Activation of 4-1BB using multivalent and tumour targeted bicyclic peptides

ABSTRACT# 3257

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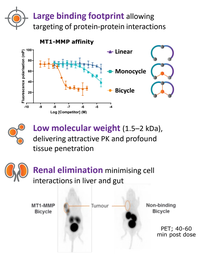
ABSTRACT

- 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 antibodies (1). Two agonistic antibodies are currently 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 proprietary Bicycle technology. Further chemical optimisation allowed systematic generation of a matrix of dimeric, trimeric and tetrameric CD137 synthetic agonists. The modular nature of our *Bicycle*® platform has allowed us to generate fully synthetic bispecific molecules linking CD137 to a broad range of tumour antigens.
- Tunable PK-properties of *Bicycles*® will enable the determination of the required time of target engagement for optimal CD137 agonism/superagonism -induced biological activity.
- Multimeric and bispecific CD137 agonists demonstrate a broad range of cell-activity properties in CD137 expressing reporter cells, primary T-cells and ex vivo patient tumour cultures. Activity of the bispecific CD137 agonists is dependent on tumour target expression.
- CD137 synthetic multimers maintain cell activity after washout consistent with high avidity to the trimeric CD137 receptor complex. Treatment with CD137 synthetic multimers lead to anti-tumour activity correlating to increased T-cell infiltration in MC38 syngeneic tumour bearing mice.
- We hypothesise that engaging CD137 only in the context of the tumour will safely allow for local superagonism.

WHY *BICYCLES*®

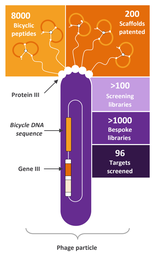
New Class of Therapies

Novel modality delivers high affinity, favourable PK and rapid clearance.



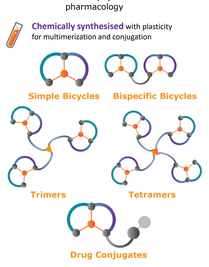
Unique Screening Platform

Proprietary screening platform using powerful evolution-driven informed-selection.



Flexible, Modular Technology

Bicycles can be used as stand-alone therapies or linked together to deliver diverse payloads or combinatorial pharmacology



Bicycles® are a new class of drugs - fully synthetic, constrained bicyclic peptides with high affinity binding and exquisite target specificity (2). 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 and bispecific targeted CD137 Bicycles.

RESULTS

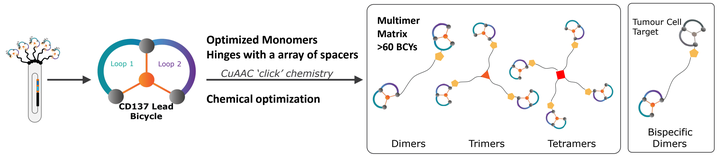


Figure 1: Phage screening identified initial CD137 binders in the μM range followed by affinity maturation. The lead peptide showed KD=5 nM (SPR) after chemical optimization. Monomeric peptides were attached to different hinges to generate dimers, trimers and tetramers with flexible spacer lengths. The multimer matrix enabled generation of molecules with a broad range of cell activity properties. An array of bispecific dimers has been generated with optimized CD137 Bicycles and tumour targeting Bicycles through chemically optimized hinges and spacers.

RESULTS MULTIVALENT *BICYCLES*®

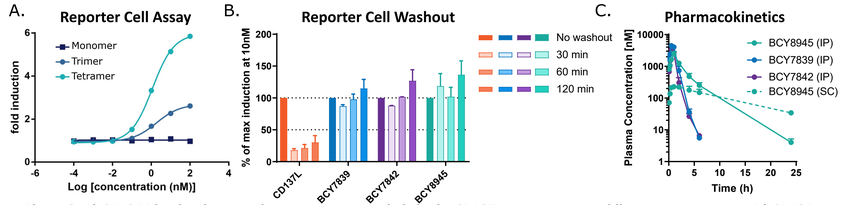


Figure 2: A) CD137 Bicycle trimers and tetramers are agonistic in the CD137 reporter assay, while monomers are not. B) CD137 Bicycle multimers maintain activity after washout. Cell activity was monitored using Jurkat cells overexpressing CD137 and carrying a CD137-dependent NF-kB luciferase reporter. C) Tunable PK properties of CD137 Bicycle multimers enable studies defining the required target engagement kinetics for optimal biological activity. The plasma concentration of BCY8945 (tetramer), BCY7839 (trimer), and BCY7842 (tetramer) following dosing at 30 mpk i.p. or s.c. is shown.

