BT5528: A Bicycle Toxin Conjugate Targeting EphA2 for the Treatment of Solid Tumours

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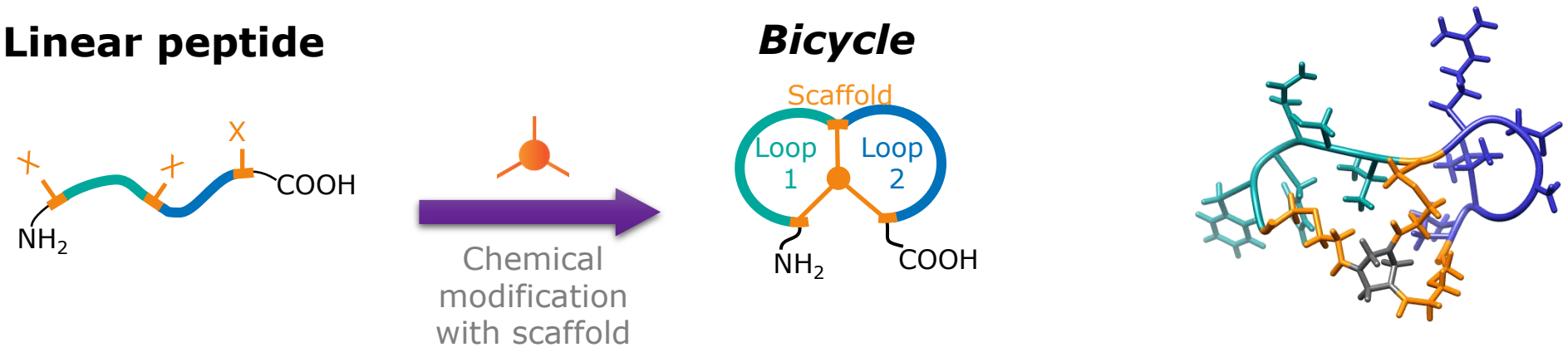
6th March 2019

Bicycles®: a new therapeutic modality



Highly constrained: high affinity, exquisite selectivity, excellent stability

Large binding footprint: disrupt protein-protein interactions

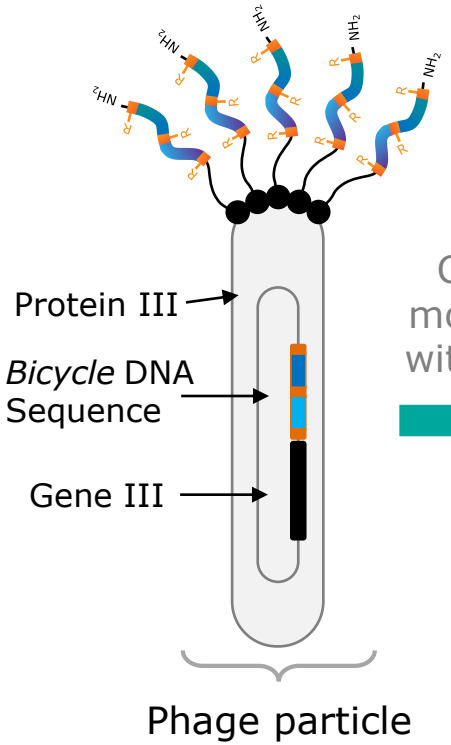
Fully synthetic: NCE classification and synthetic control

