EphA2/CD137 *Bicycle*® tumor-targeted immune cell agonists (TICAs™) induce tumor regressions, immunogenic memory, and reprogramming of the tumor immune microenvironment

ABSTRACT# 700

Kristen Hurov, Johanna Lahdenranta, Gemma Mudd, Punit Upadhyaya, Elizabeth Repash, Jun Ma, Julia Kristensson, Marianna Kleyman, Jessica Kublin, Liuhong Chen, Eric Haines, Sailaja Battula, Kevin McDonnell, Philip E. Brandish, and Nicholas Keen

ABSTRACT

- CD137/4-1BB is a member of the TNF receptor superfamily involved in the stimulation of several immune cell types, including T and NK cells.
- Despite compelling preclinical data, agonistic anti-CD137 antibodies have been hampered by failure to delineate hepatoxicity from efficacy in clinical studies [1,2].
- A new generation of both systemic and targeted CD137 agonists that are now entering clinical development rely on biologic agents with suboptimal properties for CD137 agonism due to their relatively large sizes and long circulating half-lives [3-5]. These properties may limit their tissue penetration and cause overstimulation and activation-induce cell death of lymphocytes due to continuous exposure.
- BCY12491 is a tumor-targeted immune cell agonist (TICA™) that exemplifies a new class of fully synthetic immunomodulators with constrained bicyclic peptides (*Bicycles*®) targeting a tumor antigen and a co-stimulatory molecule. We developed this new class of synthetic molecules with antibody-like affinities and target selectivity to circumvent the before mentioned barriers to optimal targeted CD137 agonistic therapeutics.
- BCY12491, an EphA2/CD137 TICA, is designed to deliver a highly potent CD137 agonist to EphA2 overexpressing tumors, including pancreatic, gastric, and colorectal, among others.
- BCY12491 is a potent EphA2-dependent CD137 agonist with optimal binding, pharmacologic, and pharmacokinetic properties that enable anti-tumor TME remodeling and complete responses *in vivo* without the need for continuous exposure.
- This work unleashes a new and tractable avenue to testing a novel class of therapeutic CD137 agonists in humans for the treatment of cancer.

INTRODUCTION

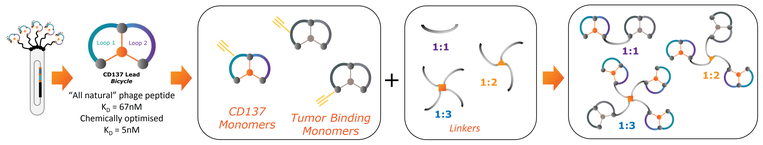
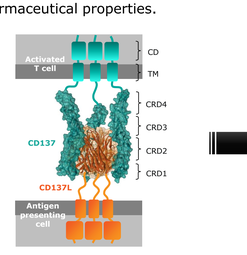
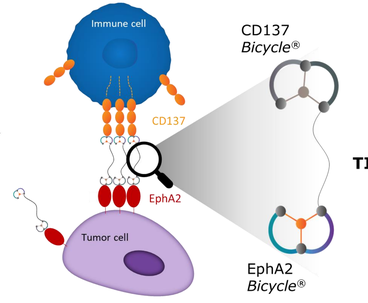
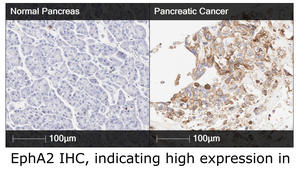


Figure 1: A schematic of the process for generating CD137 TICA molecules using *Bicycles*. Phage screening identified CD137 binders with nM potency. The lead peptide was chemically optimized to achieve K_D=5 nM (SPR). CD137 and tumor targeting monomers were synthesized with varying attachment points, affinities, 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.







EphA2 IHC, indicating high expression in pancreatic cancer

Figure 2: The concept of a *Bicycle* tumor targeted immune cell agonist (TICA™). CD137 is a member of the TNFR superfamily and requires trimerization for its activation. CD137 is depicted here bound by its ligand, CD137L. An alternative approach to achieve CD137 clustering: linking a CD137 *Bicycle* to a *Bicycle* targeting a highly expressed tumor antigen, i.e. EphA2. Binding of these molecules would result in a multivalent array of CD137 engaging *Bicycles*, enabling the clustering of the CD137 receptors in a tumor antigen-dependent manner.

