Activation of CD137 using multivalent and tumour targeted bicyclic peptides

Punit Upadhyaya

Peptide Congress 2019

bicycle therapeutics

Bicycle Therapeutics

- Founded by Sir Gregory Winter & Prof. Christian Heinis
- UK & US based (Cambridge, UK; Boston, USA)
- Internal focus on oncology
  - BT1718 – Phase 1/2a (Cancer Research UK)
  - 2nd Generation *Bicycle Toxin Conjugates*® in pre-clinical development
  - *Bicycle*® T cell modulators and *Bicycle*® targeted innate immune activators in lead optimization
- Key strategic partnerships outside oncology

CANCER RESEARCH UK

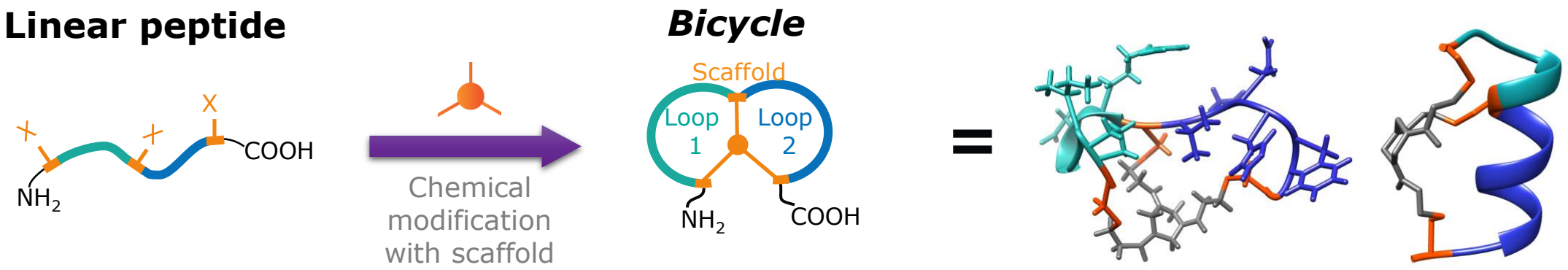
Bioverativ

OXURION

AstraZeneca

Innovate UK

Bicycles®: a new therapeutic modality



Highly constrained: high affinity, exquisite selectivity, excellent stability

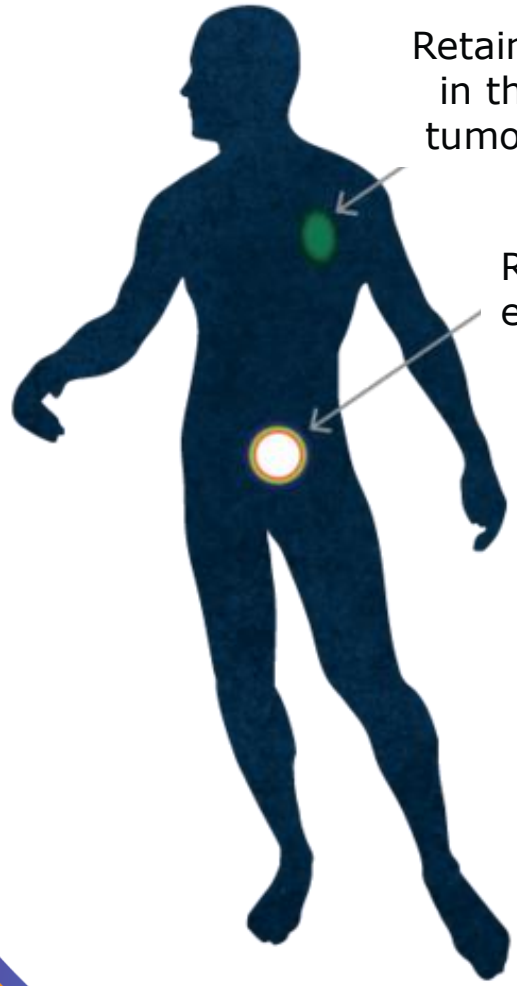
Large binding footprint: disrupt protein-protein interactions

Fully synthetic: NCE classification and synthetic control

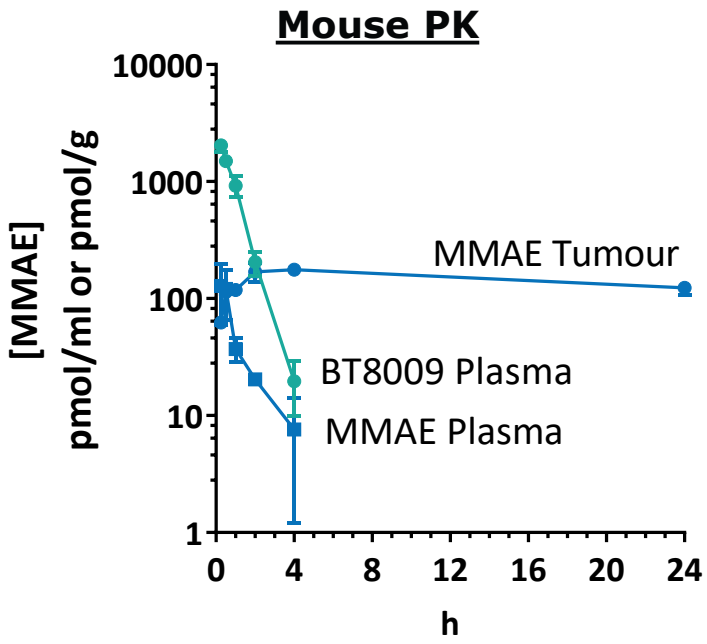
Highly flexible modality: modular building blocks retain pharmacology

Adjustable PK: excellent tissue penetration, renal elimination, tuneable $T_{1/2}$

Bicycle Toxin Conjugates® : hit and run delivery of toxins to tumour cells



BT8009



BT8009 affords rapid and long lasting MMAE retention with rapid plasma clearance of toxin and parent.

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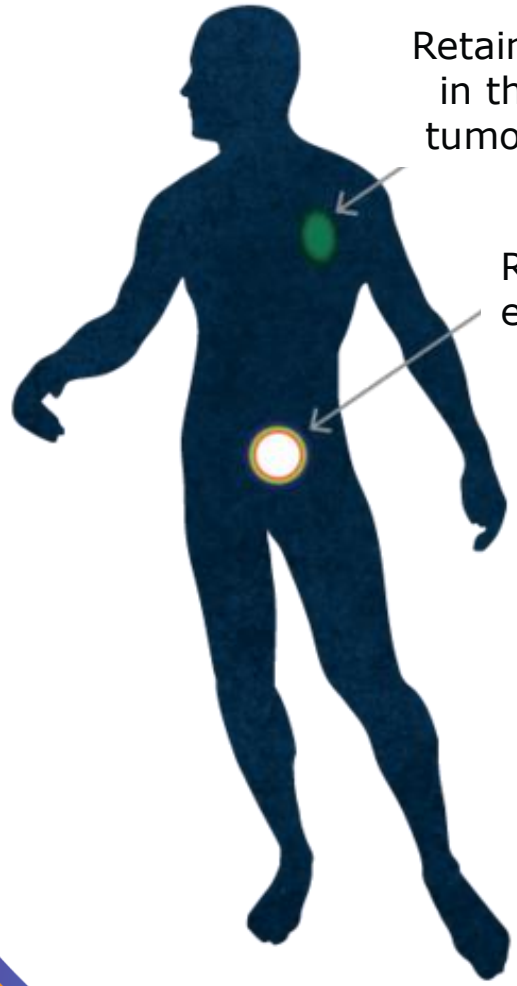
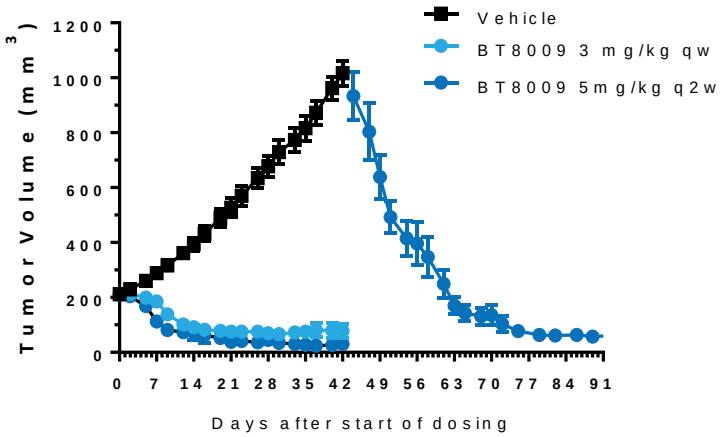
Bicycle Toxin Conjugates® : hit and run delivery of toxins to tumour cells