Highly flexible modality: modular building blocks retain pharmacology

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

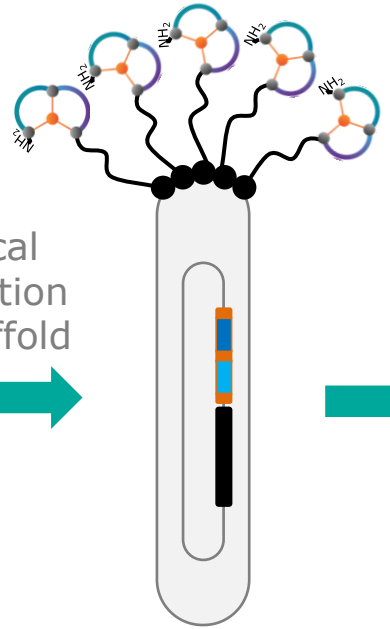
Proven platform using phage display

Linear peptide

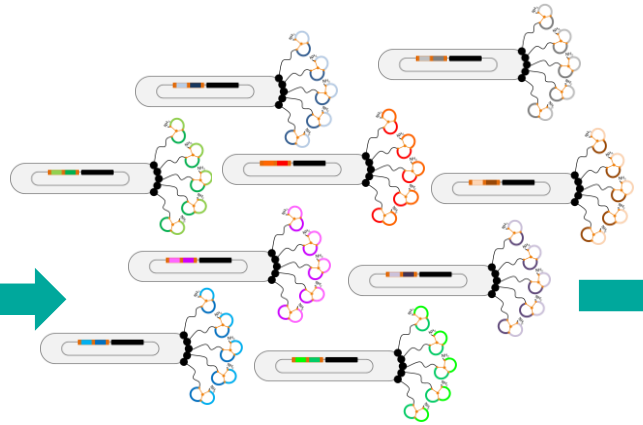


Chemical modification with scaffold

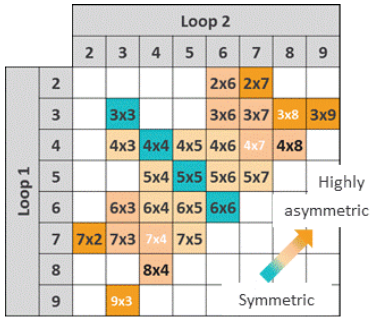
Bicycle

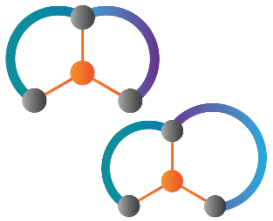


Diverse *Bicycle* phage libraries (>10^{15})

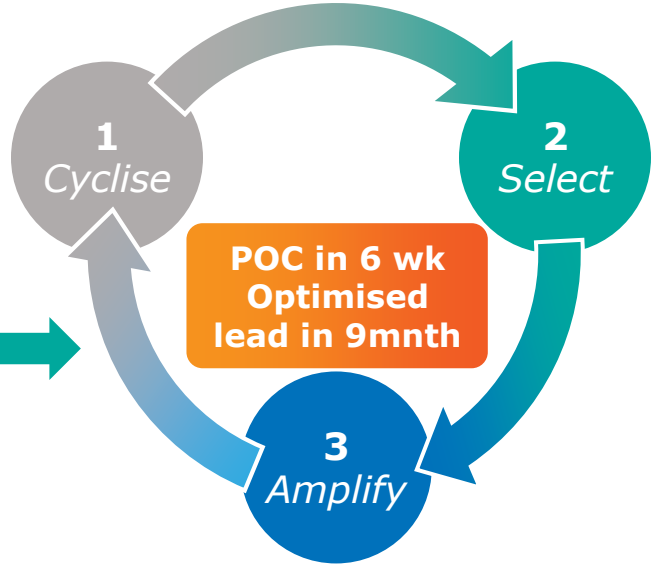


Extremely large and diverse chemical library
Low synthetic burden





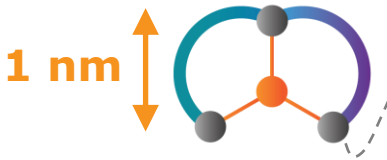
Evolution driven, informed selection



>80 diverse targets screened; >80% success rate
Antagonists, agonists and neutral binders

Bicycles® have built-in tolerance to conjugation

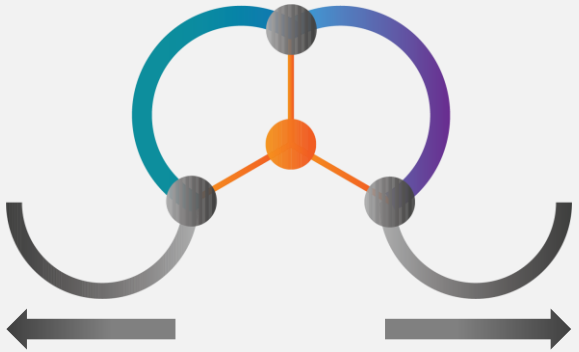
Bicycle



Tag to specific target

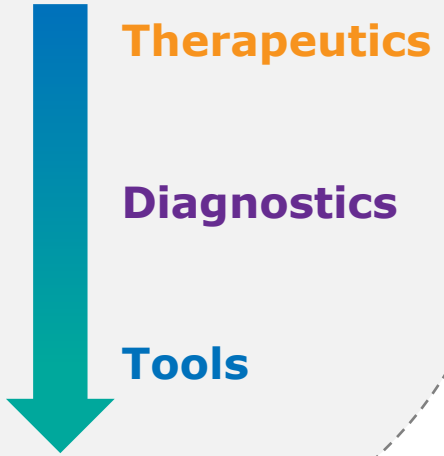
Bacteriophage

900 nm x 7 nm



Replace phage bulk with:

- Toxins
- Small molecule drugs
- Radionuclides
- Multimerised *Bicycles*
- PET ligand chelators
- PK extenders
- Fluorescent dyes
- Biotin and affinity tags

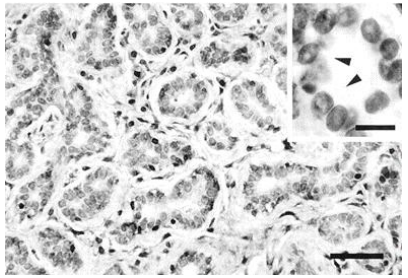


EphA2: Biological rationale

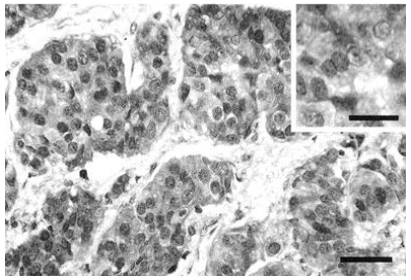
- Erythropoietin-producing hepatocellular A2 receptor: member of Eph subfamily of receptor tyrosine kinases
- Regulates cell migration, adhesion proliferation and differentiation
- Overexpression in human cancers, correlates with tumour progression
- Key area for pharma companies, multiple programs in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

