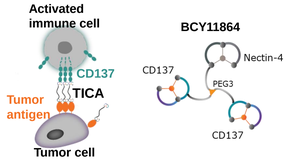


Figure 1: Activating CD137 through receptor cross-linking across the immune synapse: When co-ligated by Nectin-4 on the surface of tumor cells, BCY11864 (a close analogue of BT7480) is hypothesized to induce oligomerization and activation of CD137 via its high affinity CD137 binding *Bicycles*.

Nectin-4/CD137 TICA BT7480 modulates tumor immune microenvironment

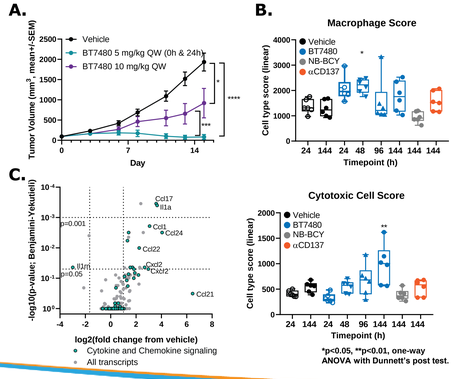
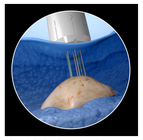


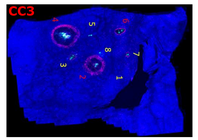
Figure 2: Nectin-4/CD137 TICA BT7480 leads to modulation of tumor immune microenvironment and anti-tumor activity in Nectin-4 expressing MC38 tumor model. **(A)** MC38-Nectin-4 tumor growth in huCD137 C57Bl/6 mice with weekly or twice a week dosing of BT7480 (n=6/cohort; *p<0.5, ***<0.001, ****p<0.0001 Mixed effects analysis with Turkey's post test, days 0–15). **(B)** NanoString analysis of tumors show the effect of BT7480 from 24h to 144h and anti-CD137 agonist antibody at 144h on the cytotoxic cell and macrophage content. **(C)** Transcriptional changes induced by BT7480 at 24h timepoint. Gray circles represent all measured transcripts and aqua circles identify transcripts belonging to the gene set for "Cytokine and chemokine signaling". Note that y-axis denotes adjusted (Benjamini-Yekutieli) p-values.

See our poster #1728 for more details on BT7480.

MATERIALS AND METHODS



CIVO injects drug microdoses directly into patient tumor as trackable drug columns

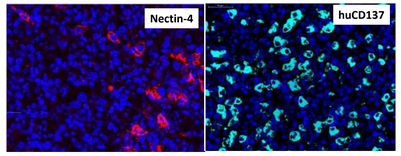


Tissue is surgically removed and analyzed (IHC, MIBI, RNA ISH, GeoMX et cetera)

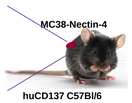
Comparative In Vivo Oncology (CIVO) platform

- **Simultaneous microdosing of multiple drugs into the same tumor**
- **Drug combinations can be investigated in the same tumor**
- **MMAE containing Bicycle Toxin conjugates are under clinical development, therefore MMAE was included to investigate impacts of MMAE on TICA mechanism of action**
- **Known MOA to dosed drugs demonstrated in clinic (7)**
- **Analysis platform for immune profiles/PD Biomarkers ex situ**

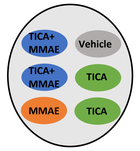
MC38-Nectin-4 tumor in huCD137-C57Bl/6 mouse



6.8%±0.3% cells Nectin-4+ **18.9%±0.4% cells huCD137+**



CIVO Microinjection device



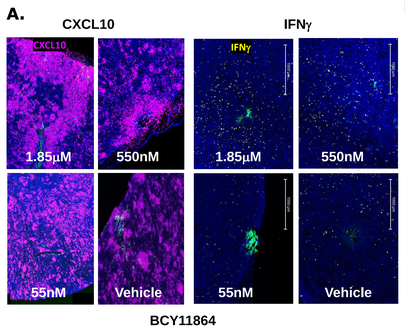
Needle array Injection volume: 2 μL

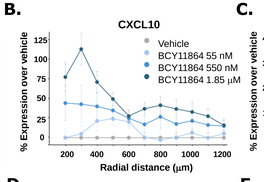
Microinjections: MC38-Nectin-4 tumors in huCD137-C57Bl/6 mice were microdosed with a set of compounds using the CIVO microinjection device. Two different configurations of compounds were loaded in the needle arrays:
Needle array 1: Vehicle (25mM histidine, 10% sucrose pH7), 550nM BCY11864, 55nM BCY11864, 120μM MMAE, 550nM BCY11864 + MMAE and 55nM BCY11864+MMAE. **Needle array 2:** Vehicle, 1.85μM BCY11864 (2 needles), 120μM MMAE, BCY11864 + MMAE (2 needles)

