A novel fully synthetic dual targeted EphA2/4-1BB *Bicycle®* peptide induces tumor localized 4-1BB agonism

ABSTRACT# P794

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ABSTRACT

- 4-1BB (CD137) is a member of TNF superfamily involved in the stimulation of several immune cells. Agonism of this receptor is a promising immunotherapeutic approach with agonistic anti–CD137 antibodies showing efficacy in pre-clinical models[1] with limited success in clinical trials due to hepatotoxicity.
- *Bicycles®* represent a new therapeutic modality - fully synthetic, constrained bicyclic peptides. We recently showed that *Bicycle®* CD137 agonists with rapid clearance, minimal liver exposure induce CD137 mediated anti-tumor activity while avoiding liver toxicity. Moreover, our platform allows to rapidly develop a portfolio of fully synthetic tumor-targeted immune cell agonists (TICAs).
- Erythropoietin-producing hepatocellular A2 receptor (EphA2) is a tumor antigen which is overexpressed in human cancers and correlates with poor prognosis.
- Here, we present substantial preclinical data demonstrating the potent immunomodulatory activity of EphA2/CD137 TICAs which engage EphA2 and CD137 simultaneously with high affinity resulting in picomolar potency. EphA2/CD137 TICAs potentiate tumor target dependent cytokine secretion in immune co-culture experiments and promote caspase activity in T cell mediated cell killing assays.
- In vivo testing of EphA2/CD137 TICA in PBMC-humanized mice bearing HT29 xenografts showed an increased percentage of CD8⁺ T cells in tumor tissue but not in the circulation, suggesting a local tumor target specific stimulation of T cells without systemic CD137 agonism. Intermittent dosing of EphA2/CD137 TICA showed a robust anti-tumor activity in a syngeneic MC38 mouse model.
- Taken together, the unique ability of EphA2/CD137 dual targeting *Bicycles®* to precisely and potently stimulate immune cells in tumors without systemic immune stimulation is very promising and provides us a rationale for developing first-in-class *Bicycles®* to target EphA2+ cancers.

INTRODUCTION

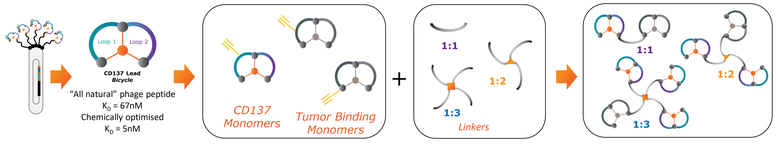


Figure 1A: Phage screening identified initial CD137 binders in the nM range following affinity maturation. The lead peptide was chemically optimized to achieve K_D=5 nM (SPR). CD137 and EphA2 monomers were designed to vary attachment point, affinity and physicochemical properties. 1:1, 1:2 and 1:3 linkers deliver different architectures and contribute to overall physicochemical properties. We identified characteristics that lead to extended pharmacokinetics and further optimized the molecules to achieve balanced properties

Figure 1B: *Bicycle®* TICAs enable optimum spacing compared to bulkier biologics. A typical immune cell receptor complex spacing formed through interaction of the TCR and MHC is 140Å. A representative *Bicycle®* TICA fits very well into this space, unlike most biologic bispecific molecules.

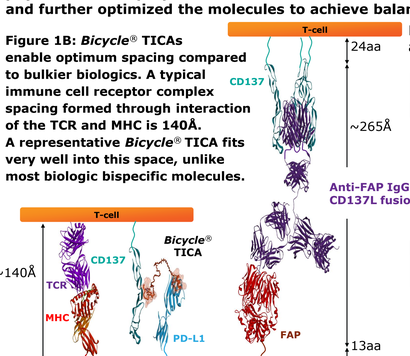
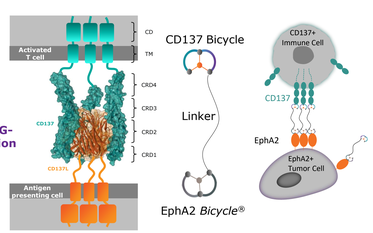


Figure 1C: Tumor/CD137 binding *Bicycles®* as potent and targeted T-cell agonists



As a member of the TNF superfamily of receptors, CD137 requires trimerization for stimulation. An alternative way to achieve CD137 clustering is by connecting the CD137 Bicycle to a Bicycle targeting a highly expressed tumor antigen. In this dual targeting format, CD137 could be effectively clustered across the immune synapse.

