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

Kristen Hurov, Punit Upadhyaya, Marianna Kleyman, Jessica Kublin, Tom Stephen, Jun Ma, Elizabeth Repash, Julia Kristensson, Sophie Watcham, Liuhong Chen, Sailaja Battula, Johanna Lahdenranta, Kevin McDonnell, and Nicholas Keen

ABSTRACT# P782

ABSTRACT

- 4-1BB (CD137/TNFRSF9) is a costimulatory receptor belonging to the TNF receptor superfamily
- Despite compelling preclinical data, 4-1BB agonistic antibodies have been hampered by failure to delineate hepatotoxicity from efficacy in the clinic [1,2]. Next generation strategies are focused on bispecific approaches aimed at promoting target-mediated clustering of 4-1BB to limit systemic and liver toxicities [3,4]
- *Bicycles*® represent a new class of drugs - fully synthetic, constrained bicyclic peptides that have antibody-like affinity and selectivity to their targets. Unlike traditional biologic approaches, the small size (~2 kDa) and tunable PK parameters of *Bicycles*® enable superior tumor penetration and allow exploration into the relationship between pulsatile dosing and 4-1BB activation while de-risking hepatotoxicity concerns due to a differentiated renal clearance mechanism combined with tumor-localized activation
- Nectin-4/PVRL4 is highly expressed on numerous tumors, including bladder, pancreatic, and lung
- We are developing tumor-targeted immune cell agonists (TICAs) targeting Nectin-4 and agonizing CD137
- Nectin-4/CD137 TICAs exhibit extremely potent and Nectin-4-dependent CD137 agonism in an engineered CD137 reporter system and induce robust production of pro-inflammatory cytokines in PBMC/tumor cell co-cultures and ex-vivo patient-derived tumor samples
- Nectin-4/CD137 TICAs induce complete regressions and resistance to re-challenge in immune competent models with intermittent dosing

INTRODUCTION

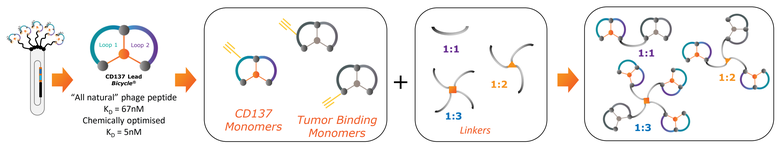
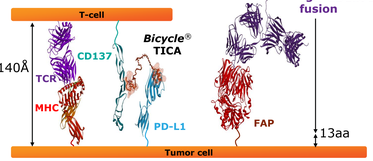
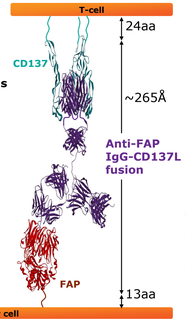


Figure 1: Phage screening, affinity maturation, and chemical optimization resulted in the lead CD137-binding *Bicycle*®. The modular nature of the *Bicycle*® platform allows us to rapidly generate fully synthetic molecules linking the CD137 *Bicycle*® to a broad range of tumor antigen-binding *Bicycles*® at different stoichiometries.

Figure 2: *Bicycle*® TICAs enable optimum spacing compared to bulkier biologics. The 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, whereas most biologic bispecific molecules do not.





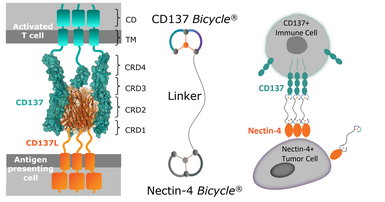


Figure 3: CD137 is a highly validated immunotherapy target that is expressed on T cells, NK cells, as well as several other immune cells. CD137 requires trimerization and clustering for its activation. *Bicycle*® TICAs offer a therapeutic modality to activate CD137.

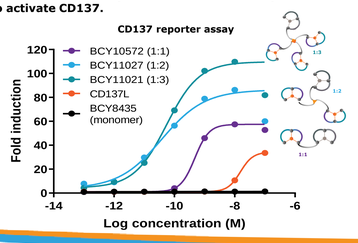


Figure 4: Nectin-4/CD137 TICAs are potent CD137 agonists. CD137-expressing Jurkat cells were co-cultured with Nectin-4-expressing HT1376 cells and NFκB-driven luciferase is monitored after 6 hours of treatment with the indicated molecules. A CD137 *Bicycle*® monomer (BCY8435) served as a negative control and the activity of CD137L is shown for comparison. The modular nature of the platform enabled us to investigate the effects of changing the valency of the CD137 binding part of the molecule.