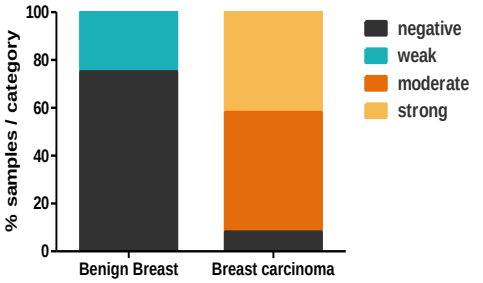
"The bleeding and coagulation events observed in humans showed similarities to those evident in rats and monkeys. In all three species, increased activated partial thromboplastin time, increased fibrinogen/fibrin degradation product, and increased fibrin D-dimer were reported. Monkeys had red/ blood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

Benign Breast



Invasive ductal carcinoma

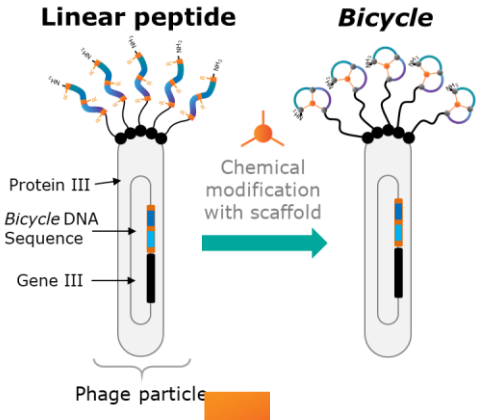




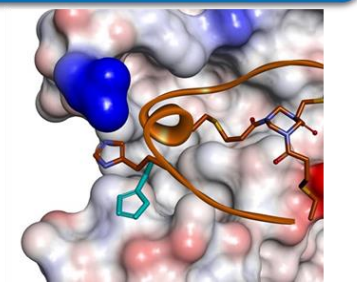
bicycle therapeutics

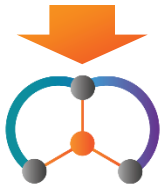
Synthesis of a matrix of Bicycle Toxin Conjugates

EphA2-Targeting *Bicycle*

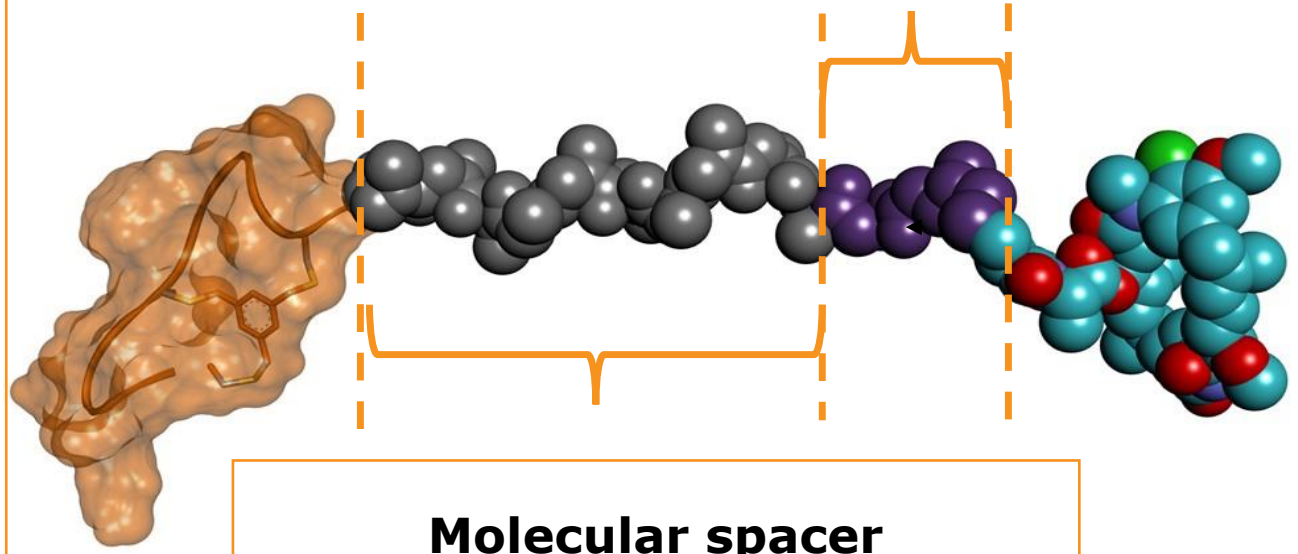


Peptide & Medicinal Chemistry





Cleavable Linker
Peptidic: val-cit, val-lys, val-ala
Plasmin-, legumain-, MMP- sensitive
Disulphide: -SS-, -SSMe-
Acidic: Hydrazone
Other: eg glucuronide, "non-cleavable"