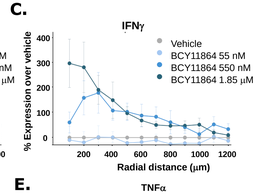
Tissue analysis: 4h and 24h after the microdosing, tumors were harvested, fixed and processed for FFPE blocks for IHC and ISH analysis.
IHC analysis was performed for CD8/Granzyme B (GzB), CD11b, Nectin-4 and huCD137/CD3
ISH analysis was performed for Cxcl10, IFNγ, TNFα and Klrb1c
Image analysis is performed from 6 sections from 3 tumors with a 1500μm diameter region of interest radiating outward from the injection site

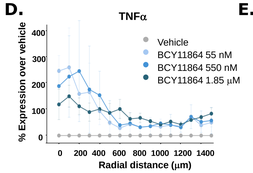
RESULTS

BCY11864 induces immune activation markers CXCL10 and IFNγ in a dose dependent manner









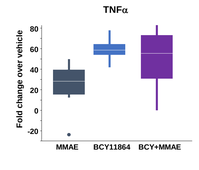


Figure 3: Dose dependent induction of CXCL10 and IFNγ after microdosing of BCY11864 in MC38-Nectin-4 tumors. MC38-Nectin-4 tumor bearing huCD137-C57Bl/6 mice were microinjected with BCY11864 at different concentrations and the initial (4h) tumor response to BCY11864 was evaluated by determining the level of CXCL10 (A and B) and IFNγ (A and C) expression by ISH. In addition to the dependence of CXCL10 and IFNγ -induction on the injected dose, radial distance from the injection site adds an additional level of information on the dose dependence of cytokine induction after BCY11864 dosing. (D) TNFα was induced by BCY11864 at all doses. (E) MMAE also induced cytokine expression at 4h timepoint. TNFα induction by BCY11864, MMAE or BCY11864+MMAE microinjection at 4h timepoint is shown.

RESULTS

BCY11864 increases the numbers of cytotoxic CD8+ T cells

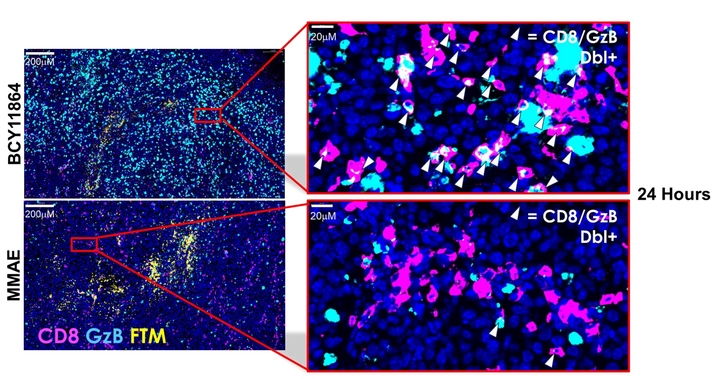


Figure 4: BCY11864 increases the numbers of cytotoxic CD8+ T cells in contrast to vehicle and MMAE. MC38-Nectin-4 tumors injected with BCY11864 and MMAE were evaluated for the presence of cytotoxic T cells (CD8+/GzB+) by IHC 24 hours after microinjection. (A) Elevation of CD8/GzB double positive cells (white, see arrows) is observed in BCY11864 injection sites compared to vehicle or MMAE injection sites. (C) Increase in activated T cells is driven by BCY11864 exposure. Data is normalized to total T cells. FTM: Fluorescent tracking marker

CONCLUSIONS/SUMMARY

- We have validated pre-clinically the CIVO platform for investigating target engagement and the mechanism of action of tumor-targeted immune cell agonists in accessible tumor lesions
- Microinjection of BCY11864 into Nectin-4-expressing tumor induces immune activation markers in a dose dependent manner
- BCY11864 activates cytotoxic T cells around the BCY11864 injection area by 24 hours

References: [1] Hinner et al, Clin Cancer Res (2019); [2] Claus et al, *Sci Transl Med* (2019); [3] Eskiocak et al, JCI Insight (2020); [4] Hurov et al. Meeting abstract, *Cancer Res* (2020); [5] Upadhyaya et al, *JITC* (2021); [6] Klinghoffer et al. *Sci Transl Med* (2015); [7] Gundle et al. *Clin Cancer Res* (2020)

Questions? Contact IR@bicycletx.com

Bicycle Therapeutics
4 Hartwell Place, Lexington, MA 02421
www.bicycletherapeutics.com