Small Synthetic, Multivalent Bicyclic Peptides That Activate T Cell Costimulatory Protein CD137

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ABSTRACT# 268

bicycle therapeutics

ABSTRACT

- CD137 agonism is a promising immunotherapeutic approach and there are currently two agonistic antibodies in clinical trials.
- CD137 receptors on immune cells form trimeric complexes in the activated state. 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 targeting synthetic multimers were shown to be stable in plasma and to have in vivo half-life of approximately 30 minutes.
- BCY7835 (Bicycle® trimer) and BCY7838 (Bicycle® tetramer) may prevent syngeneic tumour growth in the hCD137 mouse model.

INTRODUCTION

CD137 (4-1BB/TNFRSF9)

CD137 belongs to the TNF receptor superfamily & 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). These effects are mainly mediated by cytotoxic T cells and generate long lasting, memory responses.

Two human anti-CD137 antibodies Urelumab (BMS) and Utomilumab (Pfizer) are currently undergoing clinical testing. Urelumab has shown several single-agent partial responses, but its use has been hampered by on-target hepatotoxicity (2). The agonist antibody Utomilumab lacks hepatotoxicity, but has shown little or no single agent activity in solid tumours (3).

As CD137 activation requires receptor crosslinking and we aimed to generate synthetic multimers that would emulate the natural trimeric ligand.

Amino acid substitutions of parental lead peptide can reveal potential attachment sites for generation of synthetic multimers.

Bicycles® - A new therapeutic and diagnostic modality

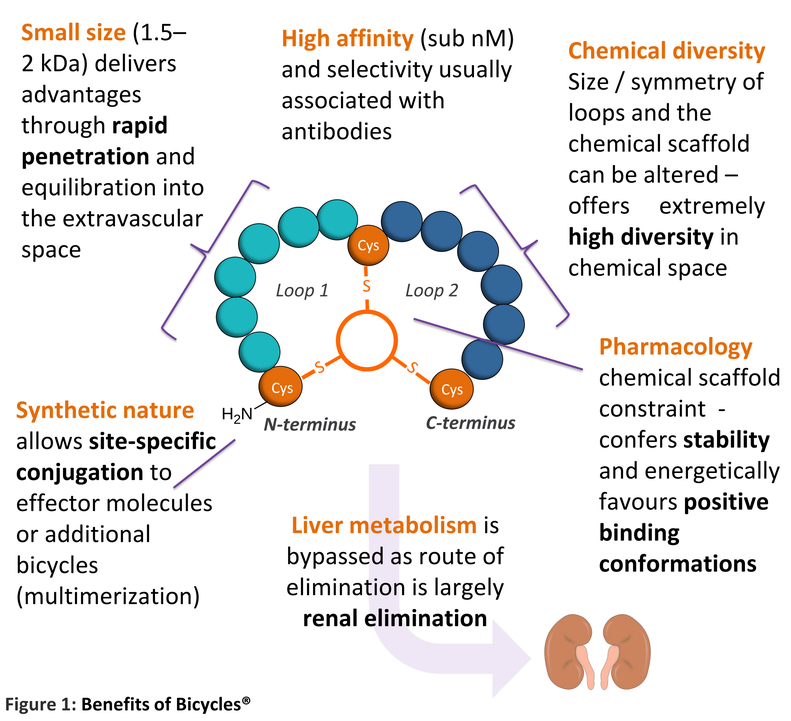

Figure 1: Benefits of Bicycles®

METHODS

Phage selection: Bicycle phage libraries were used to identify binding peptides to the target human CD137 protein, followed by further rounds of affinity maturation. Hits were characterized by alphascreen and pyrosequencing.

Chemistry: The CD137 binding peptides were further optimized to improve peptide stability by substitutions with non-natural amino acids. Monomeric peptides with different attachment points were generated based on peptide SAR information. The peptides were linked using discrete PEG spacers of various lengths to generate fully synthetic dimers, trimers and tetramers.