RESULTS

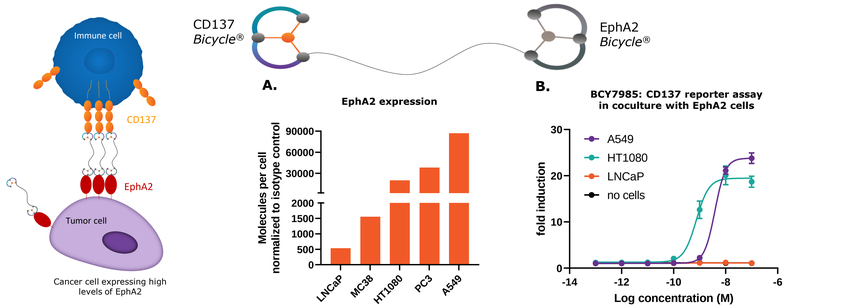


Figure 2: CD137 agonism is EphA2 dependent as demonstrated in CD137 reporter assays. (A) Quantification of multiple cell lines for EphA2 expression via flow cytometry. (B) CD137-expressing Jurkat cells were co-cultured with cancer cells expressing varying levels of EphA2 expression and NFkB-driven luciferase was monitored after treatment with EphA2/CD137 TICA (BCY7985). Luciferase quantification showed that the level of CD137 agonism to BCY7985 is dependent on EphA2 expression in the co-culture cell line (error bars = SD).

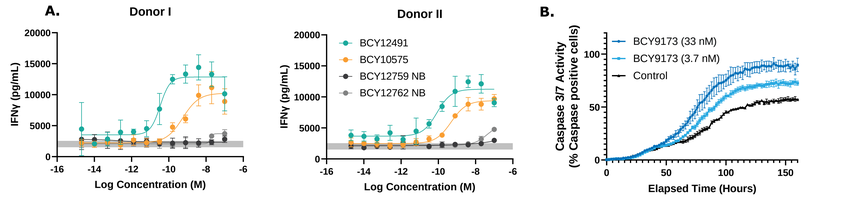
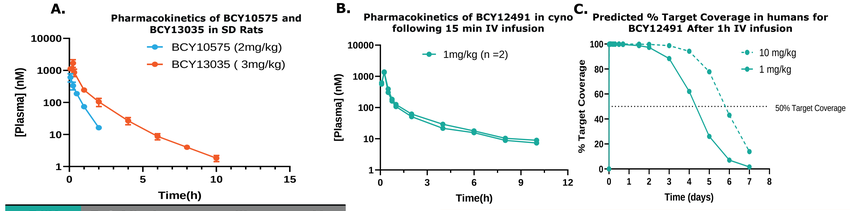


Figure 3: EphA2/CD137 TICAs promote cytokine secretion and caspase activity in a target-dependent manner. (A) PBMCs from healthy donors were co-cultured with tumor cells (5:1) in presence of anti-CD3, test molecules, and supernatants were analyzed for IFNγ by Luminex, data represented from two individual donors. (B) EphA2/CD137 TICAs induced A549 tumor cell killing by CD3-stimulated PBMCs. Cell killing was measured by quantitating Caspase 3/7 activity in cancer cells by Incucyte (error bars = SD).



BCY	EphA2 Ki (nM)	$t_{1/2}$ (h)	CLp (mL/min/kg)	Vss (L/kg)
BCY10575	2.2	0.43	22	0.71
BCY13035	0.8	1.6	10	0.48

Species	Cyno and predicted human PK parameters: in vitro EC50(nM)	$t_{1/2}$(h)	CLp (mL/min/kg)	Veff (L/kg)
Cyno	-	5.3	3.3	0.6
Human	0.085	11	1.1	1.0

Figure 4: (A) Optimization of EphA2/CD137 TICAs by improving physicochemical properties leads to molecules with longer half-life and lower clearance. (B) Plasma concentration- time profile for BCY12491 in cyno. (C) Projections to human PK coupled with in-vitro potency from primary assays suggests a potential for weekly dosing of these molecules in the clinic.

