BT5528, an EphA2-targeting *Bicycle*® Toxin Conjugate

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World ADC congress 2019

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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

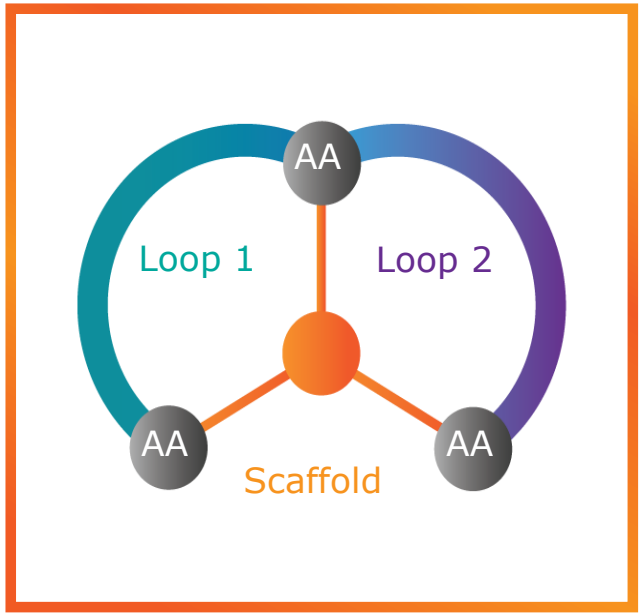
OXURION®

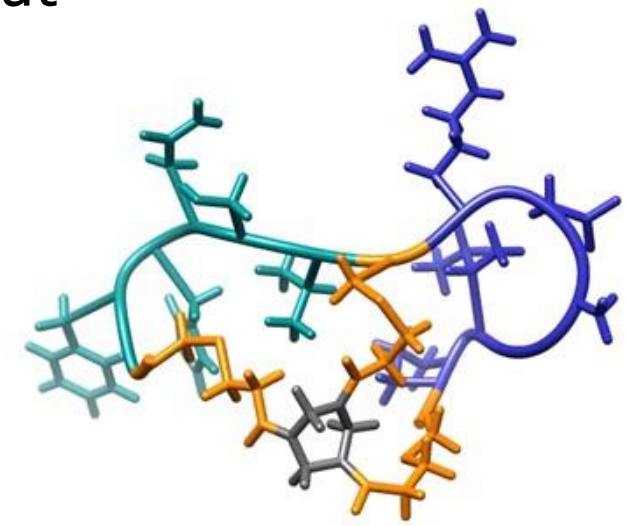
AstraZeneca

Innovate UK

Bicycles®: a new therapeutic modality

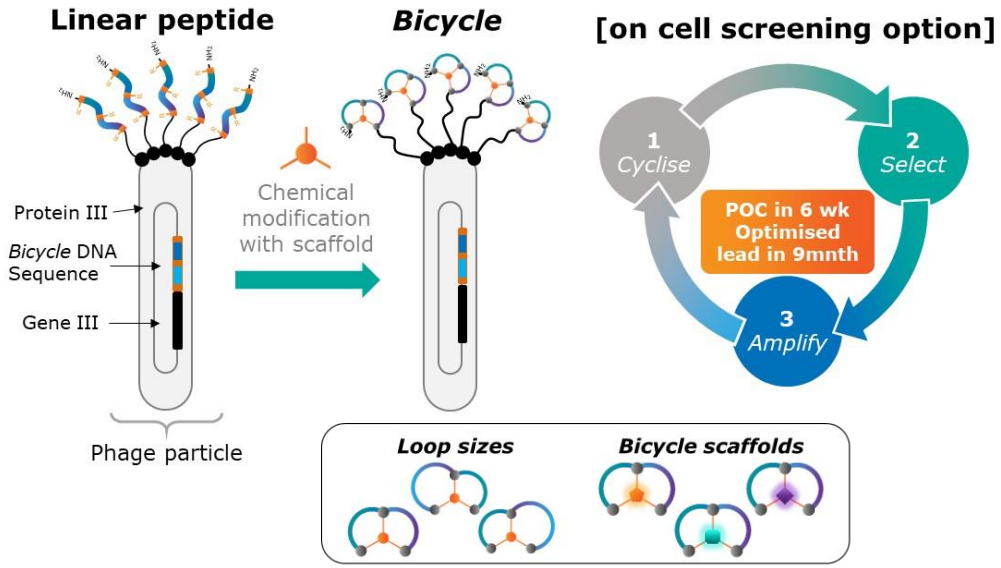
- Chemically synthesised, Low MWt (1.5-2kDa)
- Large binding footprint allowing targeting of protein-protein interactions
- Small molecule like PK and tumour penetration
- Renal elimination minimising bystander cell interactions in liver and gut





Proprietary screening platform: *Bicycles*® optimised using phage display and medicinal chemistry, informed by structural biology

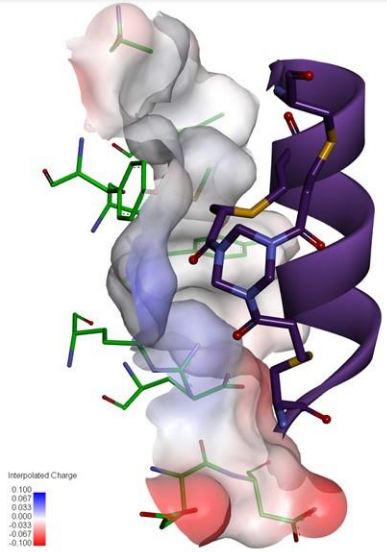
***Bicycle* Phage Display**



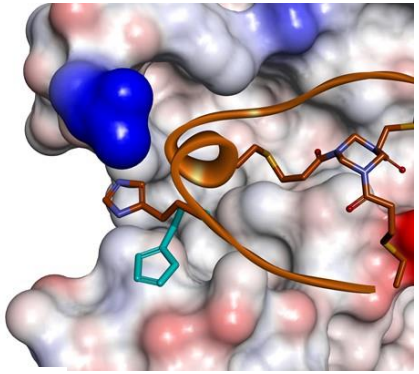
Optimize binder & capture IP

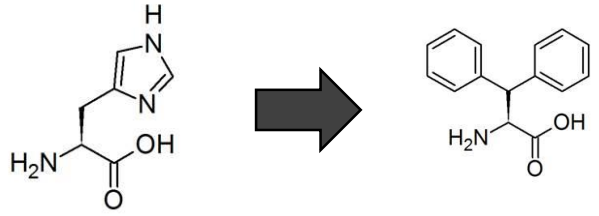
Natural Amino Acids

Structural Biology



Peptide & Medicinal Chemistry





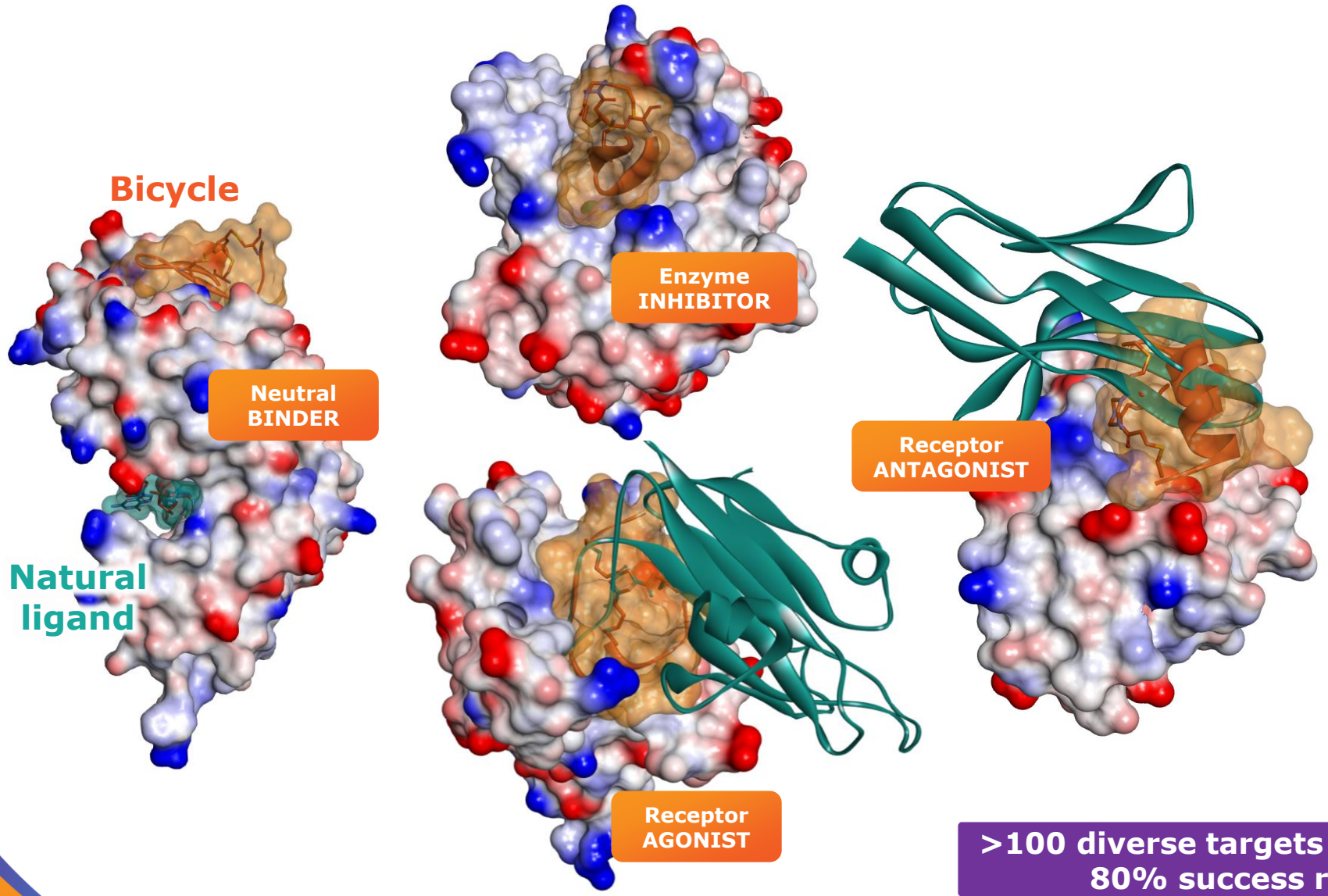
Histidine
Ki=11nM

3,3-diphenylalanine (3,3-DPA)
Ki=0.9nM

Dial in desired drug-like properties and PK profile

Non-natural Amino Acids

Bicycles® can deliver distinct modes of action

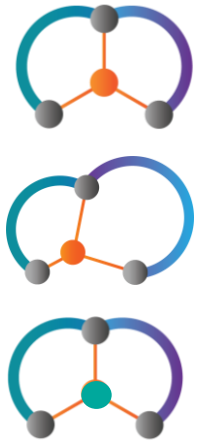


Tractable target classes	
Enzymes	Serine proteases
	Other proteases
	Metalloenzymes
	Matrix metalloproteinases
	Coagulation factors
	Other enzymes
Immune checkpoint	**TNFR superfamily members**
	IG domain receptors
Signalling	**Receptor Tyrosine kinases**
	Interleukin receptors
	Interleukins
	Growth Factors
	Cytokines
Adhesion	**Integrins**
	Other cell adhesion proteins
GPCRs	**Chemokine receptors**
	Adrenergic receptors
Other	Heat shock proteins
	Serum proteins