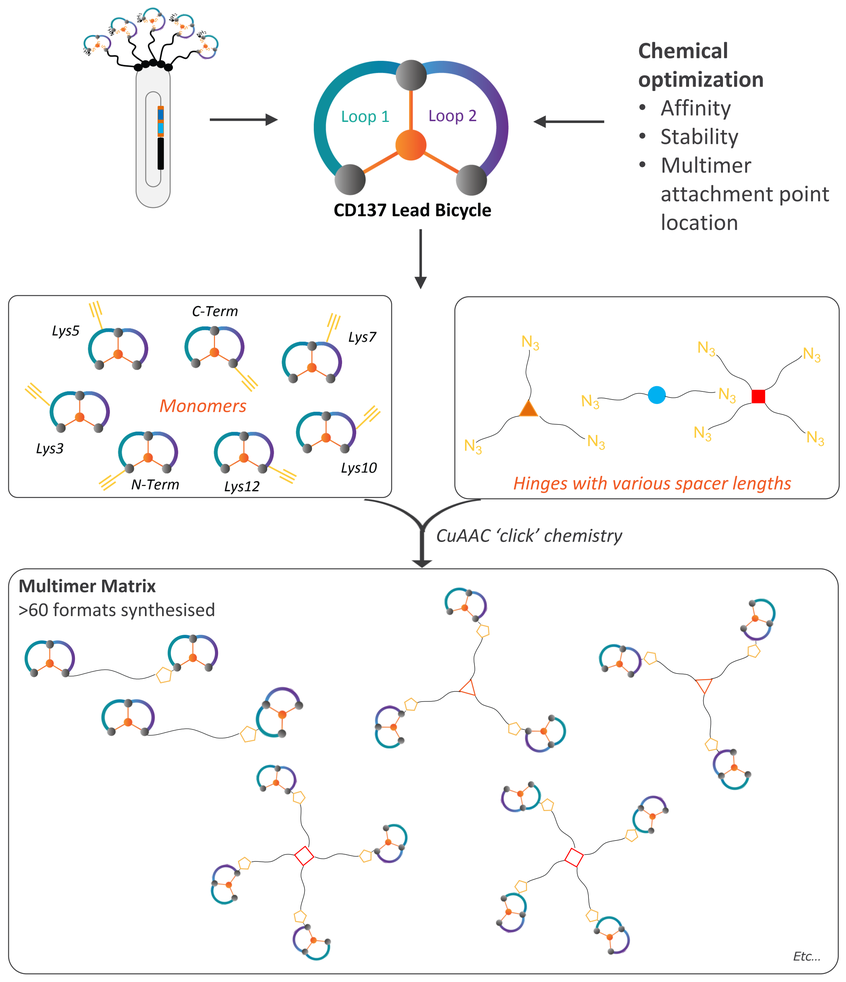

Figure 2: Modular synthesis of dimeric/multimeric Bicycle peptide complexes. Monomeric peptides were attached to different hinges to generate dimers, trimers and tetramers with flexible spacer lengths.

Protein binding: Binding affinities were determined by fluorescent polarization (FP) of Fluorescein-labelled peptides & surface plasma resonance (SPR) Biacore was used for determination of KD-values.

In vitro activity: Cell-activity was analysed using CD137 NF-kB luciferase reporter assay cells (Promega). The activity was normalized to untreated cells as fold induction.

In vivo profiling: For efficacy studies, hCD137 (Biocytogen) mice were inoculated s.c. with MC38 cells and when the tumours reached ~100 mm2 the mice were dosed with CD137 agonists. Synthetic multimers were dosed i.v. at 20-30 mg/kg biweekly and compared to Urelumab dosed i.v. at 3 mg/kg biweekly.

RESULTS

Initial hits from phage display (in the µM range) underwent affinity maturation, identifying peptides binding to CD137 with affinities below 100 nM.

After chemical optimization, a high affinity lead BCY3814 (KD ~30 nM) 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.

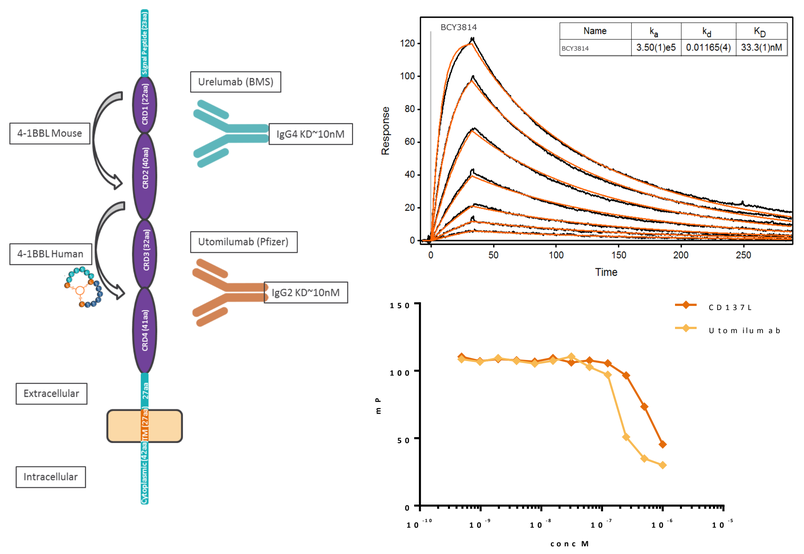

Figure 3: Top: Phage screening identified initial hits from the 6x6 phage library in the uM range that that underwent affinity maturation. The lead peptide BCY3814 showed KD=33.3 nM (SPR) after chemical optimization.
Bottom: The general structure of the human CD137 protein indicating the binding sites of agonist antibodies. The parental lead peptide bound to the CD137L-binding site in the CD137 receptor as shown by FP competition against CD137L and Utomilumab.

