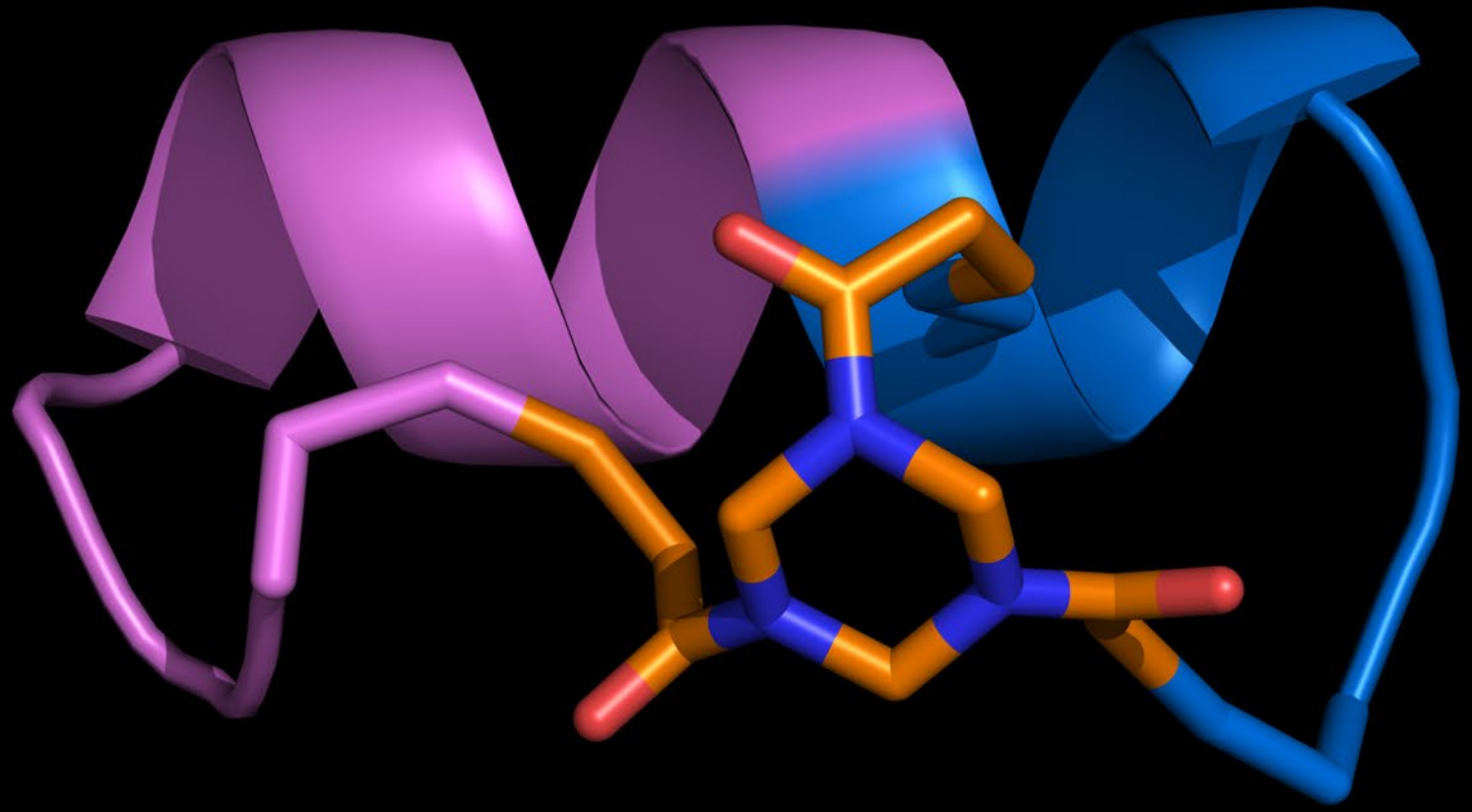
Highlighting the Potential of Bicycle Conjugates to Target Solid Tumours

World ADC London 2023— Arnaud Tiberghien, Associate Director Chemistry



Bicycle®

Bicycle Therapeutics

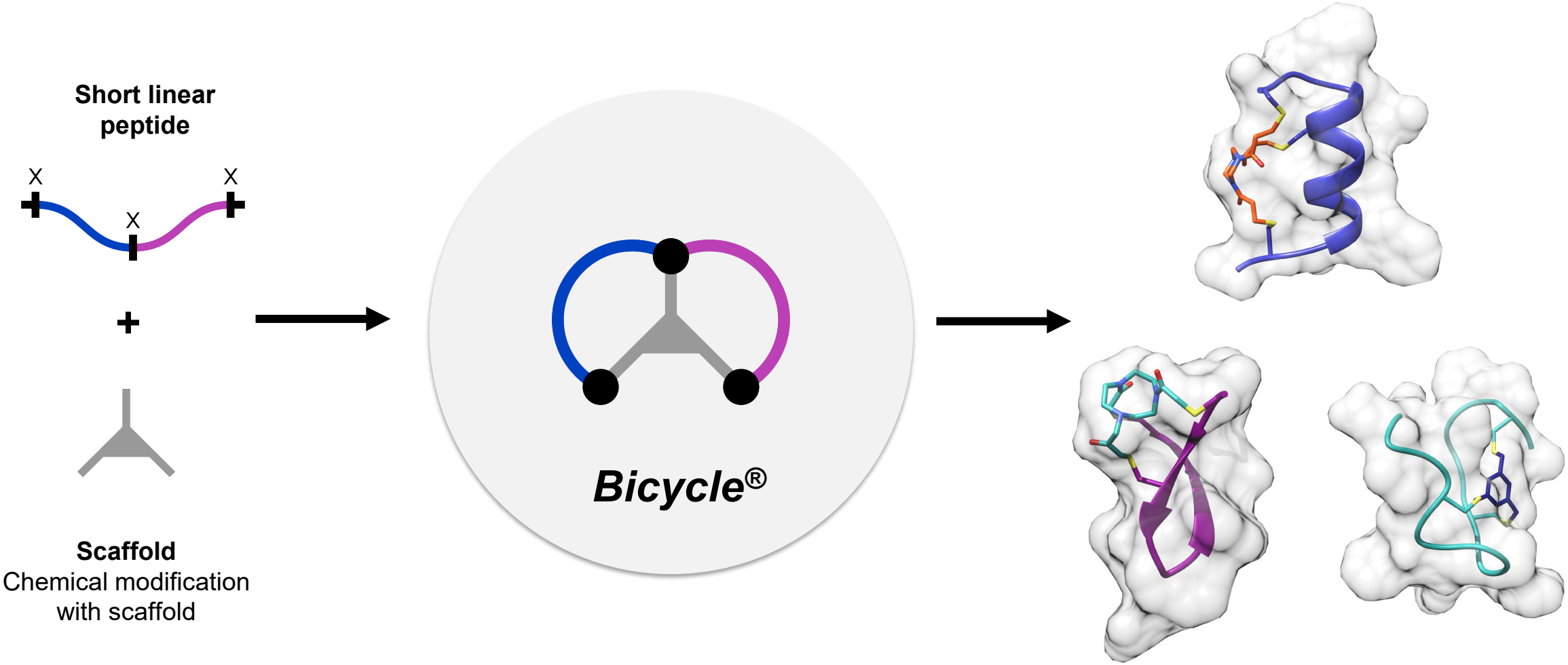
Founded by Sir Gregory Winter & Prof. Christian Heinis

UK & US based (Cambridge, UK; Boston, USA)

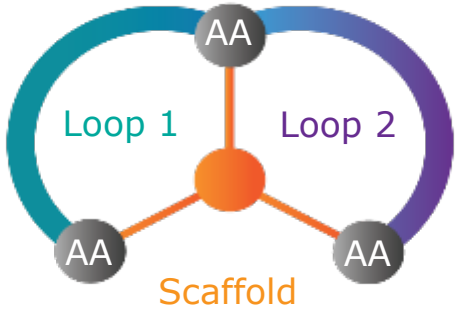
2018 Nobel Prize in Chemistry
"for the phage display of peptides and antibodies"

Target / Product	Partner / Sponsor	Indication	Modality	Preclinical	IND-enabling	Phase I	Phase II/ Expansion	Phase III
Internal Programs								
BT5528 (EphA2)		Oncology	Bicycle® Toxin Conjugate					
BT8009 (Nectin-4)		Oncology	Bicycle® Toxin Conjugate					
BT7480 (Nectin-4/CD137)		Immuno-oncology	Bicycle TICA™					
BT7455 (EphA2/CD137)		Immuno-oncology	Bicycle TICA™					
Partnered Programs								
THR-149 (Kallikrein inhibitor)	OXURION	Ophthalmology						
BT1718 (MT1-MMP)	CANCER RESEARCH UK	Oncology	Bicycle® Toxin Conjugate					
BT7401 (multivalent CD137 system agonist)	CANCER RESEARCH UK	Immuno-oncology						
Undisclosed	Genentech A Member of the Roche Group	Immuno-oncology						
Multiple targets	AstraZeneca	Cardiovascular, metabolic, respiratory						
Novel anti-infectives	Innovate UK	Anti-infectives						
Novel CNS targets	Dementia Discovery Fund IONIS	CNS						
Novel neuromuscular targets	IONIS	Neuromuscular						

Bicycles are short peptides chemically constrained with a central scaffold that can induce diverse structures