BT8009



BT8009 shows excellent efficacy in large and small MDA-MB-468 xenografts

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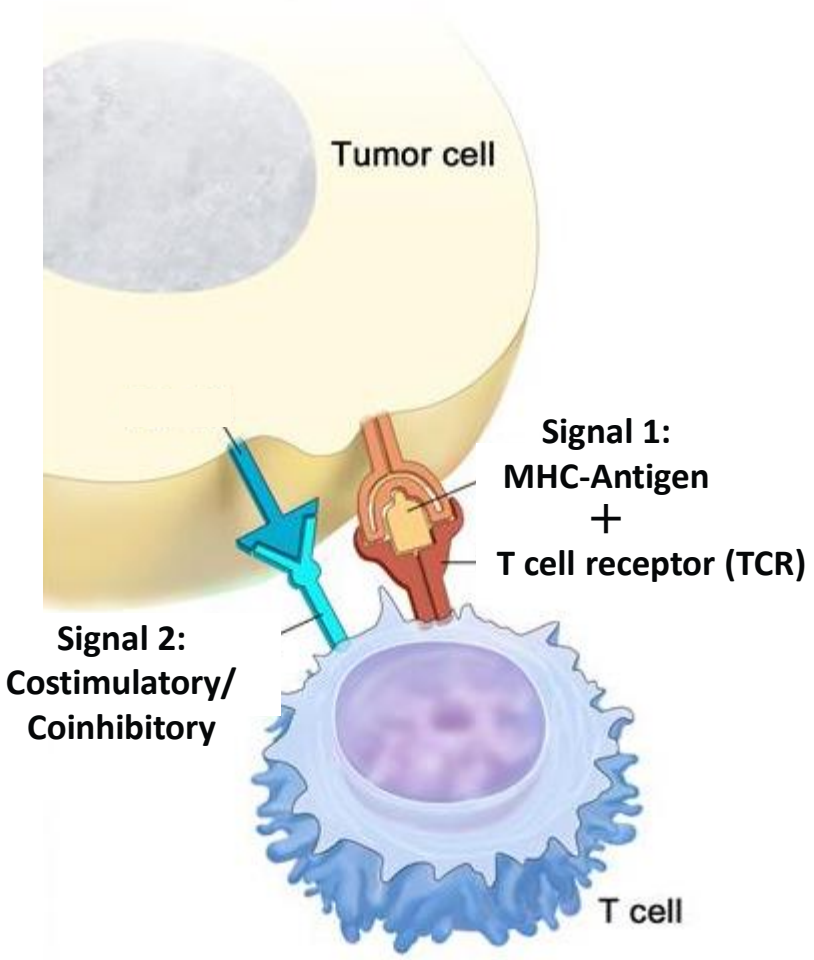
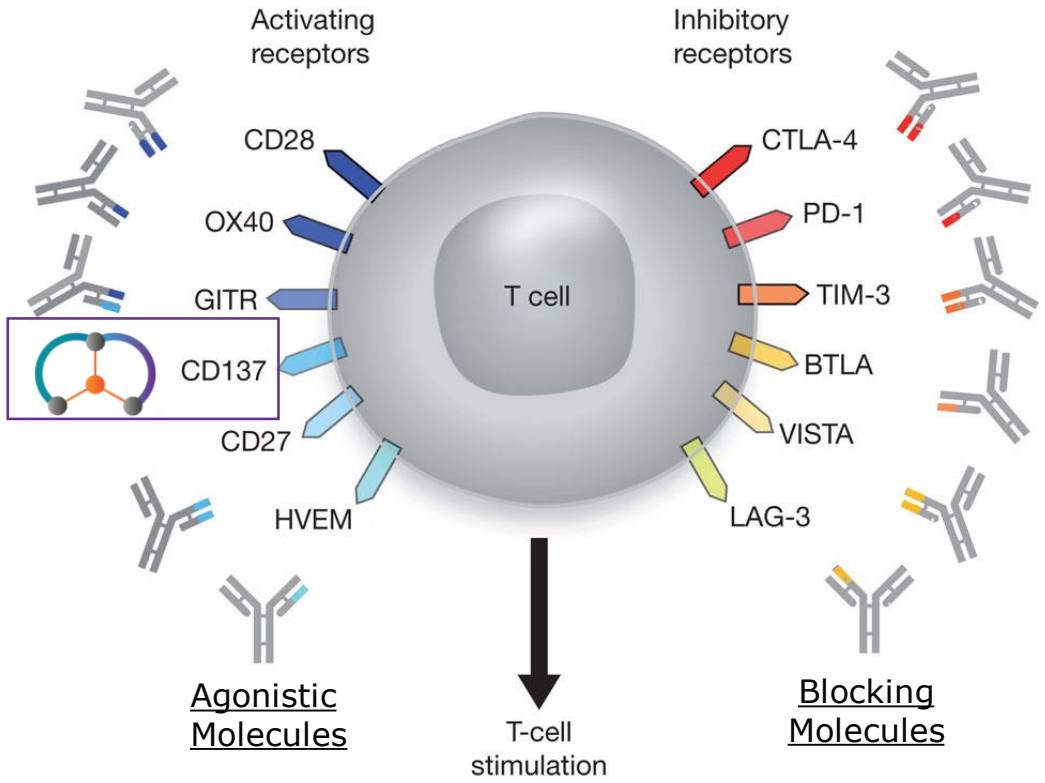
Exploring *Bicycles*® as T cell agonists

Antigen Presenting Cell: T cell Interaction



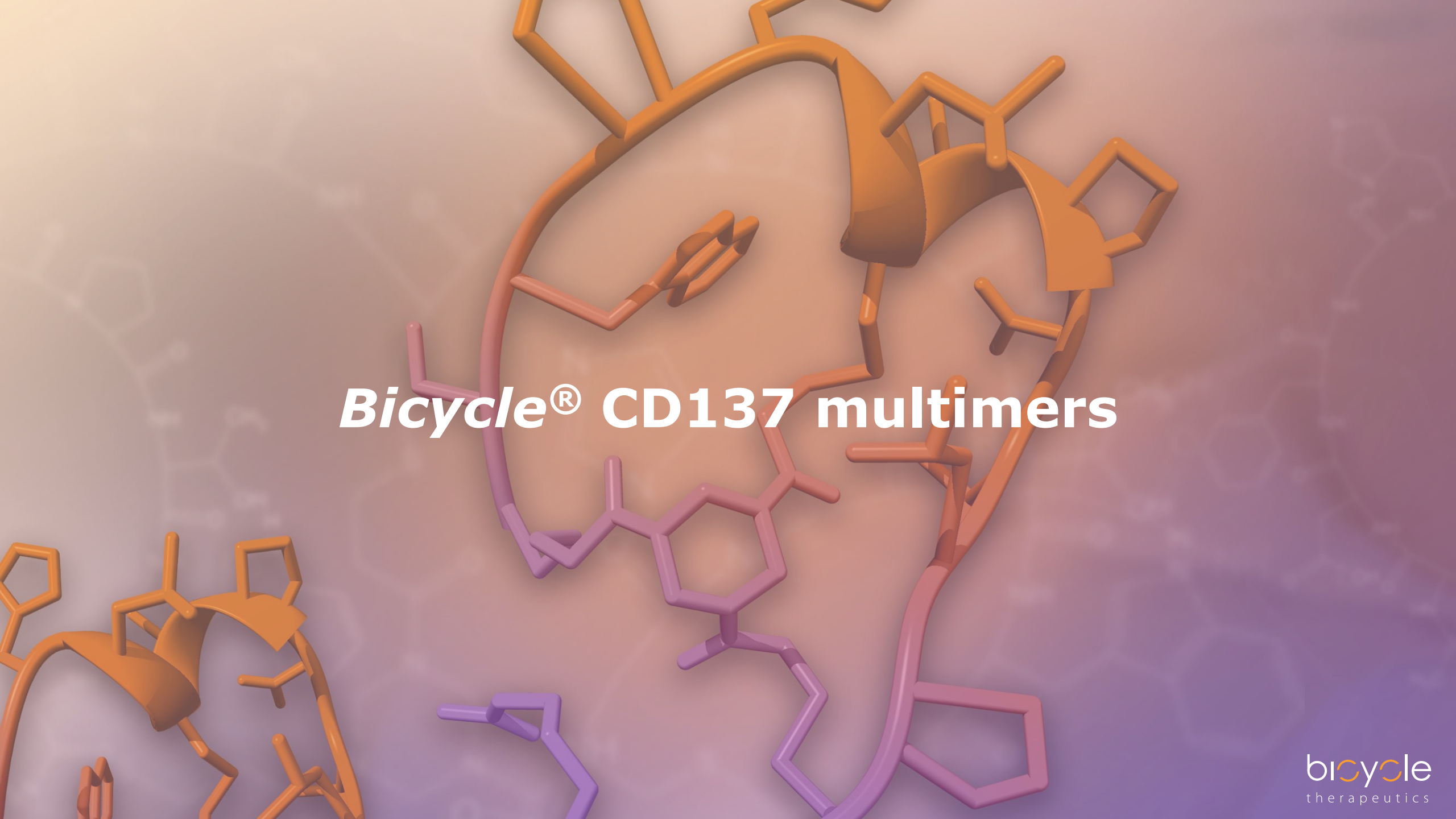
Costimulatory/Coinhibitory Signal



Adapted from Mellman *et al. Nature* **480**, 480-489 (2011)

Short acting *Bicycles* have been validated as toxin conjugates, will they offer advantages as T cell modulators?

Bicycle® CD137 multimers



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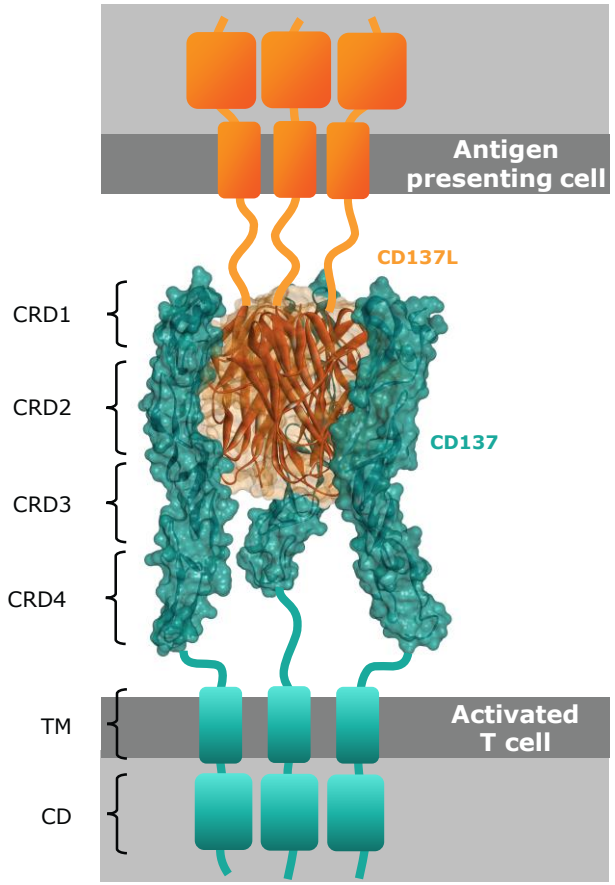
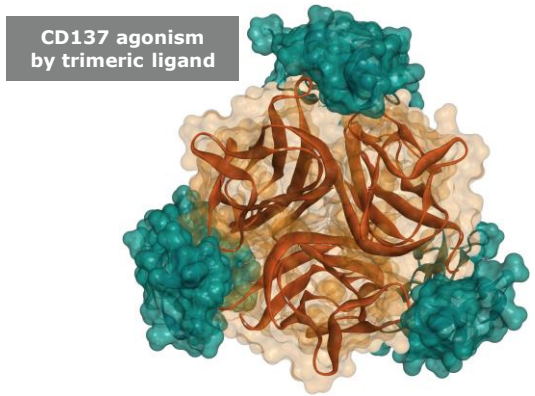
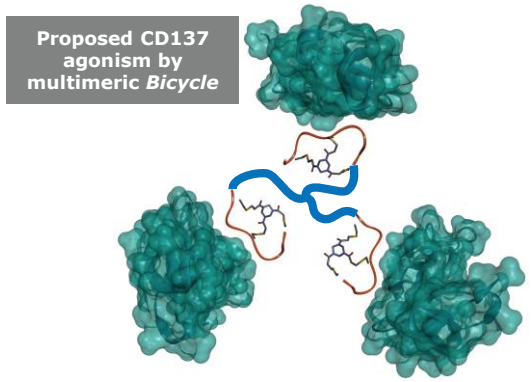
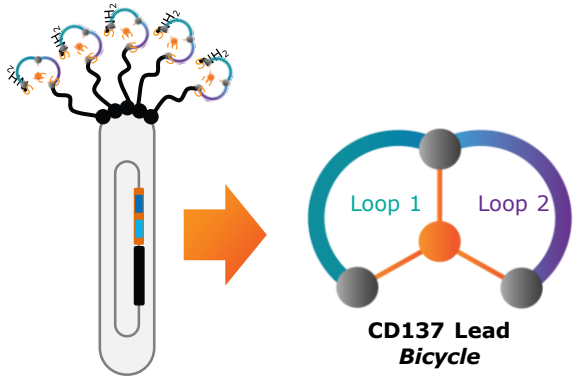
Receptor complexity fits *Bicycles'*® tolerance for multimerization

