BT5528, a Bicycle Toxin Conjugate (BTC) targeting EphA2 has potent anti-tumour activity without bleeding or coagulation abnormalities in animal models

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ABSTRACT#
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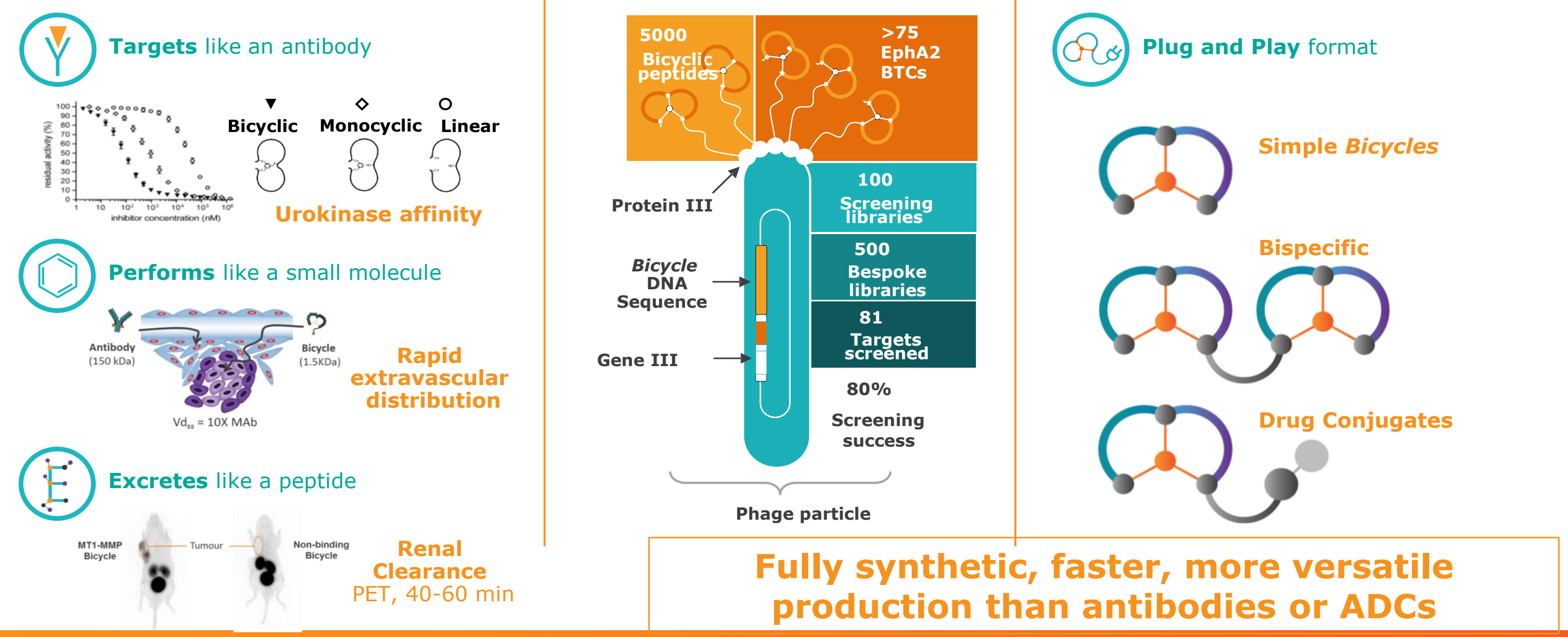
WHY *BICYCLES*

Novel Drug Modality
Combines attributes of three other modalities delivering high affinity, good PK and rapid clearance.

***Bicycle* Platform**
Proprietary screening platform using evolution-driven informed-selection.

Multiple Applications
Bicycles can be used in isolation or linked together to deliver diverse payloads.



Fully synthetic, faster, more versatile production than antibodies or ADCs

ABSTRACT

- BT5528 is a *Bicycle* Toxin Conjugate targeting EphA2-positive tumours
- BT5528 shows profound efficacy in a range of Epha2-expressing tumour models
- BT5528 maintains efficacy even in large (>1000mm3), heterogeneous PDX tumours
- BT5528 shows no bleeding or coagulation toxicity in rats or NHPs

INTRODUCTION

Bicycles® are novel therapeutic agents: bicyclic peptides constrained via a chemical scaffold, which confer structural stability leading to high affinity and selectivity comparable with antibodies. The small size of *Bicycles* (1.5-3 kDa) allows rapid tissue penetration and extravasation. *Bicycles* are fully synthetic, allowing simple conjugation to form a Bicycle Toxin Conjugate, allowing targeted delivery of a cytotoxic payload.

Ephrin receptor A2 (EphA2) is a member of the Ephrin receptor family of cell-cell junction proteins highly overexpressed in several solid tumours and associated with poor prognosis in patients. This profile has made EphA2 an attractive drug target for antibody-based therapies, including Antibody Drug Conjugates (ADCs).

