A novel fully synthetic dual targeted EphA2/CD137 *Bicycle®* peptide induces tumor localized CD137 agonism

ABSTRACT# 4613 (Poster #: 4703)

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ABSTRACT

- 4-1BB (CD137) is a member of the TNFR superfamily involved in stimulation of several immune cell types, including T cells and NK cells. CD137 is well validated pre-clinically(1), as agonism with anti-CD137 antibodies is effective in vivo, however, clinical utility to date has been limited by dose dependent hepatotoxicity(2).
- We hypothesized that the unique properties of *Bicycles®* are amenable to develop tumor targeted agonists that would offer significant advantage in terms of safety over other approaches. This led to the development of 'tumor-targeted immune cell agonists' (TICA™), that stimulate immune cells exclusively at the tumor site.
- EphA2 is highly expressed in several tumor types of high unmet medical need. EphA2/CD137 TICA's contain two *Bicycle* arms, one targeting EphA2 (tumor target arm) and the other targeting CD137 (immune arm).
- Here, we present substantial preclinical data demonstrating the potent immunomodulatory activity of EphA2/CD137 TICA's.
- EphA2/CD137 TICA's leads to an increase in $CD8^+$ T-cells locally in tumor tissue in vivo. Intermittent dosing of the molecule leads to a robust anti-tumor activity in MC38 syngeneic mouse model, and more importantly leads to the development of immunogenic memory.
- These findings provide a strong rationale to further develop first-in-class Bicycle TICA's to potentially treat EphA2 expressing cancers.

INTRODUCTION

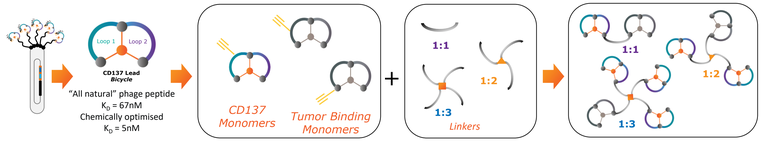
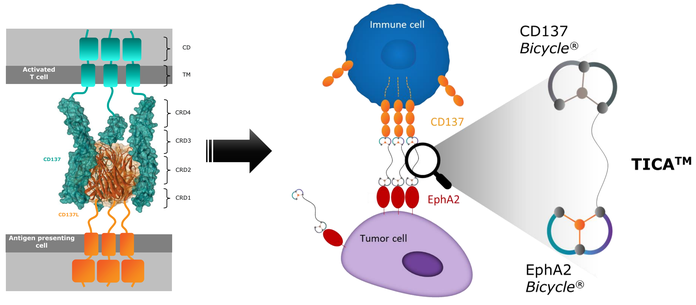


Figure 1A: Schematic of the process for generating CD137 TICA molecules using *Bicycles*: Phage screening identified CD137 binders with nM potency. The lead peptide was chemically optimized to achieve K_D=5 nM (SPR). CD137 and tumor targeting monomers were synthsised with varying attachment points, affinites, physicochemical properties. TICA's of varying valency (1:1, 1:2 and 1:3) were constructed using different linkers. TICA's were optimized to obtain the desired PK and pharmaceutical properties.

Figure 1B: The concept of Bicycle tumor targeted immune cell agonist (TICA™).



CD137 is a member of TNFR superfamily and requires trimerization for activation.

Alternative approach to achieve CD137 clustering: linking a CD137 *Bicycle* to a *Bicycle* targeting a highly expressed tumor antigen (eg. EphA2). Binding of these molecules to the tumor cells would result in a multivalent array of CD137 engaging *Bicycles*, enabling the clustering of the CD137 receptors in a tumor antigen dependent manner.

RESULTS

Reporter assay

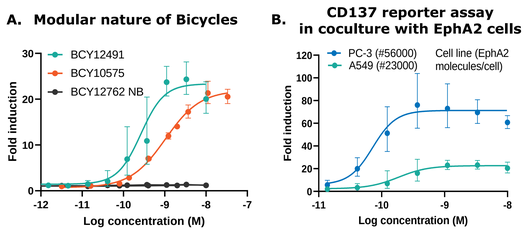


Figure 2: EphA2/CD137 TICA's display EphA2 dependent CD137 activation as measured in reporter assays. NF-κB-Luc2/4-1BB Jurkat reporter cells were co-cultured with EphA2 expressing cancer cells and the downstream CD137 mediated NF-κB activation was measured by luminescence after treatment with EphA2/CD137 TICA's. **(A)** The modular nature of Bicycle platform enabled generation of molecules with varying potencies. The non-binder (NB) control shows minimal activity **(B)** The fold NF-κB activation with BCY12491 is dependent on the levels of EphA2 expression in the co-culture cell line (mean ± SD).

hPBMC co-culture assay

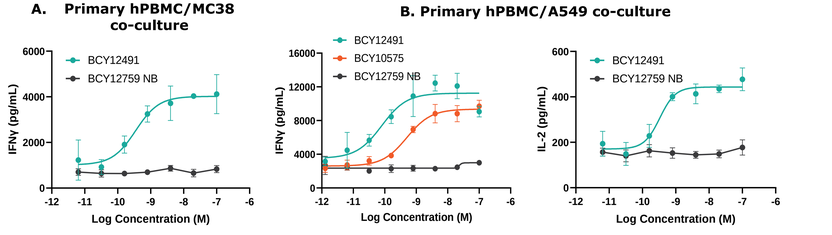


Figure 3: EphA2/CD137 TICA's promote cytokine secretion in PBMC / cancer cell co-culture experiments. PBMCs from healthy donors were co-cultured with EphA2 expressing cancer cells, **(A)** MC38 and **(B)** A549, in presence of anti-CD3 and test molecules. Supernatants were analyzed for cytokines by Luminex, figures are representative data using PBMCs from one donor (from a total of n=5). The non-binder (NB) control showed minimal activity whereas EphA2/CD137 TICA's show potent immune stimulation (mean ± SD).