>100 diverse targets screened
80% success rate

Bicycles® diversity drives hit rate & chemical optionality

4 points of variation generate enormous diversity



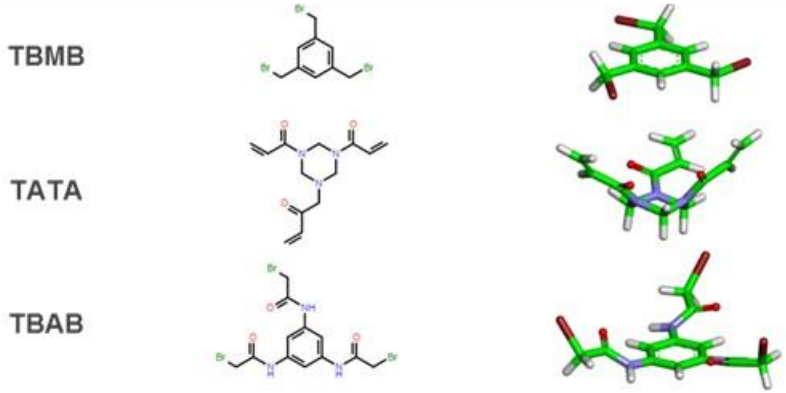
- Amino acid content (n=20)
- Loop size (n=30)
- Loop symmetry (n=3)
- Scaffold (n>6)

Diversity per scaffold up to 10^{17}

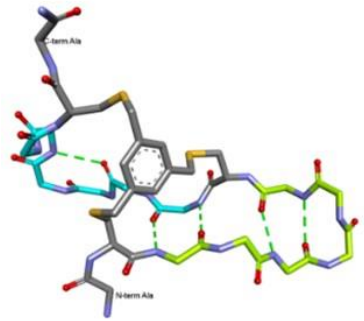
2019

Loop 1 \ Loop 2	2	3	4	5	6	7	8	9
2					2x6	2x7		
3		3x3			3x6	3x7	3x8	3x9
4		4x3	4x4	4x5	4x6		4x8	
5			5x4	5x5	5x6	5x7		
6		6x3	6x4	6x5	6x6			
7	7x2	7x3		7x5				
8	8x2	8x3	8x4					
9								

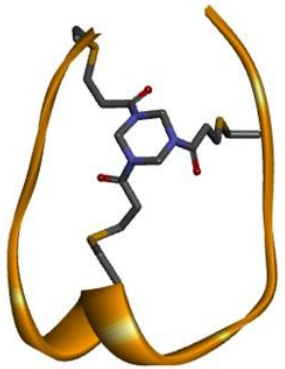
Scaffolds provide optionality to med chem



17 scaffold patents covering >200 proprietary scaffolds

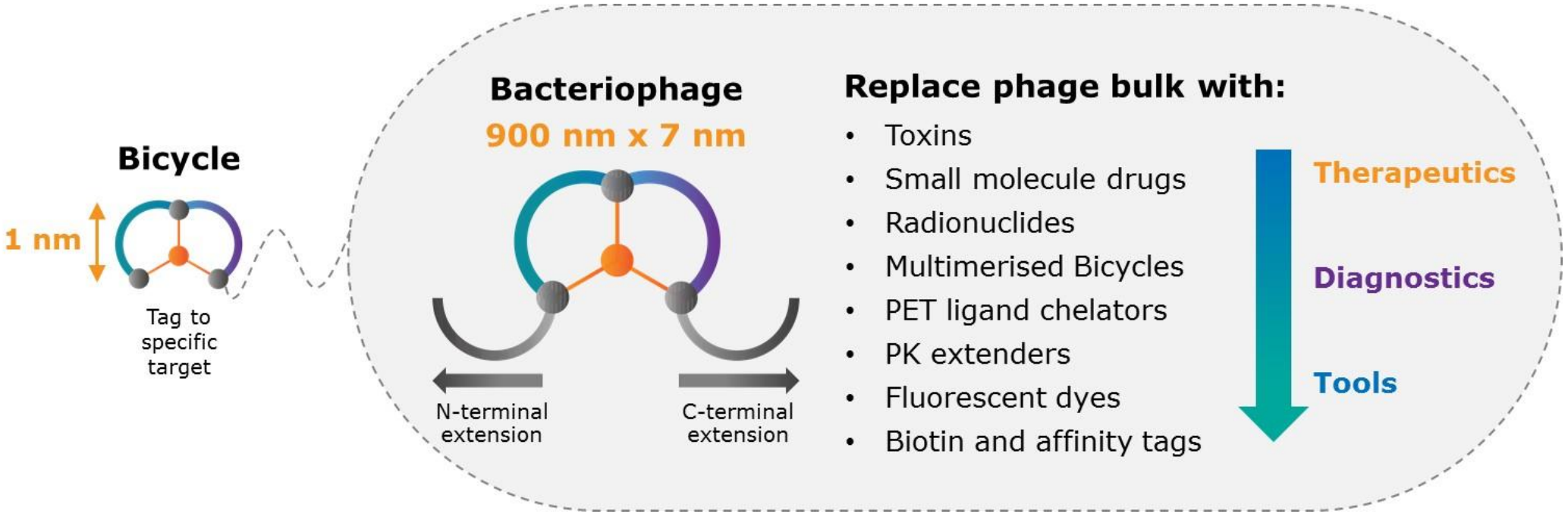


TBMB EphA2
forms β-hairpin

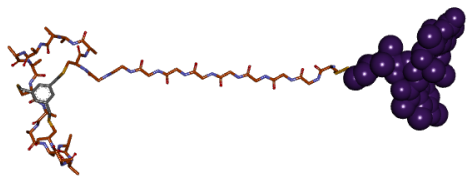


TATA EphA2
forms α-Helix

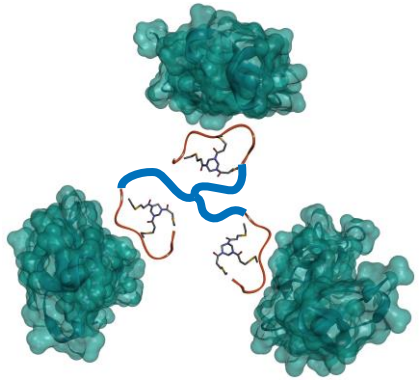
Bicycles® have built-in tolerance to conjugation



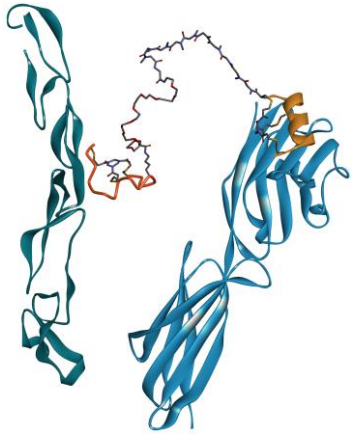
First Generation
Payload delivery



Second Generation
Receptor complexing



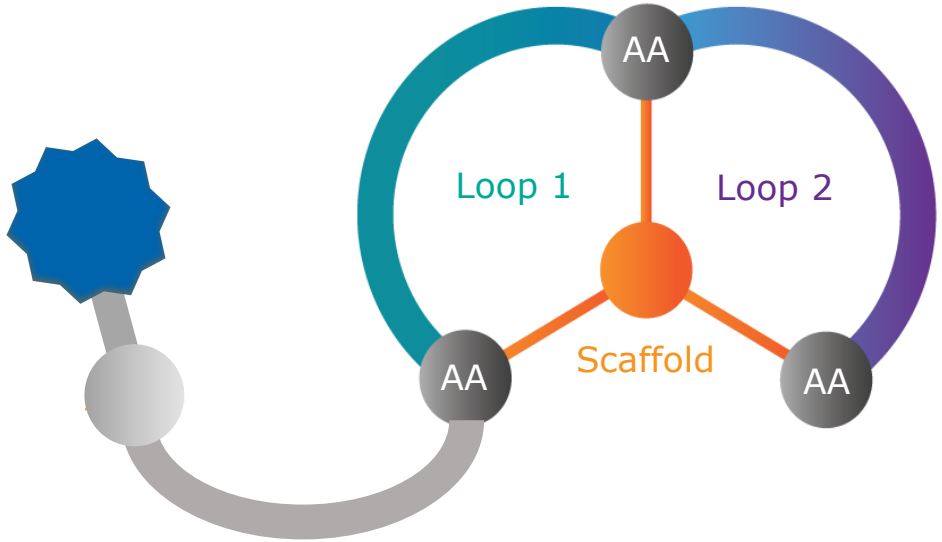
Third Generation
Dual Pharmacology



Tumour targeted *Bicycle* toxin conjugates

Cell permeable
Cytotoxin

- Too potent to be dosed alone
- Not toxic once conjugated



Bicycle selectively binds tumour

- Targets tumour antigen
- Neutral binding site

Tumour-selective
Cleavable Linker

- Negligible drug release outside tumour microenvironment
- Payload released extracellularly

