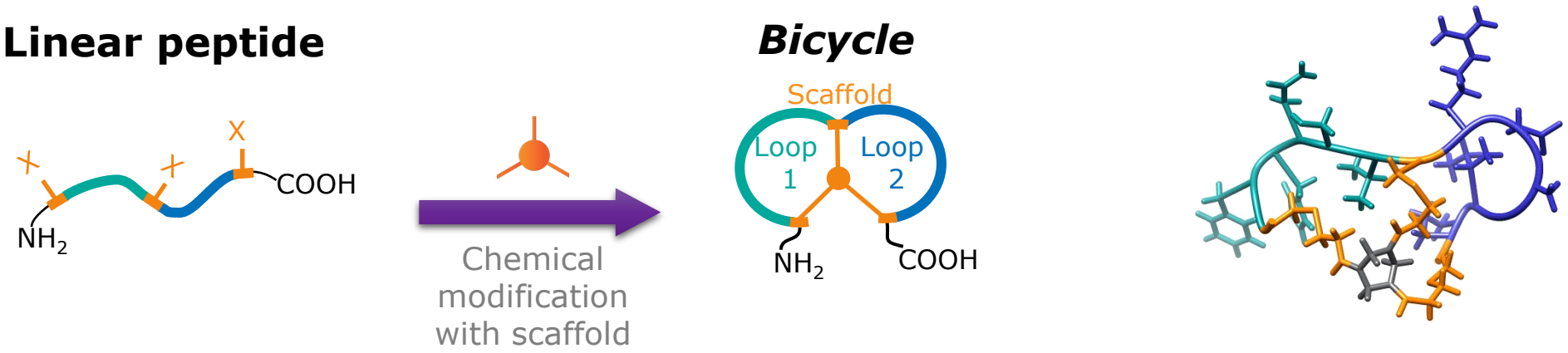
BT5528, an EphA2-targeting *Bicycle*® Toxin Conjugate (BTC): Profound efficacy without bleeding and coagulation abnormalities in animal models

Gavin Bennett

AACR Annual Meeting 2019 Atlanta 4481

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

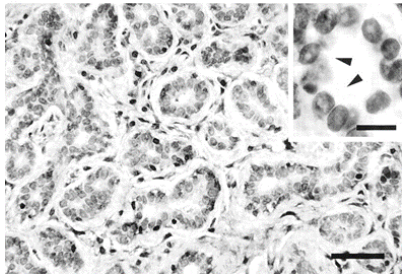
Adjustable PK: excellent tissue penetration, renal elimination, tuneable $T_{1/2}$

EphA2: Biological rationale

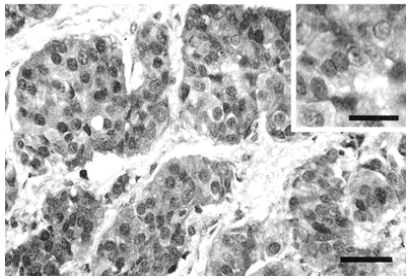
- Erythropoietin-producing hepatocellular A2 receptor: member of Eph subfamily of receptor tyrosine kinases
- Regulates cell migration, adhesion proliferation and differentiation
- Overexpression in human cancers, correlates with tumour progression
- Key area for pharma companies, multiple programs in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

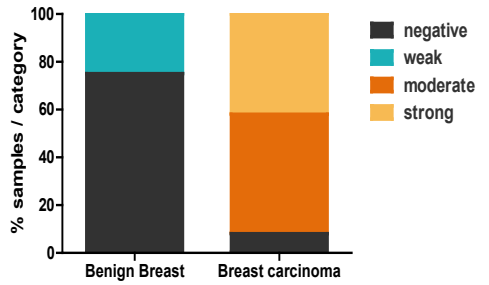
"The bleeding and coagulation events observed in humans showed similarities to those evident in rats and monkeys. In all three species, increased activated partial thromboplastin time, increased fibrinogen/fibrin degradation product, and increased fibrin D-dimer were reported. Monkeys had red/ blood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