RESULTS

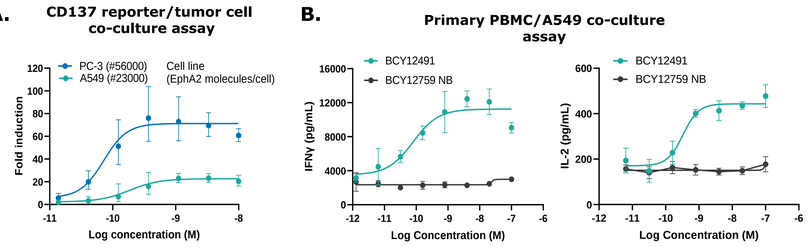
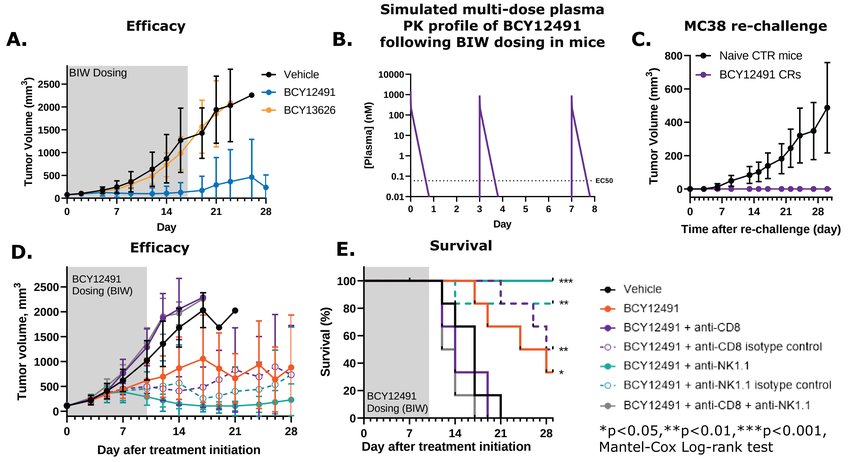


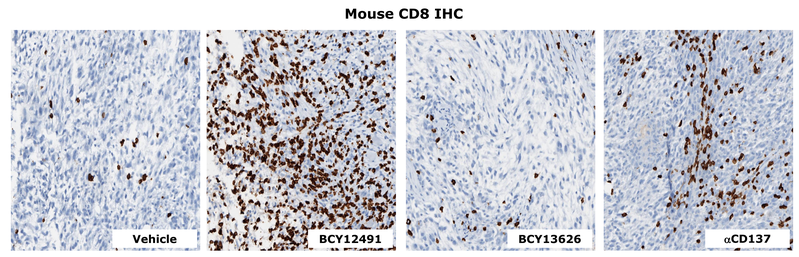
Figure 3: BCY12491 led to potent EphA2-dependent activity in CD137 reporter and primary immune cell co-culture assays. A) NF-κB-Luc2/CD137 Jurkat reporter cells were co-cultured with EphA2 expressing cancer cells and the downstream CD137 mediated NF-κB activation was measured by luminescence after treatment with BCY12491. Fold induction by BCY12491 is dependent on the levels of EphA2 expression in the co-culture cell line (mean ± SD). **B)** BCY12491 promoted cytokine secretion in PBMC / cancer cell co-culture experiments. PBMCs from healthy donors were co-cultured with EphA2 expressing A549 cells in the 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 (mean ± SD).