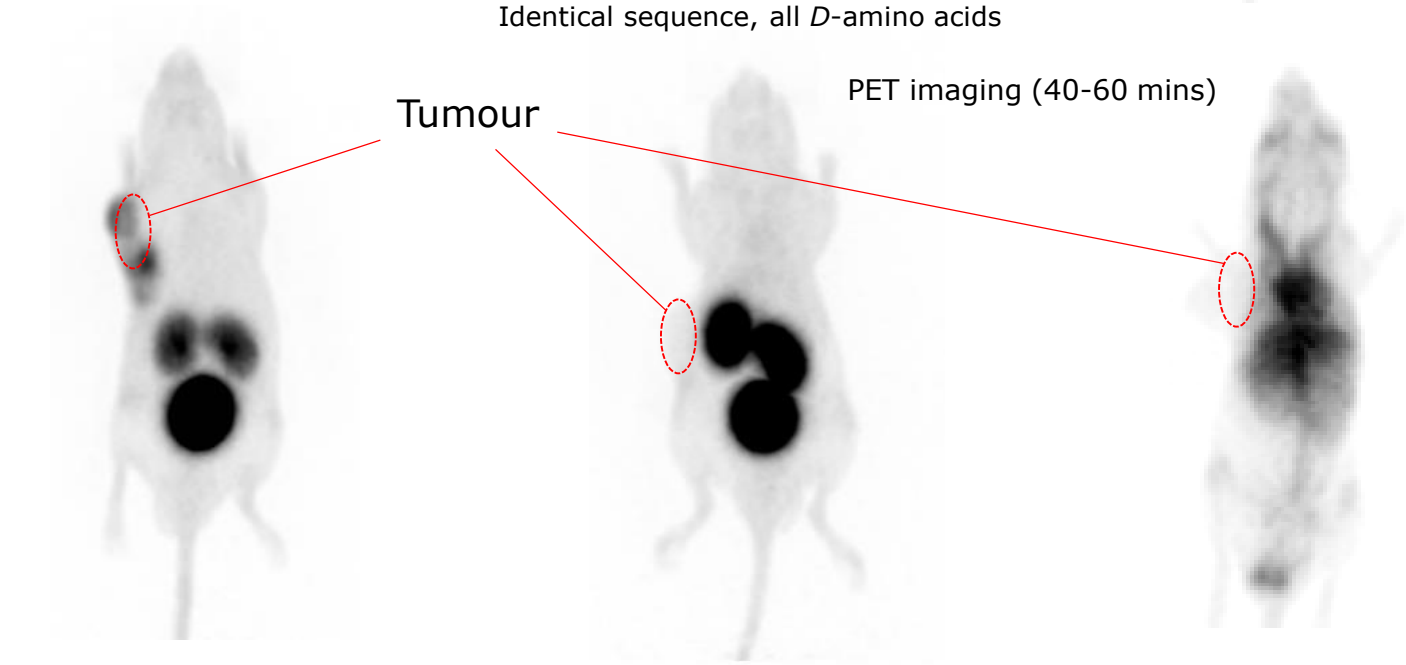
Tumour antigen binding *Bicycles*® rapidly and specifically bind within tumours, and are renally eliminated.

MT1-MMP binding *Bicycle*

Non-binding *Bicycle*

Identical sequence, all *D*-amino acids

MT1-MMP antibody

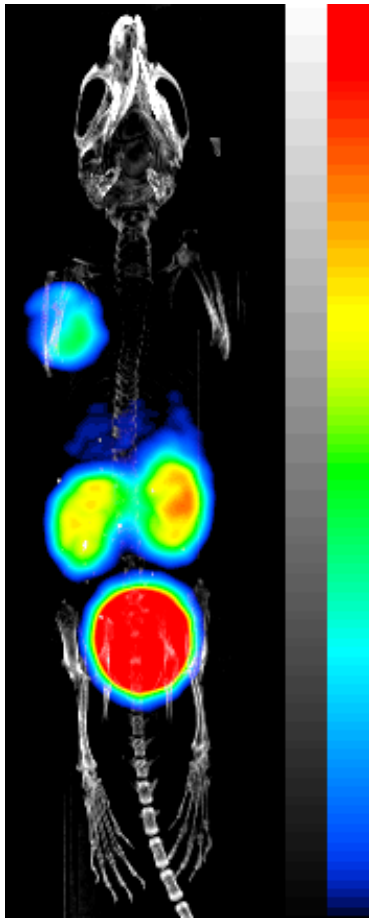


15-20%* ID/g delivered into tumour

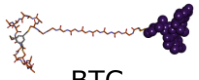
* Note close to theoretical maximum based on molecular diameter and affinity. Wittrup, K. D., Thurber, G. M., Schmidt, M. M., and Rhoden, J. J. (2012) Practical theoretic guidance for the design of tumor-targeting agents. *Meth. Enzymol.* **503**, 255–68

Rapid and target specific localization of a ^{68}Ga conjugated MT1-MMP binding bicycle to an MT1-MMP expressing tumour was observed. A non targeting control bicycle comparator does not localize to the tumour. Free labelled bicycle is only observed in the kidney and bladder consistent with renal elimination. The antibody shows no tumour penetration, and significant non-MT1-MMP1 expressing tissue accumulation (mostly liver in this image)

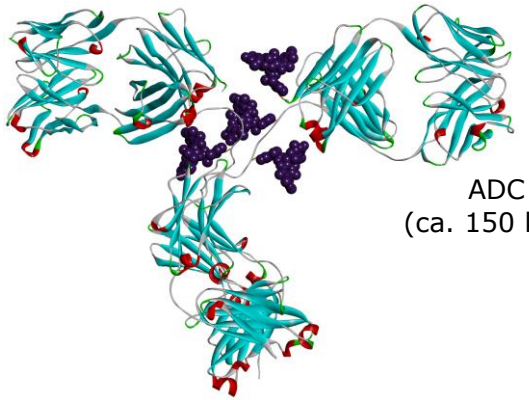
EphA2 binding *Bicycle*



Bicycle® toxin conjugates offer dramatically different ADME profile to antibodies and ADCs



BTC
(ca. 3.5 kDa)



ADC
(ca. 150 kDa)

Molecule	Vd_{ss} mL/kg	Cl mL/h/kg	$t_{1/2}$ h	AUC, dose-corrected h·ng/mL/(mg/kg)
ADC (Kadcyla[a])	57	0.67	58	504000
BTC (BT1718)	205	490	0.4	2070

NHP data, dose normalised

[a] Poon *et al*, Toxicol & Applied Pharmacol 2013

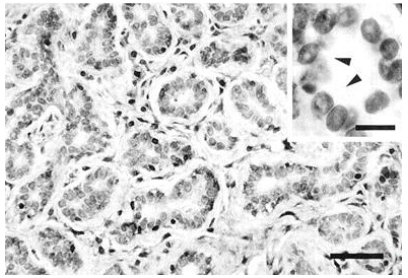
- Half-life 60-600x lower than antibodies
- AUC 100-1000x lower than antibodies

EphA2: Biological rationale

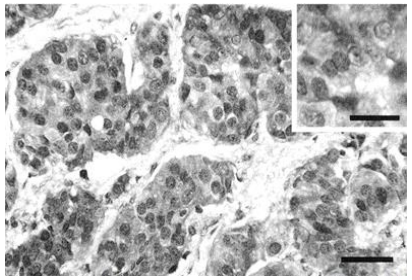
- Erythropoietin-producing hepatocellular A2 receptor: member of Eph subfamily of receptor tyrosine kinases
- Regulates cell migration, adhesion proliferation and differentiation
- Overexpression in human cancers, correlates with tumour progression
- Key area for pharma companies, multiple programs in discovery, and clinical stages but…
  - Development of MEDI-547 (MedImmune) in ovarian cancer was halted following on target bleeding events in phase I.

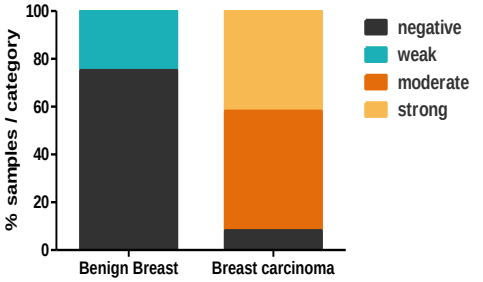
"The bleeding and coagulation events observed in humans showed similarities to those evident in rats and monkeys. In all three species, increased activated partial thromboplastin time, increased fibrinogen/fibrin degradation product, and increased fibrin D-dimer were reported. Monkeys had red/ blood discharge from the nose, mouth, gums."

Benign Breast



Invasive ductal carcinoma

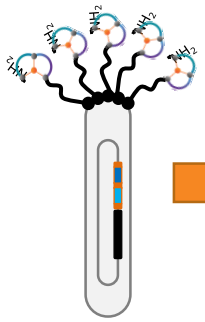




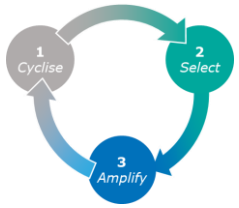
Phase 1, open-label study of MEDI-547 in patients with relapsed or refractory solid tumors

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

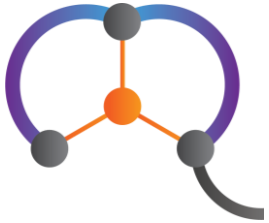
Identification of a high affinity *Bicycle*® targeting EphA2