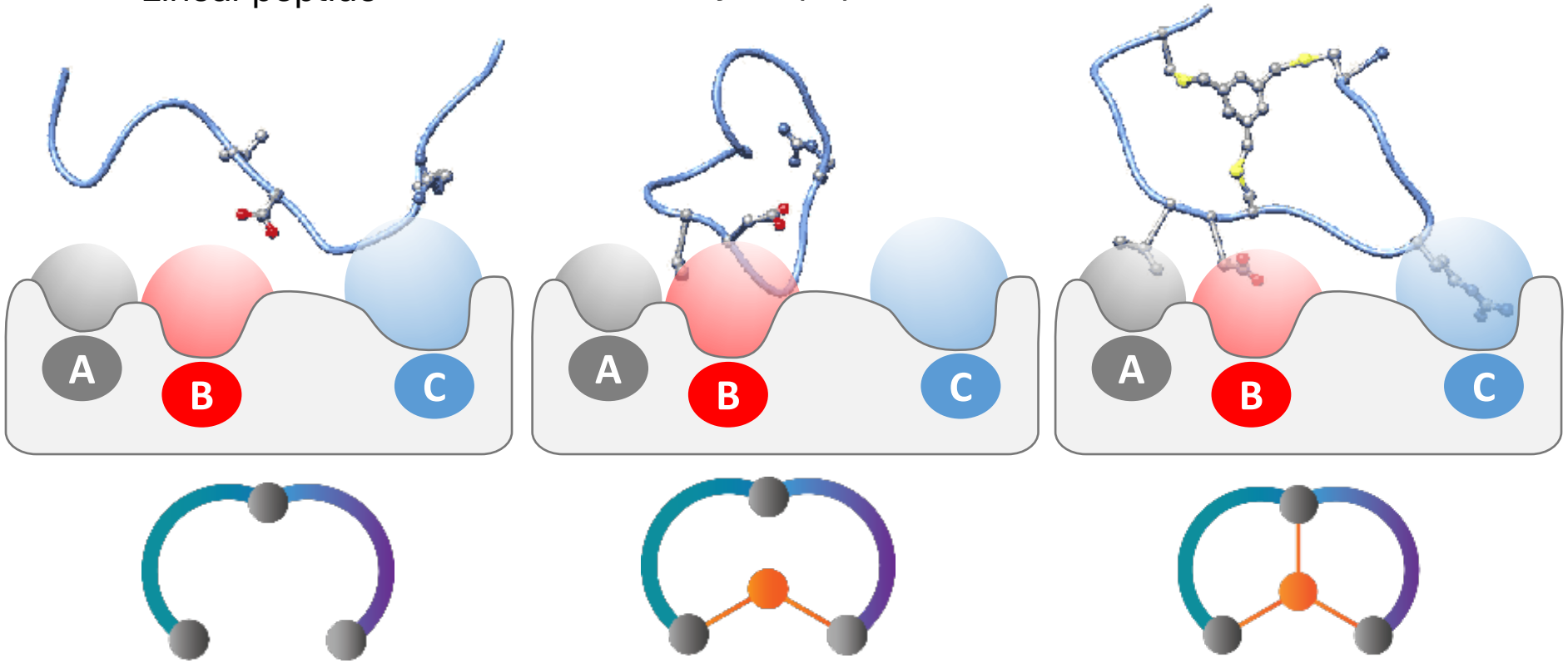
Structural constraints create *Bicycle®* advantage



Linear peptide

Cyclic peptide

Constrained Bicycle



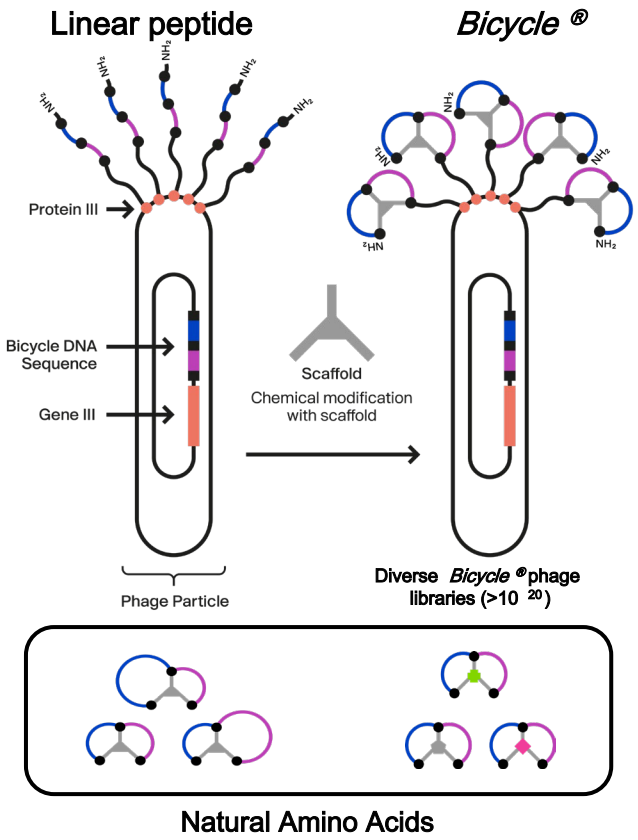
MT1-MMP affinity (FP competition)

BCY00009863 (MT1-MMP) K_i (nM)	BCY00009862 (MT1-MMP) K_i (nM)	BCY00009520 (MT1-MMP) K_i (nM)
>10000 (n=2)	115.1 ± 22.9 (n=2)	1.15 ± 0.07 (n=2)

Bicycle®

Bicycle® platform delivers a toolkit of building blocks to create novel precision guided medicines

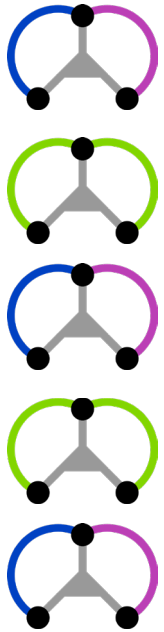
Bicycle ®Phage Display - Discovery



Peptide & Medicinal Chemistry

Optimize *Bicycle®* *monomers*

Non-natural Amino Acids



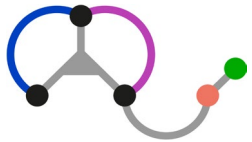
Targeting and Effector *Bicycles*

Build and Optimize Therapeutic *Bicycles*

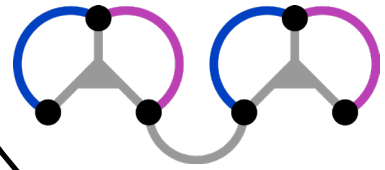
Easy conjugation of Linkers and Payloads

Potential *Bicycle* ® Medicines

Monomeric *Bicycles*



Targeted Drug Conjugates



Targeted/ Multi -specific *Bicycles*

Phage display process means Bicycles are Self-selecting for tolerance to conjugation

Bicycle®

Tag to specific target

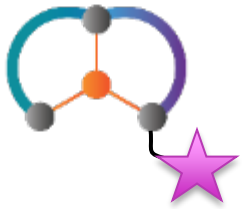
Bacteriophage

900 nm x 7 nm

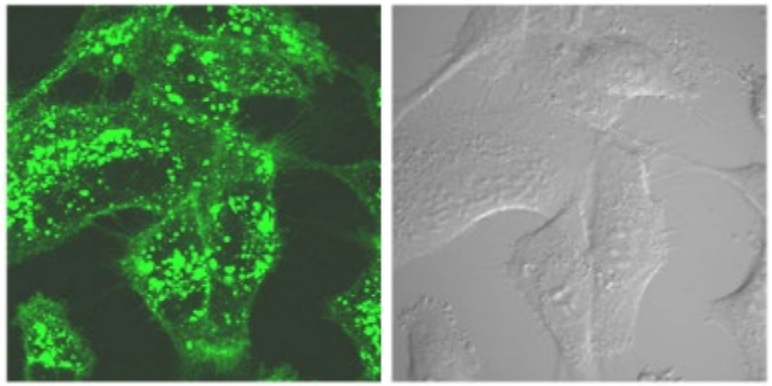
Phage bulk readily replaced without compromising binding

- Small molecule drugs
- Other *Bicycles* (tandems)
- Chelated radionuclides (PET)
- Fluorescent dyes
- Affinity tags
- PK extenders
- **Cytotoxic agents for cancer therapy**

