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.

RESULTS

10^7 Bicycle® were screened on phage against human recombinant CD137 protein. Initial hits in the whole human serum underwent affinity maturation which identified peptides binding to CD137 with improved affinity of 100 nM. After chemical optimisation, the lead Bicycle® (Kd ~30 nM SPR) was selected. BCY8314 competed for binding with the CD137 ligand and Urelumab (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).

In conclusion, Bicycle® multimers have been generated with the potential to improve affinity and physicochemical properties. Through novel optimisation to rapidly identify and improve peptide binders for affinity and physicochemical properties. Through novel chemistry, 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.

CONCLUSIONS

- Novel Bicycle® peptides specific for human CD137 were identified by phage screening.
- Trimers and tetramers showed potent cell activation 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.

REFERENCES

5. BicycleTx Limited, Building 900, Babraham Research Campus, Cambridge CB22 3AT, United Kingdom
6. Bicycle Therapeutics Inc, 4 Hartwell Place, Lexington, Massachusetts, United States

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