1 scaffold x 5 libraries
➔3 peptide families



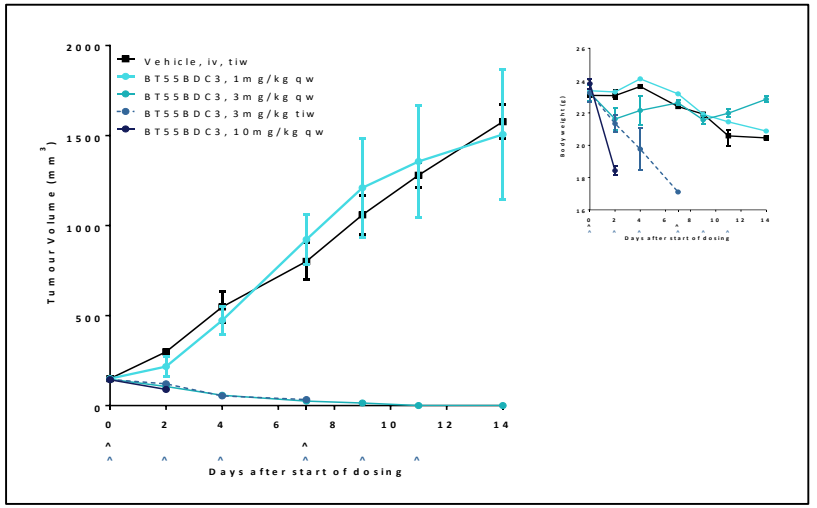
K_i=70 nM

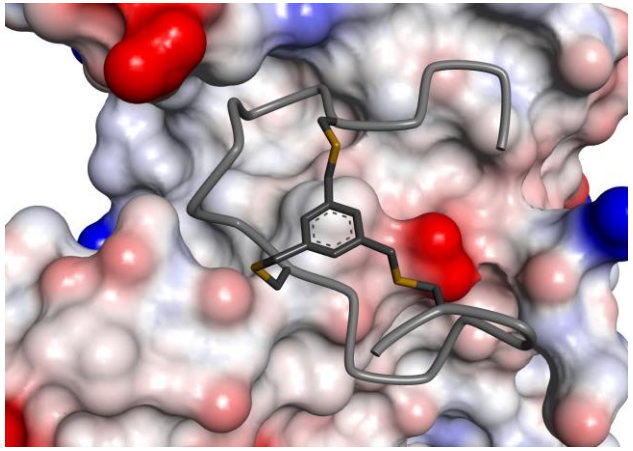


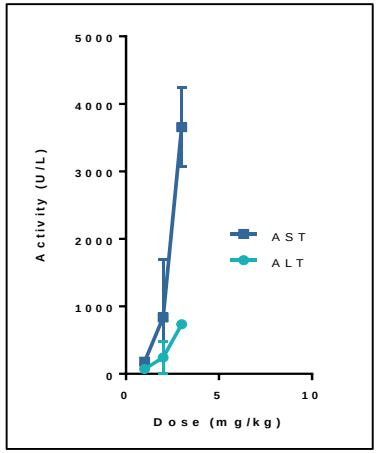
Med Chem

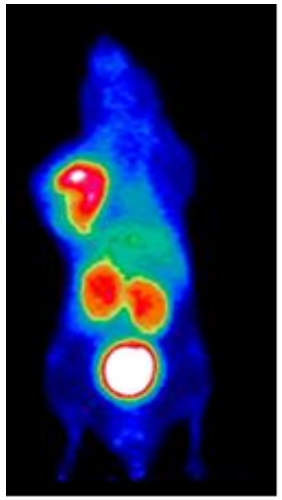
K_i=2.8 nM
CHI LogP = 1.79
PPB = >99.9%

Conjugation







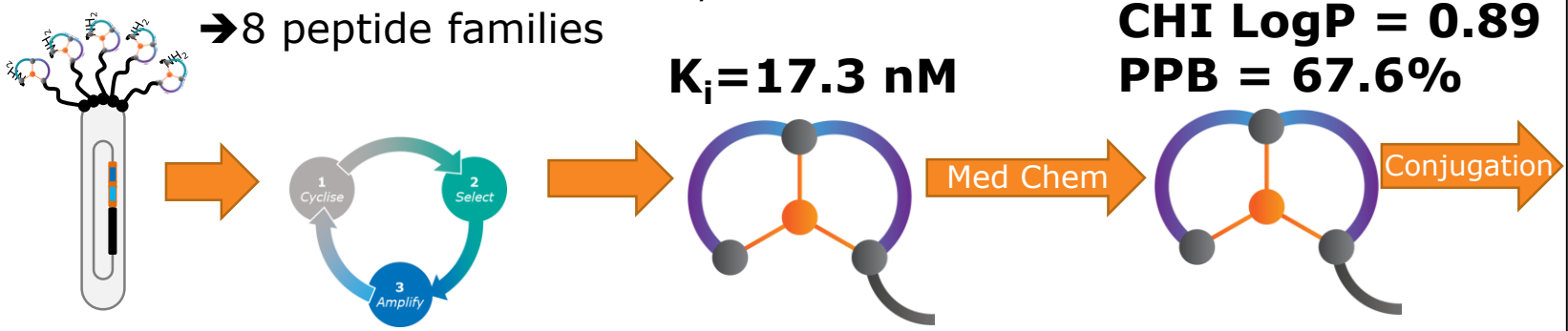


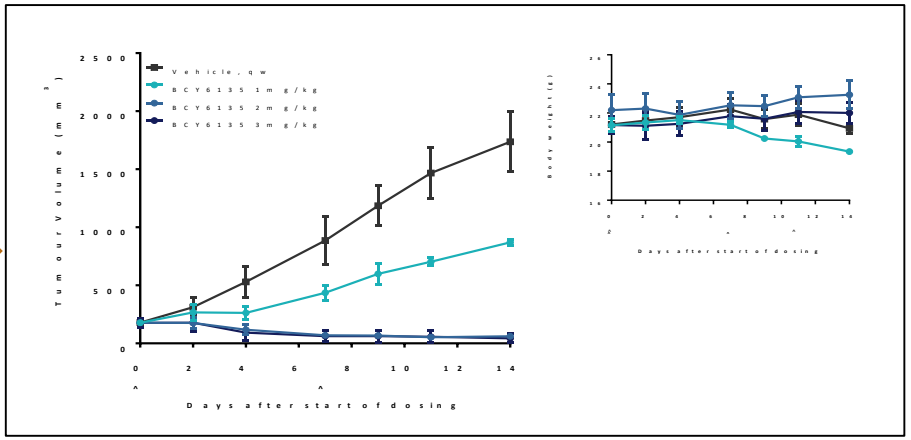
Biodistribution
HT-1080 xenograft
Bicycle-DOTA (Ga-68)
60 min

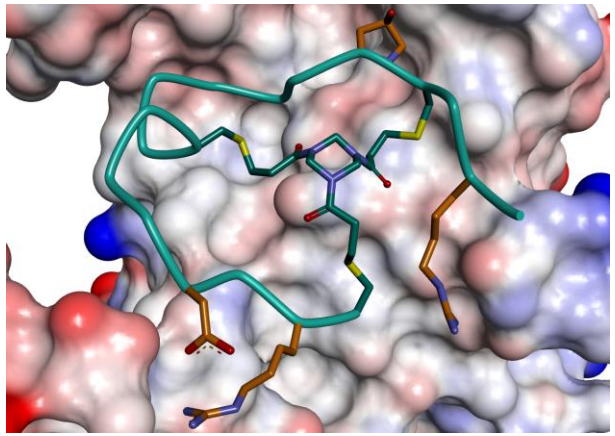
- Early phage selection and chemical optimisation yielded a high affinity EphA2 Bicycle
- Good tumour targeting but phys-chem properties of Bicycle lead to unwanted liver distribution

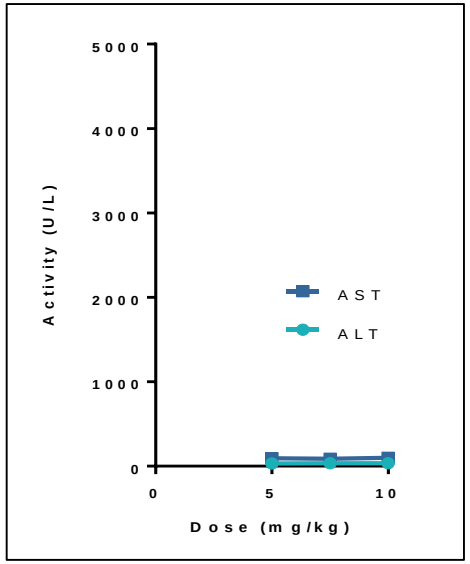
Switching scaffolds improves physical properties and therapeutic index

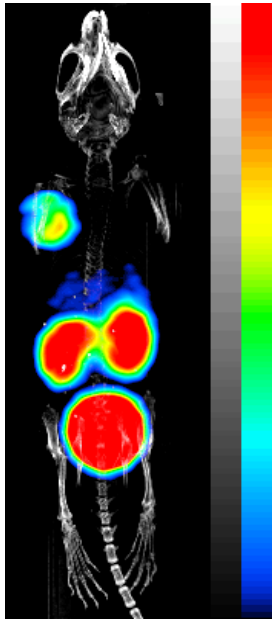
2 scaffolds x 10 libraries,
➔8 peptide families







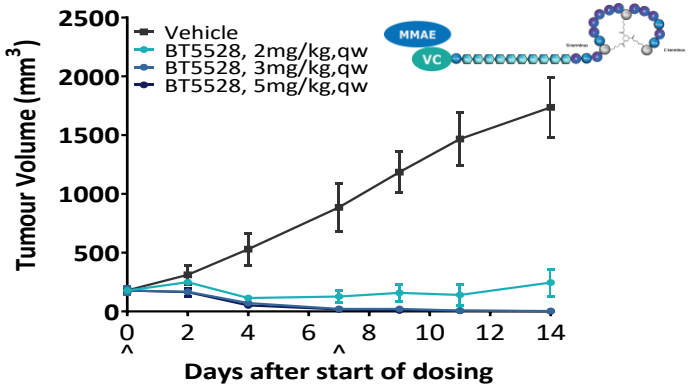


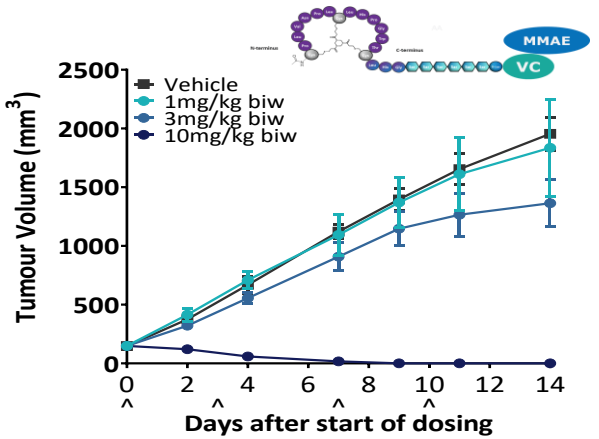


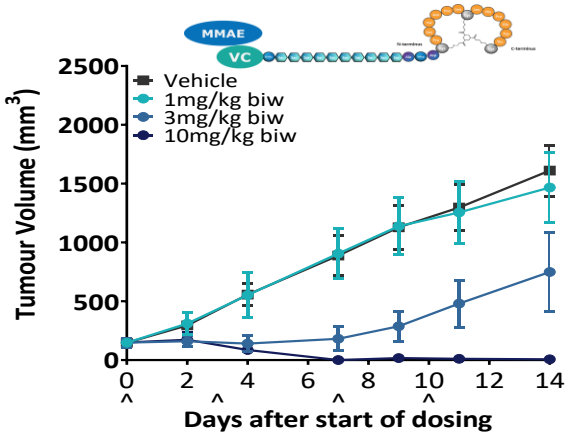
Biodistribution
HT-1080 xenograft
Bicycle-DOTA (Ga-68)
60 min