Molecular spacer
Sarcosine (Sar0-Sar10), PEG
Acidic/basic group, saccharide
Other

Toxin

Auristatins:
MMAE MMAF

Maytansinoids:
DM1, DM3, DM4
Other derivatives

Camptothecins, SN38

PBDs

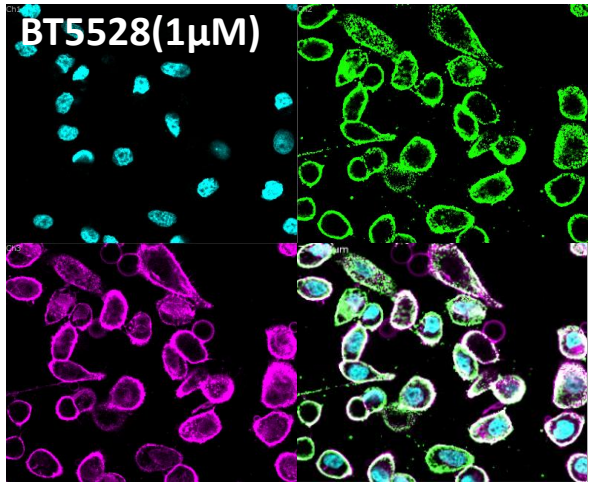
Other: non-toxins

bicycle therapeutics

Profile of Bicycle conjugates

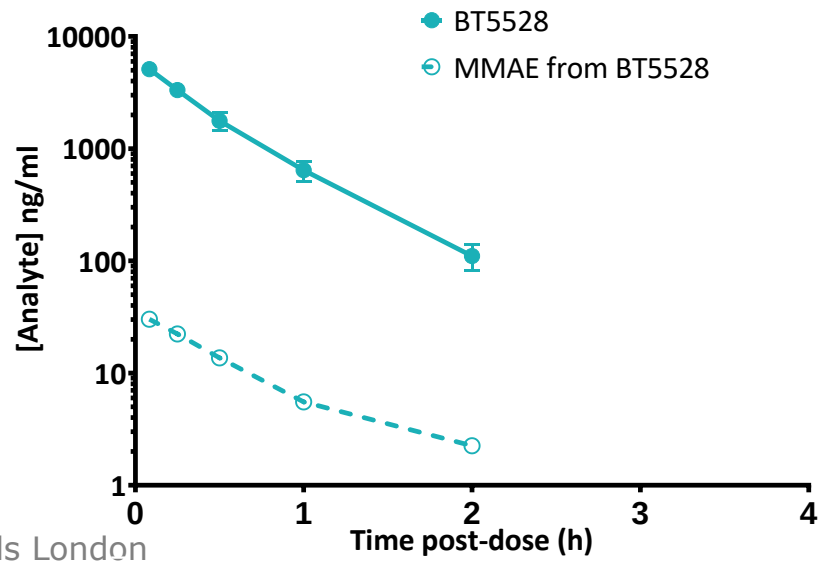
High affinity binding to EphA2 protein across species & on cells. Species cross-reactivity, high selectivity.

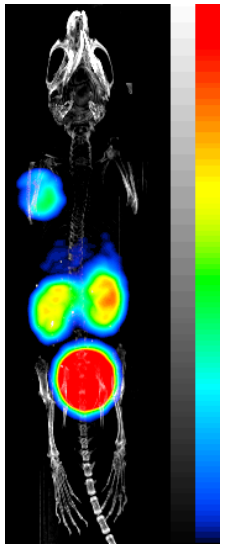
BT5528 affinity	Human	Mouse	Rat	NHP
FP comp (K_i, nM)	1.9 ± 0.9 n=29	5.2 ± 1.9 n=16	1.9 ± 1.3 n=10	
SPR (K_D, nM)	0.9 ± 0.4 n=2	2.0 ± 0.8 n=2	2.7 ± 0.4 n=2	1.0 n=1
Cell binding by HCS ($K_{b\,app}$, nM)	**14.8 ± 10.5**			



Ligand-binding domain	%identity to hEphA2	Binding affinity (SPR, using BT5528, K_D nM)
	Human	Human
EphA1	54	> @ 5uM
EphA2	**100**	**0.9**
EphA3	58	> @ 5uM
EphA4	55	> @ 5uM
EphA5	56	> @ 25uM
EphA6	56	> @ 20uM
EphA7	56	> @ 20uM
EphB1	49	
EphB4	39	> @ 20uM

Short half-life *in vivo,* rapid & selective binding to EphA2-expressing tumour, renal elimination





PET imaging (60min)

