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Novel Bicyclic Peptide Multimers Activate T Cell Costimulatory Protein CD137

ABSTRACT#

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ABSTRACT

- CD137 agonism represents a promising immunotherapeutic approach with two agonistic antibodies in clinical trials.
- Peptides binding to the human CD137 ligand-binding site were identified by phage screening using proprietary Bicycle® technology.
- A matrix of dimeric, trimeric and tetrameric CD137 synthetic agonists were generated with a broad range of cell-activity properties.
- CD137 synthetic multimers displayed rapid half-life *in vivo*.
- Tetramer BCY7838 indicated efficacious potential in preventing syngeneic tumour growth in a hCD137 mouse model.

INTRODUCTION

CD137 (4-1BB/TNFRSF9) belongs to the TNF receptor superfamily and provides costimulatory signalling for T cells and NK cells. Agonistic anti-CD137 antibodies have shown potent, often curative anti-tumour activity in preclinical mouse models (1). Two human anti-CD137 antibodies Urelumab (BMS) and Utomilumab (Pfizer) are currently undergoing clinical testing. Urelumab has shown single-agent partial responses, but demonstrates significant hepatotoxicity (2). The agonist antibody Utomilumab lacks hepatotoxicity, but has shown little or no single agent activity in solid tumours (3).

Bicycles® are a new class of drugs - fully synthetic, constrained bicyclic peptides that show antibody-like high affinity binding and exquisite target specificity (4). The Bicycle® platform uses phage display and chemical optimisation to rapidly identify and improve peptide binders for affinity and physicochemical properties. Through novel chemical approaches, peptides can be linked to generate agonistic multimers that cross-link and thus activate trimeric complexes on immune cells. We hypothesised that fully synthetic Bicycle CD137 agonist multimers may induce agonism as a novel immunotherapeutic approach.

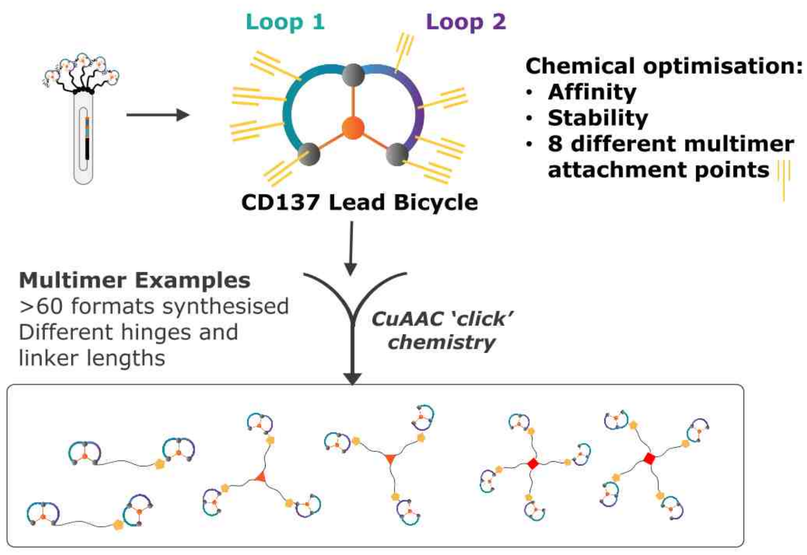


Figure 1. Modular synthesis of dimeric/multimeric Bicycle peptide complexes. These agonists enable Bicycle® peptides to target trimeric TNFR-superfamily immune receptors exemplified by CD137.

Flexibility of the Bicycle® peptide format allows different functionalities.