RESULTS

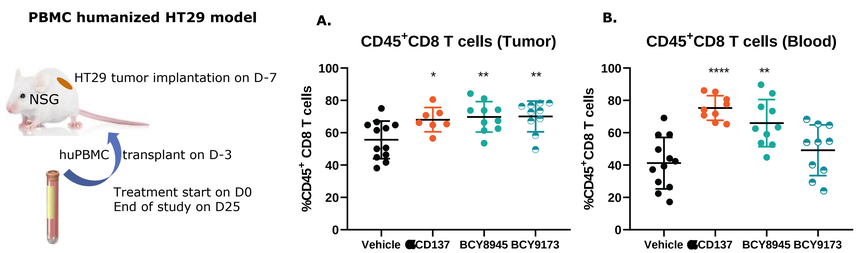


Figure 5: EphA2/CD137 TICA leads to an increase in CD8⁺ T-cells locally in tumor in HT-29/PBMC engraftment model. HT29 tumor cell line has high endogenous expression of EphA2. Before initiation of treatment, NSG mice were implanted with HT-29 tumor cells and human PBMCs. Vehicle, αCD137 (Urelumab analogue), BCY8945 (CD137 multimer agonist) and BCY9173 (EphA2/CD137 TICA) were the treatment groups. At the end of treatment (D25), immune profiling of tumor and whole blood was carried out by Flow cytometry. αCD137 and BCY8945 displayed a systemic agonist profile with increase in CD8⁺ T cells both systemically and at tumor site (A) whereas EphA2-targeted BCY9173 demonstrated only tumor localized CD137 agonism (B).

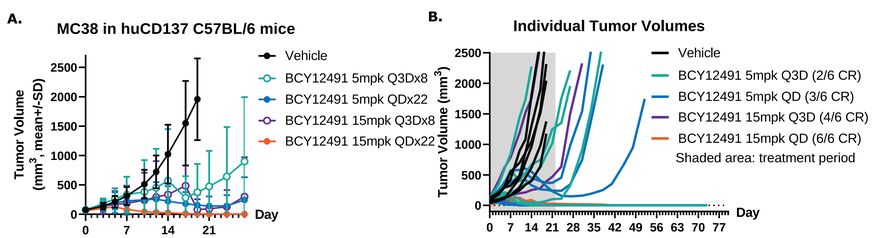


Figure 6: Intermittent dosing of EphA2/CD137 leads to a robust anti-tumor activity in syngeneic MC38 mouse model. CD137 *Bicycle®* is human specific thus humanised CD137 (huCD137) C57BL/6 mice were used. Treatment was initiated when the tumor volume is ~60mm³. BCY12491 was administered for a period of 22 days at two different doses and two different dosing schedules (QD and Q3D). (A) All treatment groups displayed anti-tumor activity (n=6 per group). (B) Individual tumor volumes are displayed with the corresponding number of complete responders (CR) in each group. Subsequently, CRs are being enrolled into re-challenge study to evaluate the potential for EphA2/CD137 TICA induced immune agonism to elicit memory T cell response.

CONCLUSIONS

- We have successfully synthesized EphA2/CD137 TICAs that engage EphA2 and CD137 simultaneously with high affinity resulting in picomolar potency
- EphA2/CD137 Bicycles are highly potent in both reporter cell and primary T-cell assays in a tumor–target dependent manner.
- EphA2/CD137 Bicycles show robust anti-tumor activity in HT-29/PBMC engraftment model and syngeneic MC38 mouse model.
- PK/PD modeling predicts a potential for weekly dosing of these molecules in the clinic.
- The *Bicycle®* platform enables a unique opportunity to rapidly generate fully synthetic *TICA* molecules with tuneable PK-properties across wide range of tumor targets as well as other immune cell receptors

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

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