Benign Breast

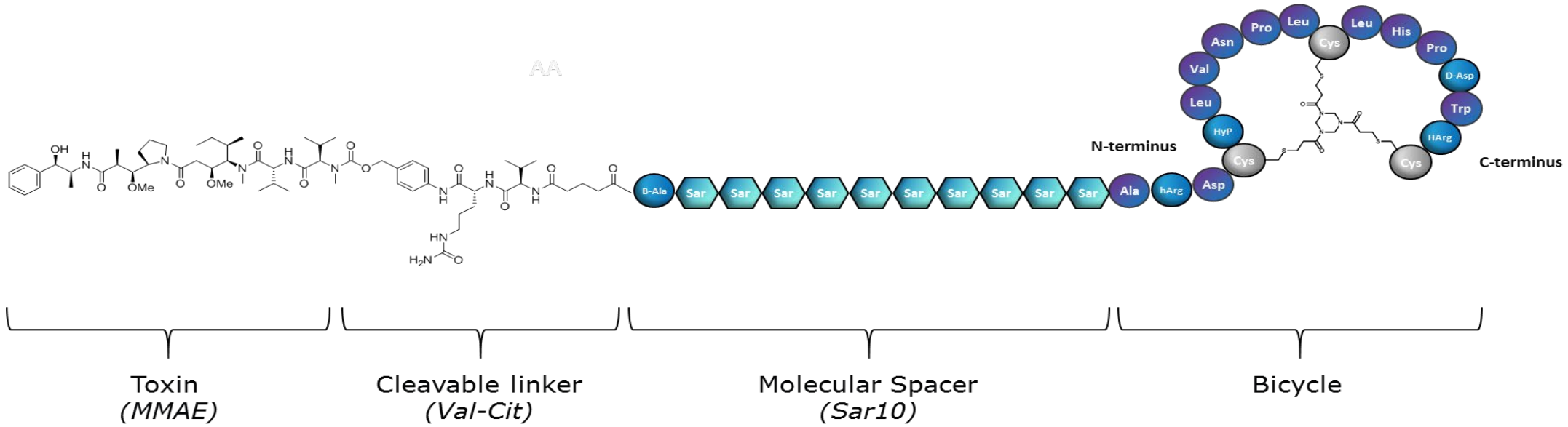


Invasive ductal carcinoma



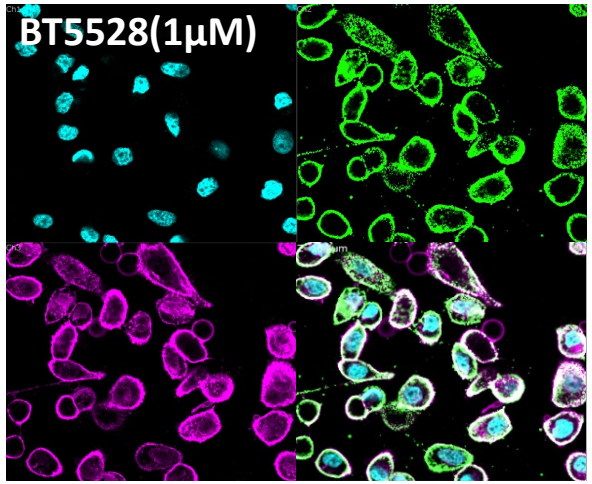


BT5528: structure & profile



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			

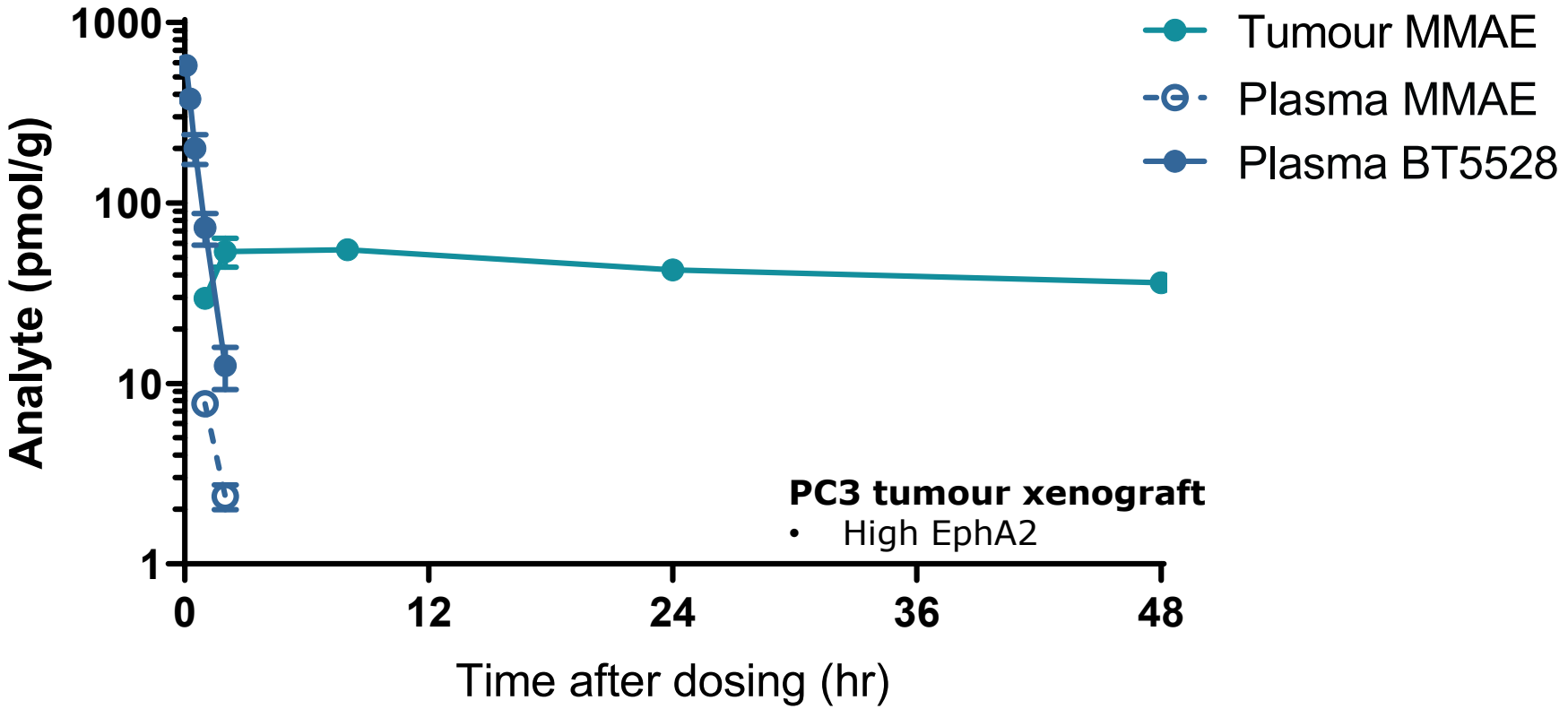


Ligand-binding domain	%identity to hEphA2	Binding affinity (SPR, using BT5528, K_D nM)
	Human	Human
EphA1	54	> @ 5uM
EphA2	**100**	**0.9**
EphA3	58	> @ 5uM
EphA4	55	> @ 5uM
EphA5	56	> @ 25uM
EphA6	56	> @ 20uM
EphA7	56	> @ 20uM
EphB1	49	
EphB4	39	> @ 20uM