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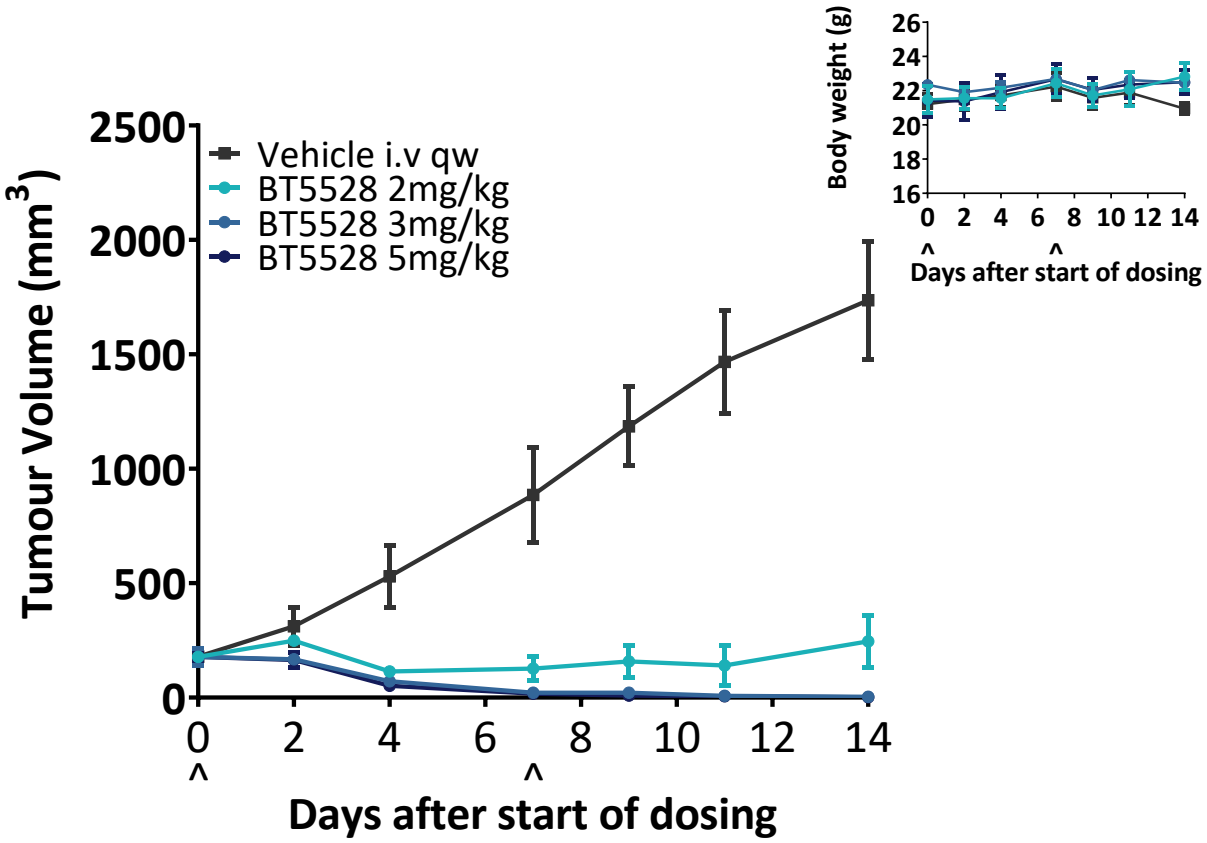
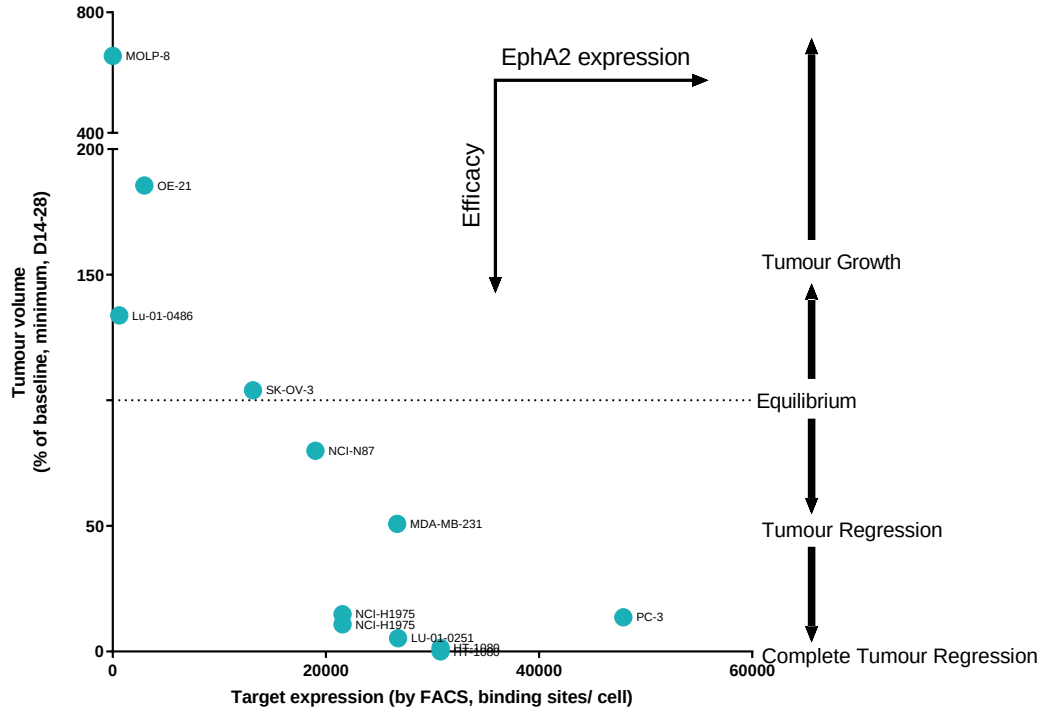
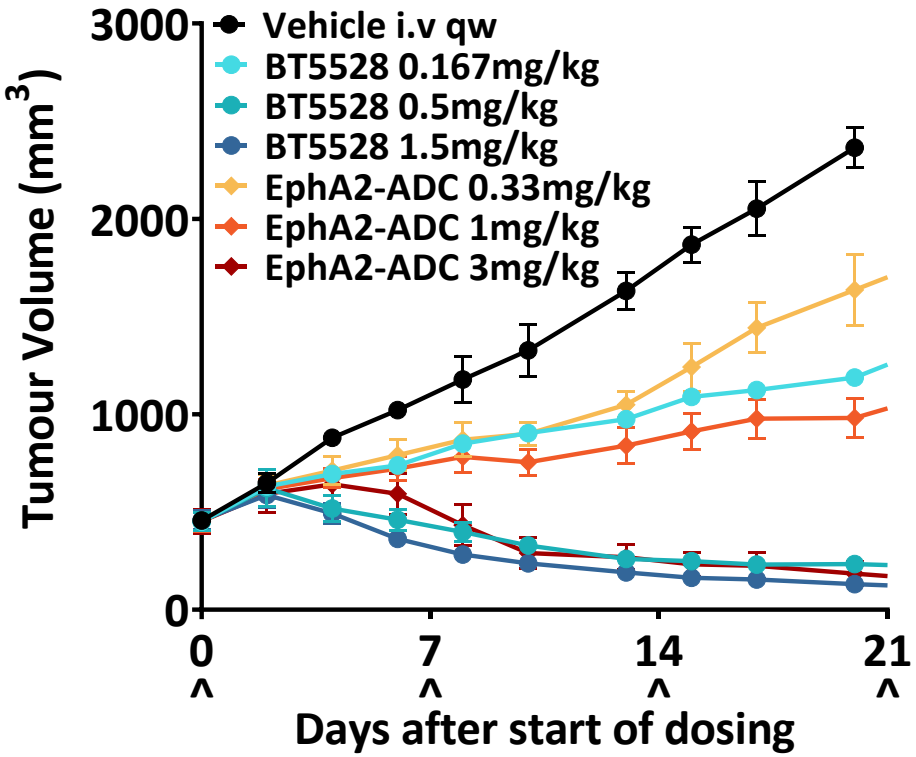
Efficacy is EphA2-dependent across tumour types