- CD137 is member of TNF superfamily, requires trimerisation for activation









Human Specific
No Rodent
X-reactivity

Phage campaign identifies lead CD137 *Bicycle*

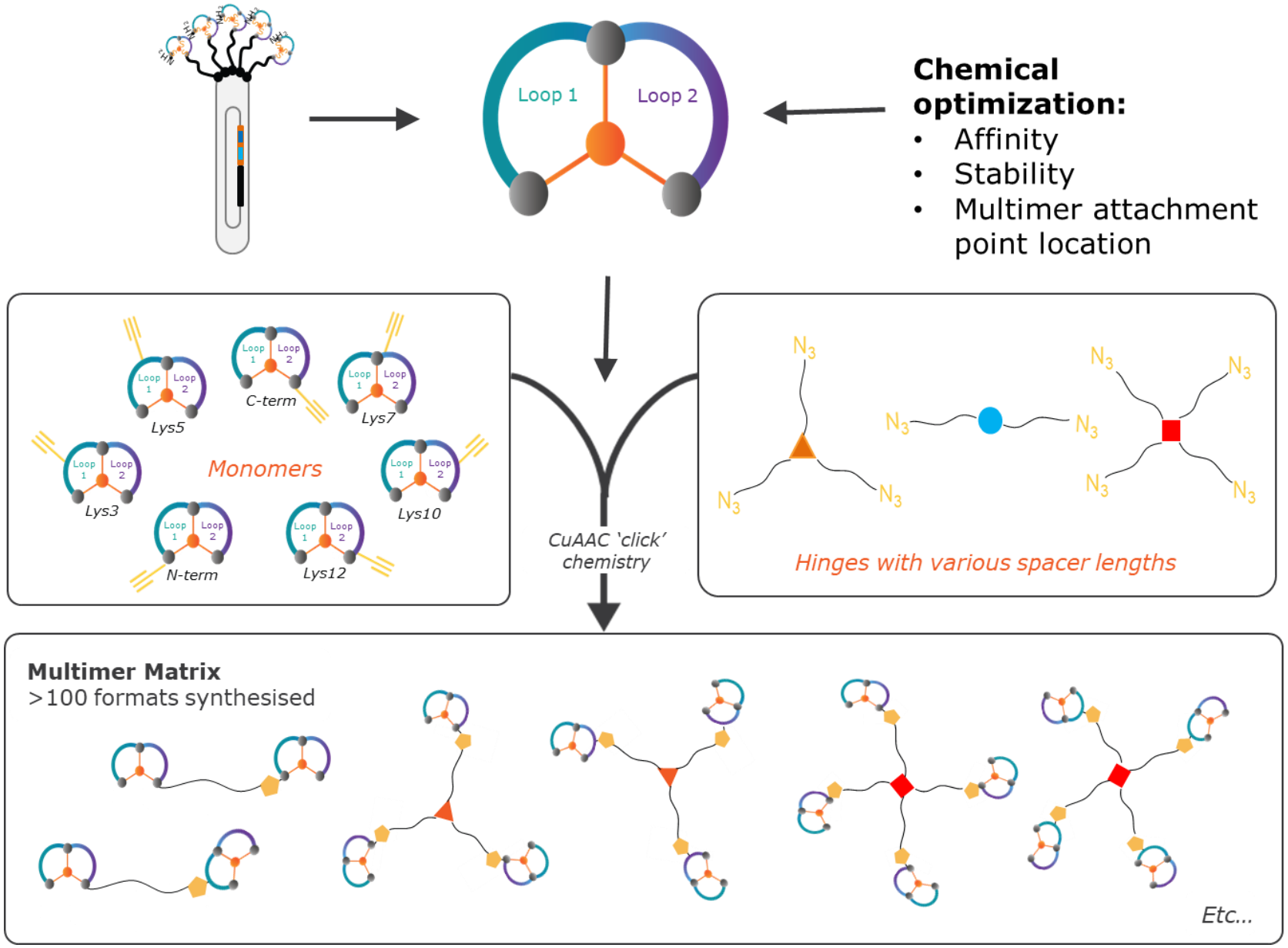
Naïve phage peptide KD = 1.4uM

Affinity matured phage peptide KD = 67nM (Wild Type: WT)

Chemically optimised KD = 5nM (High Affinity: HA)

Adapted from Chin *et al. Nat Commun* **9**, 1-13 (2018)

Chemically enabled optimization of CD137 multimers



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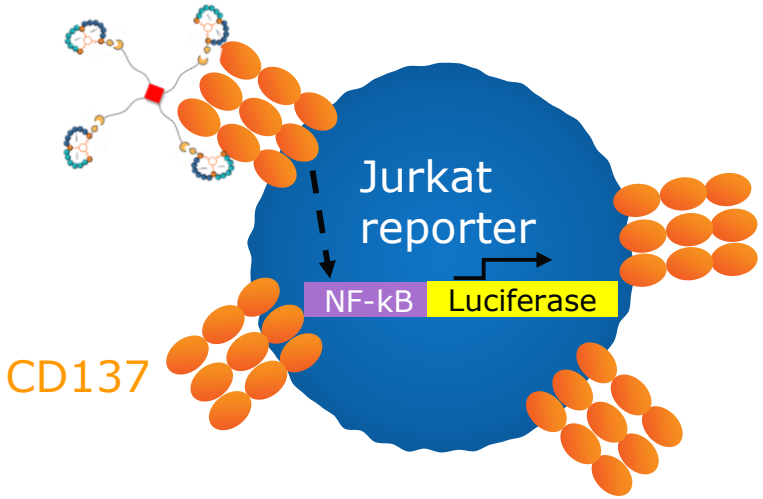
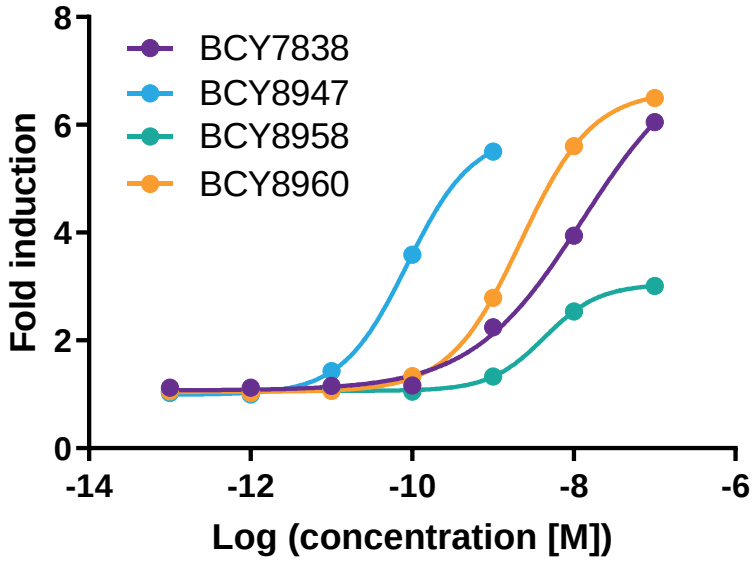
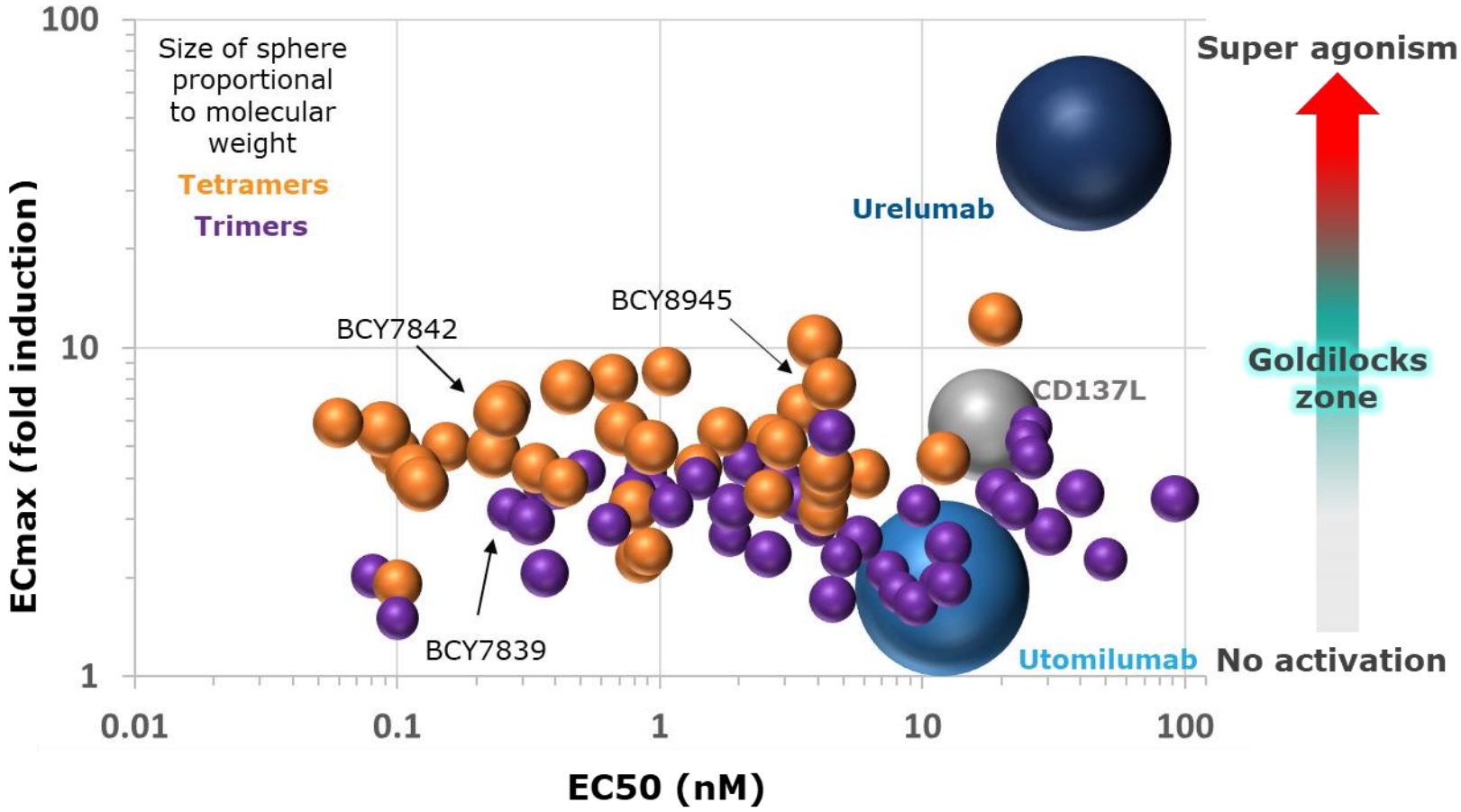
Reporter cell based screening of CD137 multimers