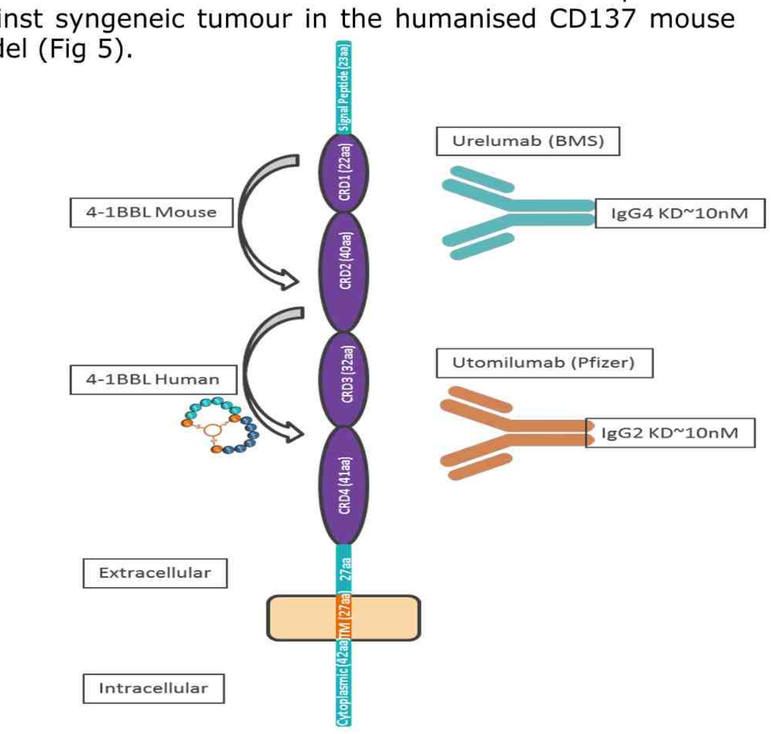
METHODS

Phage selection: Bicycle® phage libraries were used to identify binding peptides to human CD137 protein. The hits were characterised by signal/background screening and pyrosequencing.
Chemistry: Peptides were further optimised by substitutions with non-natural amino acids and linked using discrete PEG spacers of various lengths to generate fully synthetic dimers, trimers and tetramers.
Protein binding: Binding affinities were determined by surface plasma resonance (SPR).
In vitro activity: Cell-activity was measured using CD137 NF-kB luciferase reporter assay cells (Promega).
In vivo profiling: Multimer half-life upon i.v. dosing 5 mg/kg was determined by LC-MS/MS. For efficacy studies, hCD137 (Biocytogen) mice were inoculated s.c. with MC38 cells and when the tumours reached ~100 mm^2 the mice were dosed i.v. with CD137 agonists.

RESULTS

10^{15} Bicycles® were screened on phage against human recombinant CD137 protein. Initial hits in the μM range underwent affinity maturation which identified peptides binding to CD137 with improved affinity of <100 nM. After chemical optimisation, the lead BCY3814 (K_D ~30 nM SPR) was selected. BCY3814 competed for binding with the CD137 ligand and Utomilumab (known to bind to CD137 ligand binding site) but did not compete with Urelumab which binds an alternative epitope (Fig 2). CD137 activation requires receptor crosslinking and we aimed to generate synthetic multimers that would emulate the natural trimeric ligand. The versatility of the Bicycle format allowed us to rapidly generate more than 60 different dimer, trimer and tetramer assemblies of BCY3814 (Fig 1). Different linker lengths and attachment points were explored while maintaining a compact molecular size (4-15 kDa). The multimers exhibited strong avidity-driven binding.

Several of these synthetic Bicycle CD137 agonists were more potent than the clinical antibodies or the natural ligand in the cell reporter assay (Fig 3). Two multimers showed an *in vitro* cell activity profile similar to CD137L. The *in vivo* PK profile of the two Bicycle multimers indicated rapid kinetics typical of peptide metabolism (Table 1, Fig 4). The tetramer BCY7838 demonstrated anti-tumour potential against syngeneic tumour in the humanised CD137 mouse model (Fig 5).