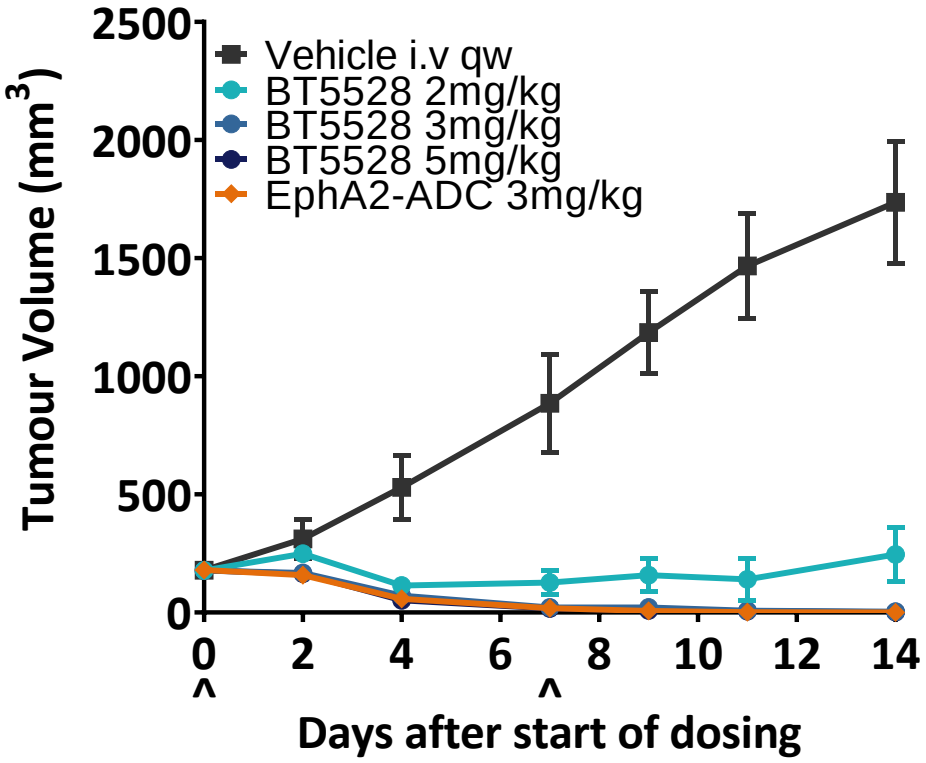


Comparison to MEDI-547 (EphA2 targeting ADC)