PBMC humanized HT29 model

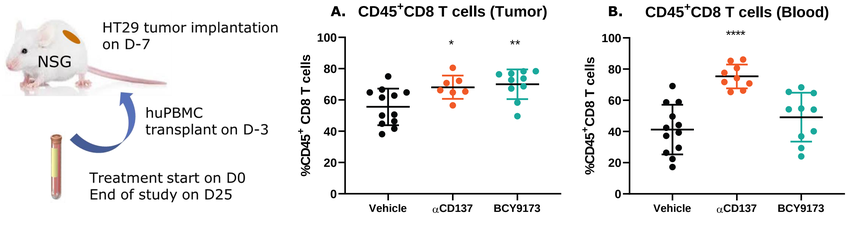
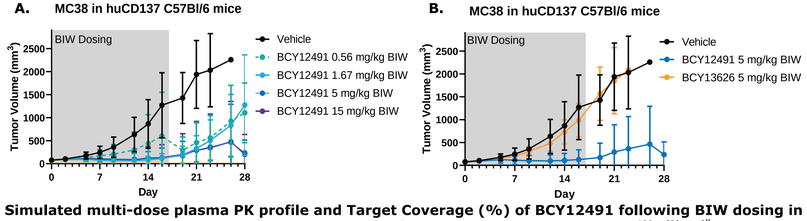


Figure 4: EphA2/CD137 TICA leads to tumor localized increase in CD8+ T cells in HT-29/PBMC engraftment model. NSG mice were implanted with HT-29 (endogenous EphA2+) tumor cells and human PBMCs prior to treatment with αCD137 (Urelumab analogue) and BCY9173 (EphA2/CD137 TICA). Immune profiling of **(A)** tumor and **(B)** whole blood by flow cytometry demonstrated a systemic agonist response with αCD137, an increase in CD8+ T cells both systemically and at tumor site. EphA2-targeted BCY9173 demonstrated only tumor localized CD137 agonism.

RESULTS

Syngeneic MC38 mouse model



C. Simulated multi-dose plasma PK profile and Target Coverage (%) of BCY12491 following BIW dosing in mice

PK parameters	IV
Dose (mg/kg)	5.0
Vss (L/kg)	1.5
CL (mL/min/kg)	17
Terminal t½ (h)	1.1

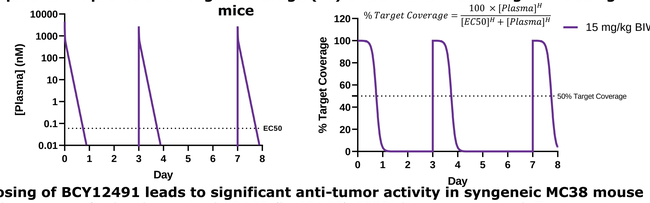


Figure 5: Intermittent dosing of BCY12491 leads to significant anti-tumor activity in syngeneic MC38 mouse model. CD137 Bicycle® is human specific thus humanized CD137 (huCD137) C57BL/6 mice were used. Treatment was initiated when the tumor volume was ~60mm^3. Different doses of BCY12491 were administered BIW over 22 days. (A) All BCY12491 treatment groups displayed anti-tumor activity (n=6 per group) whereas (B) the non-binding control BCY13626 showed no anti-tumor activity. (C) PK parameters obtained from single IV bolus dose of BCY12491 were used to simulate plasma concentrations and target coverage (%) following repeat doses of 15 mg/kg IV BIW. The EC50 for target coverage was based on the mean EC50 for INFγ secretion (determined *in vitro* from 2 donors using the mc38-hPBMC coculture assay). Between doses, trough plasma concentrations of BCY12491 were below the corresponding *in vitro* EC50 for substantial periods, demonstrating intermittent plasma exposure of an EphA2/CD137 TICA produces robust anti-tumor activity.

MC38 tumor rechallenge

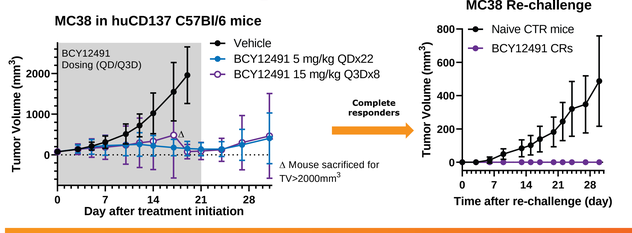


Figure 6: Complete responder animals to BCY12491 treatment were re-inoculated with MC38 tumor cells 81 days after the initial treatment initiation. Unlike in matched naïve control mice (100% tumor growth), no tumor growth was observed in complete responder mice implying a development of immunogenic memory. (Tumor volumes are mean ± SD)

CONCLUSIONS

- EphA2/CD137 TICA's exhibit highly potent, tumor localized EphA2 dependent stimulation of CD137 in preclinical models.
- Intermittent dosing of an EphA2/CD137 TICA leads to robust anti-tumor activity. PK/PD simulations indicate that sustained plasma exposure and continuous target coverage is not required for efficacy.
- Based on these experiments, the pharmacokinetic and biological properties of TICA's are potentially suitable for once weekly dosing in the clinic.
- Bicycle Therapeutics are advancing an EphA2/CD137 TICA clinical candidate.

References:

(1) Melero et al, Nat Med 3(6): 682-5 (1997)

(2) Segal et al, Clin Cancer Res 23(8): 1929-36 (2017)

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