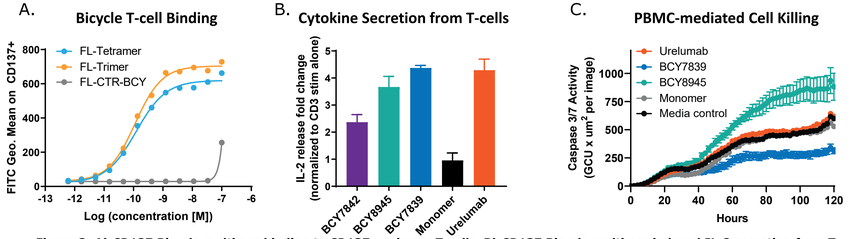


Figure 3: A) CD137 Bicycle multimer binding to CD137+ primary T-cells. B) CD137 Bicycle multimer induced IL-2 secretion from T-cells. CD137 expression is induced on T cells (isolated from human PBMCs) using anti-CD3 antibody. T cells are then treated with CD137 multimers (5μM), CD137 monomer (5μM) or Urelumab (0.5μM) for 48 hours and IL-2 levels were measured in the supernatant using a HTRF assay. C) CD137 Bicycle multimer (1μM) induced A549 tumour cell killing by CD3-stimulated PBMCs. Cell death was measured by quantitating Caspase 3/7 activity on PBMC/tumour cell co-cultures by Incucyte.

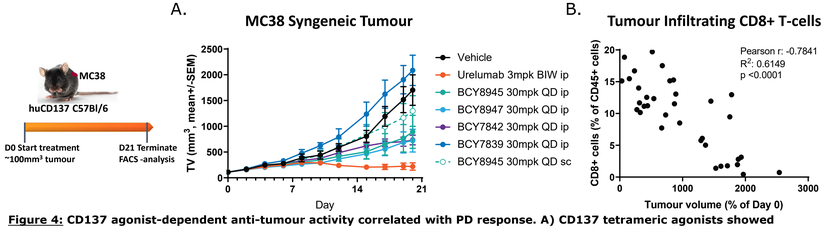


Figure 4: CD137 agonist-dependent anti-tumour activity correlated with PD response. A) CD137 tetrameric agonists showed efficacious potential in MC38 mouse tumour model in syngeneic C57Bl/6 mouse with knocked-in huCD137 ECD (Biocytogen). Mice were treated with either vehicle, BCY (30mpk ip or sc), or Urelumab (3mpk ip, twice a week). B) CD137 tetrameric agonists led to increased tumour infiltrating CD8+T cells at the end of the study and this response correlated with tumour shrinkage.

RESULTS BISPECIFIC *BICYCLES*®

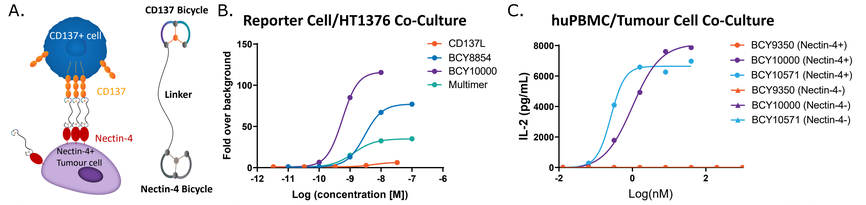


Figure 5: A) Modular nature of the Bicycle platform allows us to rapidly generate fully synthetic bispecific molecules linking CD137 agonist to a broad range of tumour antigen binding peptides. Nectin-4/CD137 Bicycle is shown as an example. B) Bispecific Nectin-4/CD137 Bicycles are agonistic in the CD137 reporter assay in the presence of Nectin-4 expressing tumour cells. C) Bispecific Nectin-4/CD137 Bicycles induce IL-2 secretion from human T-cells only in the presence of Nectin-4 expressing tumour cells.