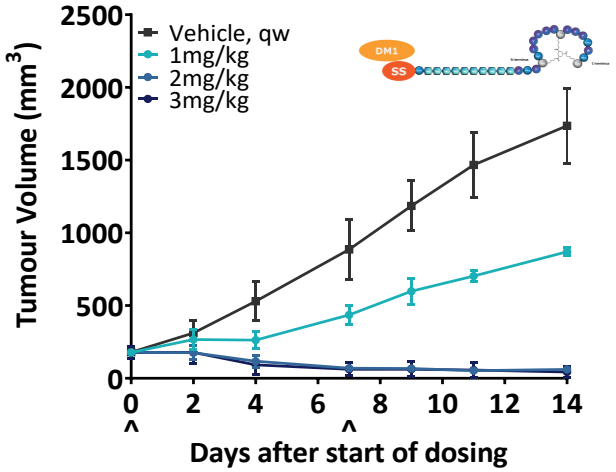
- 2nd generation phage selection with more diverse library and chemical optimisation yielded a equally high affinity EphA2 Bicycle
- Improved phys-chem properties of Bicycle eliminated liver exposure while retaining strong tumour targeting

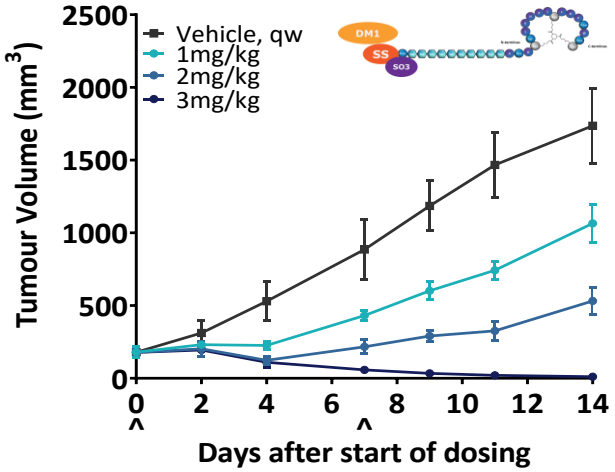
Lead Optimisation of EphA2 BTC evaluated payload, linker and Bicycle components

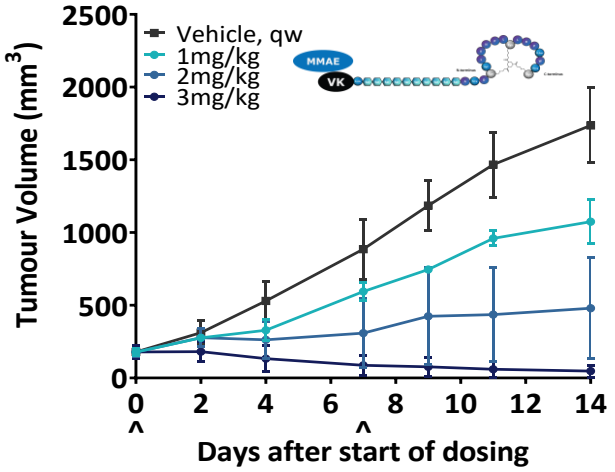






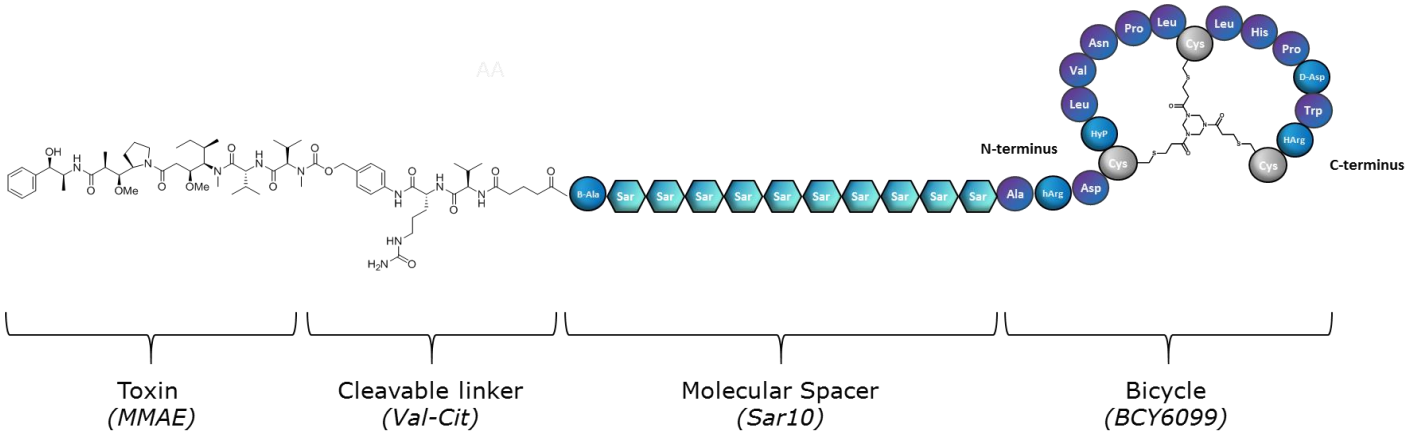


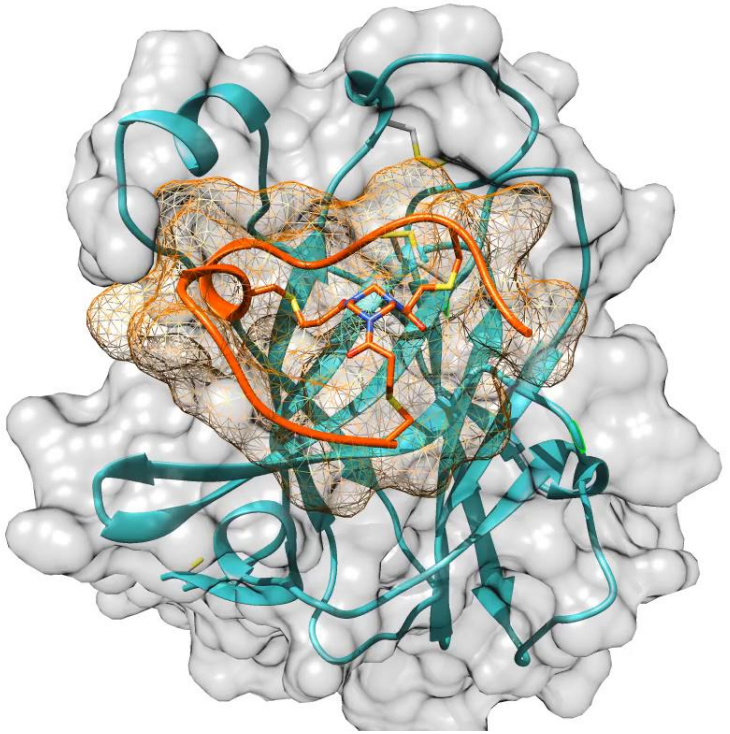




A diverse array of Bicycle conjugates were evaluated in vivo Vs CTX and PDX models to establish SAR and STR

BT5528: structure & profile





High affinity binding to EphA2 protein across species & on cells. Species cross-reactivity, high selectivity.

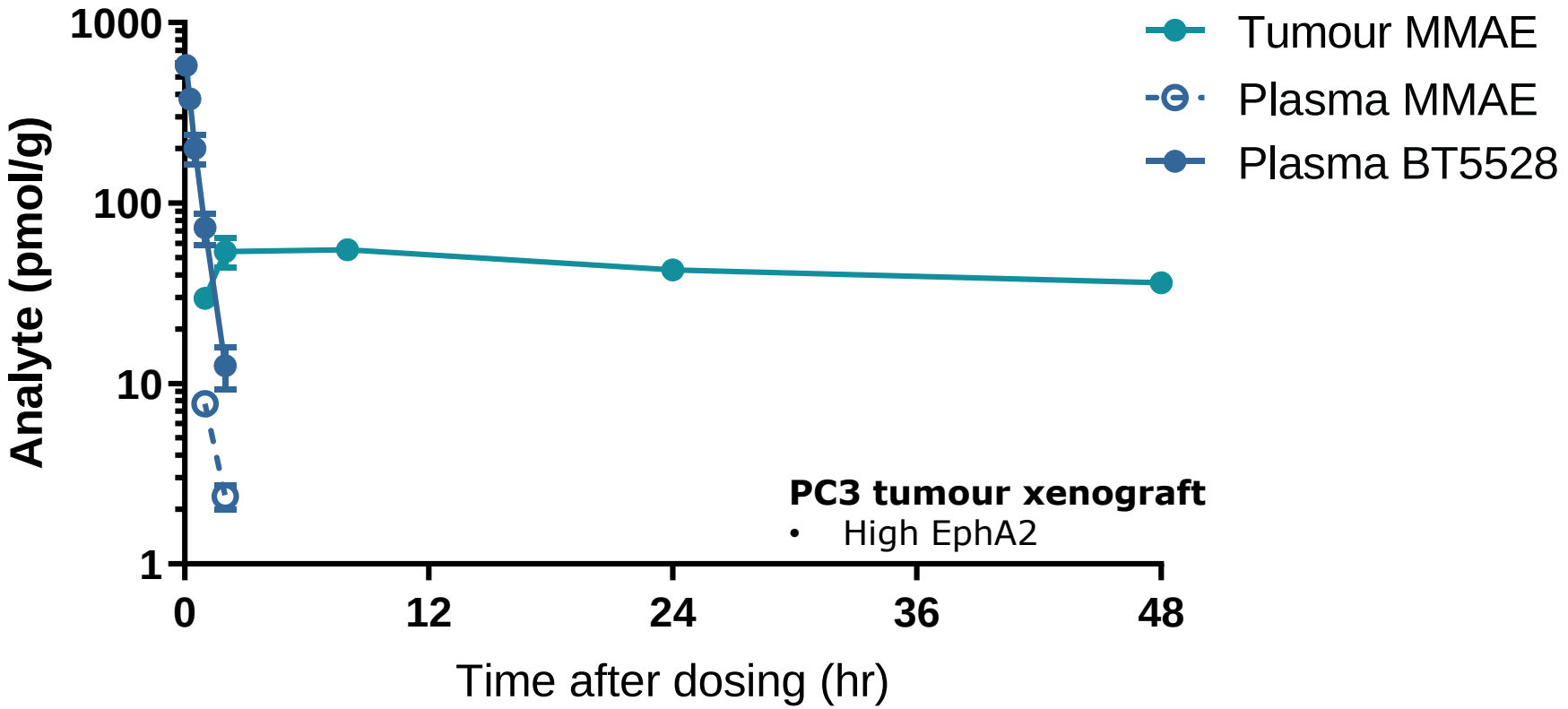
BT5528 affinity	Human	Mouse	Rat	NHP
FP comp (K_i, nM)	1.9 ± 0.9 n=29	5.2 ± 1.9 n=16	1.9 ± 1.3 n=10	
SPR (K_D, nM)	0.9 ± 0.4 n=2	2.0 ± 0.8 n=2	2.7 ± 0.4 n=2	1.0 n=1
Cell binding by HCS ($K_{b\,app}$, nM)	14.8 ± 10.5			

