An integrative approach to optimize a synthetic EphA2-dependent CD137 agonist: Balancing potency, physiochemical properties, and pharmacokinetics to achieve robust anti-tumor activity

ABSTRACT# 888

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ABSTRACT

- CD137 (4-1BB) is a resurging target in immunotherapy after the first generation of monoclonal antibodies were limited by hepatotoxicity [1] or lack of efficacy [2]. A new generation of CD137 agonists are now in clinical development but they exclusively utilize large molecules derived from recombinant technology with long circulating half-lives [3-6]. Intermittent target engagement which mimics the physiologic context of T-cell costimulation has not yet been explored by current modalities targeting CD137.
- Bicyclic peptides or *Bicycles* are a class of small (MW~2kDa), highly constrained peptides characterized by formation of two loops cyclized around a symmetric scaffold. EphA2/CD137 *Bicycle*® tumor-targeted immune cell agonists (*Bicycle* TICAs) were synthesized by linking *Bicycle* binders to EphA2, a highly expressed tumor antigen expressed in several tumor types of high unmet medical need[7], to those binding CD137 [8].
- Integrating structure activity relationship (SAR) data from biochemical binding studies as well as *in vitro* and *in vivo* models led to an understanding of the relationship between plasma exposure, target engagement and efficacy in mouse tumor models. The findings from these analyses suggest a strong rationale to develop EphA2/CD137 *Bicycle* TICAs with non-continuous plasma exposure (Cmin=0) to potentially treat EphA2 expressing cancers.

INTRODUCTION

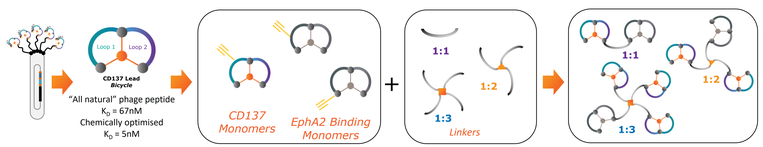
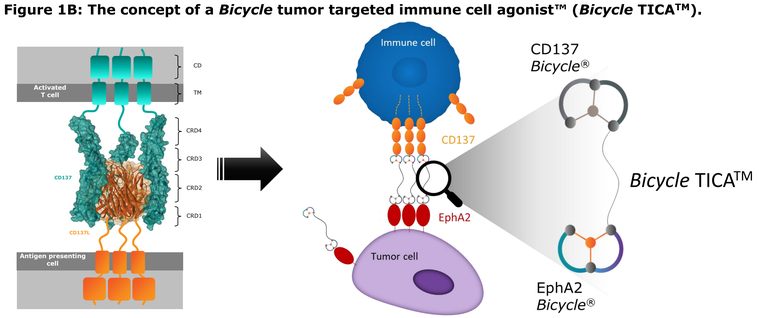


Figure 1A: Schematic of the process for generating CD137 *Bicycle* TICA™ molecules using *Bicycles*: Phage screening identified CD137 binders with nM potency. The lead peptide was chemically optimized to achieve K_D=5 nM (SPR). CD137 and tumor targeting monomers were synthesized with varying attachment points, affinities, physicochemical properties. *Bicycle* TICAs of varying valency (1:1, 1:2 and 1:3) were constructed using different linkers. *Bicycle* TICAs with a range of *in vitro* potency, physiochemical properties and pharmacokinetics were evaluated in efficacy models.

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



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

Alternative approach to achieve CD137 clustering: linking a CD137 binding *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

CD137 Reporter Assay

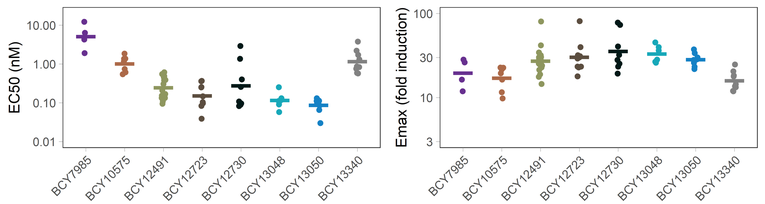


Figure 2: The modular nature of Bicycle® platform enabled generation of EphA2/CD137 *Bicycle* TICAs with a range of potencies in the reporter assay. NF-κB-Luc2/4-1BB Jurkat reporter cells were co-cultured with EphA2 expressing cancer cells (A549) and the downstream CD137 mediated NF-κB activation was measured by luminescence after treatment with EphA2/CD137 TICAs. EC50 and Emax (fold induction over background) from each experiment were reported as individual data points with the mean as the crossbar. The modular nature of the Bicycle® platform enabled generation of molecules with varying potencies as shown above. The non-binder (NB) control incorporating non-binding versions of either the EphA2 or CD137 binders shows minimal activity in this assay (data not shown)