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

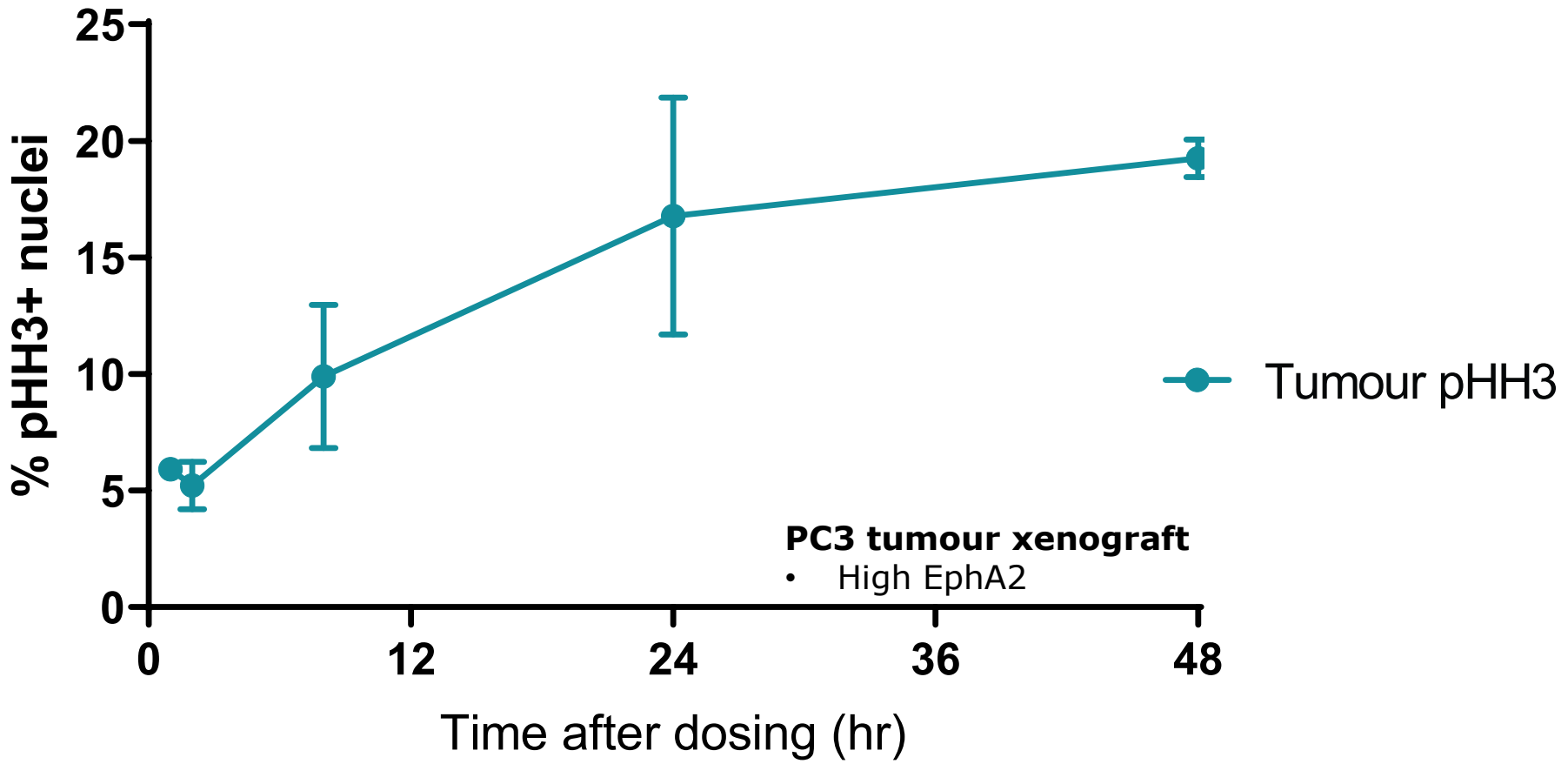


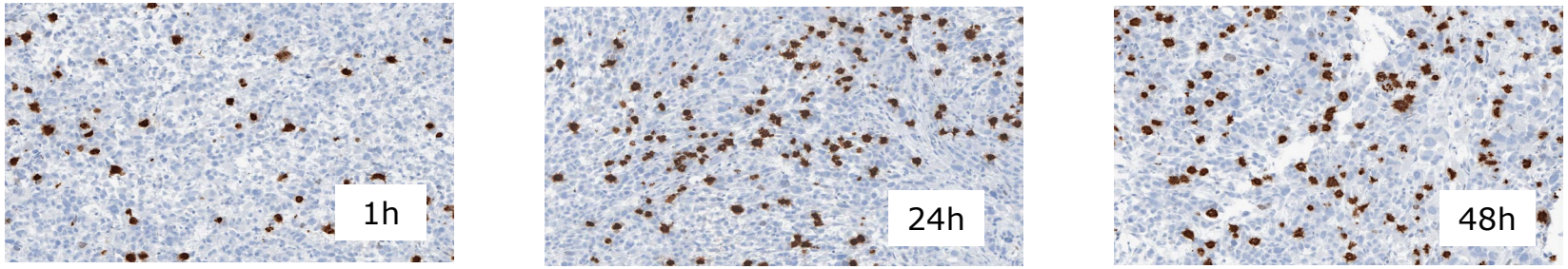
BT5528 PK Parameters	Mouse	Rat	NHP
C_{max} (ng/mL)	6321	4048	7643
$T_{1/2}$ (h)	0.4	0.3	~0.6h
Vd_{ss} (L/kg)	0.18	0.33	0.21
Cl (mL/min/kg)	6.2	15.5	4.9
$AUC_{0\text{-last}}$ (ng.h/mL)	2643	998	3516