We rapidly generated more than 60 different dimer, trimer and tetramer assemblies of BCY3814 to mimic the trimeric nature of the CD137.

The multimers exhibited strong avidity driven binding, characterised by extremely slow off-rates typical of higher order multimers. The linker lengths and attachment points were explored while maintaining a compact molecular size (4-15 kDa).

Several of these synthetic Bicycle CD137 agonists were more potent than the clinical antibodies or the natural ligand in the cell reporter assay.

Two multimers containing attachment points at the third amino acid position (Lys3) in the peptide that showed potent Emax-values and in vitro cell activity profile similar to CD137L, were selected for mouse proof-of-concept experiments. The in vivo PK profile of the two Bicycle multimers indicated rapid kinetics typical of peptide metabolism.

The lysine 3 trimer and tetramer demonstrated a trend towards anti-tumour efficacy against the MC38 syngeneic tumour in the humanized CD137 mouse model.

BCY ID	Multimer	Attachment Point	MW (Da)	EC50 nM (in vitro)	Emax (in vitro)
7835	Trimer	Lys3	8691	19.8	5.55
7838	Tetramer	Lys3	13816	13.7	11.2
CD137L	Trimer		60800	16.7	6.8

Table 1: The two Lysine 3 attachment point multimers selected for in vivo profiling compared to the natural ligand CD137L.

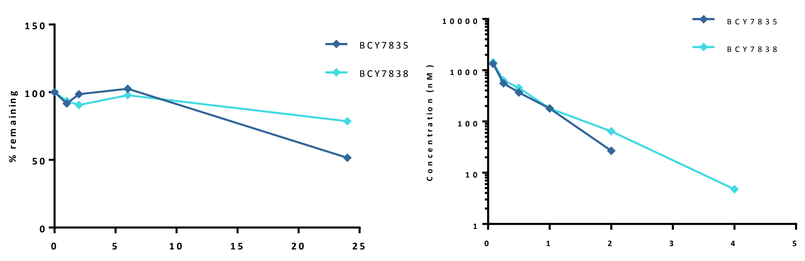

Figure 4. The left panel shows the stability of the two Lysine 3 multimers BCY7835 (trimer) and BCY7838 (tetramer) in mouse plasma over time measured by LC-MS/MS. The data was normalized relative to Time 0.
The right panel shows the plasma concentration of the BCY7835 and BCY7838 after dosing at 5 mg/kg i.v. The multimer half-life was calculated to be 20-30 minutes, typical of the rapid clearance kinetics of peptides.

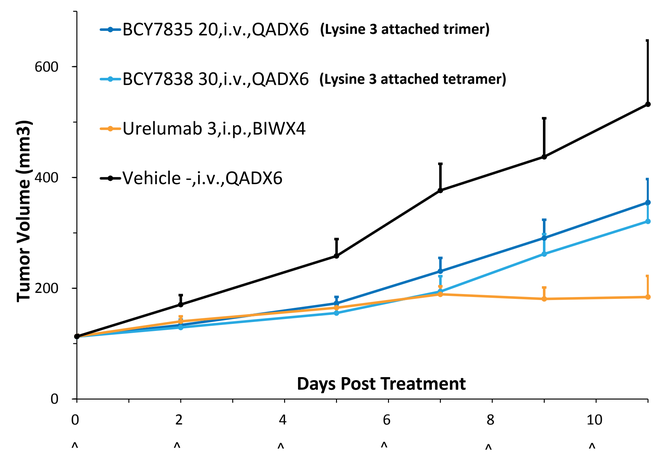

Figure 5. CD137 Bicycle multimers may inhibit tumour growth in humanized hCD137 syngeneic mouse tumour model.
The hCD137 mice were inoculated s.c. with syngeneic MC38 cells. Mice were treated when the tumours reached a size of ~100 mm³. The Bicycle multimers were dosed i.v. at 20-30 mg/kg every other day. Urelumab (3 mg/kg, twice a week) was used as a positive control and vehicle as a negative control.

CONCLUSION/SUMMARY

- Bicycle® peptides specific for human CD137 protein were identified by phage screening.
- Different attachment points and linkers were explored as a matrix to comprehensively investigate the biological properties of synthetic multimers. Trimers and tetramers showed potent cell-activity agonism that was comparable or better than the natural ligand and clinical antibodies.
- Lysine 3 side-chain attached trimer BCY7835 and tetramer BCY7838 showed anti-tumour activity in MC38 syngeneic tumour model in a humanized CD137 knock-in mouse model.
- These results demonstrated the in vivo proof-of-concept (POC) of short-acting CD137 peptide agonists as a promising, novel cancer immunotherapeutic candidate.

REFERENCES

1) Cariad Chester et al. Blood 4;131(1):49-57 (2018)
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