Activation of the T cell costimulatory protein CD137 using multivalent bicyclic peptides

ABSTRACT# P398

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ABSTRACT

- CD137 (4-1BB/TNFRSF9) is a costimulatory receptor belonging to the TNF receptor superfamily.
- CD137 agonism is a promising immunotherapeutic approach as indicated by anti-tumour effects in mouse models with agonistic monoclonal antibody therapy (1).
- There are currently two agonistic antibodies in clinical trials, however they have been limited by hepatoxicity and/or suboptimal activity.
- Peptides binding to human CD137 ligand-binding site were identified by phage screening using proprietary Bicycle technology.
- Further chemical optimisation allowed systematic generation of a matrix of dimeric, trimeric, and tetrameric CD137 synthetic agonists with a broad range of cell-activity properties.
- CD137 synthetic multimers maintain cell activity after washout consistent with high avidity to the trimeric CD137 receptor complex.
- CD137 synthetic multimers were shown to have an *in vivo* half-life of approximately 30 minutes.
- A tetramer using the Lysine 3 attachment point showed trends towards preventing syngeneic tumour growth in the hCD137 mouse model.

INTRODUCTION

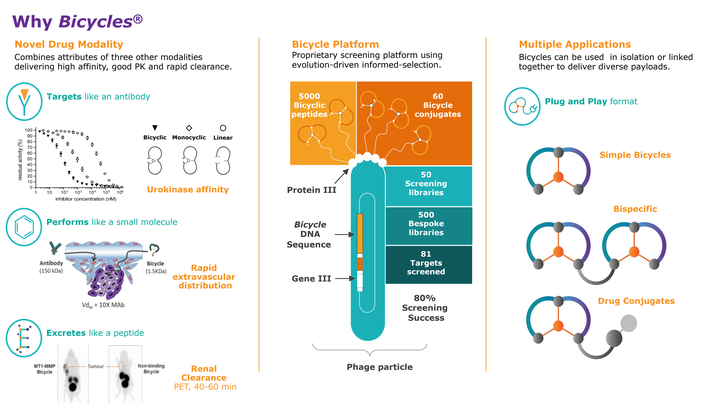

Figure 1: Bicycles® are a new class of drugs - fully synthetic, constrained bicyclic peptides that show antibody-like high affinity binding and exquisite target specificity (2). The Bicycle platform uses phage display and chemical optimization to rapidly identify and improve CD137 binders for affinity and physicochemical properties. Through novel chemical approaches, peptides can be attached to generate agonistic multimers that cross-link and thus activate the trimeric CD137 receptor complex on immune cells.

- **Large (>10^{15}) & diverse libraries generate multiple chemical start points.**
- **Fully synthetic, faster, more versatile production than antibodies.**
- **Bicyclic multimers have a different *in vivo* profile compared to antibodies. The clinical development of Urelumab has been hampered by on-target hepatotoxicity. We expect the risk of liver inflammation to be minimal with CD137 synthetic multimers.**

RESULTS

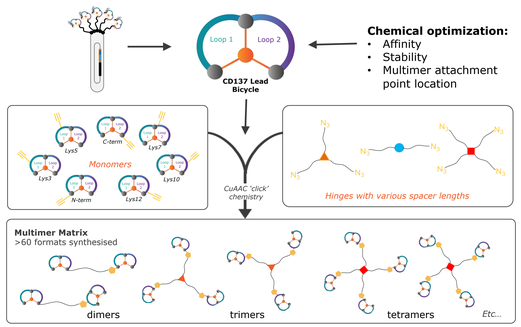

Figure 2: Phage screening identified initial CD137 binders in the uM range that then underwent affinity maturation. The lead peptide BCY3814 showed KD=33.3 nM (SPR) after chemical optimization. Monomeric peptides were attached to different hinges to generate dimers, trimers, and tetramers with flexible spacer lengths.

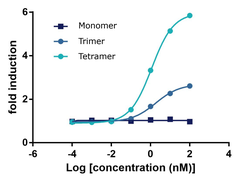

Figure 3: CD137 Bicycle trimers and tetramers are agonistic in the CD137 reporter assay, while monomers are not. Cell activity was monitored using Jurkat cells overexpressing CD137 and carrying a CD137-dependent NF-kB luciferase reporter (Promega). The activity was normalized to untreated cells as fold induction.

A.

B.