RESULTS

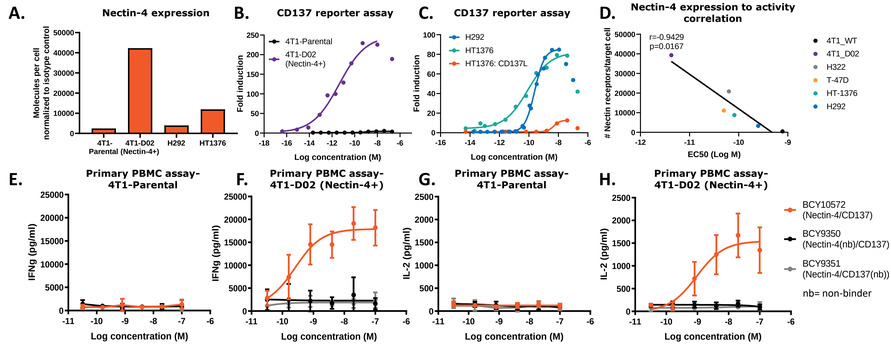
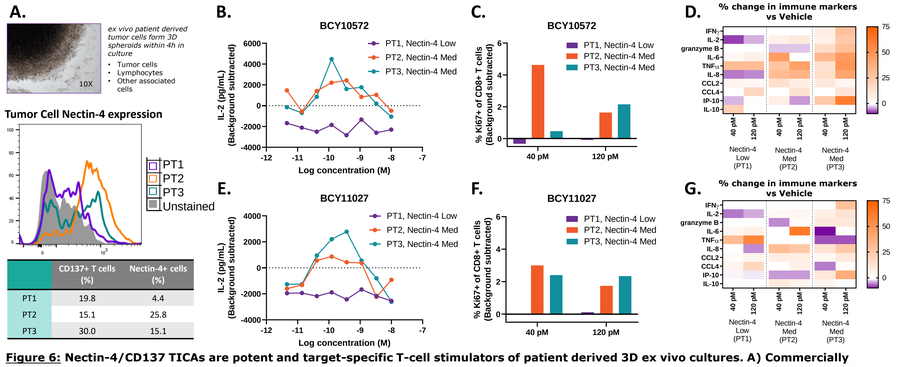


Figure 5: Nectin-4/CD137 TICAs displayed robust Nectin-4-dependent activity in both a CD137 reporter assay and in a primary immune cell assay. A) Quantification of Nectin-4 expression in several tumor cell lines by flow cytometry. B) and C) BCY11863 is agonistic in the CD137 reporter assay in the presence of Nectin-4-expressing cells. D) BCY11863 activity in the CD137 reporter assay correlates with Nectin-4 expression of the co-culture cells (Spearman correlation). E) and F) Human PBMCs were co-cultured with 4T1 cells or 4T1 cells that were engineered to overexpress Nectin-4 and interferon gamma (IFNg) levels in the media were measured at 48 hours by Luminex. Activity is dependent on Nectin-4 expression and on the molecule binding to both Nectin-4 and CD137. Error bars represent the standard deviation of biological duplicates in each of three individual donors (n=6). G) and H) Same as E) and F) except interleukin-2 (IL-2) was measured.



	CD137+ T cells (%)	Nectin-4+ cells (%)
PT1	19.8	4.4
PT2	15.1	25.8
PT3	30.0	15.1

Figure 6: Nectin-4/CD137 TICAs are potent and target-specific T-cell stimulators of patient derived 3D ex vivo cultures. A) Commercially obtained NSCLC tumor –derived single cell suspensions were profiled for immune cell content and tumor cell expression of Nectin-4. NSCLC cell suspensions were used to generate 3D structures (magnetic 3D cell culture, n3D) that were treated with Nectin-4/CD137 TICAs for 48h. Nectin-4/CD137 TICAs induce dose-responsive IL-2 secretion (B, E), CD8+ T-cell proliferation (C, F), and modulation of several immune markers (D, G) in ex vivo patient samples that have at least a medium level of tumor cell Nectin-4 expression.