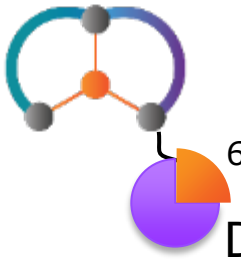
In vitro tools

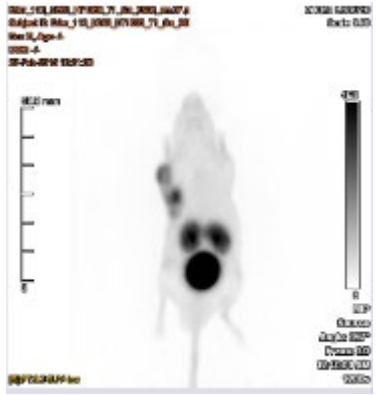


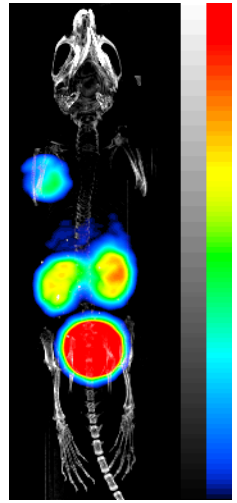
Fluorescent probe



In vivo tools/ diagnostics







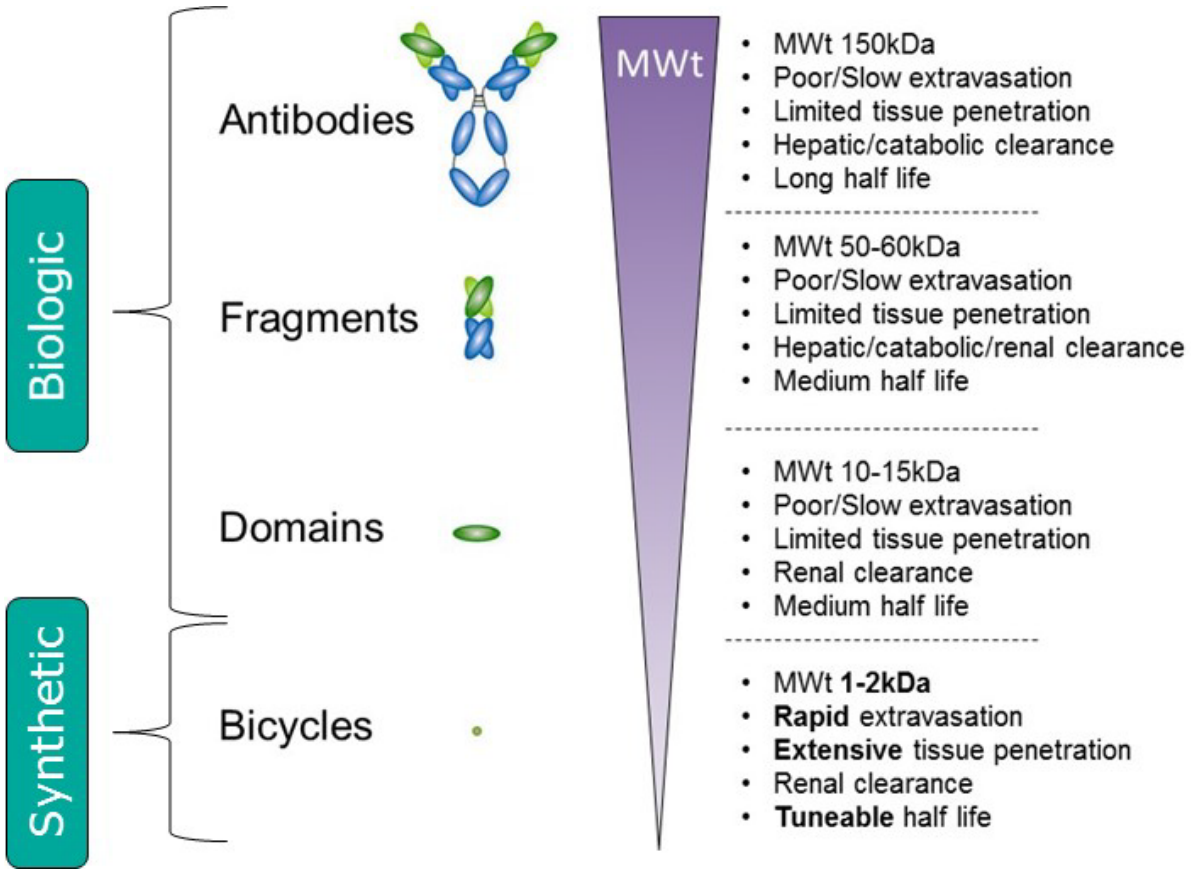
60min post inj.

Drug delivery: Size matters
Smaller & faster / more precise / power to weight ratio?

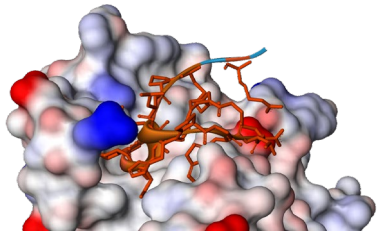
OR

OR

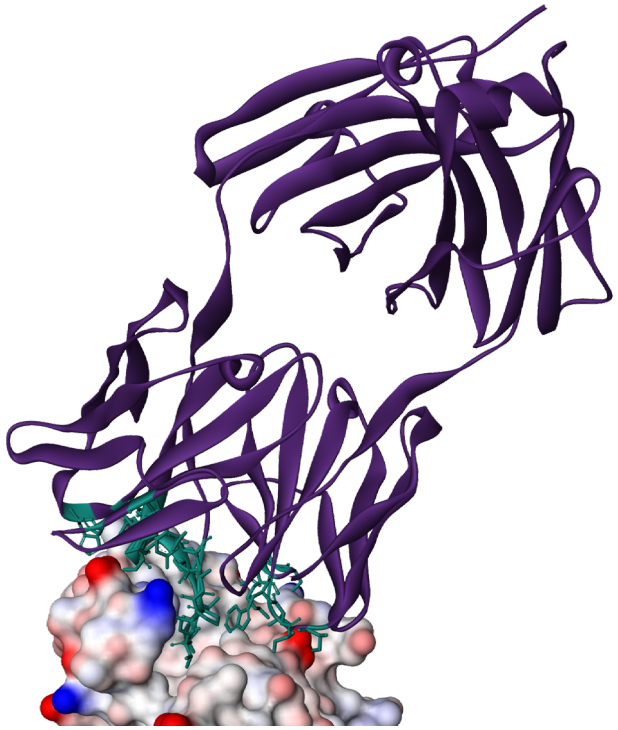
Bicycles are chemically efficient, precision guided and fit for purpose delivery vehicles



	Bicycles	Fab
Weight	2.3 kDa	48 kDa
Size	19 aa	445 aa
Binding residues	16 aa (85%)	24 aa (5%)

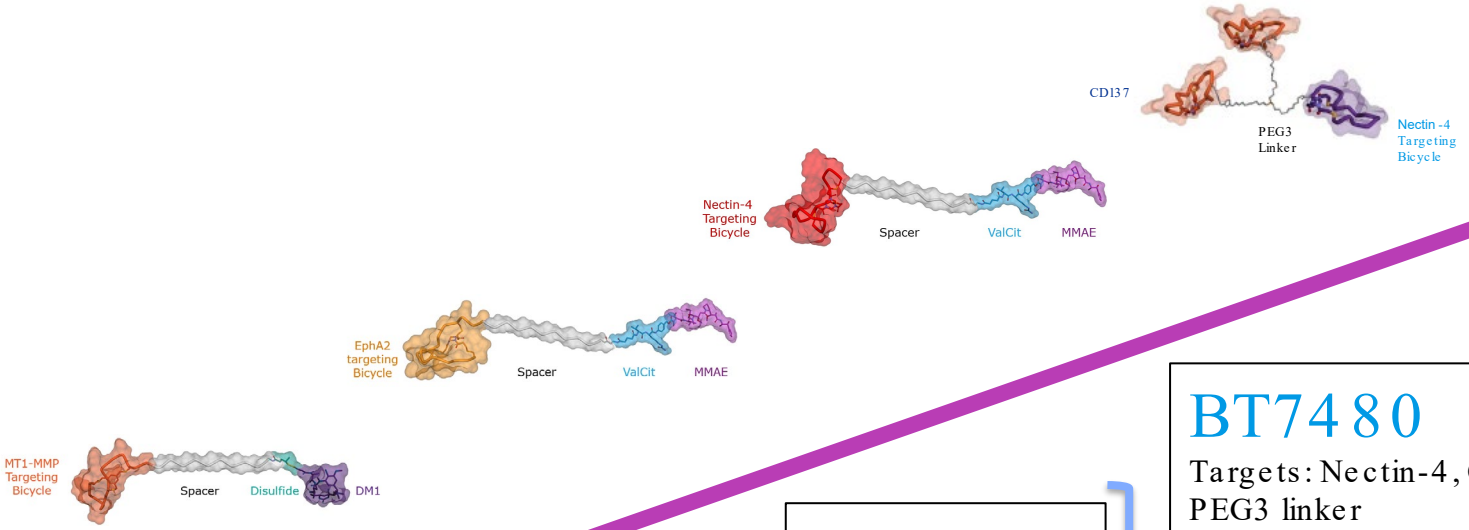


EphA2-binding *Bicycle®*



EphA2-binding **Fab**

Diversifying the *Bicycle*® platform



Targeted ASO/SiRNA delivery

BT1718
Target: MT1-MMP
DM1 payload
Disulfide linker

BT5528
Target: EphA2
MMAE payload
Val-Cit linker

BT8009
Target: Nectin-4
MMAE payload
Val-Cit linker

BT7480
Targets: Nectin-4, CD137
PEG3 linker

Validation

BT7455
Targets: EphA2, CD137
PEG3 linker
Next Bicycle TICA®
scheduled for clinic

NK-TICA®

Validation

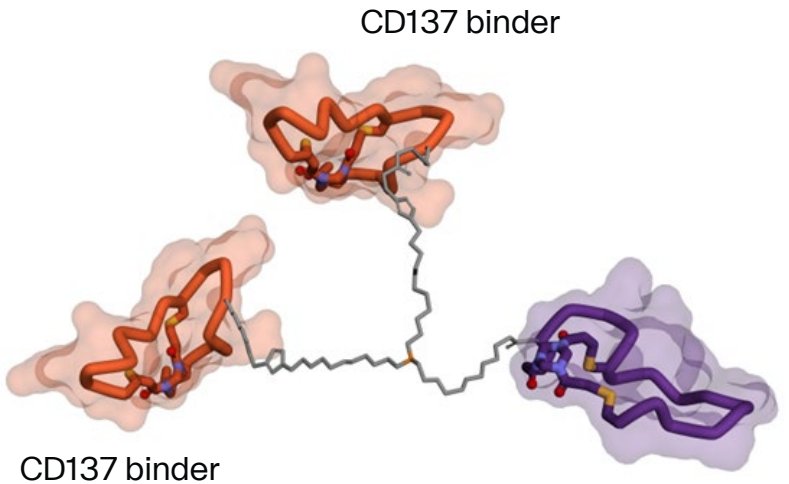
Next-gen BTCs
Different targets, linkers and payloads

Radiopharm

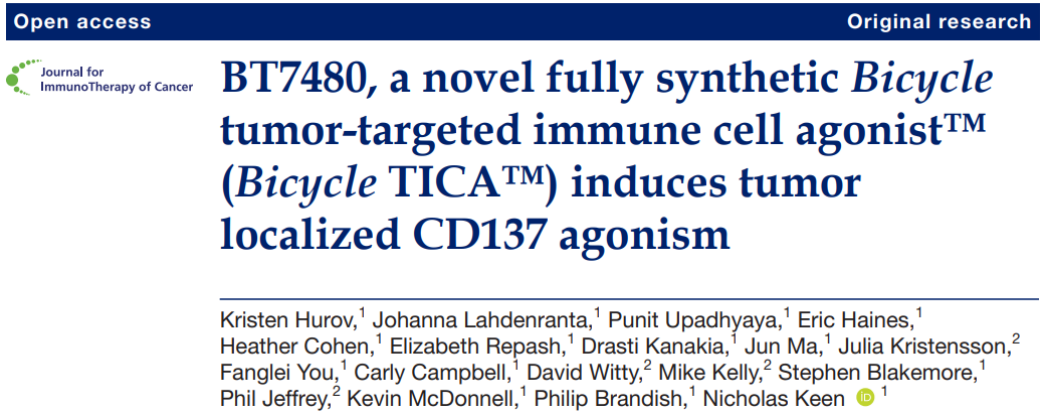
FIH 2018 2019 2020 2021 2023 and beyond

BT7480 – the first chemically synthetic, conditionally active targeted CD137 activator