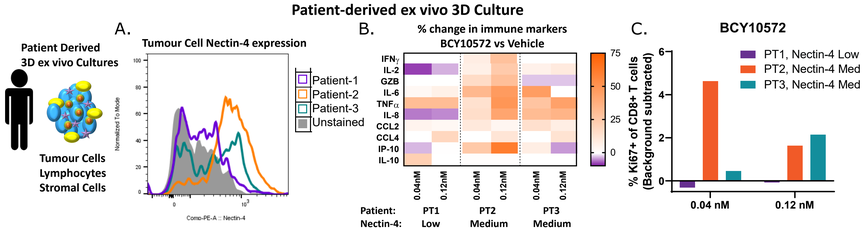


Figure 6: Nectin-4/CD137 bicpecific peptide is a potent and target-specific T-cell activator of patient derived 3D ex vivo –cultures. A) Commercially obtained NSCLC tumour –derived single cell suspensions were profiled for immune cell content and tumour cell expression of Nectin-4. B and C) NSCLC Cell suspensions we used to generate 3D structures (magnetic 3D cell culture, n3D) that were treated with Nectin-4/CD137 bispecific peptides for 48h. Nectin-4/CD137 bispecific peptides induce cytokine secretion (B) and CD8+ T-cell proliferation in ex vivo patient samples that have at least a medium level of tumour cell Nectin-4 expression.

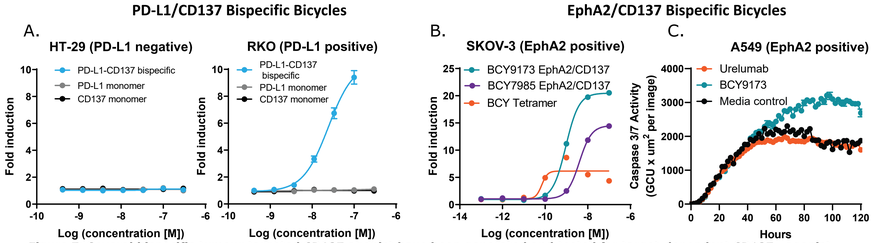


Figure 7: Several bispecific tumour targeted CD137 agonist have been generated and tested for target-dependent CD137 –agonist activity. A) Bispecific PD-L1/CD137 Bicycles are agonistic in the CD137 reporter assay in the presence of PD-L1 expressing tumour cells. B) Bispecific EphA2/CD137 Bicycles are agonistic in the CD137 reporter assay in the presence of EphA2 expressing tumour cells. C) Bispecific EphA2/CD137 Bicycle (0.1μM) induced A549 tumour cell killing by CD3-stimulated PBMCs. Cell death was measured by quantitating Caspase 3/7 activity on PBMC/tumour cell co-cultures by Incucyte.

CONCLUSIONS/SUMMARY

- Multimeric and Bispecific *Bicycle*® peptides specific for human CD137 protein were identified by phage screen and chemically optimized for desired properties. *Bicycle*® T-cell agonists have tunable PK properties
- Multimeric CD137 *Bicycle*® peptides bind human T-cells and are active in both primary T-cells and reporter cell assays. Multimeric CD137 *Bicycles*® increase tumoural CD8+ T-cell infiltration in syngeneic mouse models
- We have generated tumour targeted bispecific CD137 *Bicycles*® specific to various different tumour targets. Bispecific CD137 *Bicycles*® are highly potent in both primary T-cells and reporter cell assays in a tumour –target dependable manner
- Nectin-4/CD137 bispecific *Bicycles*® induce target dependent cytokine release in ex-vivo cultures of patient-derived lung tumours
- References (1) Melero et al, *Nat Med* 3(6): 682-5 (1997); (2) Heinis et al, *Nat Chem Biol* 5(7): 502-7 (2009)

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