RESULTS

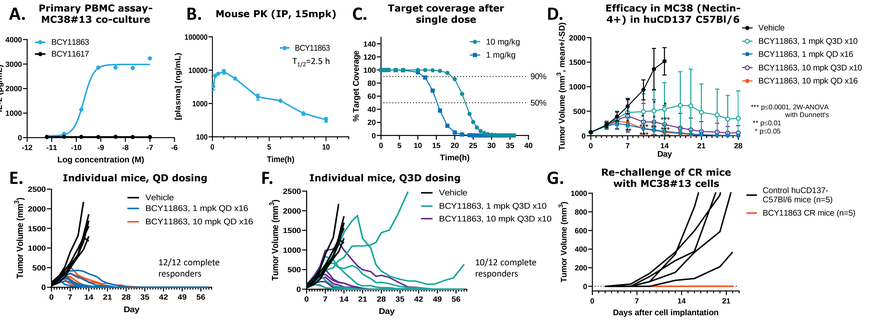


Figure 7: Intermittent dosing of a Nectin-4/CD137 TICA led to robust anti-tumor activity and resistance to re-challenge in a syngeneic mouse model. A) BCY11863 induces IL-2 release in a human PBMC assay when in co-culture with MC38 cells that were engineered to express Nectin-4 (MC38#13). B) PK in mouse after an IP dose of 15mpk. C) With a 10mpk dose, we expect to be maintaining at least 50% target coverage for ~24 h. D) In syngeneic mice carrying a MC38#13 tumor, BCY11863 led to complete responses in 22/24 mice, even when dosing frequency was reduced to Q3D. E) and F) Individual mouse plots in QD and Q3D dosing cohorts, respectively. G) Five complete responder (CR) mice were re-implanted with MC38#13 cells and 5/5 tumors were rejected, indicating an established memory response.

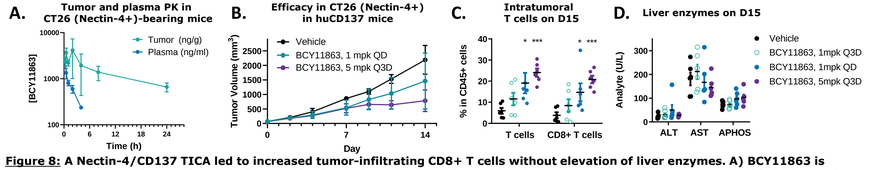
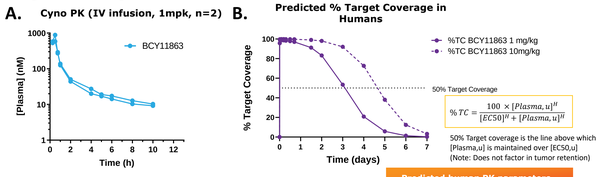


Figure 8: A Nectin-4/CD137 TICA led to increased tumor-infiltrating CD8+ T cells without elevation of liver enzymes. A) BCY11863 is retained in the tumor longer than it is detected in circulation (single IV dose of 5mpk; tumor $t_{1/2}$=13.4h; plasma $t_{1/2}$=1.7h). B) Intermittent IP dosing of BCY11863 led to anti-tumor activity in a syngeneic model carrying a CT26-Nectin-4-expressing tumor. C) Immune cell populations in the tumor were measured by flow cytometry at the end of study (day 15). D) Liver enzymes were measured on day 15 and no significant changes were observed.



Compound	Terminal half-life $t_{1/2}$ (h)	CLp (mL/min/kg)	Vss (L/kg)
BCY11863	5.3	3.3	0.62

Compound	in vitro EC50 (nM)	Terminal half-life $t_{1/2}$ (h)	CLp (mL/min/kg)	Voff (L/kg)
		Predicted human PK parameters		
BCY11863	0.47	12	1.2	1.2

Figure 9: Target coverage modeling predicts the plasma level of a Nectin-4/CD137 TICA may be maintained above the in vitro EC50 for nearly a week in humans. A) BCY11863 has a 5.3 hour terminal half-life in cynomolgus monkeys following an IV infusion. B) Using the in vitro EC50 (IL-2 release assay in PBMC/4T-1-Nectin-4 co-culture) and predicted human PK parameters (allometric scaling from cyno PK data), we modeled percent target coverage in humans with the formula shown.

CONCLUSIONS/SUMMARY

- Bicycle Therapeutics is building a new generation of chemically synthetic (NCE) tumor antigen targeted CD137 agonists
- *Bicycle*® Nectin-4/CD137 tumor-targeted immune cell agonists (TICAs) are highly potent in CD137 reporter cells, primary immune cell assays, and ex vivo patient-derived tumor cultures in a Nectin-4 dependent manner
- Intermittent dosing of a Nectin-4/CD137 TICA lead to complete regressions, resistance to re-challenge, and an increase in tumoral CD8+ T-cell infiltration in syngeneic mouse models
- Modeling predicts that a single IV dose to humans will cover the target for nearly a week, suggesting that continuous infusion in the clinic may not be necessary

References: [1] Segal et al, *Clin Cancer Res* 23(8): 1929-36 (2017); [2] Chester et al, *Blood* 131(1): 49-57 (2018);
[3] Pastor et al, *Mol Ther* 19(10): 1878-86 (2011); [4] Claus et al, *Sci Transl Med* 11(496): eaav5989

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