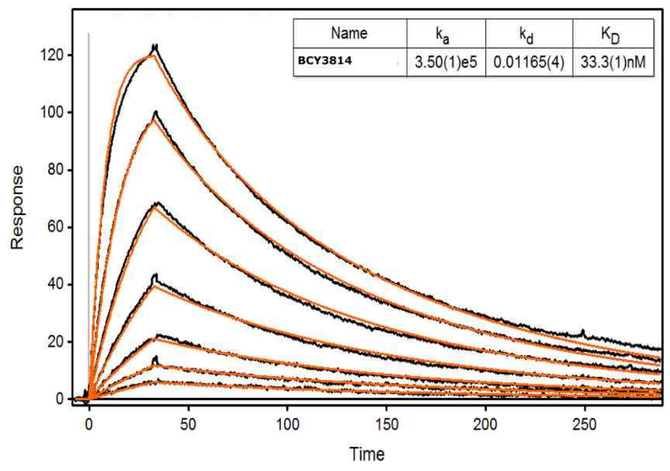


Figure 2. Top: The general structure of the human CD137 protein indicating the binding sites of agonist antibodies. The parental lead peptide bound to the CD137L-binding site in the CD137 receptor. Bottom: The lead peptide BCY3814 showed KD=33.3 nM (SPR) after chemical optimisation. Binding affinities are improved by the avidity effect of multimeric Bicycles® (data not shown).

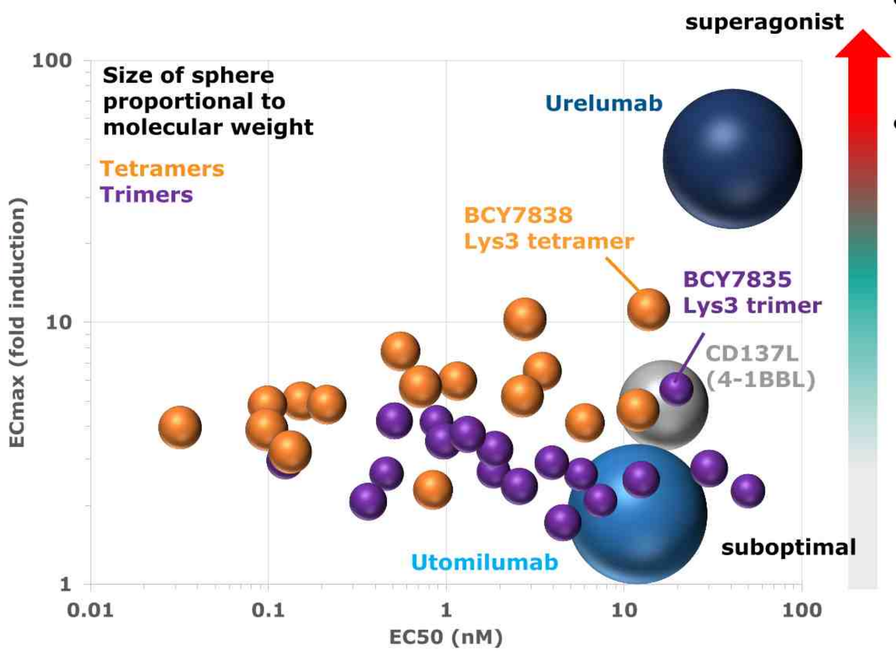


Figure 3. The cellular activity of trimers and tetramers compared to known agonists CD137L, Urelumab and Utomilumab. Synthetic dimers showed no/little biological activity *in vitro* (data not shown). There is a trend towards tetramers being more potent than trimers.

BCY ID	Multimer	Attachment point	Molecular weight (Da)	EC50 nM (*in vitro*)	Emax fold induction (*in vitro*)
BCY7835	trimer	Lysine3	8691	19.8	5.55
BCY7838	tetramer	Lysine3	13816	13.7	11.2
CD137L	trimer		60800	16.7	6.8

Table 1: The two Lysine 3 attachment point multimers and CD137L.

Bicyclic multimers have different *in vivo* profiles to antibodies. The clinical development of Urelumab has been hampered by hepatotoxicity. We expect the risk of liver inflammation to be minimal with CD137 Bicycle® multimers.