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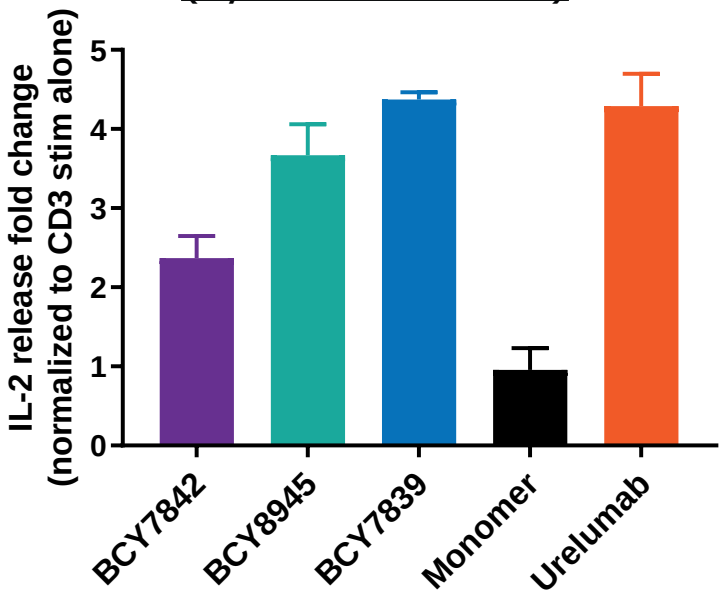
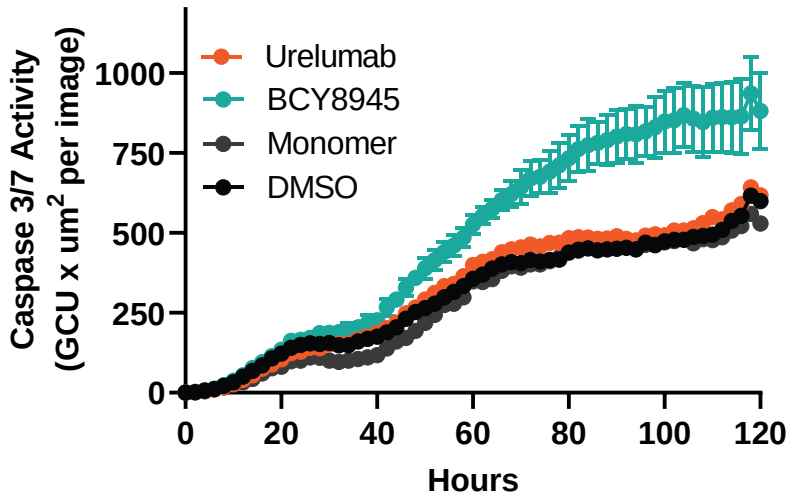
CD137 multimers are active in primary immune cell assays

Primary human T cells
(cytokine release)



Primary human immune cells
(tumour cell killing)



BCY7839 = Trimer WT affinity *Bicycle*

BCY7842 = Tetramer WT affinity *Bicycle*

BCY8945 = Tetramer High affinity *Bicycle*

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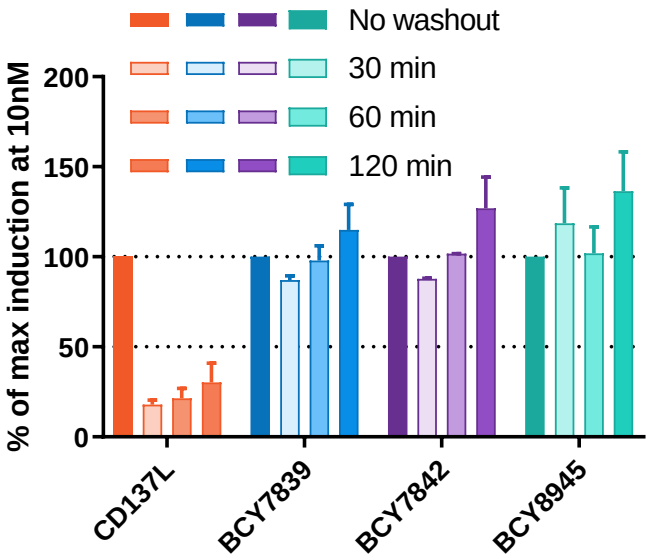
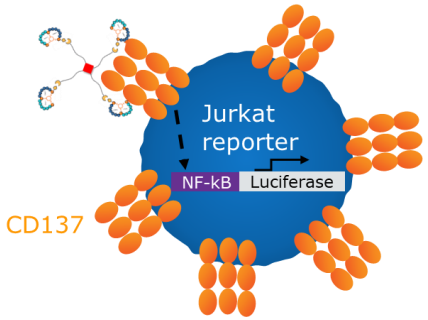
CD137 multimers have prolonged receptor engagement and tunable PK

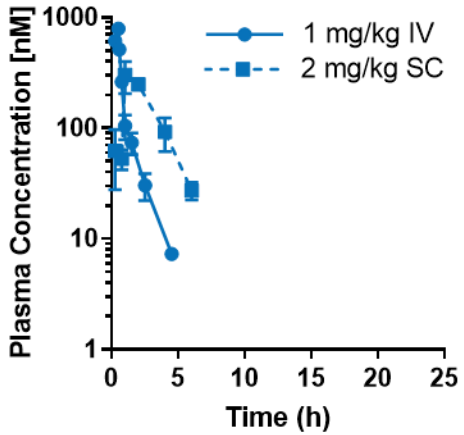
Prolonged activity



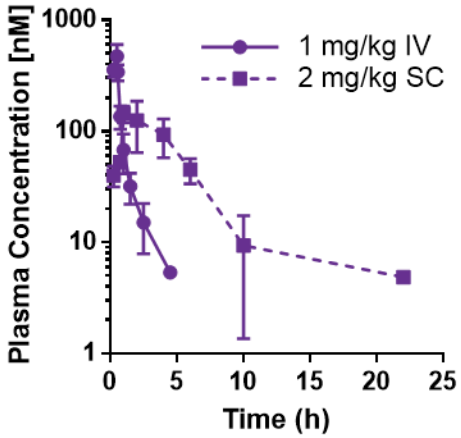


Cyno PK: Agonists with range of exposure

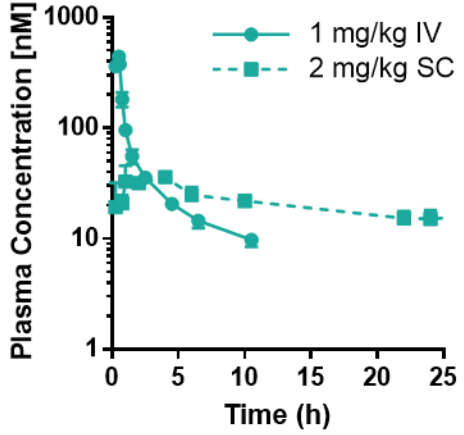
BCY7839



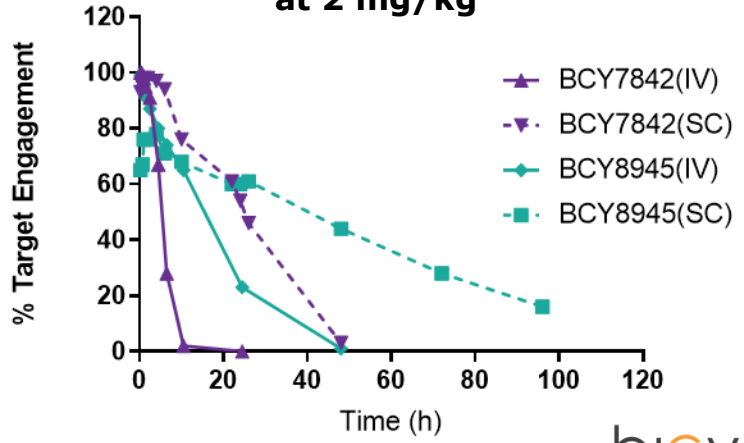
BCY7842



BCY8945



Projected Target Coverage at 2 mg/kg



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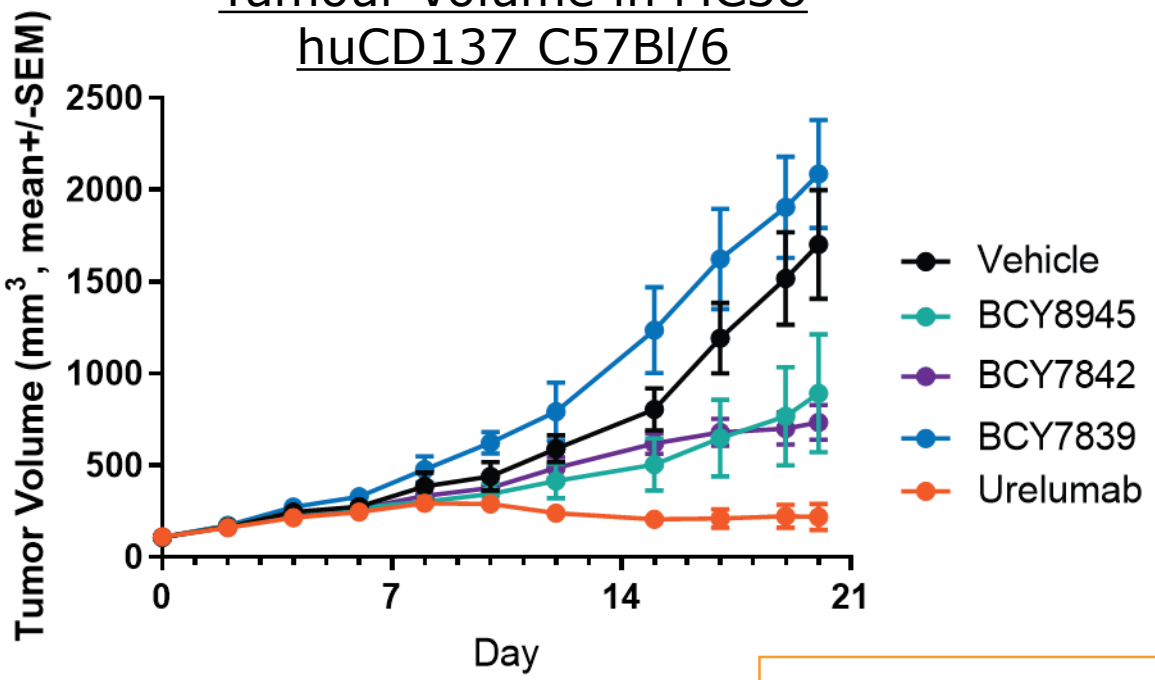
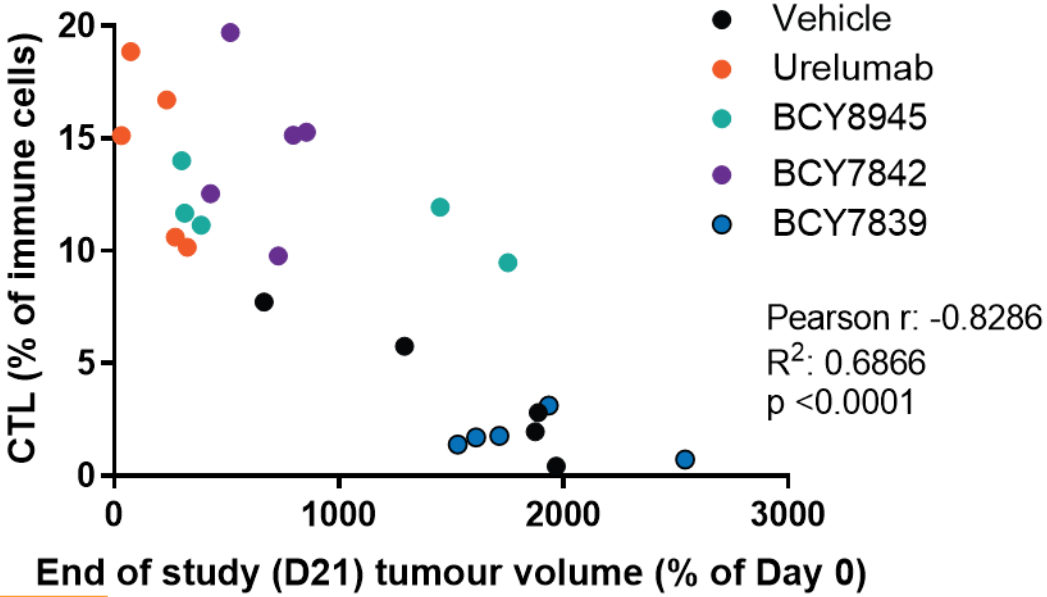
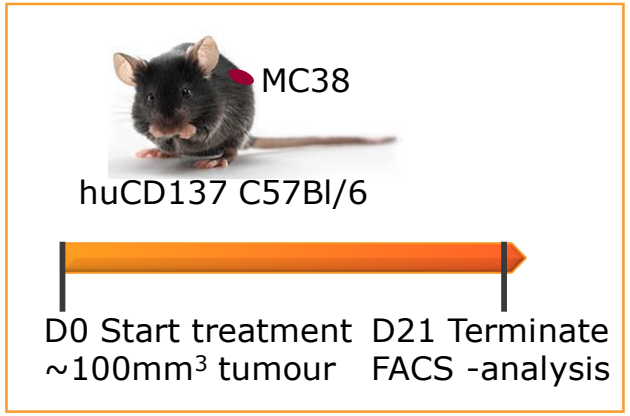
Anti-tumour activity of CD137 multimers correlates with increased tumour infiltrating lymphocytes