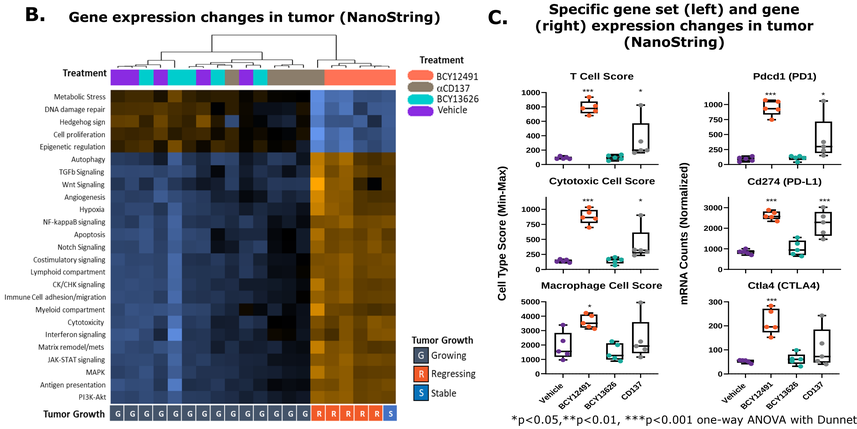


*p<0.05,**p<0.01,***p<0.001, Mantel-Cox Log-rank test

Figure 4: Intermittent dosing of BCY12491 led to significant anti-tumor activity and immunogenic memory in the syngeneic MC38 mouse model. CD137 *Bicycle* is human-specific thus humanized CD137 (huCD137) C57BL/6 mice were used. BCY12491 (5 mg/kg) was administered BIW over 17 days. **A)** BCY12491 treatment led to anti-tumor activity (n=6 per group) whereas the non-binding control BCY13626 was inactive. **B)** PK parameters obtained from single IV bolus dose of BCY12491 were used to simulate plasma concentrations following repeat doses of 5 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 PBMC/MC38 co-culture assay). Between doses, trough plasma concentrations of BCY12491 were below the corresponding *in vitro* EC50 for substantial periods, demonstrating intermittent plasma exposure of BCY12491 produces robust anti-tumor activity. **C)** Unlike in matched naïve control (CTR) mice (100% tumor growth), no tumor growth was observed in complete responder mice implying a development of immunogenic memory. **D)** BCY12491-driven anti-tumor activity is dependent on CD8+ T cells, but not NK cells. MC38 tumor-bearing mice depleted of CD8+ cells and/or NK cells or treated with vehicle or isotype-control antibodies received 4 doses of 15 mg/kg BCY12491 or vehicle BIW. **E)** Survival data corresponding to panel (A). (Tumor volumes are mean ± SD)

RESULTS





*p<0.05,**p<0.01, ***p<0.001 one-way ANOVA with Dunnett's

Figure 5: BCY12491 treatment led to increased T cell infiltration and reprogramming of the tumor immune microenvironment. MC38 tumor bearing mice (huCD137-C57Gl/6) were treated with 15 mg/kg q3d i.v. of BCY12491 or BCY13626 (enantiomeric non-binding control TICA), 2 mg/kg q3d i.v. of αCD137 antibody (Urelumab, Creative Biolabs), or vehicle. Tumors were harvested after 6 days and either processed for IHC or transcriptional analysis by NanoString. NanoString data was analyzed using nSolver software. **A)** Representative images of tissue sections from tumors stained for mouse CD8. **B)** Heatmap of pathway scores showing the immunomodulatory effect on 25 functional pathways. Orange indicates high pathway scores; blue indicates low pathway scores. Scores are displayed on the same scale via a Z-transformation. **C)** Increase in T cell score, cytotoxic cell score, macrophage cell score (left column of plots), and immune checkpoint gene expression (right column of plots) in tumor tissue upon BCY12491 treatment.

CONCLUSIONS

- BCY12491 is an EphA2/CD137 *Bicycle* TICA that exhibits highly potent, EphA2-dependent activity in *in vitro* primary immune cell assays
- BCY12491 causes tumor regression, complete responses, immunogenic memory, and significant modulation of the tumor immune microenvironment in preclinical syngeneic mouse models without continuous drug exposure in the periphery
- Bicycle Therapeutics is advancing an EphA2/CD137 TICA clinical candidate

References: [1] Segal et al, *Clin Cancer Res* 23(8): 1929-36 (2017); [2] Chester et al, *Blood* 131(1): 49-57 (2018); [3] Hinner et al, Clin Cancer Res 25(19): 5878-89 (2019); [4] Claus et al, *Sci Transl Med* 11(496): eaav5989 (2019); [5] Eskiocak et al, JCI Insight 5(5): e133647

Questions? Contact maren.killackey@bicycletx.com

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