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

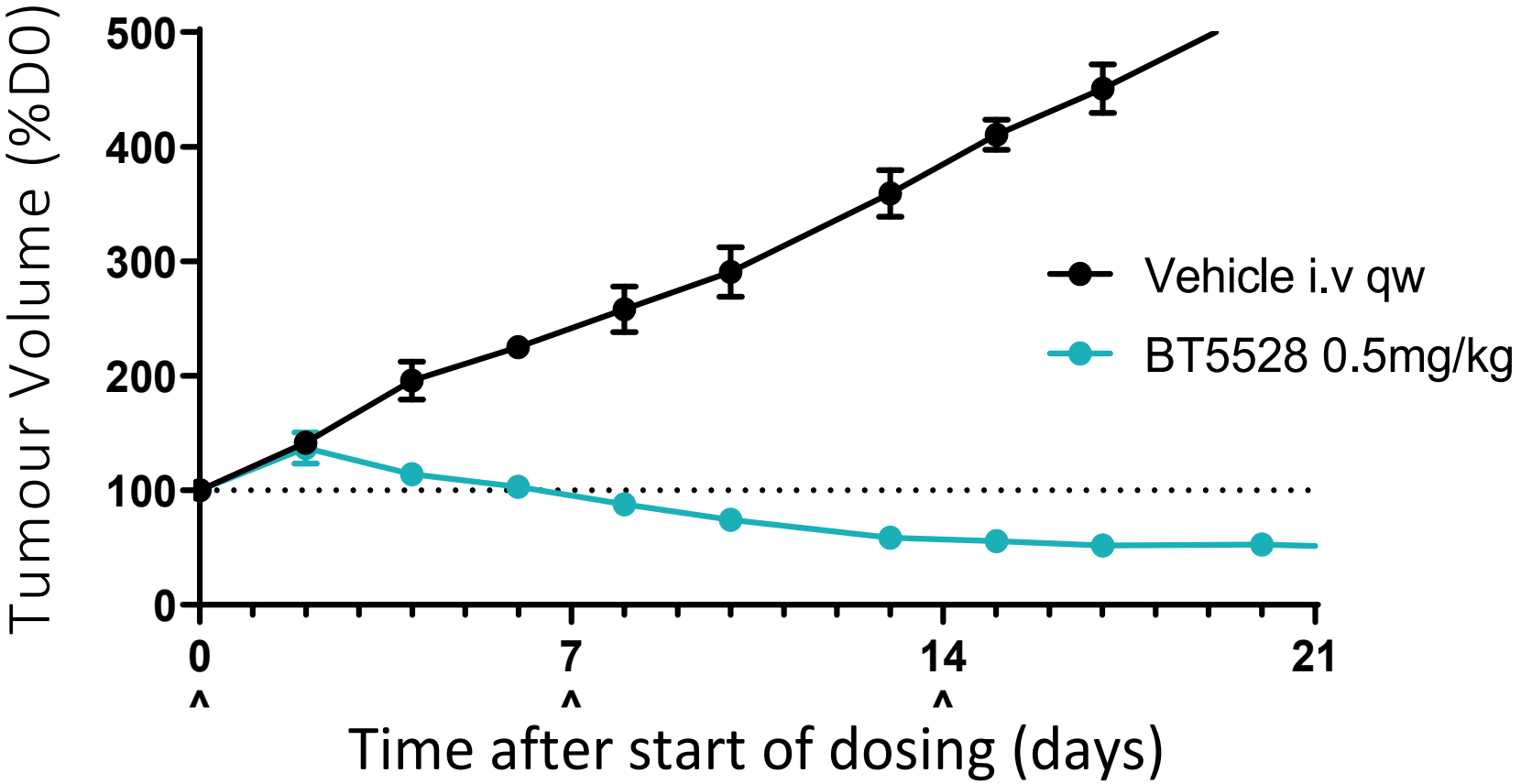




BT5528 produces tumour regression

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

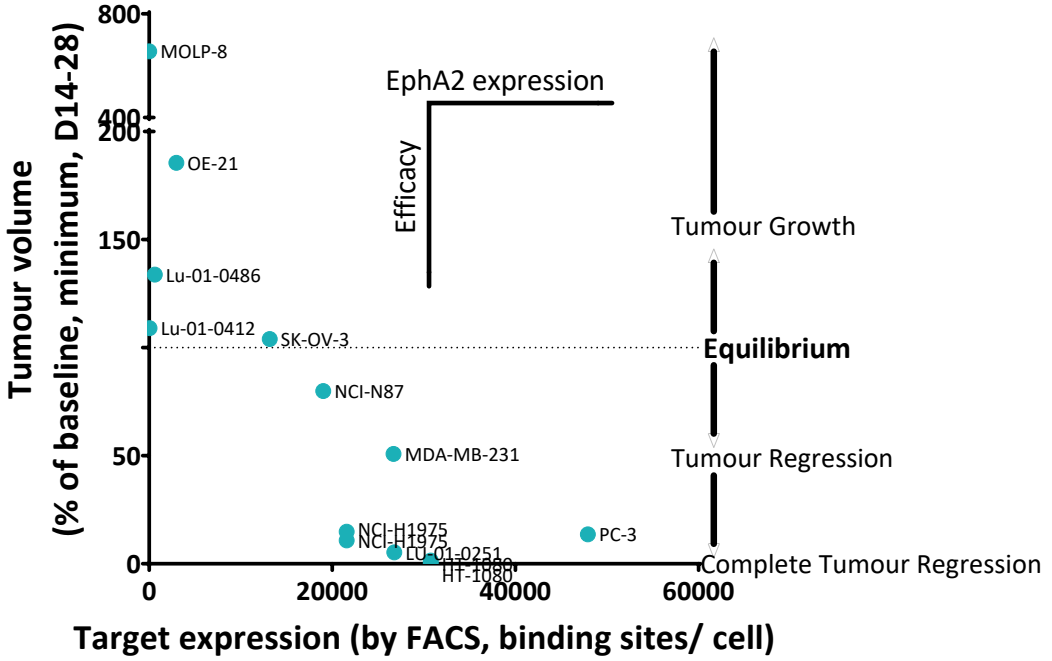