- Activity of the CD137 agonist arm is dependent on ligation of the Nectin-4 arm, leading to tumor specificity
- Causes complete regressions and anti-tumor activity with only intermittent dosing in syngeneic mouse models
- Causes an early increase in chemotactic cytokine production that precedes an increase in CD8+ T cell infiltration into the tumor
- Is well-tolerated in preclinical safety species
- Entered Phase I clinical testing in November 2021



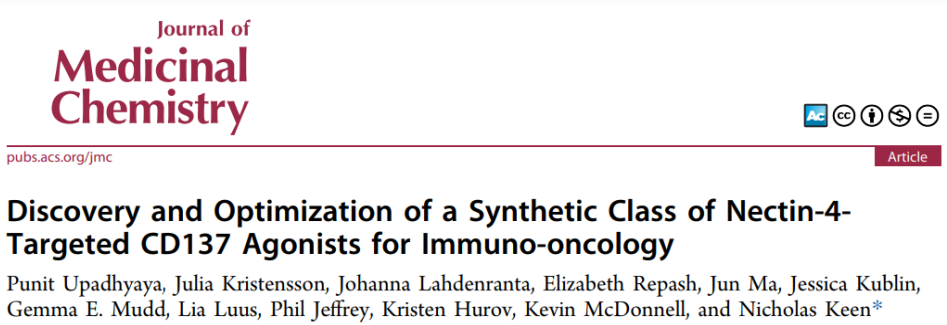
BT7480
MW = 7213.3 Da


Open access Original research

Journal for ImmunoTherapy of Cancer

BT7480, a novel fully synthetic *Bicycle* tumor-targeted immune cell agonist™ (*Bicycle* TICA™) induces tumor localized CD137 agonism

Kristen Hurov,[1] Johanna Lahdenranta,[1] Punit Upadhyaya,[1] Eric Haines,[1] Heather Cohen,[1] Elizabeth Repash,[1] Drasti Kanakia,[1] Jun Ma,[1] Julia Kristensson,[2] Fanglei You,[1] Carly Campbell,[1] David Witty,[2] Mike Kelly,[2] Stephen Blakemore,[1] Phil Jeffrey,[2] Kevin McDonnell,[1] Philip Brandish,[1] Nicholas Keen [1]


Journal of Medicinal Chemistry

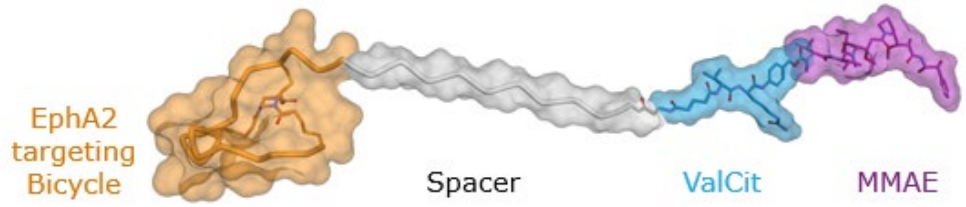
pubs.acs.org/jmc Article

Discovery and Optimization of a Synthetic Class of Nectin-4-Targeted CD137 Agonists for Immuno-oncology

Punit Upadhyaya, Julia Kristensson, Johanna Lahdenranta, Elizabeth Repash, Jun Ma, Jessica Kublin, Gemma E. Mudd, Lia Luus, Phil Jeffrey, Kristen Hurov, Kevin McDonnell, and Nicholas Keen*

Hurov K, Lahdenranta J, et al., 2021, *J Immunother Cancer*, **9**(11):e002883; Upadhyaya, et al., 2022, *J Med Chem*, **65**(14):9858-72

BT5528 is a first-in-class BTC-targeting EphA2



- BT5528 has potential to penetrate solid tumors; approximately 40X smaller than an ADC
- Toxin is released and retained in tumor cells, resulting in tumor cell death and bystander killing
- PK profile distinct from ADCs; renally eliminated, bypassing liver metabolism
- Recently completed dose escalation of Phase I clinical study

Journal of Medicinal Chemistry
pubs.acs.org/jmc Article

Identification and Optimization of EphA2-Selective Bicycles for the Delivery of Cytotoxic Payloads

Gemma E. Mudd,* Amy Brown, Liuhong Chen, Katerine van Rietschoten, Sophie Watcham, Daniel P. Teufel, Silvia Pavan, Rachid Lani, Philip Huxley, and Gavin S. Bennett

Cite This: https://dx.doi.org/10.1021/acs.jmedchem.9b02129 Read Online

Published OnlineFirst May 12, 2020; DOI: 10.1158/1535-7163.MCT-19-1092
MOLECULAR CANCER THERAPEUTICS | SMALL MOLECULE THERAPEUTICS

MMAE Delivery Using the *Bicycle* Toxin Conjugate BT5528 AC

Gavin Bennett[1], Amy Brown[1], Gemma Mudd[1], Philip Huxley[1], Katerine Van Rietschoten[1], Silvia Pavan[2], Liuhong Chen[1], Sophie Watcham[3], Johanna Lahdenranta[4], and Nicholas Keen[4]

Multiple approaches targeting EphA2-expressing tumors have failed

- MEDI-547 (MedImmune) ADC: halted following first dose-cohort coagulopathy[1]
- DS-8895a (Daiichi) antibody: limited efficacy in EphA2+ gastric and esophageal cancer, significant infusion reactions. Discontinued because of poor risk-benefit profile[2]
- MM-310 (Merrimack) antibody-targeted nanoliposome: terminated - “unable to reach optimal therapeutic index”[3]

Invest New Drugs (2013) 31:77–84
DOI 10.1007/s10637-012-9801-2

PHASE I STUDIES

Phase 1, open-label study of MEDI-547 in patients with relapsed or refractory solid tumors

Christina M. Annunziata · Elise C. Kohn · Patricia LoRusso · Nicole D. Houston · Robert L. Coleman · Manuela Buzoianu · Gabriel Robbie · Robert Lechleider

Investigational New Drugs
https://doi.org/10.1007/s10637-022-01237-3

PHASE I STUDIES

A phase 1 safety and bioimaging trial of antibody DS-8895a against EphA2 in patients with advanced or metastatic EphA2 positive cancers

Hui K. Gan[1,2,3] · Sagun Parakh[1,2,3] · F. T. Lee[1] · Niall C. Tebbutt[3] · Malaka Ameratunga[3] · Sze Ting Lee[1,2,4,5] · Graeme J. O'Keefe[1,4] · Sylvia J. Gong[1,4] · Christine Vanrenen[3] · Jaren Caine[3] · Mara Giovannetti[6] · Carmel Murone[1] · Fiona E. Scott[1,2] · Nancy Guo[1] · Ingrid J. G. Burvenich[1,2] · Cameron Paine[4] · Mary J. Macri[6] · Masakatsu Kotsuma[7] · Giorgio Senaldi[7] · Ralph Venhaus[6] · Andrew M. Scott[1,2,4,5]

Clinical Trial · J Immunother Cancer. 2019 Aug 14;7(1):219. doi: 10.1186/s40425-019-0679-9.

Safety, tolerability, pharmacokinetics, and pharmacodynamics of the afucosylated, humanized anti-EPHA2 antibody DS-8895a: a first-in-human phase I dose escalation and dose expansion study in patients with advanced solid tumors

Kohei Shitara [1], Taroh Satoh [2], Satoru Iwasa [3], Kensei Yamaguchi [4], Kei Muro [5], Yoshito Komatsu [6], Tomohiro Nishina [7], Taito Esaki [8], Jun Hasegawa [9], Yasuyuki Kakurai [9], Emi Kamiyama [9], Tomoko Nakata [9], Kota Nakamura [9], Hayato Sakaki [9], Ichinosuke Hyodo [10]

merrimack

Merrimack Discontinues Development of MM-310

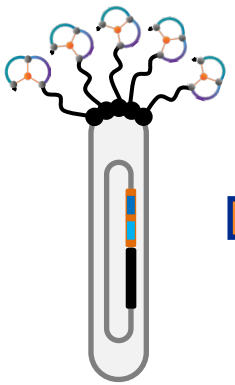
April 4, 2019

-- Safety update shows Phase 1 study unable to reach optimal therapeutic index for MM-310 due to continued observation of cumulative peripheral neuropathy --

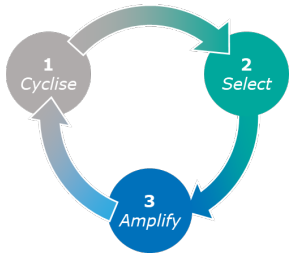
-- Company expects to reduce workforce reflective of narrowed preclinical development pipeline; continues to prudently advance programs while completing the assessment of its strategic alternatives --

1. Annunziata et al, Invest New Drugs. 2013 Feb;31(1):77-84
2. Shitara et al, Journal for ImmunoTherapy of Cancer. 2019 7: 219-230 (Ph1 study); Gan et al, Invest New Drugs. 2022 40(4) 747-755
3. Merrimack Pharmaceuticals Inc., press release April 4, 2019

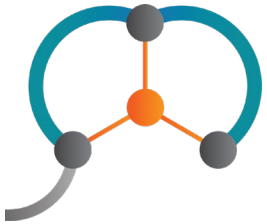
Chemical optimization of a high affinity EphA2 targeting *Bicycle*® with improved properties