Tumour Volume in MC38 huCD137 C57Bl/6



T cell infiltration *vs.* activity





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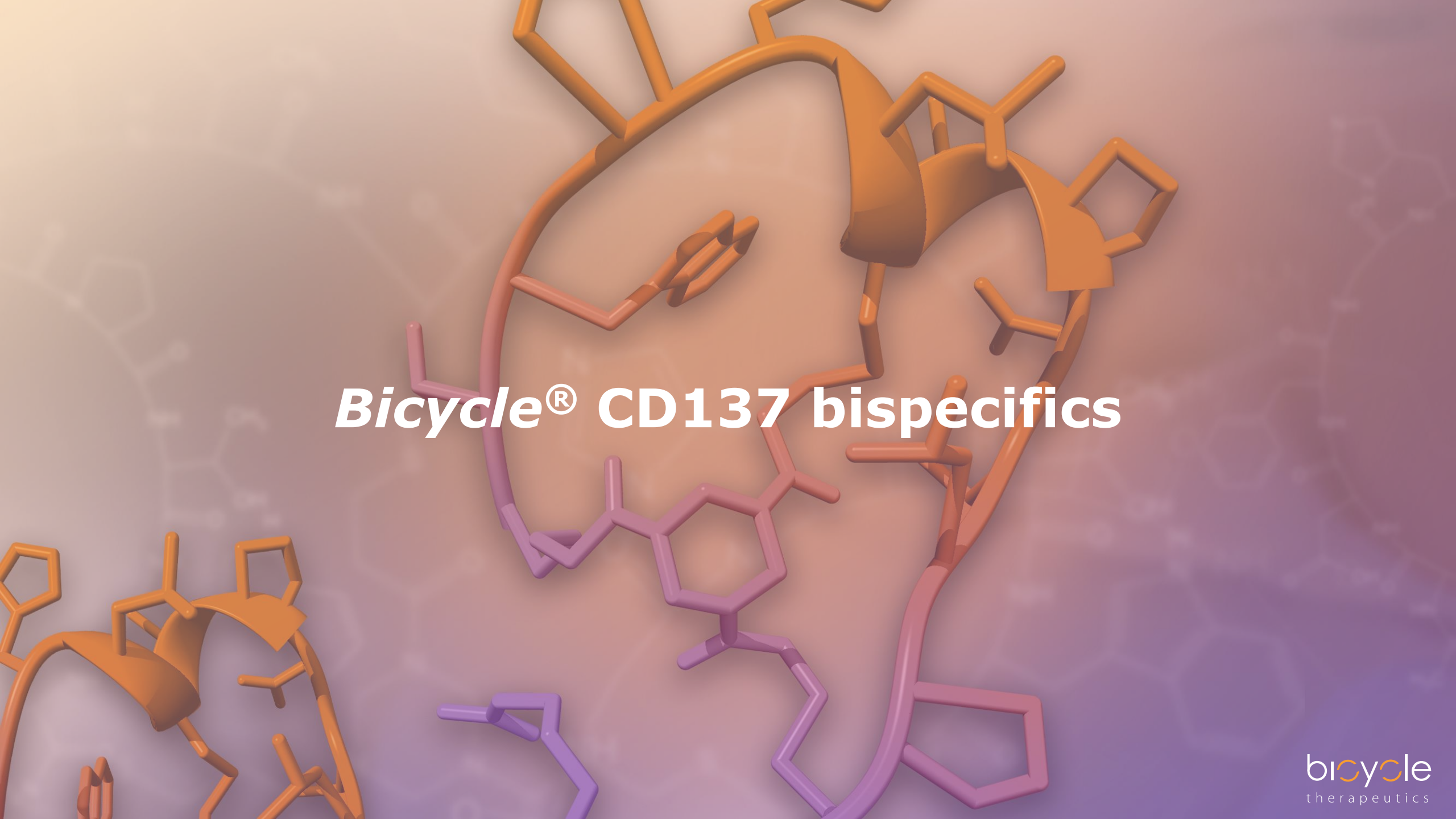

Bicycle® CD137 bispecifics

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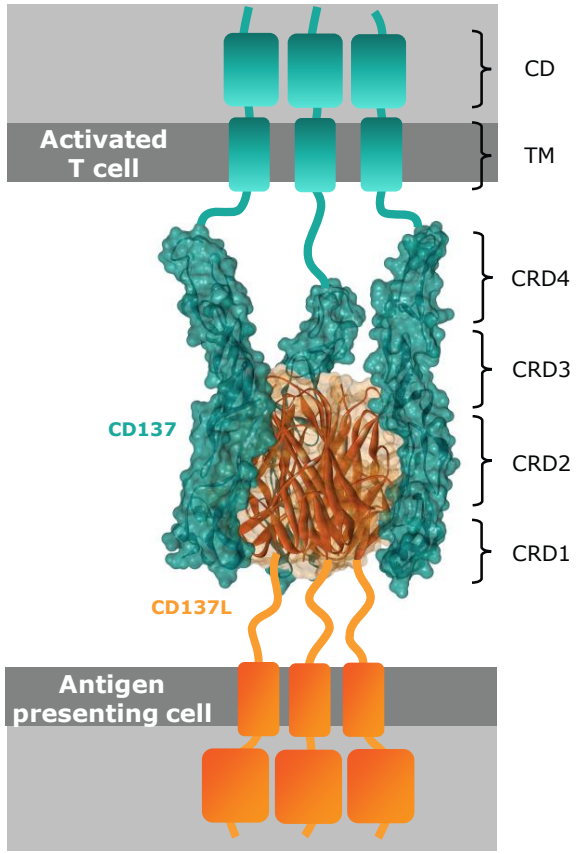
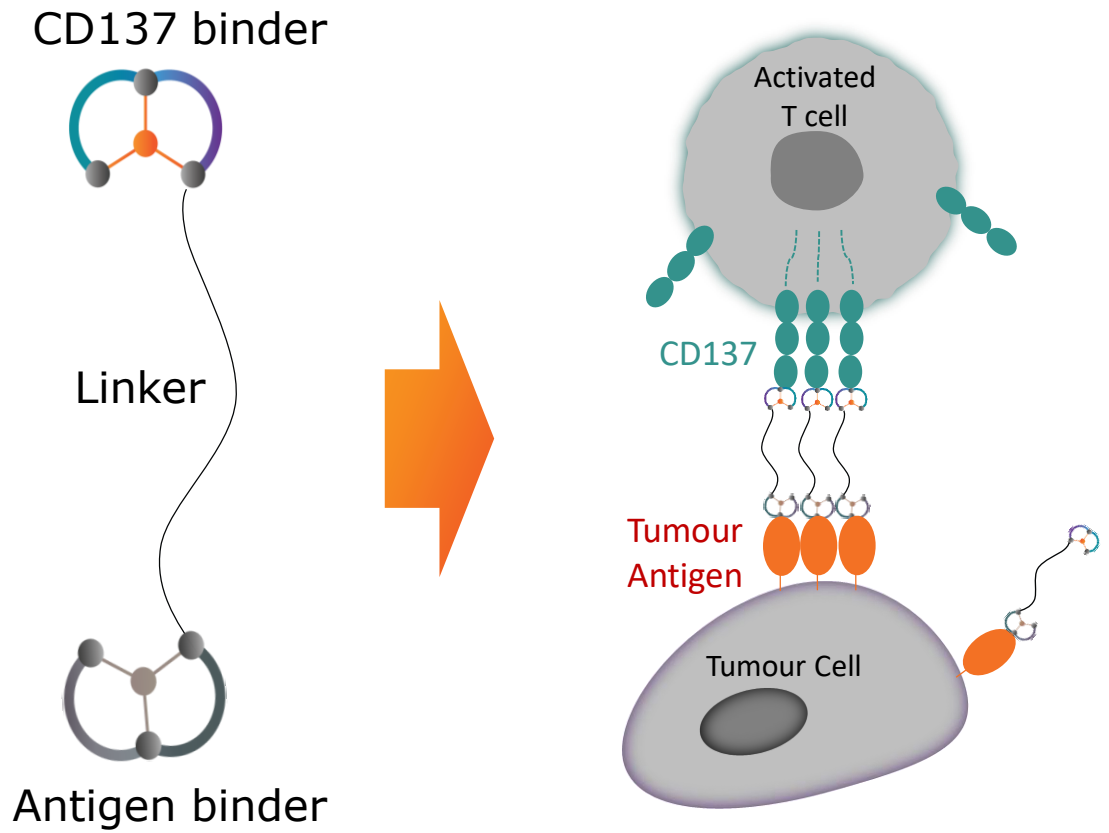
Bispecific tumour/CD137 binding *Bicycles*® as potent and targeted T cell activators





CD137 is member of TNF superfamily & requires clustering for activation

Chin *et al. Nat Commun* **9**, 1-13 (2018)

Fully synthetic molecules comprising CD137 and tumour antigen targeting *Bicycles* could achieve potent CD137 activity through receptor cross-linking across the immune synapse