Human PBMC/tumor cell co-culture assay

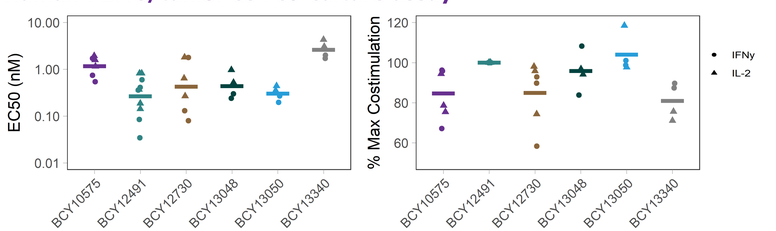
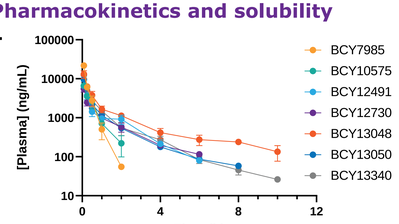


Figure 3: EphA2/CD137 *Bicycle* TICAs promote cytokine secretion in PBMC / tumor cell co-culture experiments. Anti-CD3 activated PBMCs from healthy donors were co-cultured with EphA2 expressing tumor cells (MC38) in presence of test molecules. Supernatants were analyzed for cytokines by Luminex. EC50 from each experiment for IL-2 and IFNγ secretion were reported as individual data points with means as crossbars. Max costimulation is the ratio (expressed in %) of Emax of test compound relative to BCY12491 in the same run. EphA2/CD137 *Bicycle* TICAs leads to dose dependent increase in secretion of IL-2 and IFNγ in the human immune cell/EphA2 expressing tumor cell coculture systems. The non-binder (NB) control incorporating non-binding versions of either the EphA2 or CD137 binders shows minimal activity in this assay (data not shown)

Pharmacokinetics and solubility

A.



B.

BCY	CLp (mL/min/kg)	Vd_{ss} (L/kg)	$t_{1/2}$ (h)	Solubility (mg/mL)
BCY7985	13	0.2	0.2	>20
BCY10575	24	0.8	0.4	> 4.7
BCY12491	17	1.5	1.1	2.2
BCY12723	12	0.8	1.0	>5.5
BCY12730	19	1.9	1.6	1.1
BCY13048	8	1.4	4.0	>20
BCY13050	15	1.4	2.5	2.9
BCY13340	12	0.9	2.4	>20

Figure 4: Pharmacokinetics of EphA2/CD137 *Bicycle* TICAs in mice. (A) Plasma concentration-time profile and **(B)** PK parameters of EphA2/CD137 *Bicycle* TICA™ after administration of 5 mg/kg via intravenous dose (IV Bolus) in CD-1 mice. The half-life of these molecules range from 0.12-4 h allowing us to explore the optimal exposure required for anti-tumor activity in MC38 syngeneic tumor model.