2 scaffolds x 10 libraries,
➔ 8 peptide families

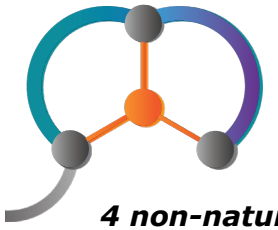


BCY6014

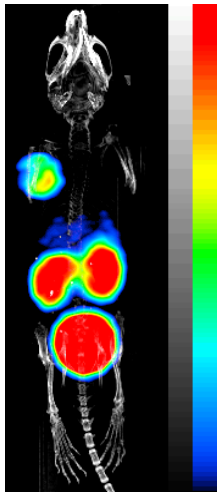


Chemistry Optimisation <40 analogs

BCY6099



4 non-natural amino acids



PET imaging of HT-1080 xenograft at 60 minutes

Modifications (*) to increase polarity, stability and/or potency

BCY6014 Ki = 16 nM – early *Bicycle*

A	R	D	C	P	L	V	N	P	L	C	L	H	P	G	W	T	C	A

BCY6099 Ki = 3 nM – newly optimised *Bicycle*

A	hR	D	C	hyP	L	V	N	P	L	C	L	H	P	dD	W	hR	C	A
	*			*										*		*		

MOLECULAR CANCER THERAPEUTICS | SMALL MOLECULE THERAPEUTICS

MMAE Delivery Using the *Bicycle* Toxin Conjugate BT5528 ADC

Gavin Bennett[1], Amy Brown[1], Gemma Mudd[1], Philip Huxley[1], Katerine Van Rietschoten[1], Silvia Pavan[2], Liuhong Chen[1], Sophie Watcham[3], Johanna Lahdenranta[4], and Nicholas Keen[4]

Journal of Medicinal Chemistry

pubs.acs.org/jmc Article

Identification and Optimization of EphA2-Selective Bicycles for the Delivery of Cytotoxic Payloads

Gemma E. Mudd,* Amy Brown, Liuhong Chen, Katerine van Rietschoten, Sophie Watcham, Daniel P. Teufel, Silvia Pavan, Rachid Lani, Philip Huxley, and Gavin S. Bennett

Cite This: https://dx.doi.org/10.1021/acs.jmedchem.9b02129 Read Online

Potential of *Bicycles* as precision guided therapeutics

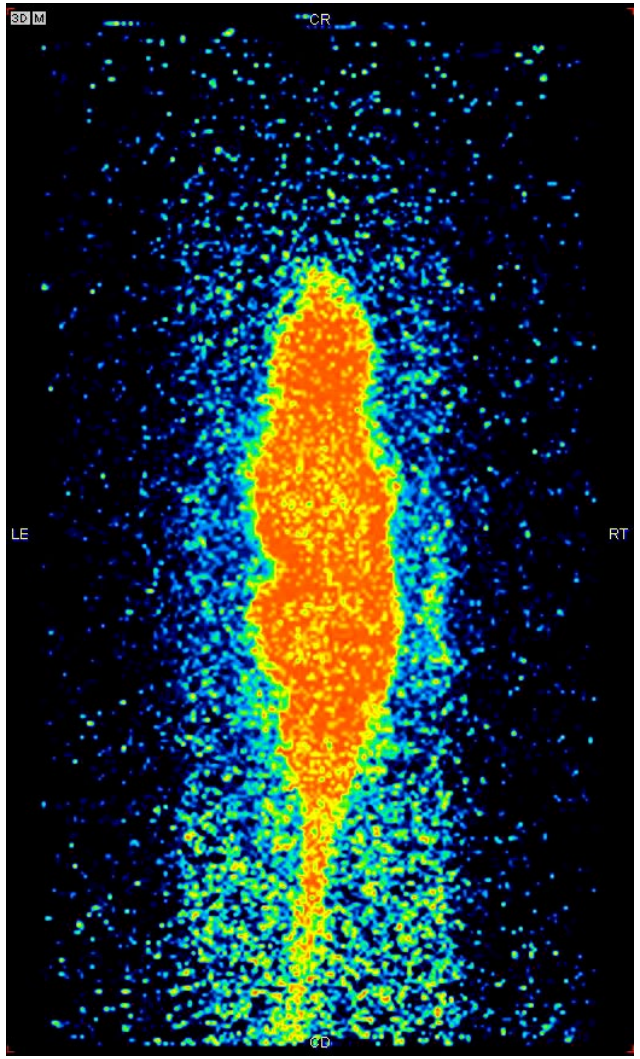
Bicycles rapidly penetrate tumour, eliminated through renal route

Short systemic exposure & tumour retention

Activity at site of action with reduced body burden

Can be used to deliver key pharmacological activity for solid tumours:

- **Cytotoxic payloads**
- **Immune-oncology**



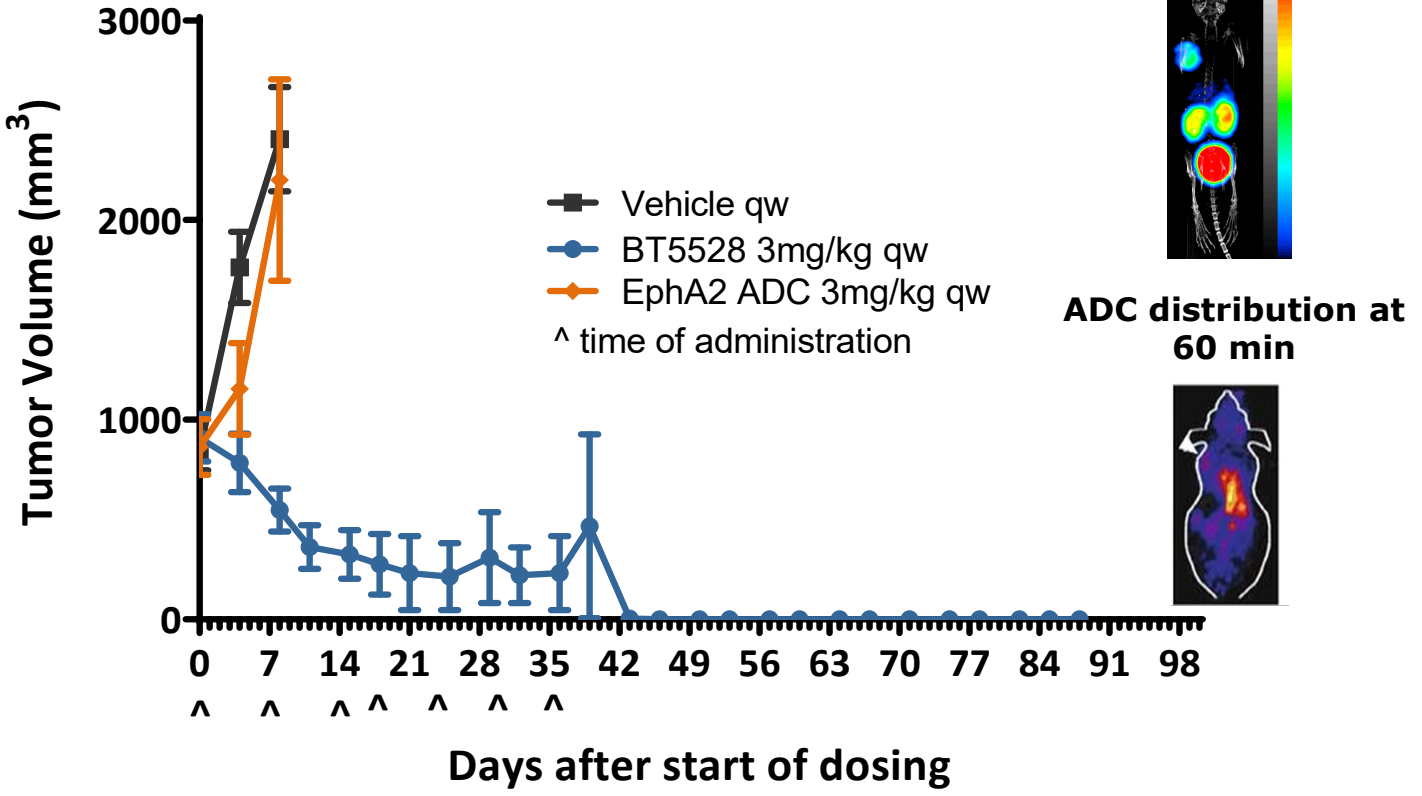
DKTK German Cancer Consortium

PET imaging of Bicycle-radioisotope conjugate, 0-60min post-injection

Imaging conducted in collaboration with
Prof. Dr. Matthias Eder
Dr. Ann-Christin Eder
Mohamed El Fakiri