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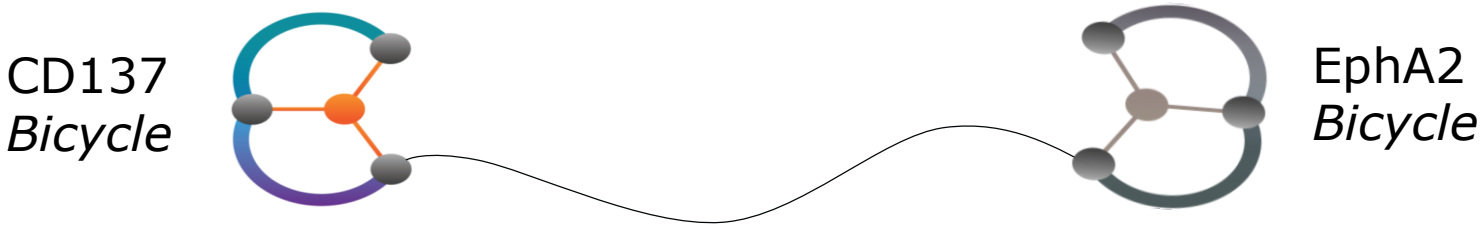
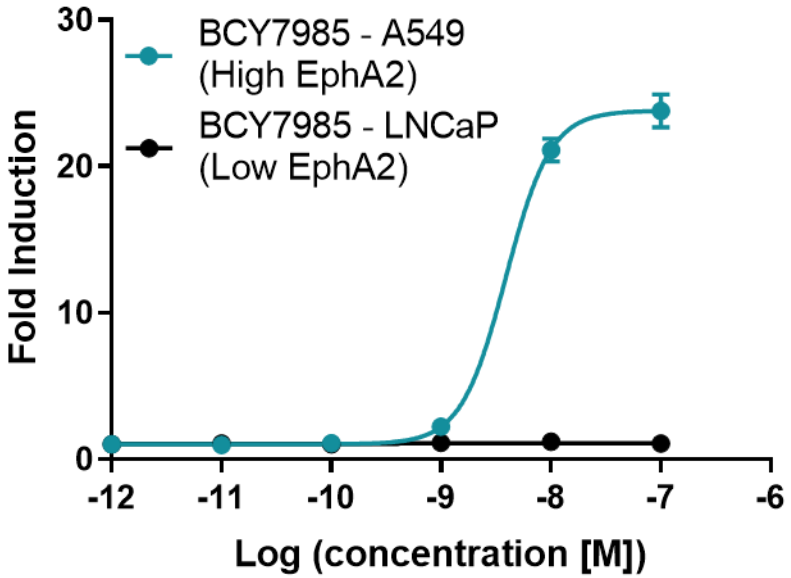
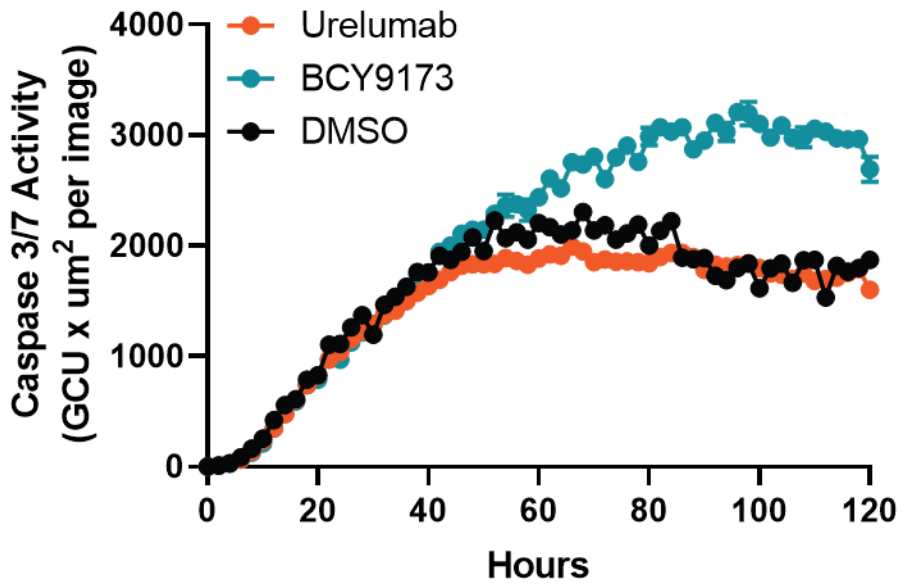
Proof of concept with the first EphA2/CD137 molecule



CD137 reporter assay co-culture



Primary human immune cells-A549 co-culture (Tumour cell killing)



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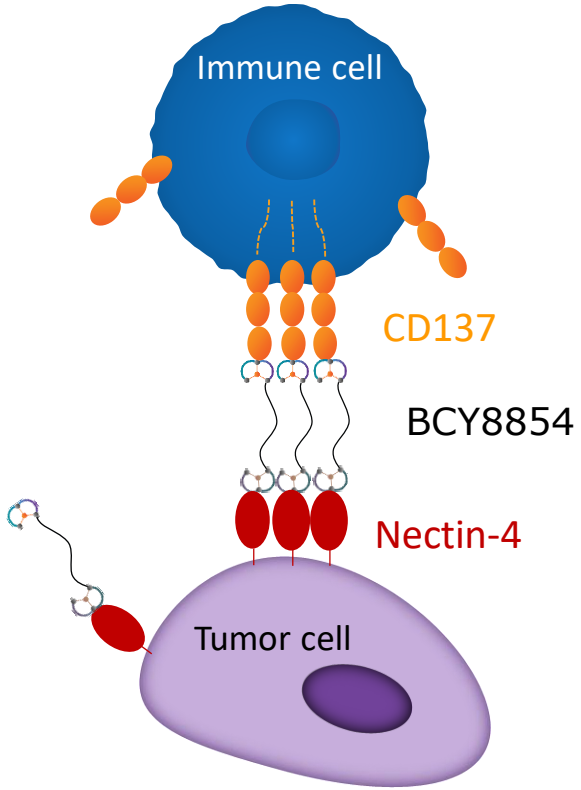
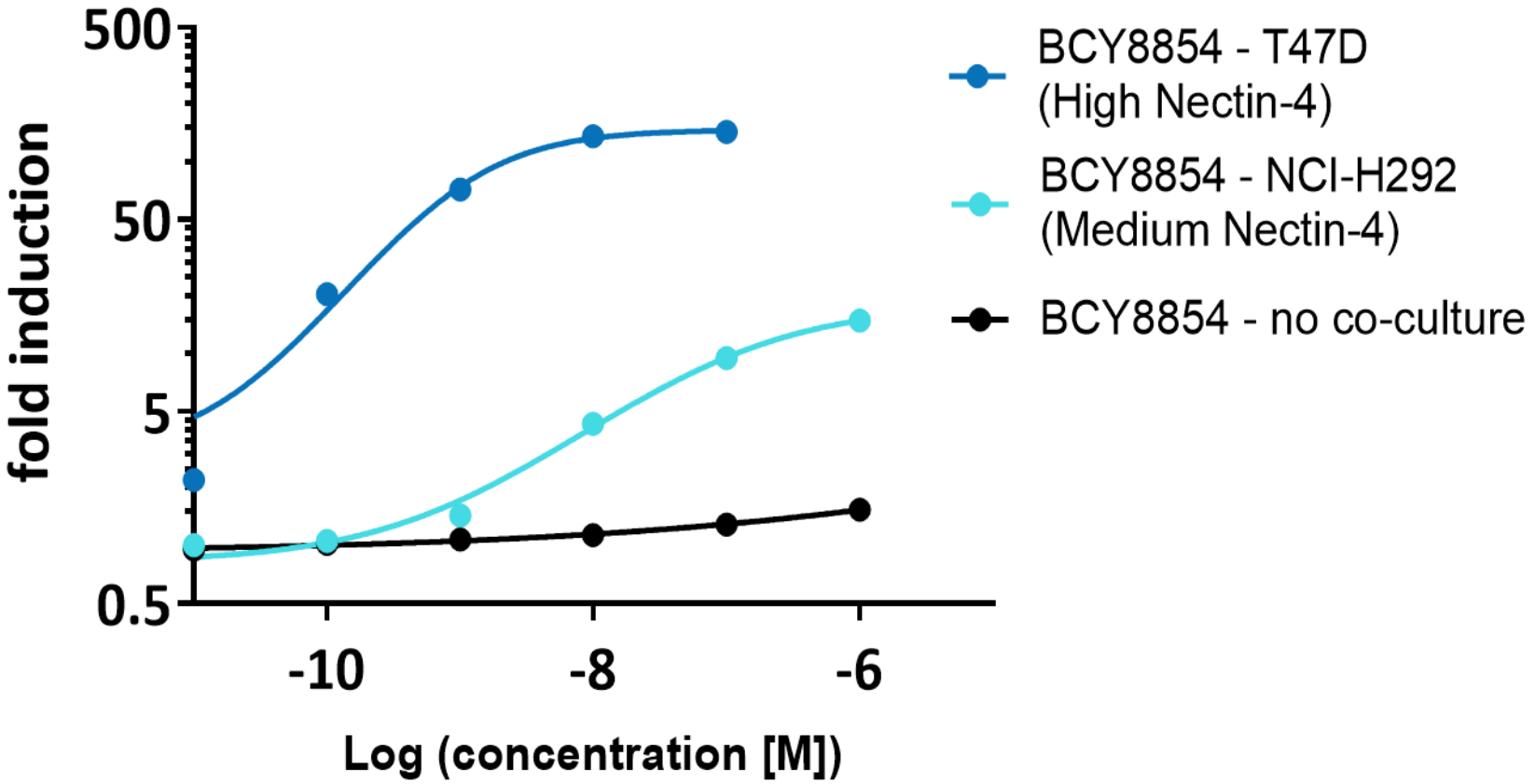
Nectin-4/CD137 bispecific as an exemplar (concept is generalizable)



Cancer cell expressing high levels of Nectin-4



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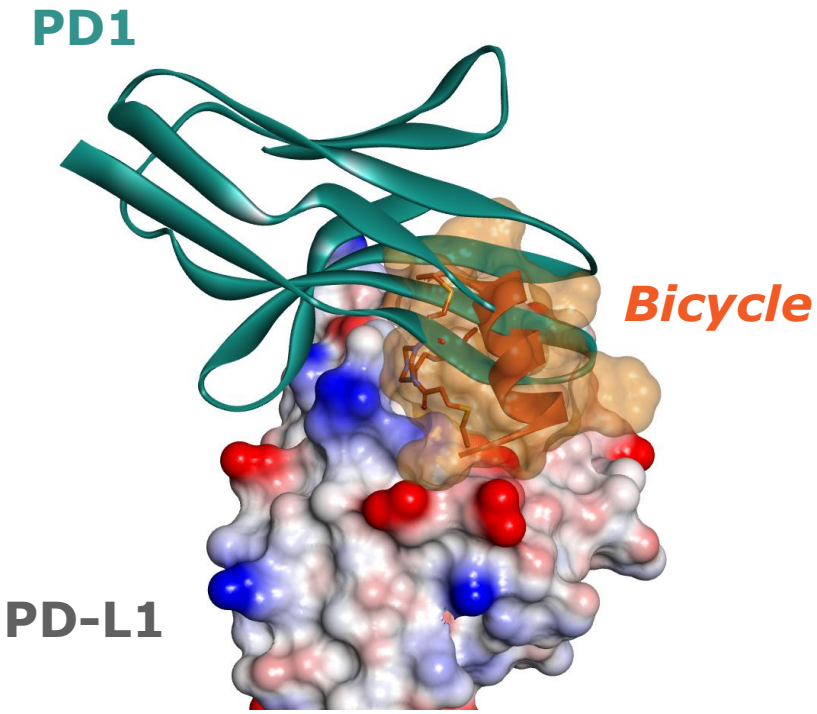
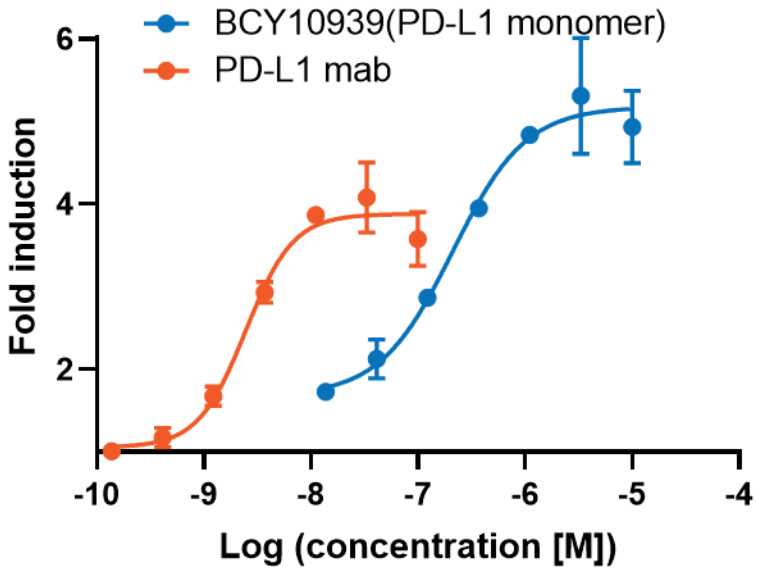
PD-L1/CD137 : 3rd bispecific exemplified