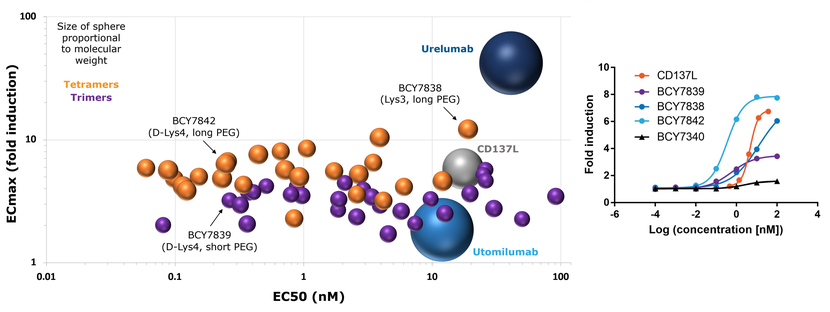

Figure 4: A) The cellular activity of trimers and tetramers compared to known agonists CD137L, urelumab and utomilumab. Data is from the CD137 reporter assay as described in Figure 3. The most potent multimers have 100-fold lower EC50-values compared to the natural ligand (below 0.1 nM compared to 10 nM). Synthetic dimers showed no/little biological activity in vitro (data not shown). There is trend towards tetramers being more potent than trimers. The multimer matrix enabled rapid generation of molecules with a broad range of cell activity properties. B) CD137 Bicycle multimers show dose responsive agonistic activity in the CD137 reporter assay. BCY7340 is a CD137 monomer and is not active.

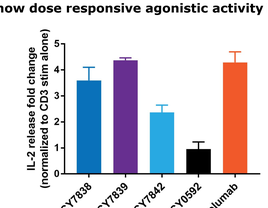

Figure 5: CD137 Bicycle multimers lead to increased cytokine secretion in a primary T cell assay. CD137 expression is induced on T cells (isolated from human PBMCs) using anti-CD3 antibody. T cells are then treated with CD137 multimers (5μM), CD137 Bicycle monomer (BCY0592; negative control; 5μM), or Urelumab (0.5μM) for 48 hours and IL-2 levels were measured in the supernatant using a HTRF assay.

BCY ID	Multimer	Attachment Point	Linker	Molecular Weight (Da)
BCY7838	Tetramer	Lysine 3	PEG23	13816
BCY7839	Trimer	D-Lysine 4	PEG10	8904
BCY7842	Tetramer	D-Lysine 4	PEG23	14100

Table 1. The properties of three CD137 multimers that were selected for in vitro and in vivo characterization.

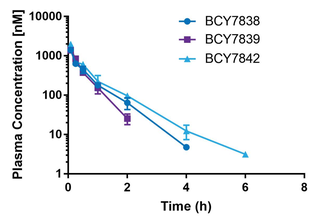

Figure 6: CD137 Bicycle multimers are cleared rapidly in mice. The plasma concentration of BCY7838 (tetramer), BCY7839 (trimer), and BCY7842 (tetramer) following dosing at 5mpk i.v. is shown. The multimer half-life was estimated to be approximately 20-40 minutes.

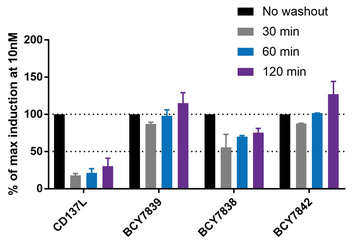

Figure 7: CD137 Bicycle multimers maintain activity after washout. CD137 reporter cells are exposed to compound for 30, 60, or 120 minutes prior to washout of the compound and activity is measured 5.5, 5, or 4 hours later, respectively. In the 'no washout' conditions, cells are exposed to the compound for the full 6 hour incubation.

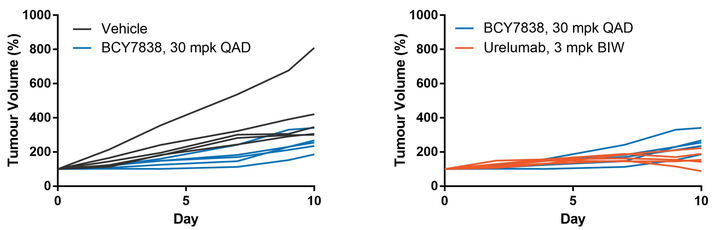

Figure 8: A CD137 tetramer agonist showed efficacious potential in the human CD137 (hCD137) syngeneic mouse tumour model. hCD137 knock-in mice were generated by Biocytogen with expression of hCD137 under the control of the endogenous mouse regulatory elements. The hCD137 mice were inoculated s.c. with syngeneic MC38 cells. When tumours reached a size of ~100mm³, mice were treated with either vehicle, BCY7838 (30mpk i.v., every other day), or Urelumab (3mpk i.p., twice a week).

CONCLUSION/SUMMARY

- Bicycle® peptides specific for human CD137 protein were identified by phage screening.
- CD137 trimers and tetramers showed cell potent agonism that was comparable or better than the natural ligand and clinical antibodies.
- Bicycle® technology generated potent, compact, and fully synthetic CD137 multimeric agonists, which represent novel cancer immunotherapeutic candidates.
- Reference (1) Melero et al, *Nat Med* 3(6): 682-5 (1997).
- Reference (2) Heinis et al, *Nat Chem Biol* 5(7): 502-7 (2009).

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