BT5528: activity in difficult-to-treat xenograft models

Superior activity to EphA2 ADC in large NSCLC xenograft models

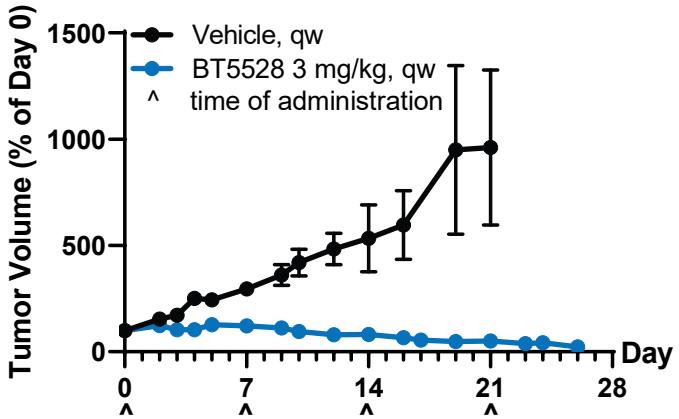


***Bicycle* distribution at 60 min**

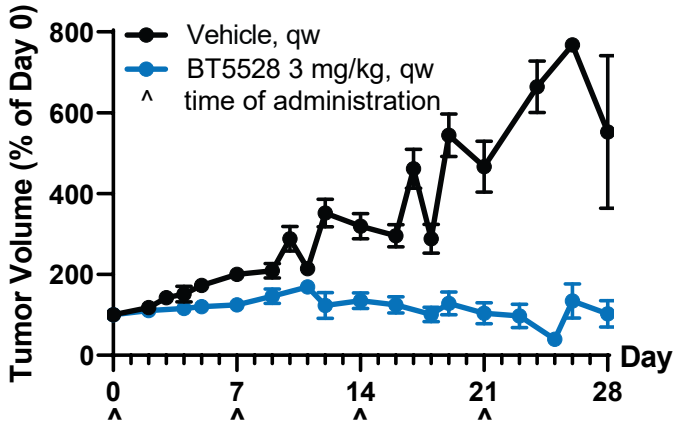
ADC distribution at 60 min

Activity in pancreatic xenograft models

PDAC1

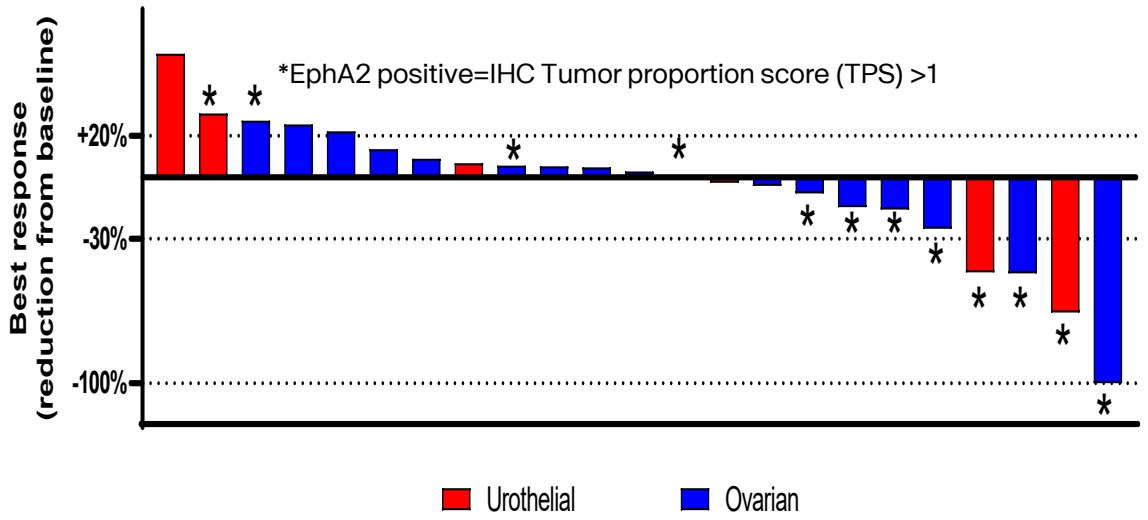


PDAC2

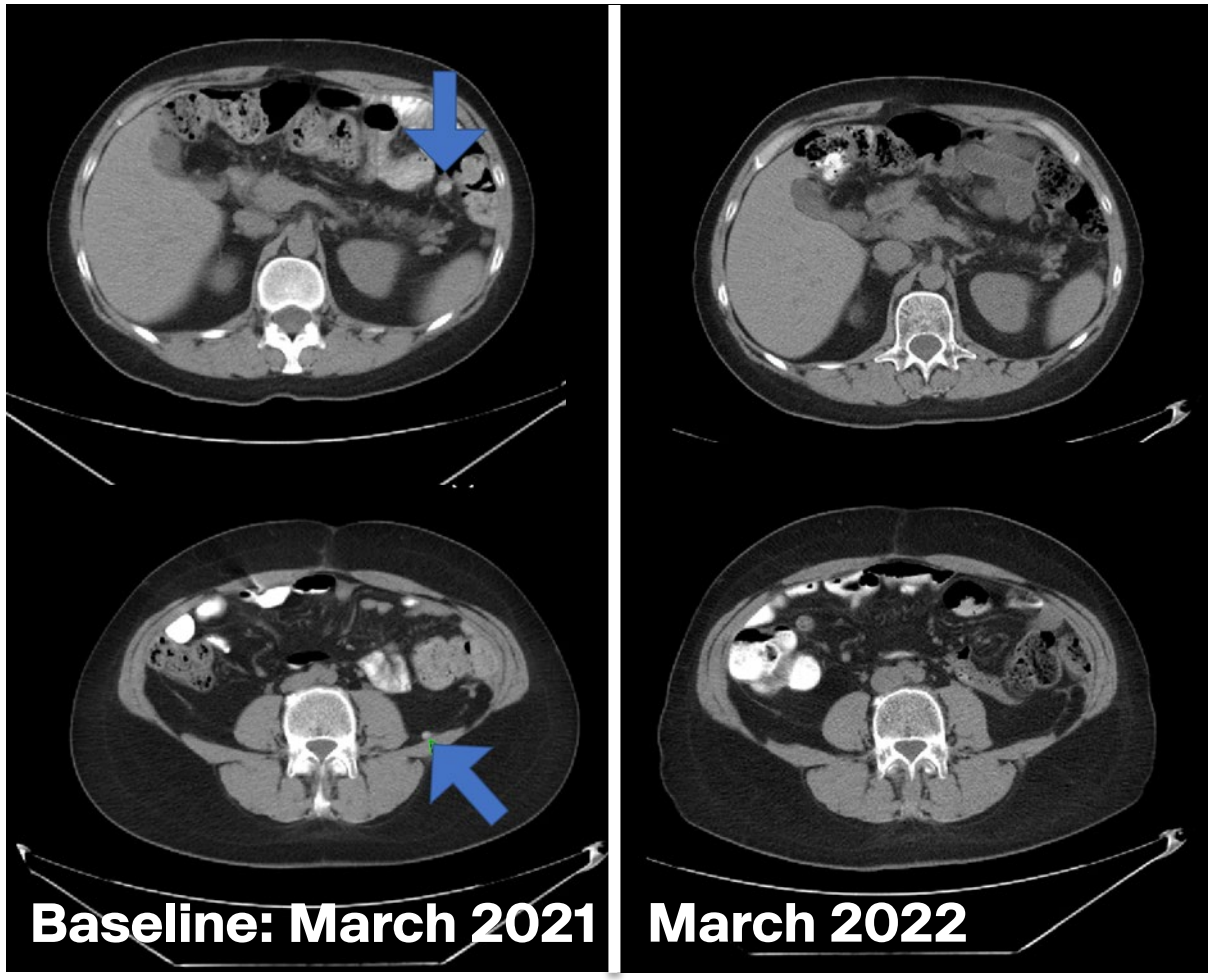


BT5528: Emerging relationship between EphA2 expression and response in ovarian and urothelial cancers

Best response by RECIST in response evaluable patients*



CT scans-abdomen. First in human dose escalation trial.



- Waterfall plot showing best response among urothelial and ovarian cancer patients in first in human study
- Immunohistochemistry data suggest EphA2-positive patients more likely to respond to BT5528
- Scan showing complete responder with ovarian cancer

Bicycle®

* End of Phase I update, Sep 22, with data cut-off Aug 22

Nectin-4 Bicycle® optimization from lead to BT8009

Parent *Bicycle*

Ac C P F G C M K N W S W P I W C

Amino Acids Important For Target Engagement

Poor solubility, short half-life. AAs required for binding identified

Ki (nM)	cLogP	$t_{1/2}$ (plasma)
18.4	-6.98	1.3h

Stabilised *Bicycle*

Ac C P F d C M hArg N W S W P I W C

Improve stability, hydrophilicity

Improvements made to half-life and hydrophilicity, whilst retaining binding AAs

Ki (nM)	cLogP	$t_{1/2}$ (plasma)
13	-6.74	>24h

Optimised *Bicycle*

Ac C P 1Nal d C M hArg D W S T P Hyp W C

+ affinity

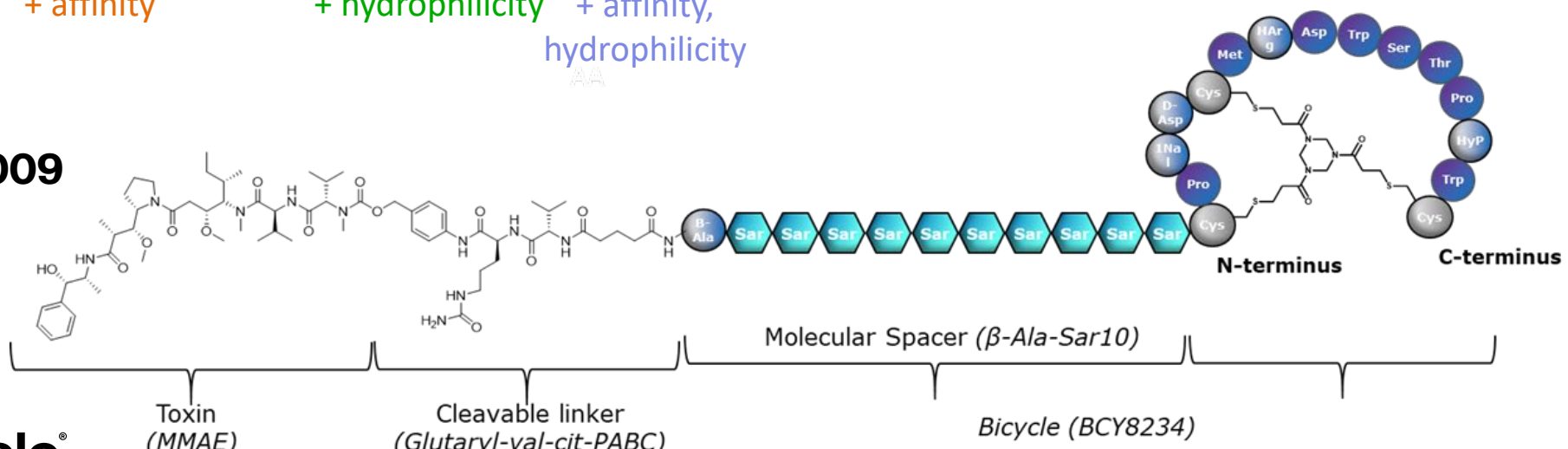
+ hydrophilicity

+ affinity, hydrophilicity

AAs further optimised to increase affinity, improve hydrophilicity. Selected as candidate peptide binder