RESULTS

Syngeneic MC38 mouse model

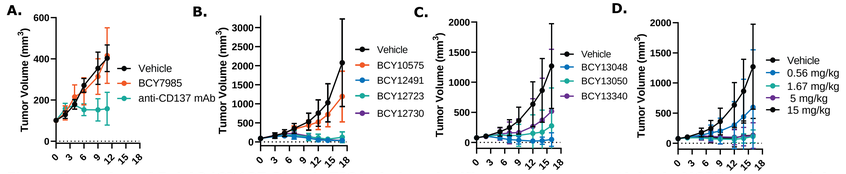


Figure 6: Dosing of EphA2/CD137 *Bicycle* TICAs led to significant anti-tumor activity in MC38 tumor model in huCD137-C57Bl/6 mice. (A) No anti-tumor activity was observed with BCY7985, a molecule with weaker potency (EC50 of 5 nM) and very short in-vivo exposure ($t_{1/2}$ of 0.2 h), when dosed at 20 mg/kg daily (QD) while 3mg/kg twice a week (BIW) anti-CD137 mAb demonstrated robust anti-tumor activity **(B)** Anti-tumor activity of BCY10575 (0/6 Complete Responses on day 28, CR), BCY12491(4/6 CR), BCY12723 (2/6 CR) and BCY12730 (3/6 CR) when dosed at 15 mg/kg Q3D (once every 3 days) show that molecules with sub-nanomolar potency in the reporter assay and half-life of ≥1 h have robust efficacy. **(C and D)** Anti-tumor activity of **(C)** BCY13048 (5/6 CR), BCY13050 (3/6 CR), BCY13340 (0/6 CR) at 5 mg/kg BIW and **(D)** BCY12491 at 0.56 (0/6), 1.7(1/6 CR), 5 (2/6 CR) and 15 (3/6 CR) mg/kg BIW. (**B,C,D**) Studies were run for total of 4 weeks with dosing stopped after week 3.

Target Coverage Model

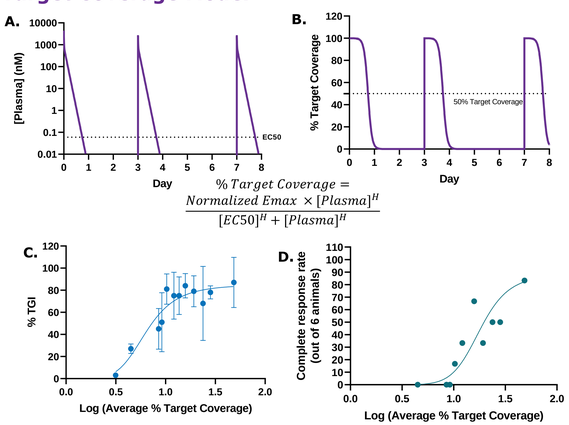


Figure 7: Continuous plasma exposure of EphA2/CD137 *Bicycle* TICAs not necessary for robust anti-tumor activity (**A**) Multi-dose plasma PK and corresponding (**B**) %Target Coverage plot were simulated for each EphA2/CD137 *Bicycle* TICA™ with example for 15 mg/kg BIW dose of BCY12491 shown. **(C and D)** Average % target coverage (AUC(% target coverage$_{0\text{-last}}$)/ AUC(100% target coverage)) was calculated based on % Target Coverage-time profile (**C**) % Tumor growth inhibition (%TGI) plotted against Average % Target coverage shows maximum anti-tumor activity is observed with intermittent target coverage. (**D**) Rate of complete responses (out of 6 mice) vs Average % Target coverage shows high complete response rate without complete target coverage throughout the dosing interval.

CONCLUSIONS

- A medicinal chemistry approach afforded an EphA2/CD137 *Bicycle* TICA™ that delivered robust anti-tumor efficacy in vivo.
- Integrated pk/pd simulations indicate that this activity is achieved despite intermittent plasma exposure of EphA2/CD137 *Bicycle* TICAs, suggesting that continuous target coverage is not required.
- Based on these experiments, the pharmacokinetic and biological properties of EphA2/CD137 *Bicycle* TICAs are potentially suitable for once weekly dosing in the clinic.

References: [1] Segal NH et al. *Clin Cancer Res*. 2017;23(8):1929-1936. [2] Segal NH et al. *Clin Cancer Res*. 2018;24(8):1816-1823. [3] Chester C et al. Blood. 2018;131(1): 49-57. [4] Hinner MJ, et al. Clin Cancer Res. 2019;25(19):5878-5889 [5] Claus C. et al. Sci Transl Med. 2019;11(496): eaav5989. [6] Eskiocak U et al. JCI Insight. 2020;5(5):e133647. [7] Mayes PA et al. Nat Rev Drug Discov 2018;17:509-27. [8]Upadhyaya P et al. J Immunother Cancer 2021;9:e001762.

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