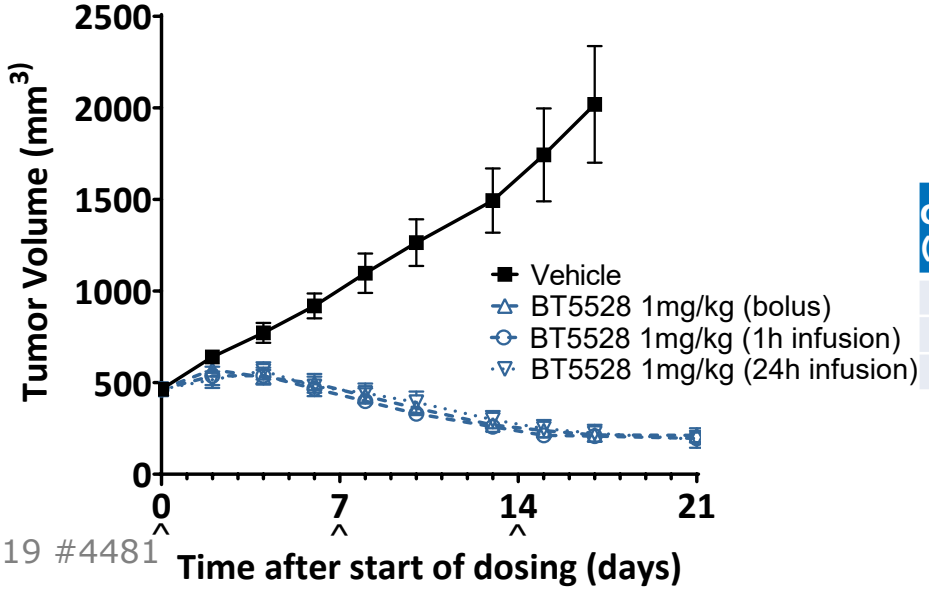
PC3 tumour xenograft

- High EphA2

BT5528 efficacy: target-mediated, flexible dosing

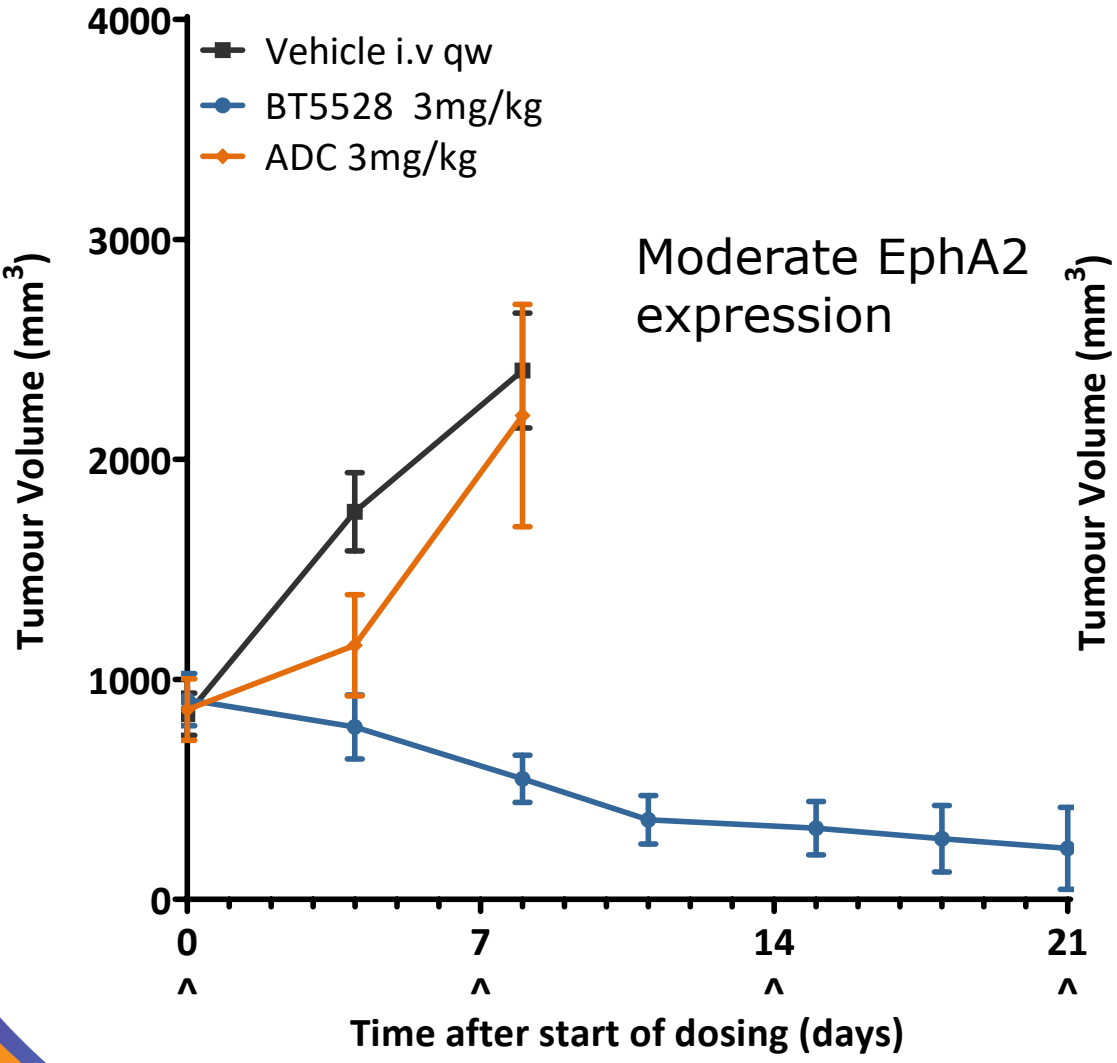
- BT5528 shows target-dependent efficacy
  - Significant regression in a wide range of EphA2-positive tumours
- BT5528 shows equivalent efficacy with a wide range of dosing paradigms
  - Bolus, 1h infusion, 24h infusion
- BT5528 efficacious with intermittent dosing
  - Efficacy also shown dosing every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