Ki (nM)	cLogP	$t_{1/2}$ (plasma)
3.2	-13.32	>24h

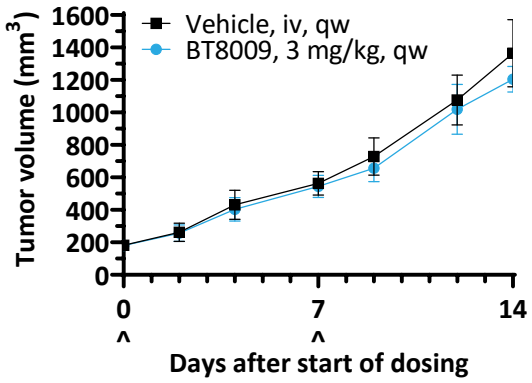
BT8009

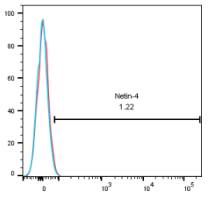


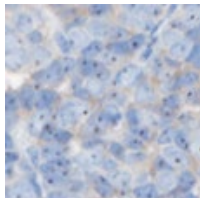
MW=4173.8

BT8009 activity generally tracks Nectin-4 expression in CDX and PDX models

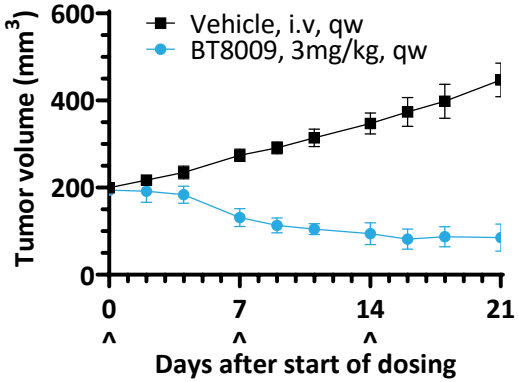
NCI-H526



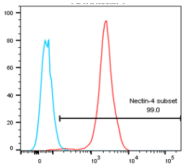


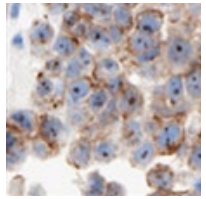


MDA-MB-468

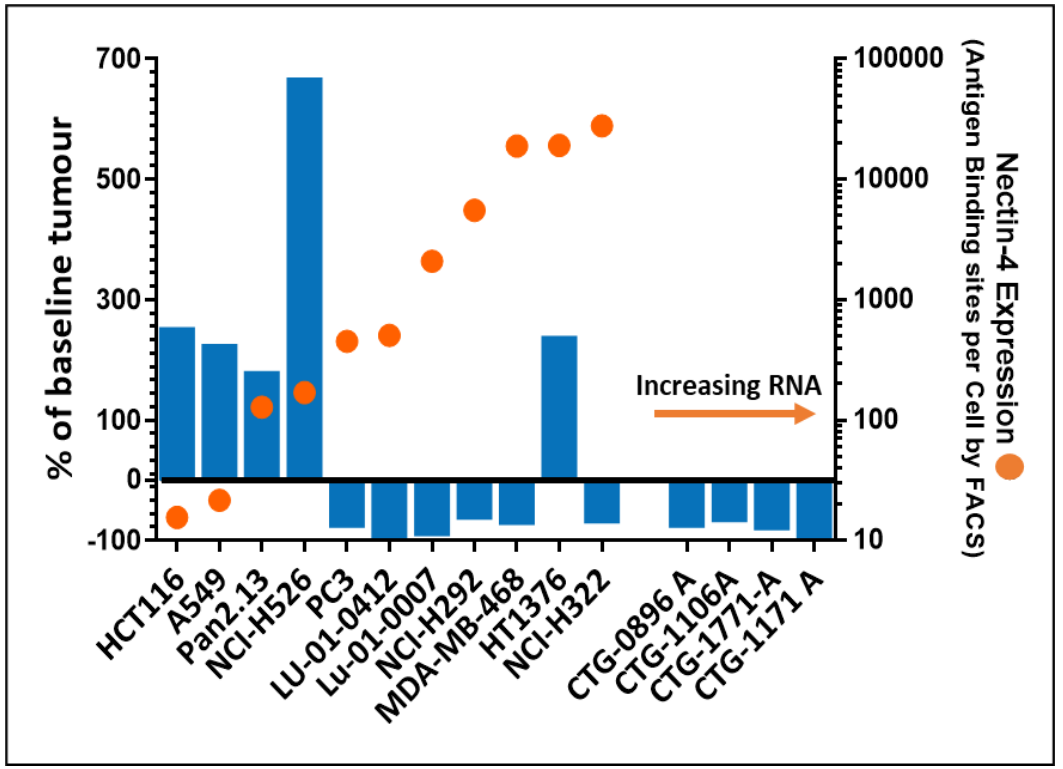


Nectin-4 FACS

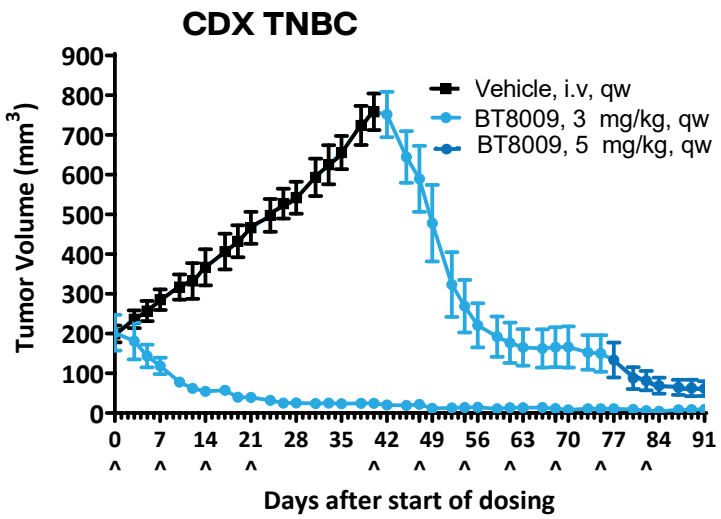


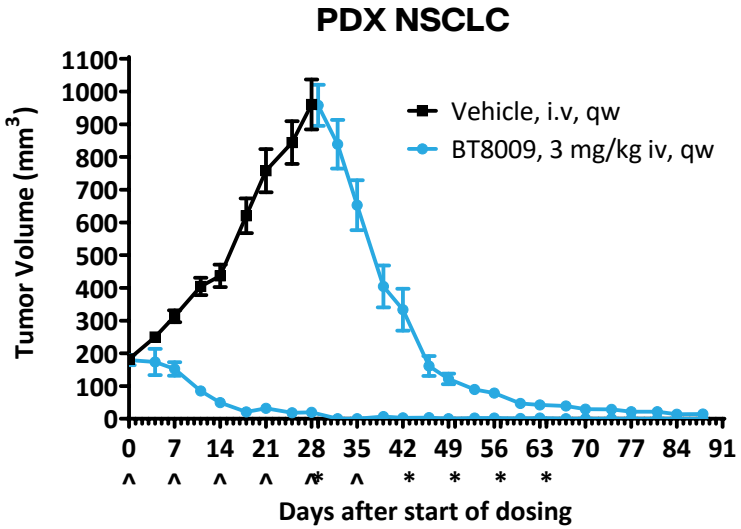


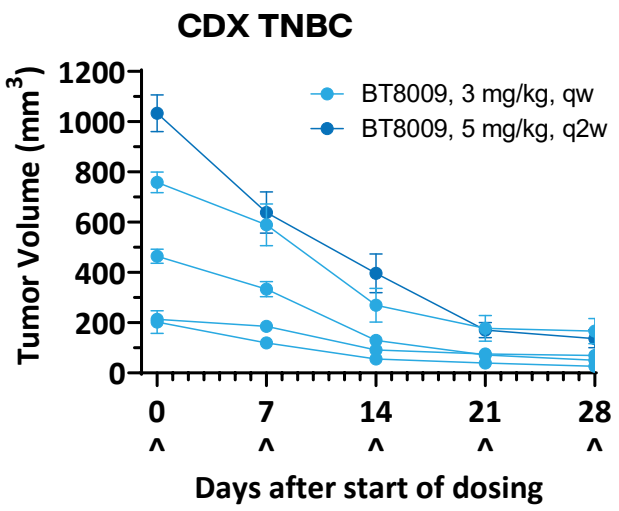
- **Optimal efficacy requires membrane expression of Nectin-4**