Ligand-binding domain	% identity to EphA2	Binding affinity (SPR K_D nM)
EphA2	100	1.2
EphA1	54	>5000
EphA3	58	>5000
EphA4	55	>5000
EphA5	56	>5000
EphA6	56	>5000
EphA7	56	>5000
EphB4	39	>5000

BT5528 delivers MMAE to tumour

Single dose of BT5528

- Produces high MMAE concentrations in tumour
  - Stable from 2h to >48h
  - Transient exposure of both BT5528 & MMAE in plasma

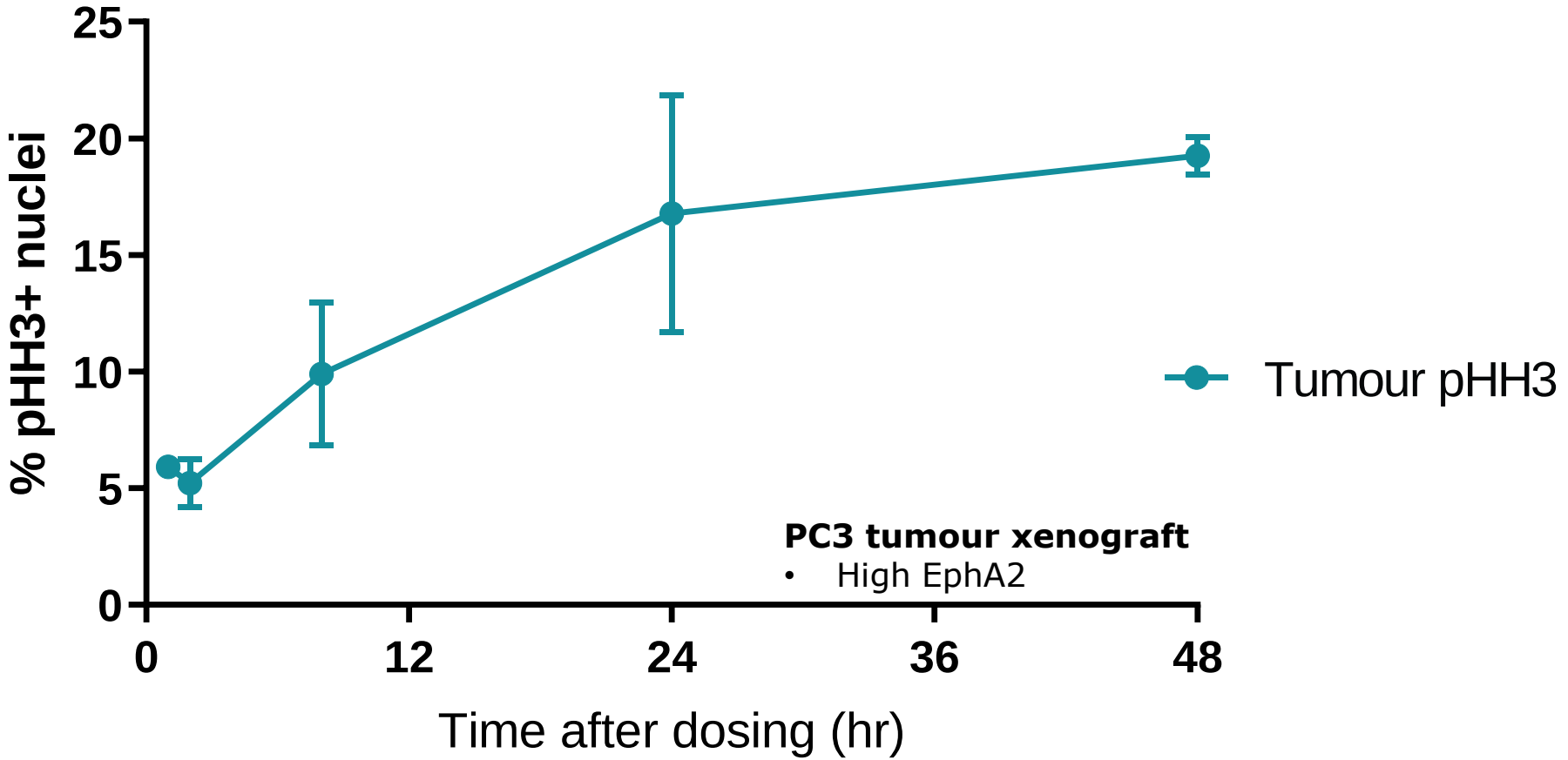


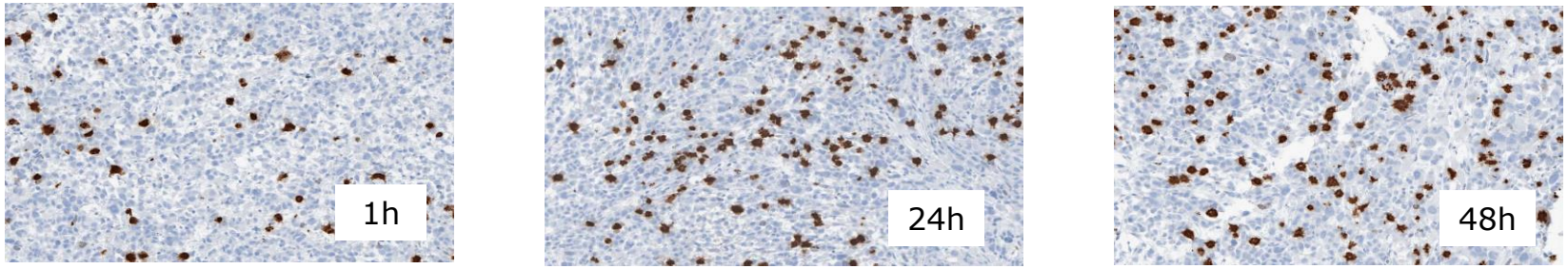
BT5528 PK Parameters	Mouse	Rat	NHP
C_{max} (ng/mL)	6321	4048	7643
$T_{1/2}$ (h)	0.4	0.3	~0.6h
Vd_{ss} (L/kg)	0.18	0.33	0.21
Cl (mL/min/kg)	6.2	15.5	4.9
$AUC_{0\text{-last}}$ (ng.h/mL)	2643	998	3516

BT5528 induces mitotic arrest in tumour

Single dose of BT5528

- Produces high MMAE concentrations in tumour
  - Stable from 2h to >48h
  - Transient exposure of both BT5528 & MMAE in plasma
- Induces mitotic arrest
  - Measurable by pHH3 IHC within 24h

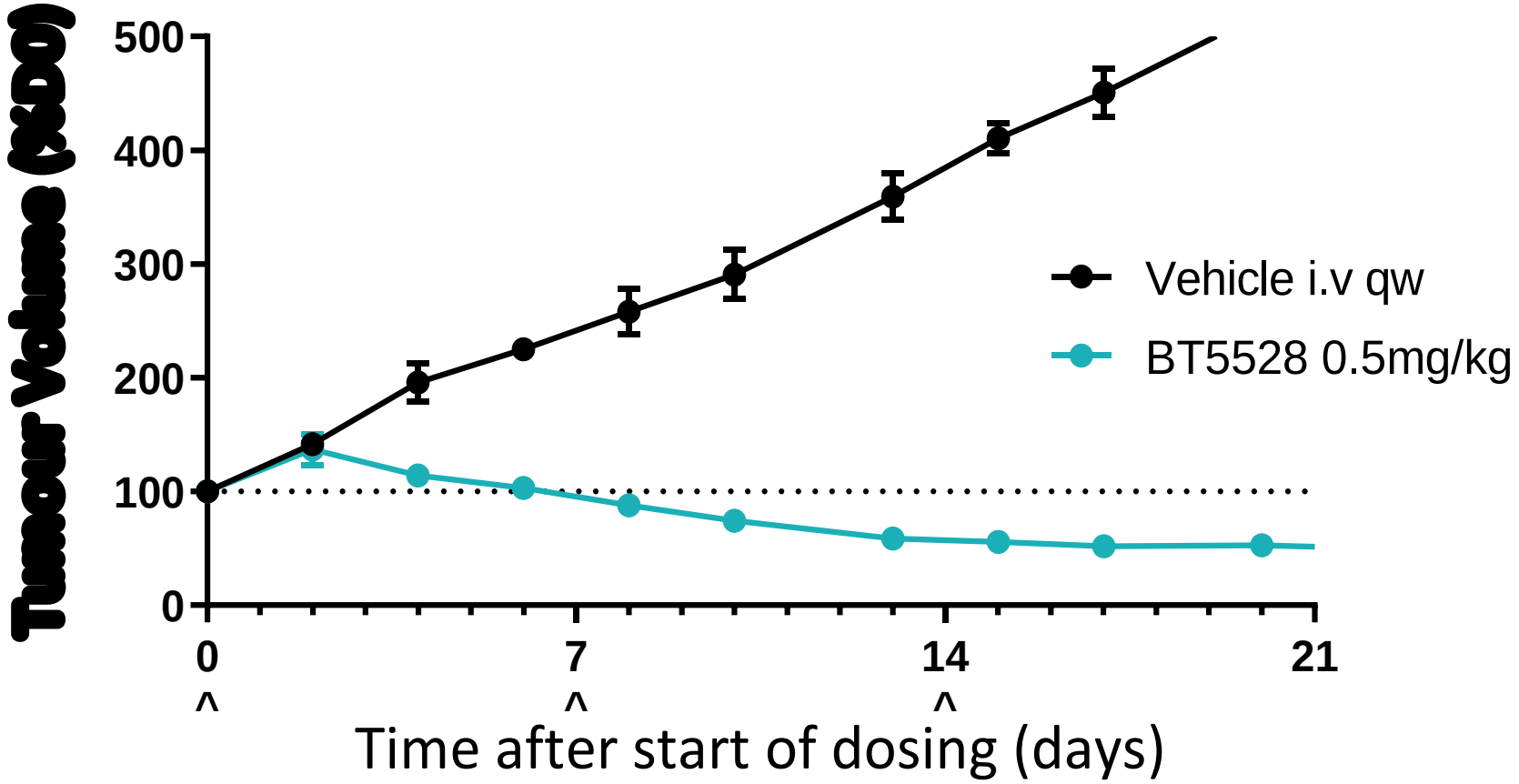




BT5528 produces tumour regression

Weekly dosing of BT5528

- Produces high MMAE concentrations in tumour
  - Stable from 2h to >48h
  - Transient exposure of both BT5528 & MMAE in plasma
- Induces mitotic arrest
  - Measurable by pHH3 IHC within 24h
- Induces tumour cell death
  - Measurable regression by day 4