PC-3 Prostate cancer



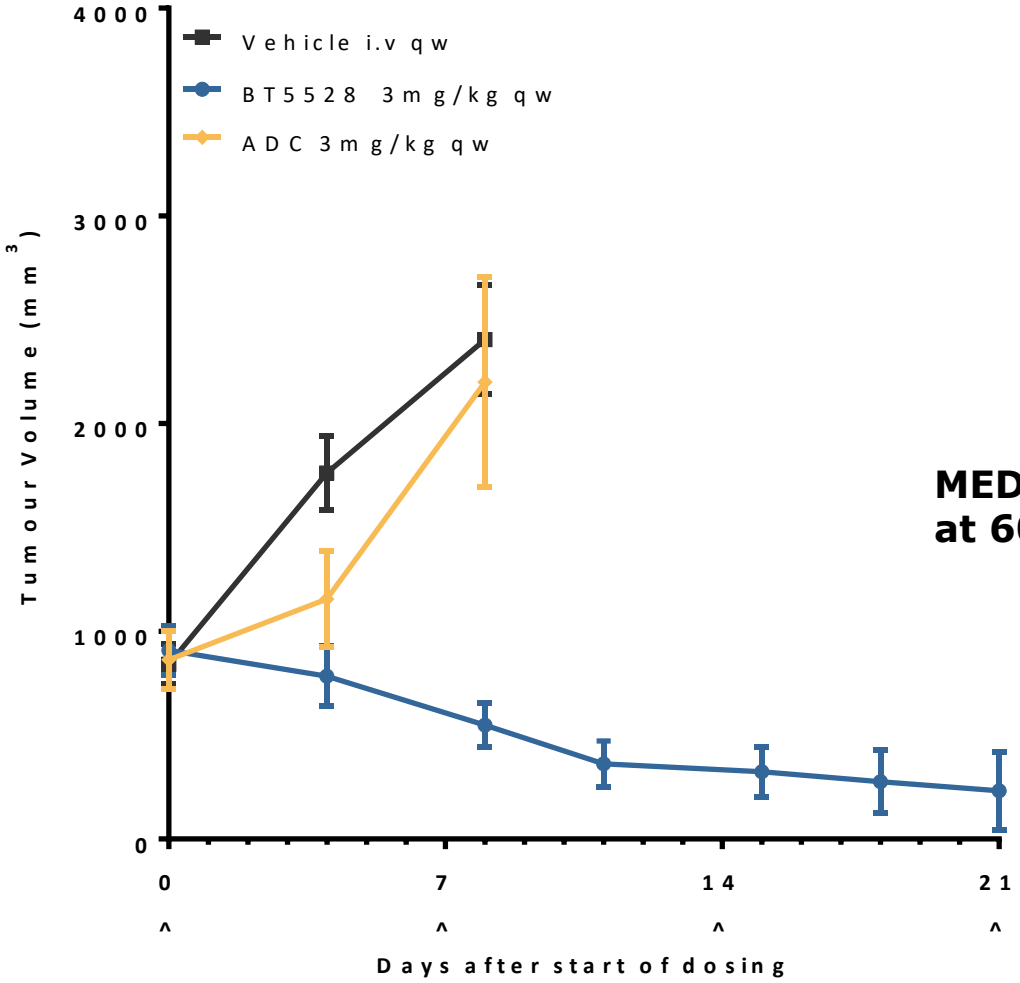
HT-1080 Fibrosarcoma



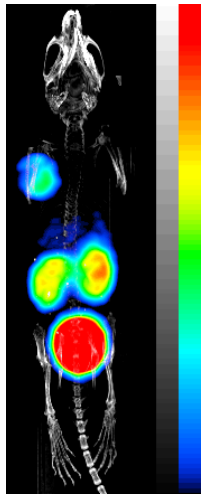
- Efficacy reported with MEDI-547 in PC-3 xenograft
  - Regression at 3mg/kg qw, in line with published paper (Lee *et* al, 2009)
- BT5528 shows efficacy in PC-3 & HT1080 models
  - Complete regression seen from 1mg/kg qw (PC-3)
  - Efficacy with *BTC* and ADC are comparable at 3mg/kg qw