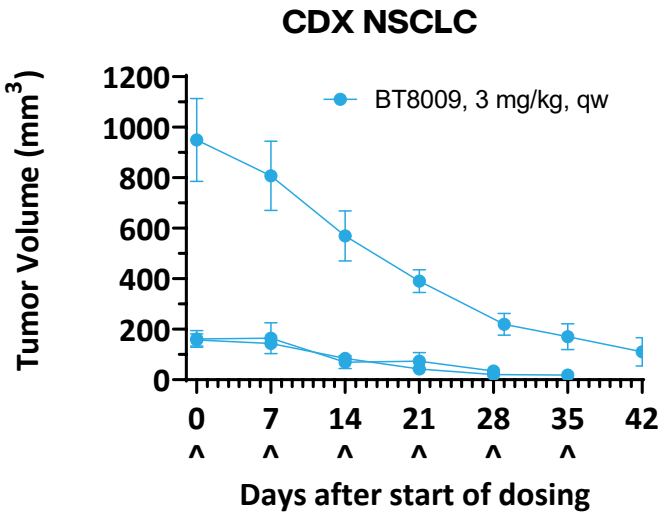


BT8009 is highly active in small and large tumors in both CDX and PDX models







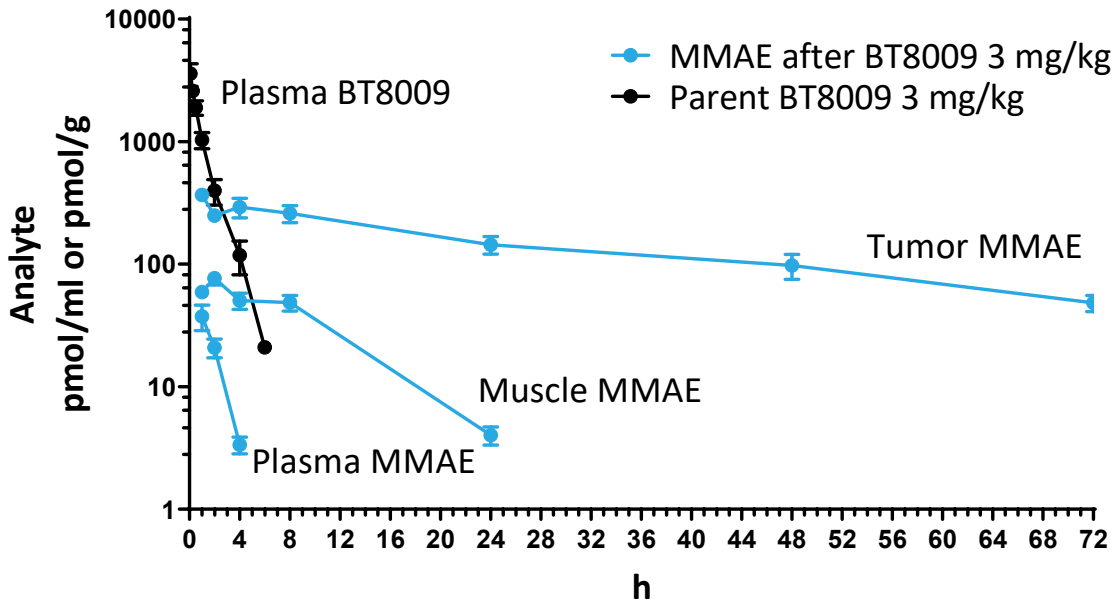


Bicycle®

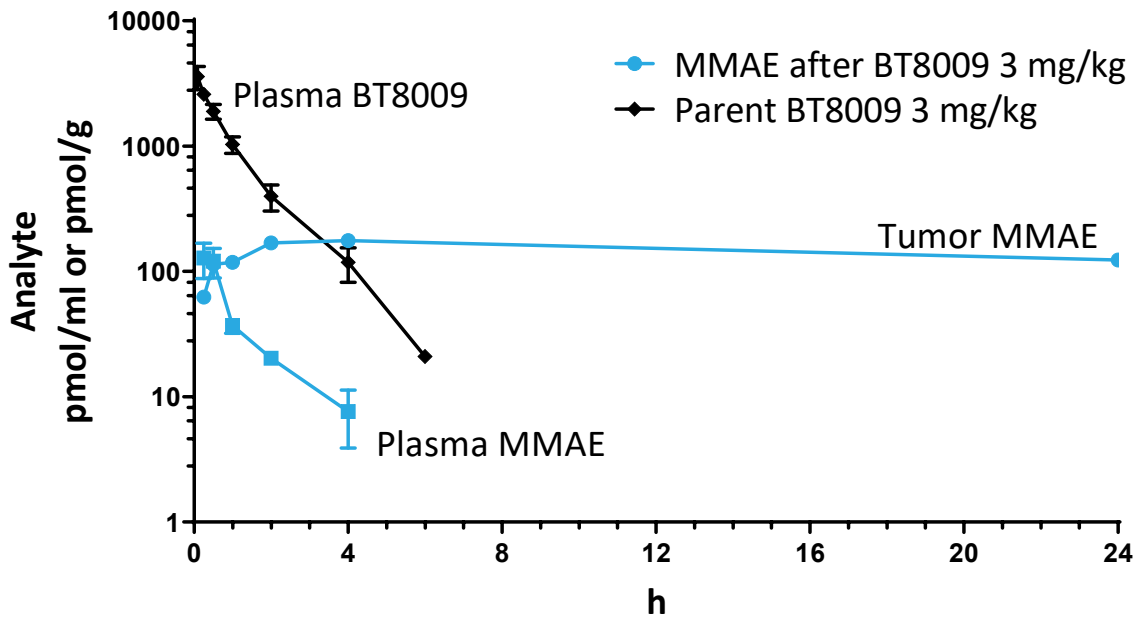
BT8009 targets tumor in CDX models and MMAE is retained there

•MMAE is retained in tumour after parent and payload are cleared from systemic circulation

NCI-H292



MDA-MB-468

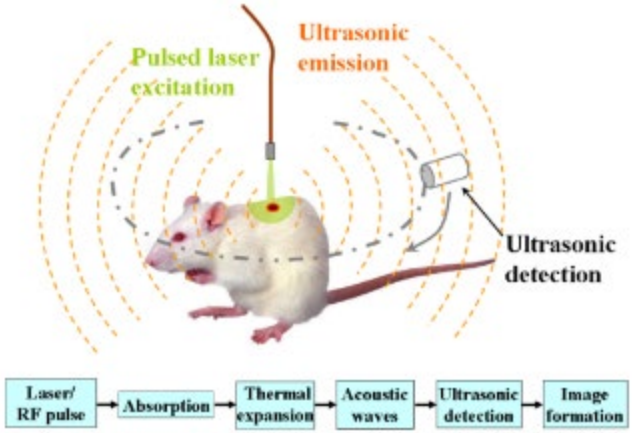


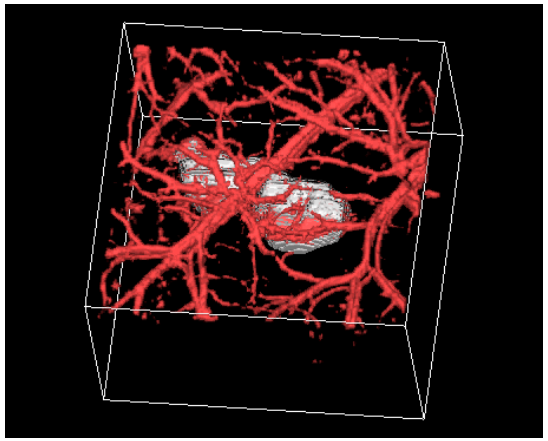
BTCs show excellent tumour penetration

High tumour penetration

Photoacoustic Imaging

laser excitation of fluorophores allows measurement of fluorophore in constrained volume.





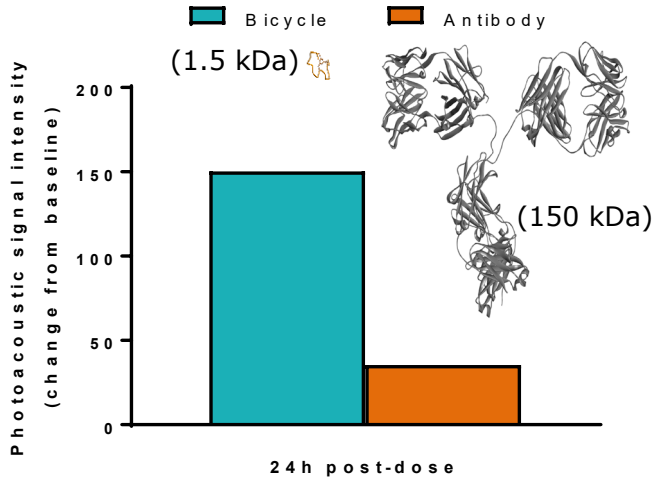
Animals co-injected with Bicycle-fluorophore conjugate and antibody-fluorophore conjugate

Different fluorophores

Comparable affinity & molar concentration

Signal measured in regions of interest

Poorly perfused region of tumour 40 um from vasculature shown

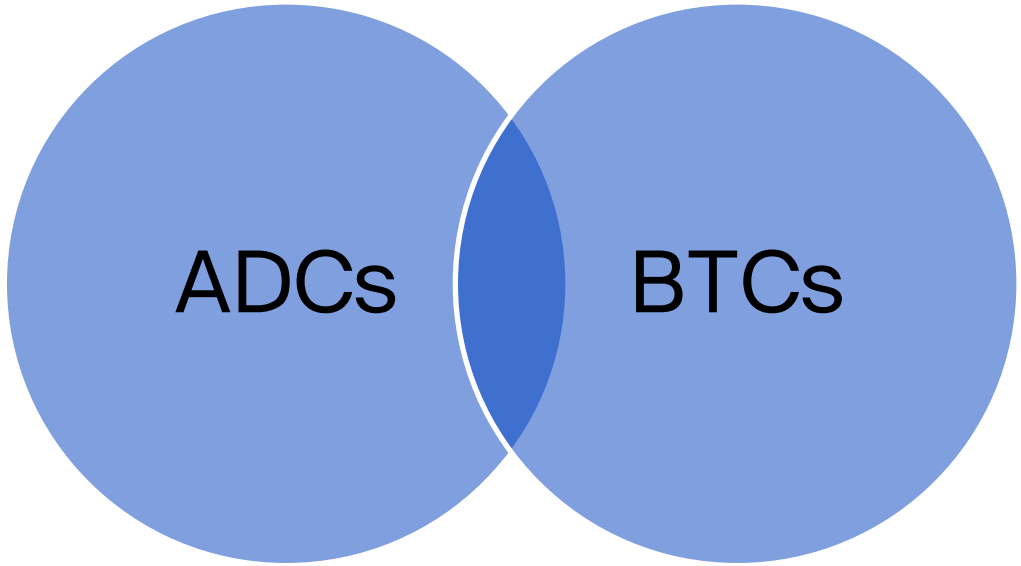


Bicycle®

Outlook: BTCs and ADCs studies

- Common factors
  - Payload (mechanism of action, toxicities, bystander effect, resistance mechanism)
  - Linker
  - Target (expression profile, internalisation rate)

- Differences
  - PK
  - Tumour penetration
  - Clearance route
  - Linker cleavage mechanisms
  - Target binding kinetics



Conclusions

- **Very encouraging data observed in preclinical and clinical BTC programs.**
- **Much remains to be investigated to understand the differences in ADME and toxicity profiles of BTCs versus ADCs.**
- **Scientists have started to write the rules of ADCs, but rules of BTCs (and PDCs) are being discovered only now:**
  - Optimum binding affinity (Kd)
  - Optimum half-life(s) (Koff and PK)
  - Warhead potency and type
  - Internalizing versus non-internalizing mechanisms

Thank you

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