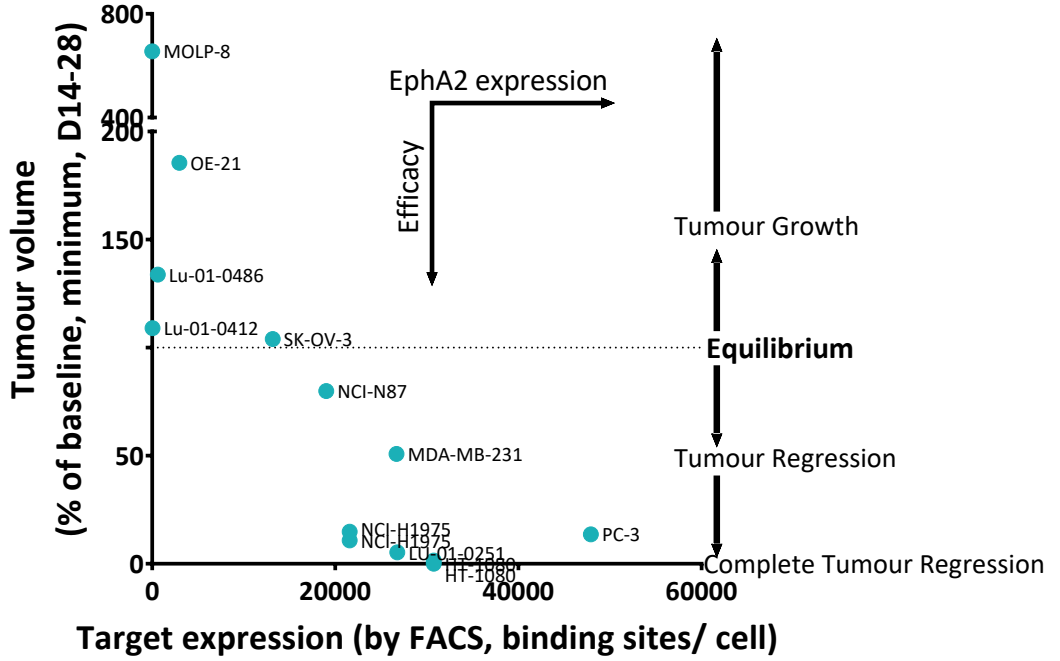


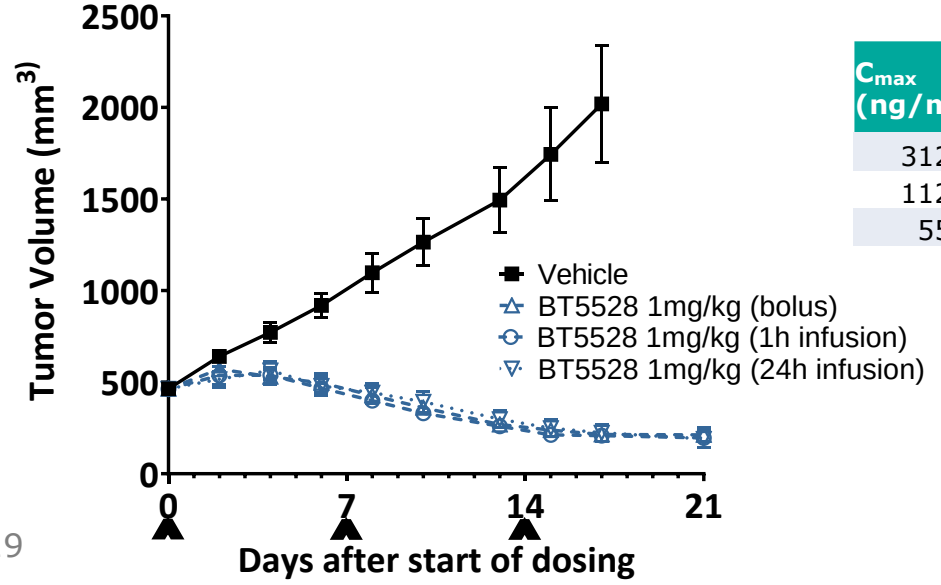
PC3 tumour xenograft

- High EphA2

BT5528 efficacy: target-mediated, flexible dosing

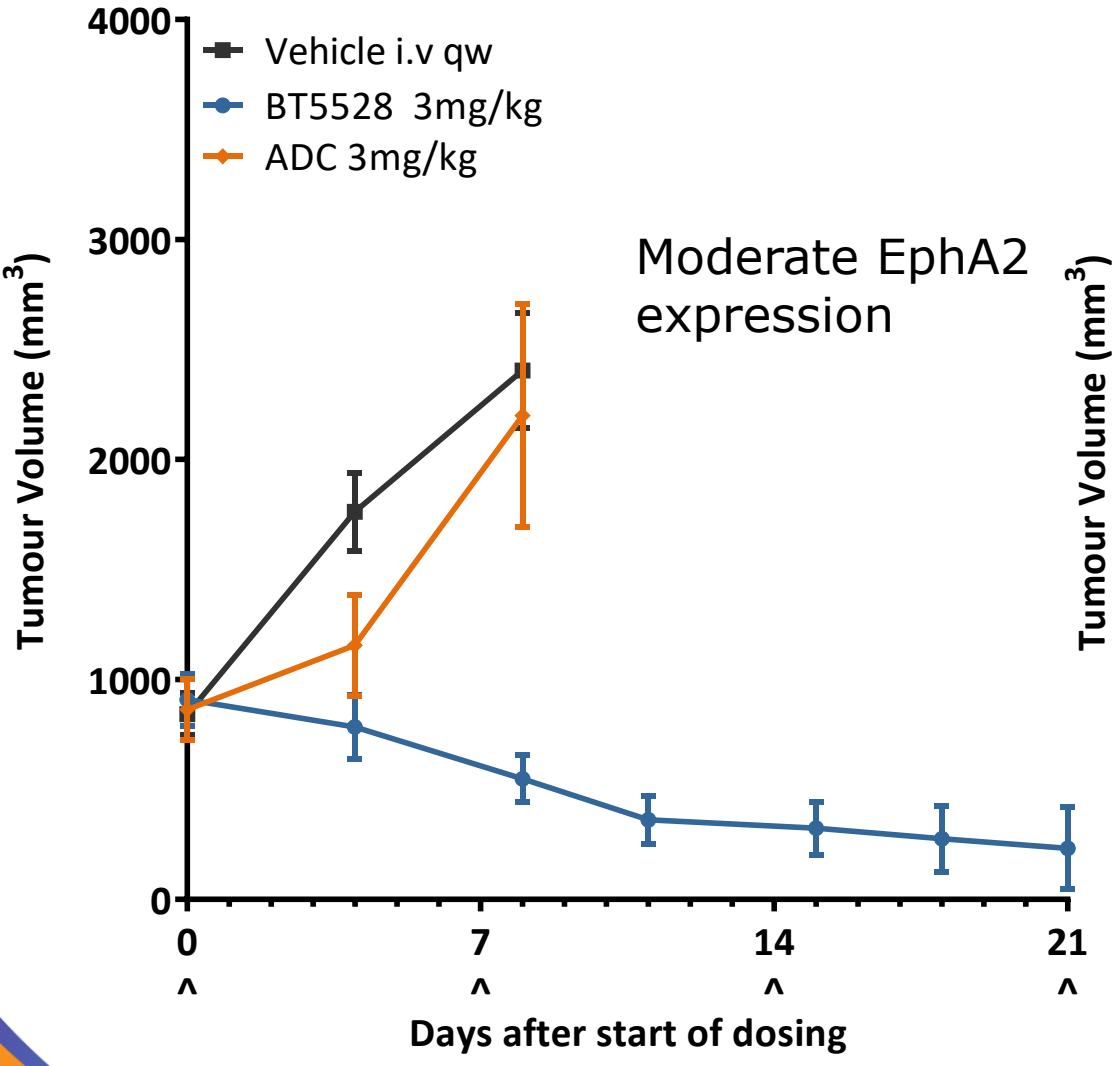
- BT5528 shows target-dependent efficacy
  - Significant regression in a wide range of EphA2-positive tumours
- BT5528 shows equivalent efficacy with a wide range of dosing paradigms
  - Bolus, 1h infusion, 24h infusion
- BT5528 efficacious with intermittent dosing
  - Efficacy also shown dosing every 2 weeks