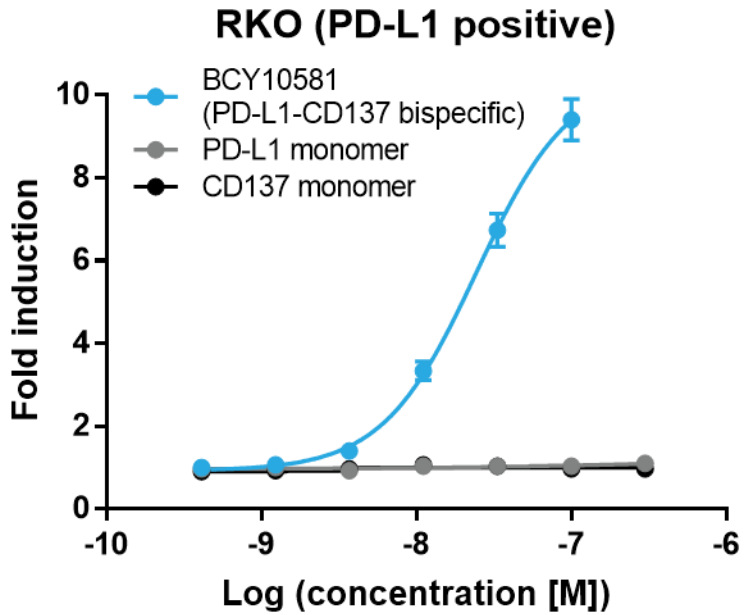
PD-L1 *Bicycle* binds to epitope that is directly competitive with PD1

PD-1/PD-L1 blockade bioassay



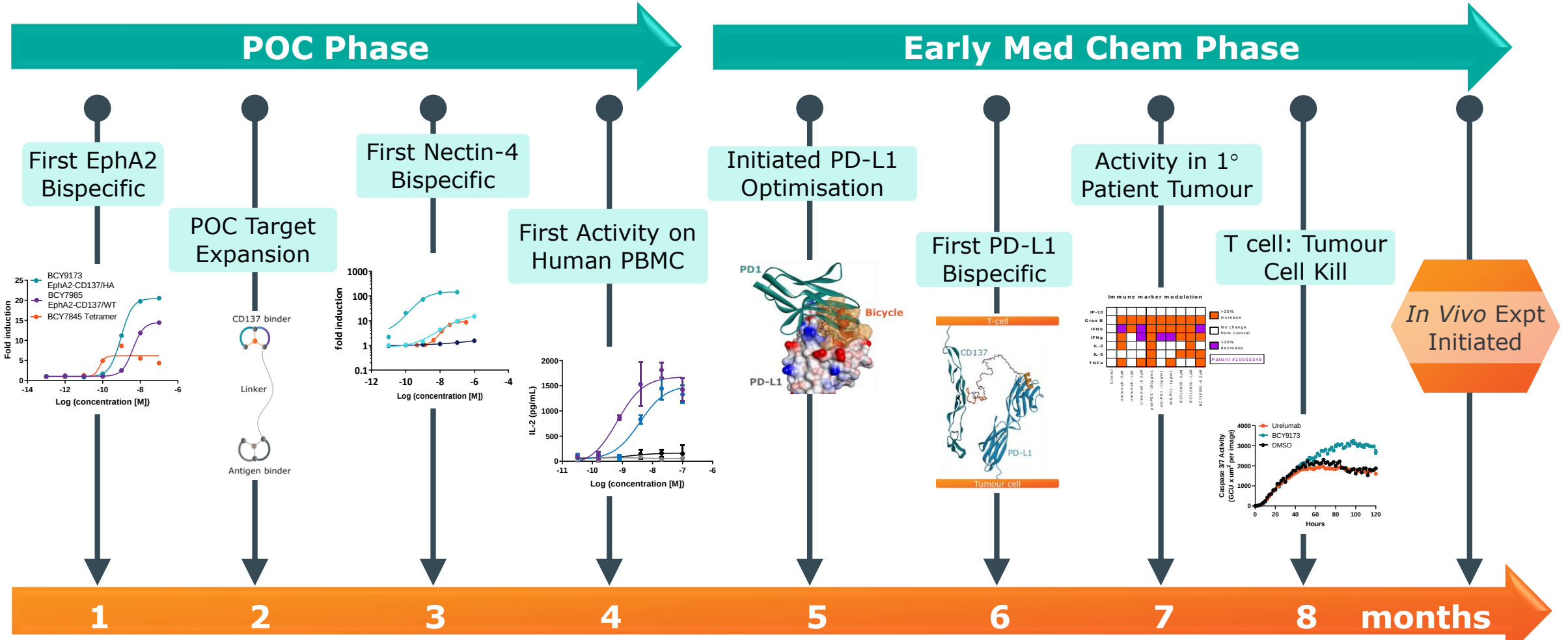
PD-L1 *Bicycle* blocks PD1/PD-L1 Interaction between PD1 expressing T cells and CHO-K1 stable expressing PD-L1

CD137 reporter assay



PD-L1/CD137 bispecifics induce agonism in CD137 reporter assay only when cocultured with PD-L1 expressing RKO cells.

CD137 bispecific chemistry: rapid progress from POC



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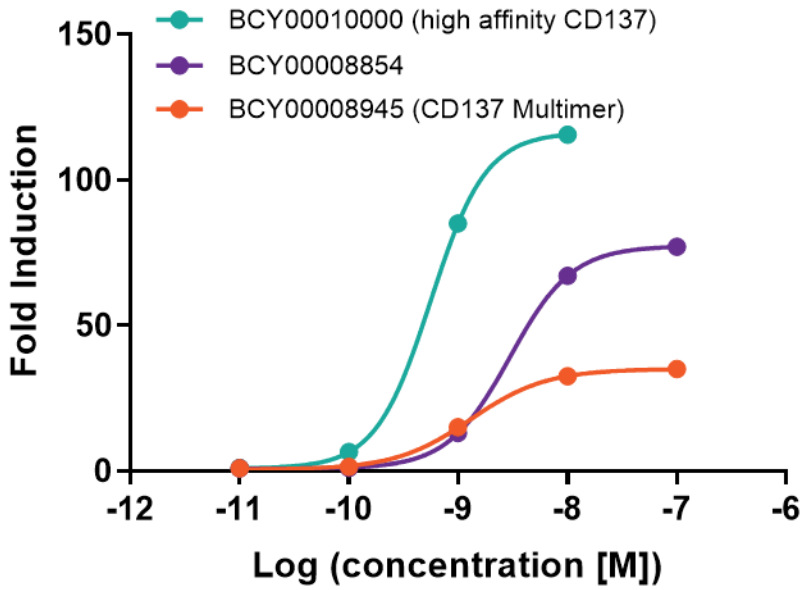
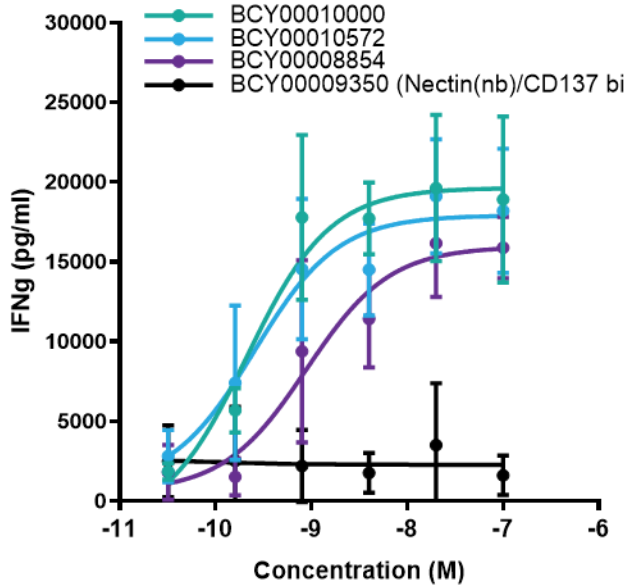
Higher affinity CD137 *Bicycle*® increases potency of Nectin-4 bispecific in reporter and human PBMC assay

CD137 Reporter Assay Coculture with H1376 Cells



Human PBMC-4T1(Nectin-4^{+}) co-culture (cytokine release)

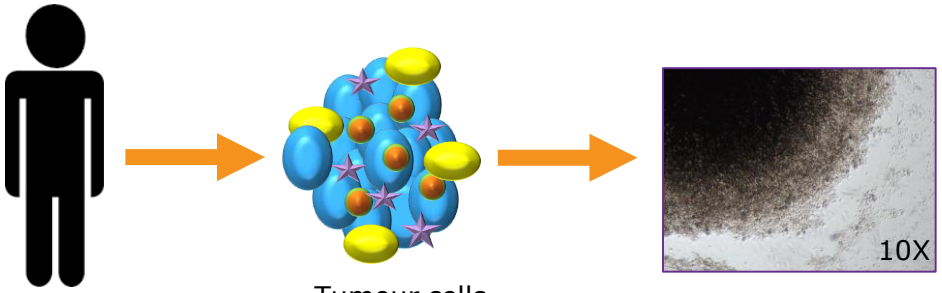


Potent Activation of human PBMCs

Compound	Molecular Description	K_D(nM) Nectin-4	K_D(nM) CD137
BCY8854	Nectin-Sar10-Peg12-CD137(WT, C-term)	2.76	108
BCY10000	Nectin-Sar10-Peg12-CD137(HA, C-term)	2.26	6.19
BCY10572	Nectin-Peg5-CD137(HA, dLys4)	ND	5.00

Nectin-4/CD137 bispecific *Bicycles*® induce target dependent cytokine release in *ex vivo* cultures of patient-derived lung tumours