Extensive tumour penetration maintains efficacy even in very large PDX model

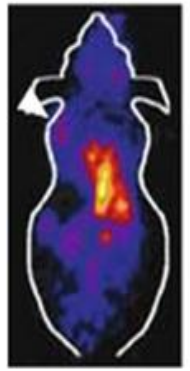
- BT5528 maintains efficacy seen in CDX models even in large PDX
  - Patient-derived xenograft
    - Lung adenocarcinoma
  - Heterogeneous tumour
  - 1000mm^3 at dosing start
- Significant regression of tumour after 21d dosing 3mg/kg qw
- ADC (MEDI-547) shows no efficacy
  - Dosed 3mg/kg qw
- PET imaging shows rapid penetration of *Bicycle* conjugate into tumour
  - ADC data shows largely vascular distribution



***Bicycle* distribution at 60 min**



MEDI-547 distribution at 60 min



Cai W *et al*, Quantitative radioimmunoPET imaging of EphA2 in tumor-bearing mice. Eur J Nucl Med Mol Imaging. 2007

bicycle therapeutics

MEDI-547 toxicity

- MEDI-547 (ADC comprising 1c1 antibody conjugated to MMAF via a VC cleavable linker)
- Ph1 terminated following unexpected bleeding/coagulation events, study published
  - Annuziata *et al,* Invest New Drugs 2013 Feb 31(1) 77-84

- 5/6 patients dosed with MEDI-547 at a dose of 0.08 mg/kg once every 3 weeks experienced bleeding and coagulation events. Three patients had haemorrhage-related events, and 2 patients reported epistaxis. Three patients had bleeding/ coagulation AEs that were also SAEs.
  - Haemorrhage/ epistaxis occurred d3-9 post treatment. One further patient received a second cycle, after which SAEs (pain & liver disorder) occurred.

- Clinical events were "not unexpected based on the preclinical toxicology findings"
  - 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 and/or anus, whereas two patients experienced epistaxis and one patient experienced an oral cavity haemorrhage.
  - Preclinical toxicology studies in the monkey identified disseminated intravascular coagulation (DIC) as the DLT. The events observed in humans were considered to be consistent with the preclinical findings, in particular to the observation of DIC.
  - The dose used in the clinical study was 10-fold lower than the highest non-severely toxic dose predicted based on extrapolations from rat studies (MedImmune, LLC, data on file), and no evidence of drug accumulation after the administration of a second dose was apparent

BT5528 (EphA2 BTC) does not exhibit the primate toxicologies (clotting defects, hepatic effects) observed with the EphA2 ADC, MEDI-547.

Findings from MEDI-547 Phase I study

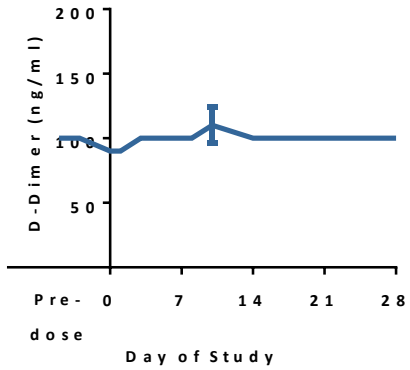
Treatment related adverse events	# events (% of patients) n of total
ALT increased	3 (50) 3/6
Haemorrhage	6 (83.3) 5/6

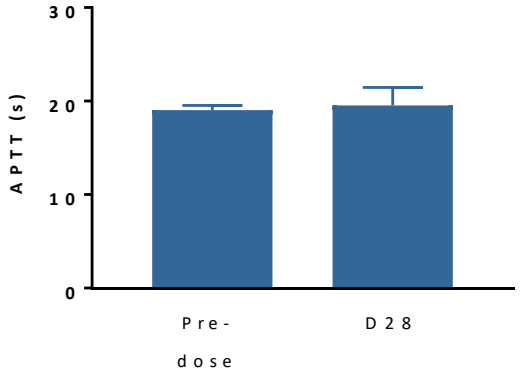
Phase 1, open-label study of MEDI-547 in patients with relapsed or refractory solid tumors

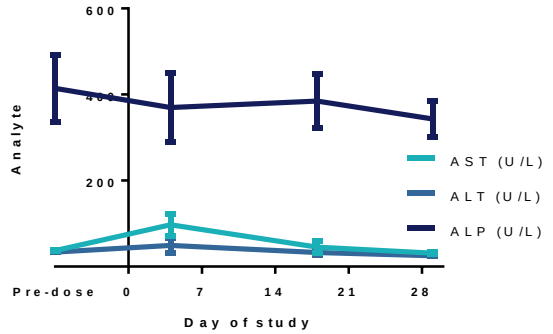
Christina M. Annunziata · Elise C. Kohn · Patricia LoRusso · Nicole D. Houston · Robert L. Coleman · Manuela Buzoianu · Gabriel Robbie · Robert Lechleider

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

Findings from BT5528 toxicology study







Animals dosed weekly, 1-10mg/kg ascending dose protocol

- Dosing: no bleeding events in primates at toxin equivalent doses >100 fold higher than the clinical 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 Bicycle Toxin Conjugate to target EphA2
  - High affinity binding
  - Short half-life and renal elimination
  - "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
  - Efficacy maintained even in large, heterogeneous PDX
  - No bleeding/ coagulation toxicity seen