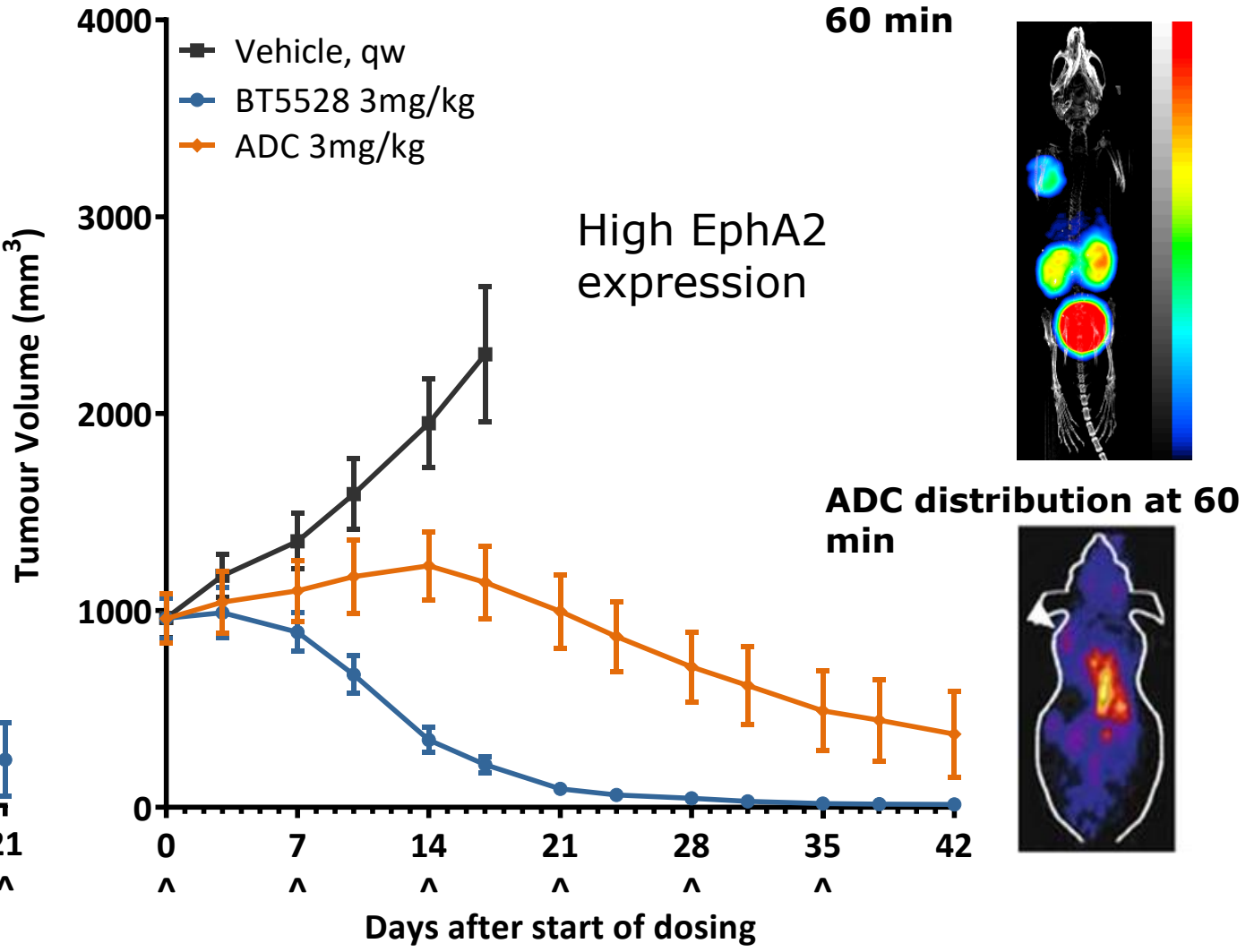




C_{max} (ng/mL)	AUC (ng.h/mL)
3120	1325
1120	1325
55	1325

BT5528: differentiation from ADC in complex PDX models





Bicycle distribution at 60 min

ADC distribution at 60 min

BT5528 is efficacious in treating metastatic disease in mouse

Metastatic PC3 xenograft model

Intracardiac implantation of PC3-Luc tumour cells on D0

High EphA2

Grows in bone

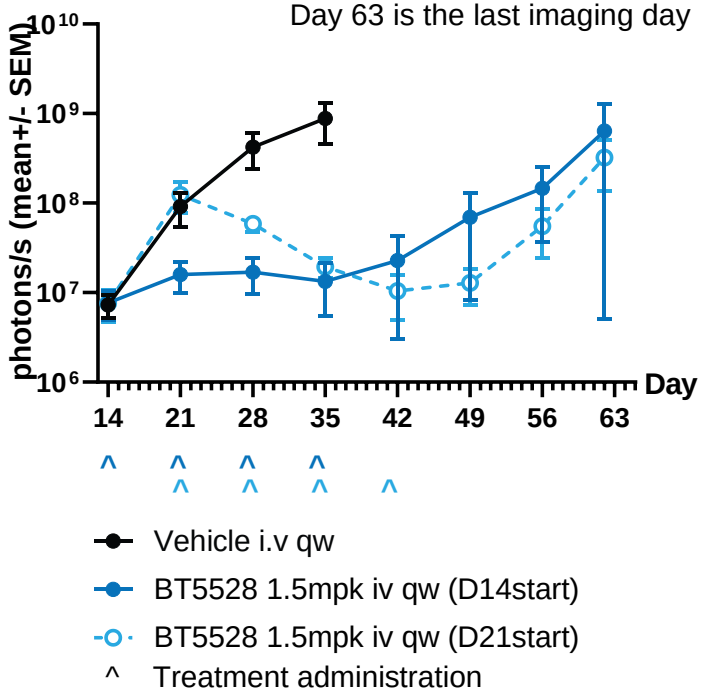
Initiate treatment on D14 or D21
QWx4

Weekly bioluminescence imaging during treatment

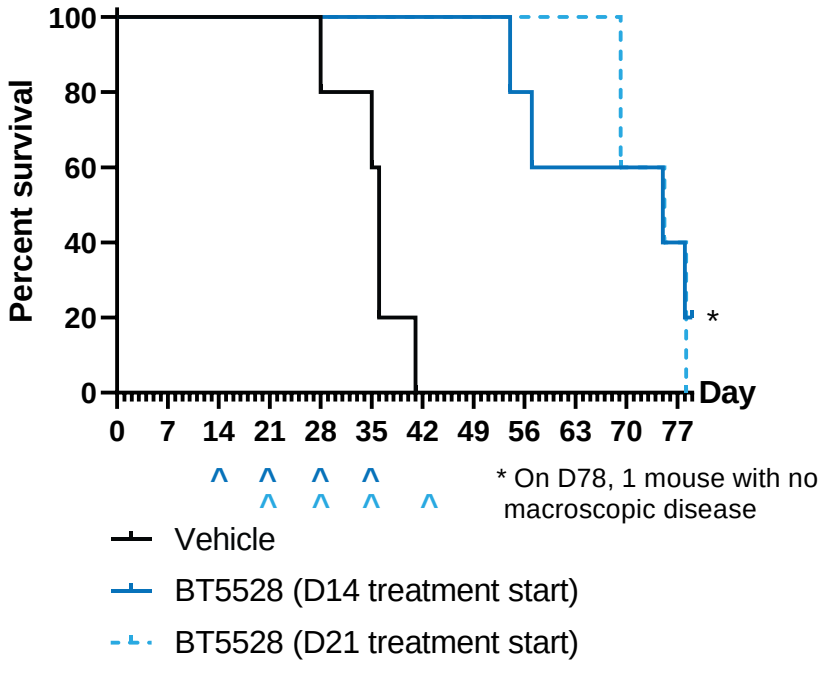
Monitor survival until D78

Total Bone Signal

Day 63 is the last imaging day



Survival



- PC3 metastatic lesions have the required enzymatic activity for payload release from BT5528 to yield significant anti-tumor activity
- 4 weekly BT5528 treatment cycles reduced the bone tumor cell burden significantly and extended the survival of the mice

BT5528: differentiation from ADC in bleeding/ coagulation & liver toxicology

Findings from MEDI-547 Phase I study

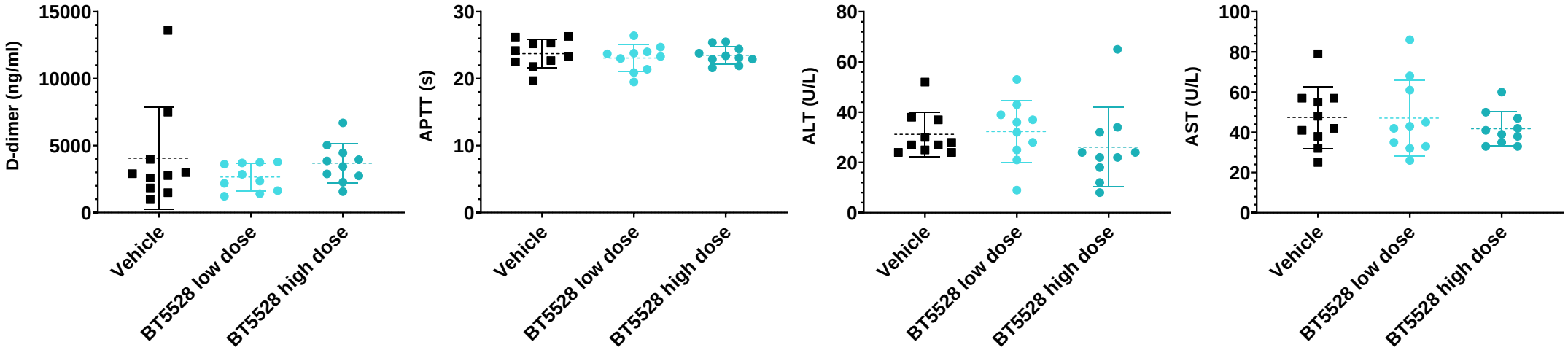
Treatment related adverse events	# events (% of patients) n of total
ALT increased	3 (50) 3/6
Haemorrhage	6 (83.3) 5/6

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

Bleeding observed on days 3-8 following a single dose of MEDI-547

Findings from BT5528 toxicology study



- No bleeding events seen in either species
  - Dosing to toxin equivalent doses >100x dose of MEDI-547 used in patients
- No significant effect on clotting parameters
- No evidence of abnormal liver function

BT5528: a Bicycle Toxin Conjugate targeting EphA2 for the Treatment of Solid Tumours

- EphA2 is highly expressed on tumour cell surface in a wide range of solid tumours

- BT5528 was developed as a BTC to target EphA2
  - High affinity binding and tumour penetration
  - Engineered short systemic half-life and renal excretion
  - "Hit and run" delivery of toxin

- BT5528 shows profound efficacy in a wide range of tumour models
  - Efficacy correlates with EphA2 expression

- BT5528 shows clear differentiation from previous ADC approaches
  - Efficacy maintained even in large, heterogeneous PDX
  - No bleeding/ coagulation toxicity observed in preclinical models