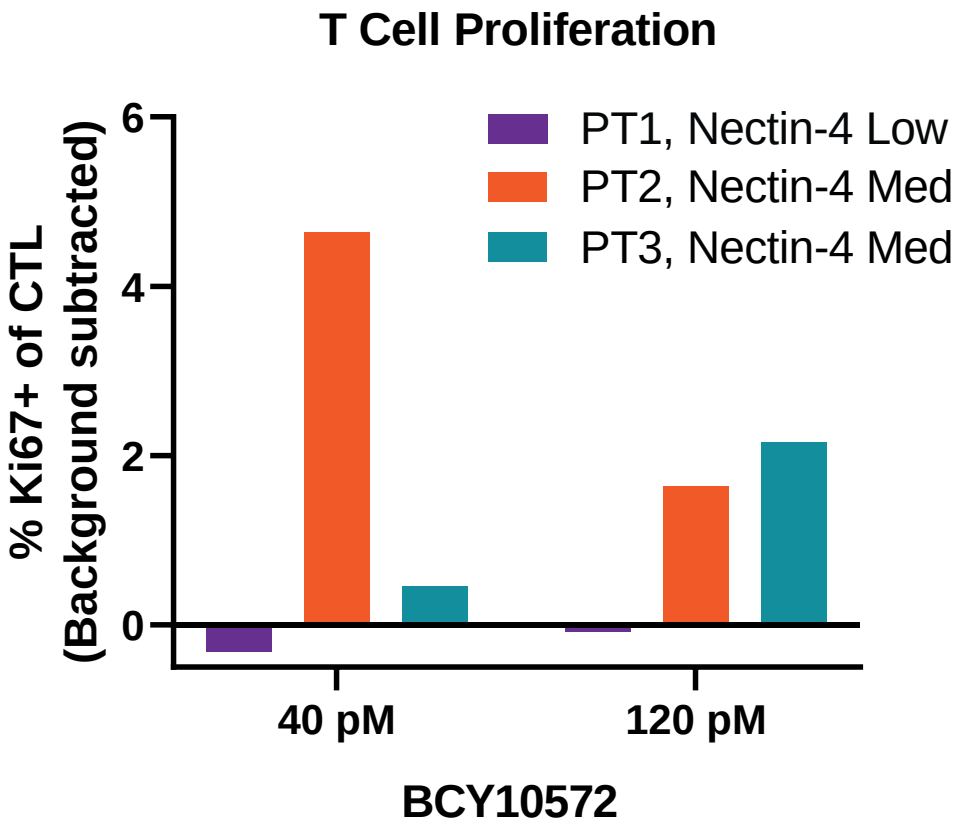
Patient derived tumour cells *ex vivo* for 48h



- Tumour cells
- Lymphocytes
- Other associated cells

ex vivo patient derived tumour cells form 3D spheroids within 4h in culture

ID	CD137+ T cells (%)	Nectin-4+ cells (%)
PT1	19.8	4.4
PT2	15.1	25.8
PT3	30.0	15.1



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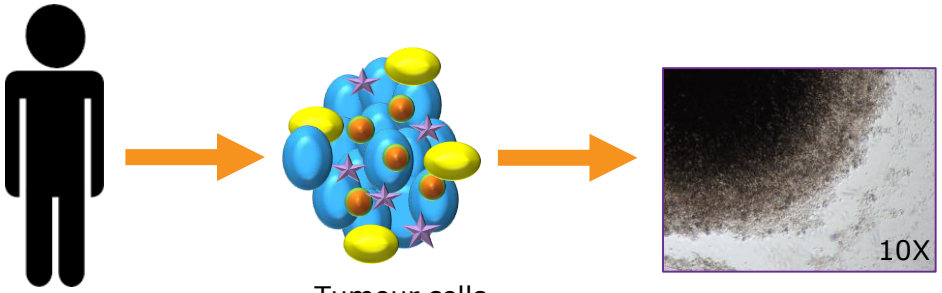

- Tumour cells
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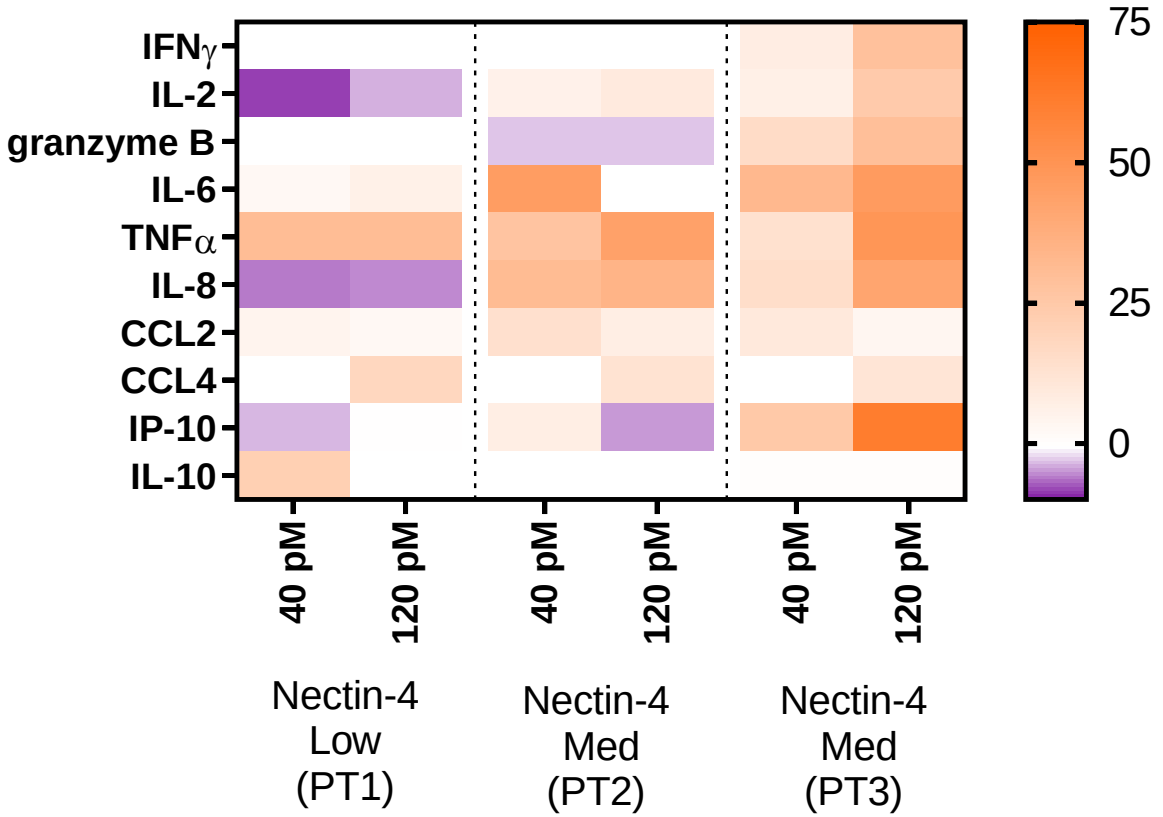
ex vivo patient derived tumour cells form 3D spheroids within 4h in culture

ID	CD137+ T cells (%)	Nectin-4+ cells (%)
PT1	19.8	4.4
PT2	15.1	25.8
PT3	30.0	15.1

% change in immune markers between BCY10572 vs Vehicle



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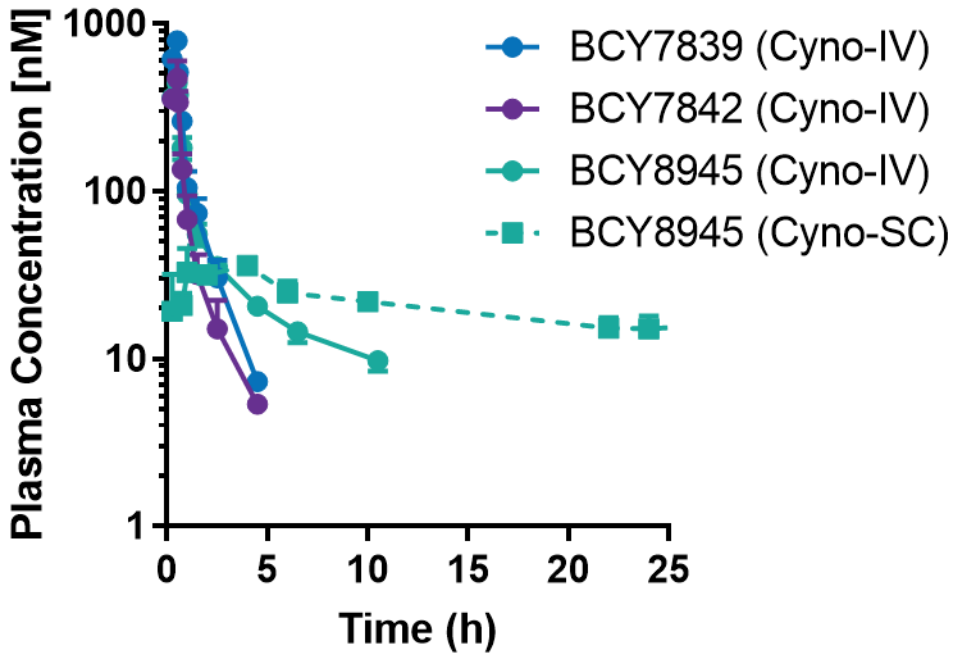
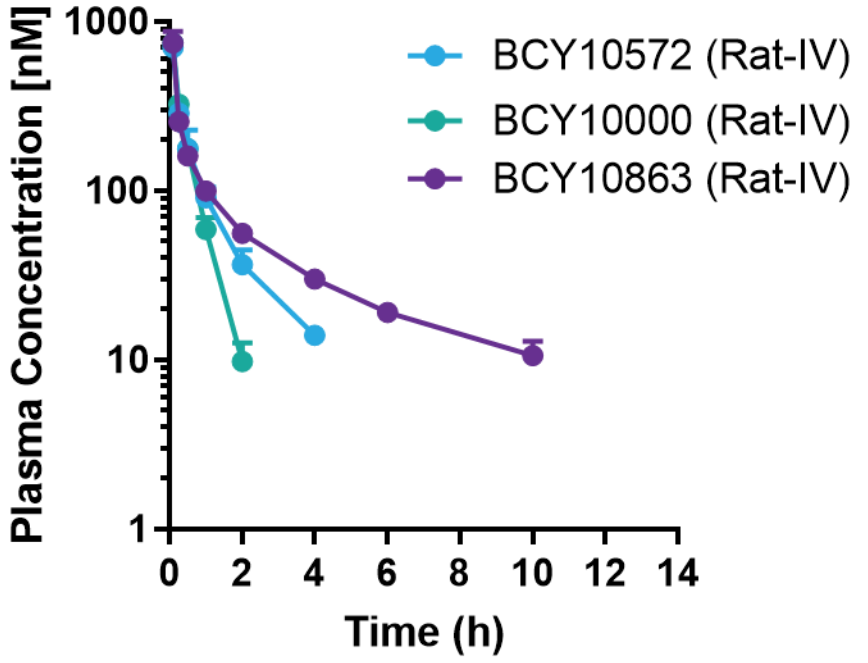
Bicycle® T cell agonists have tunable PK properties

CD137 Multimer Agonist PK



Nectin-4/CD137 Bispecific Agonist PK



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Summary

- First fully synthetic *Bicycle*® multimeric T cell activator and *Bicycle*® bispecific T cell activator platform.
- *In vivo* anti-tumour activity in humanized mouse models with *Bicycle*® CD137 Multimers.
- Profound agonist activity in primary human T cell assays, and in human tumours *ex vivo* with Nectin-4/CD137 *Bicycle*® bispecifics.
- Promising *in vitro* activity with EphA2/CD137 and PD-L1/CD137 *Bicycle*® bispecifics.

Acknowledgements

- Team at *Bicycle* UK & US


LinkedIn
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#NotWaiting