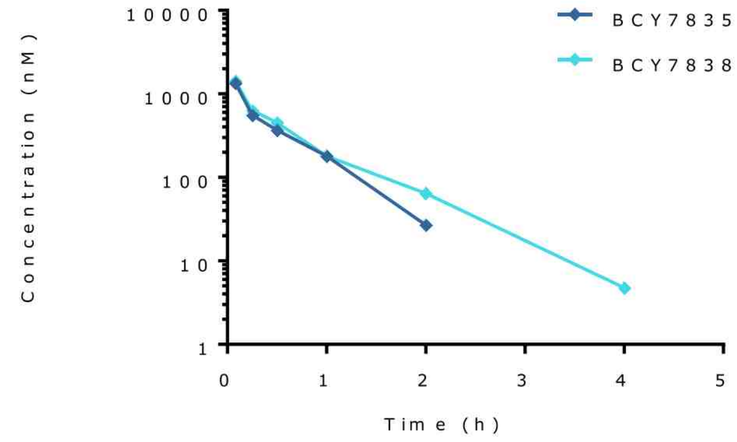


Figure 4. The plasma concentration of BCY7835 and BCY7838 after dosing at 5 mg/kg i.v. The multimer half-life was ~30 minutes, which is typical of the rapid clearance kinetics of peptides.

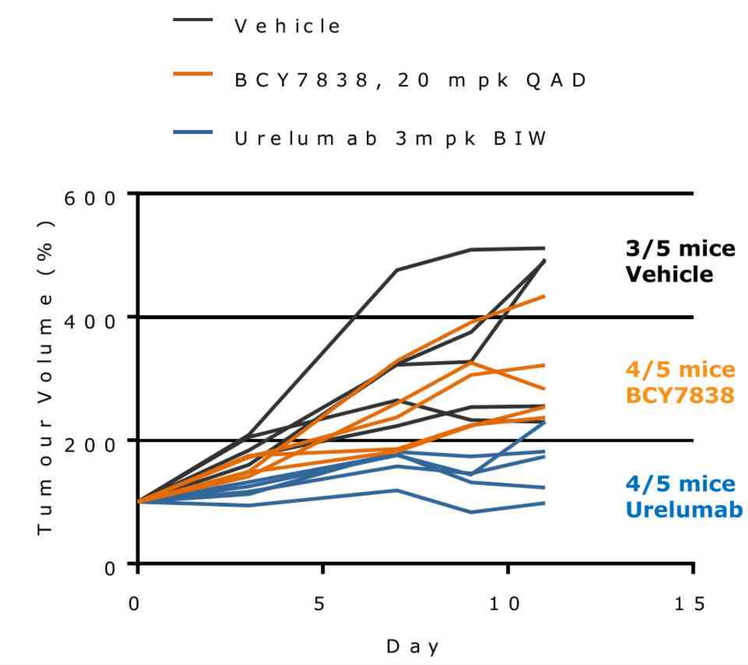


Figure 5. CD137 Bicycle tetramer BCY7838 can inhibit syngeneic tumour growth in the humanised hCD137 mouse model.
BCY7838 was dosed i.v. 20 mg/kg every other day. Urelumab (3 mg/kg, twice a week) was used as a positive control. Each line represents one individual mouse and the tumour volume is plotted relative to day 0 tumour size.

CONCLUSIONS

- Novel Bicycle® peptides specific for human CD137 were identified by phage screening.
- Trimers and tetramers showed potent cell agonism that was comparable to or better than the natural ligand and clinical antibodies.
- Lysine 3 side-chain attached multimers were rapidly cleared in mice.
- The tetramer BCY7838 showed anti-tumour potential in a syngeneic tumour model in a humanised CD137 knock-in mouse.
- **In conclusion, Bicycle® technology generated potent, compact and fully synthetic CD137 multimeric agonists, which represent novel cancer immunotherapeutic candidates.**

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