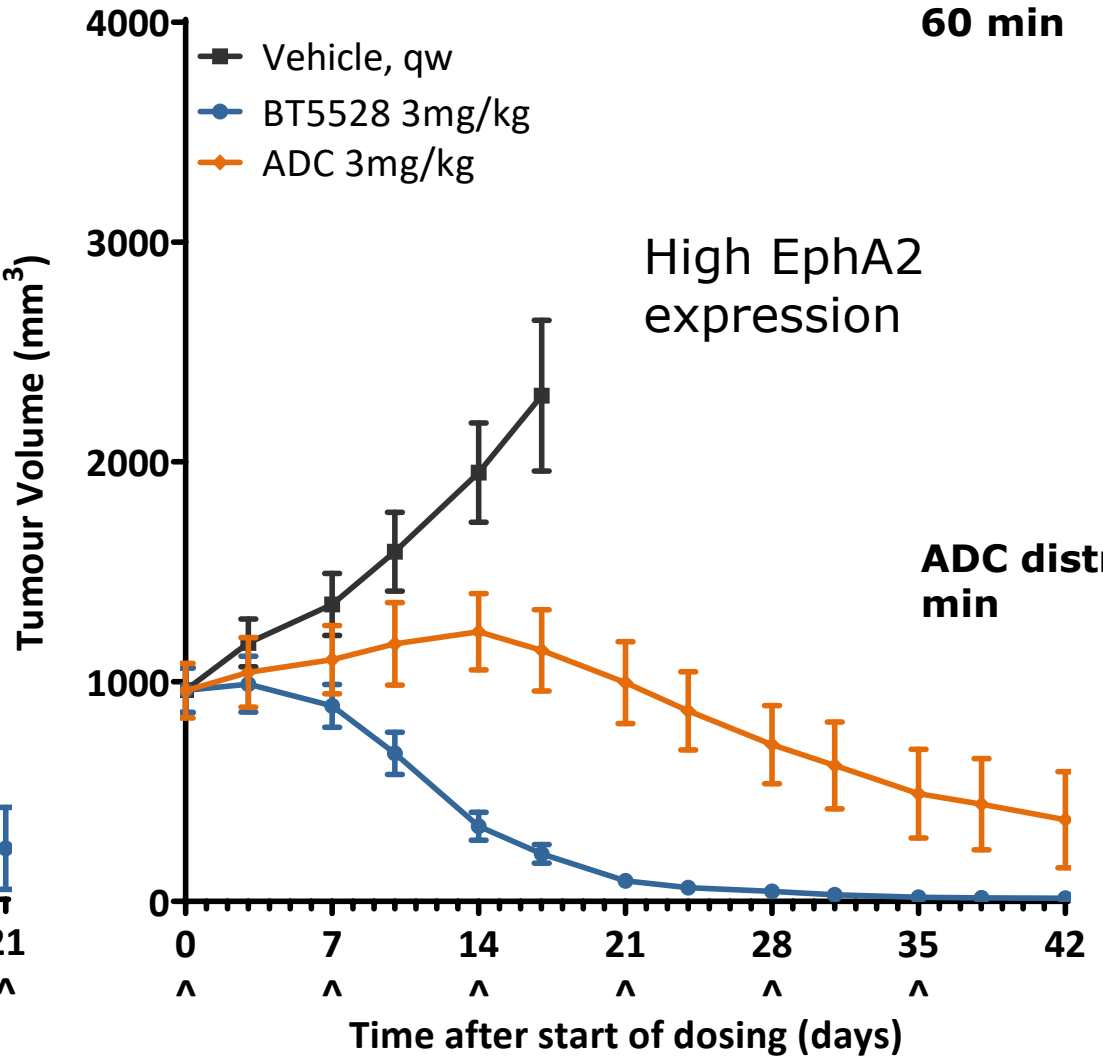




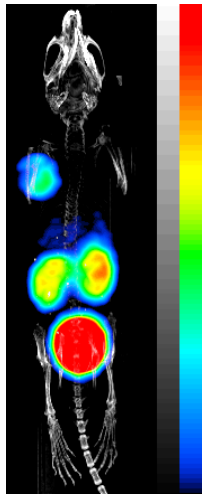
C_{max} (ng/mL)	AUC (ng.h/mL)
3120	1325
1120	1325
55	1325

BT5528: differentiation from ADC in complex PDX models

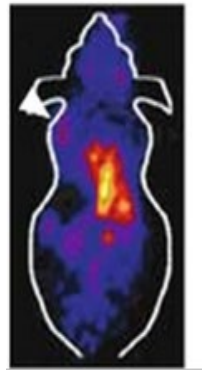




Bicycle distribution at 60 min



ADC distribution at 60 min



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

Findings from MEDI-547 Phase I study

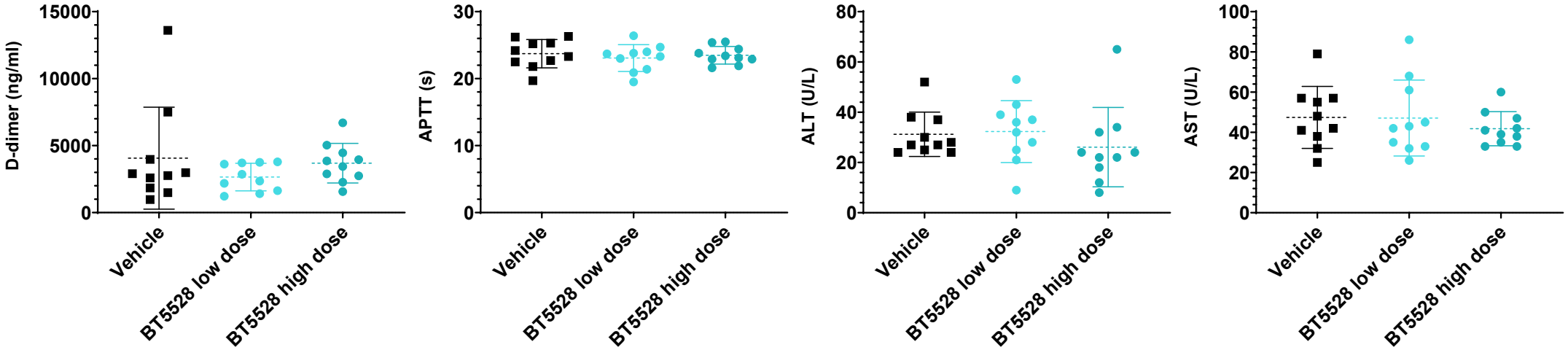
Treatment related adverse events	# events (% of patients) n of total
ALT increased	3 (50) 3/6
Haemorrhage	6 (83.3) 5/6

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
  - Short half-life and renal elimination
  - "Hit and run" delivery of toxin, minimising systemic exposure

- BT5528 shows excellent 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

- IND enabling studies ongoing