One such ADC, MEDI-547, showed good efficacy in preclinical models (Jackson *et al*, 2008) and was progressed into clinical trials. As described in Annuziata *et al* (2013), the clinical trial was terminated early, due to treatment-related bleeding and coagulation events (hemorrhage-related, n=3; epistaxis, n=2) occurring in 5/6 patients receiving the starting dose. The bleeding and coagulation events observed in humans showed some similarities to those evident in rats and monkeys. In all three species, increased activated partial thromboplastin time (APTT) and increased fibrin D-dimer were reported, together with changes in liver function parameters (ALT, AST, ALP, serum albumin). 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 preclinical and toxicology data for BT5528 offer a profoundly different PK/PD profile while targeting EphA2-positive tumours. The rapid and complete tissue penetration, plus short circulating half-life delivers a "short, sharp hit" to the tumour, without extended exposure of toxin conjugate within the vasculature. This offers differentiation from the ADC profile, minimizing the potential for bleeding and coagulation toxicity while offering profound efficacy

RESULTS

As shown in Figure 1, BT5528 binds to EphA2 with high affinity, across a range of relevant species (a). There is no significant binding to other, related receptors.

In EphA2-expressing cells, BT5528 shows high affinity binding (b), agonist activity on EphA2 (c) and cell cytotoxicity (d).

As shown in Figure 2, in *in vivo* models, BT5528 shows target-mediated efficacy after intravenous administration, with significant effects seen in the high EphA2-expressing NCI-H1975 NSCLC xenograft (a) and MDA-MB-231 TNBC xenograft (b), but no significant effects seen in the low EphA2-expressing MOLP-8 Multiple Myeloma xenograft (c).

Figure 3 shows comparative efficacy in xenograft models between BT5528 and the ADC MEDI-547. Comparable efficacy is seen at 3mg/kg qw for the two agents in HT-1080 fibrosarcoma (a) and PC-3 prostate cancer (b) xenografts (though BT5528 is also fully efficacious at 1mg/kg qw in PC3). In a larger, more heterogeneous xenograft (NSCLC PDX), BT5528 maintains efficacy, while MEDI-547 fails to show efficacy (c). This probably reflects the greater ability of BT5528 to penetrate solid tumours, as illustrated by PET imaging (d).

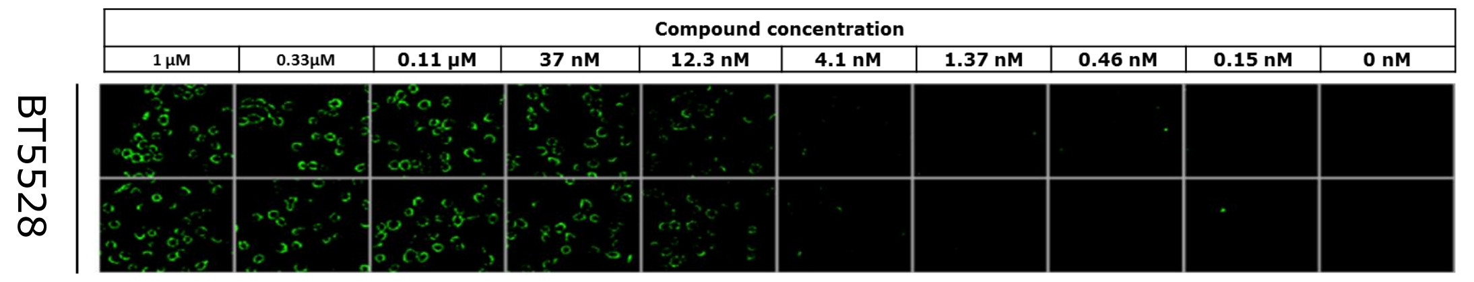
Figure 4 shows the lack of effect of BT5528 on toxicological parameters described for MEDI-547. In NHP exploratory toxicology studies, BT5528 at doses ~MTD does not produce changes in D-Dimer (a), APTT (b) or liver enzymes (c). Additionally, no signs of bleeding were seen in macro- or micro-scopic pathology analysis.

(a)

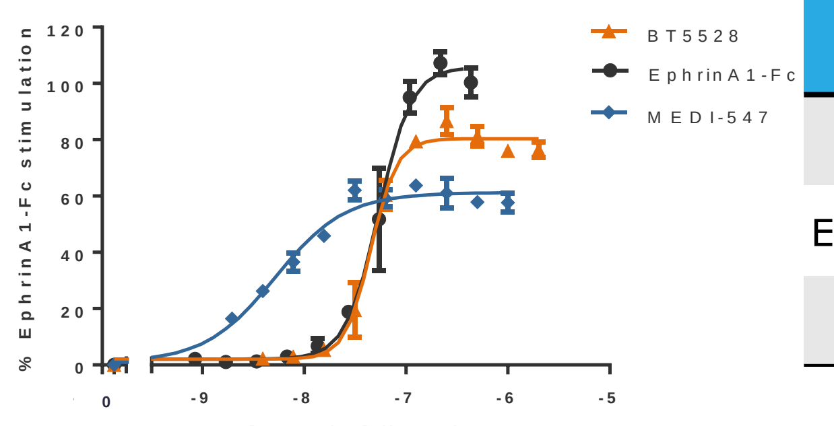
Ligand-binding domain	%identity to hEphA2	% identity to corresponding human protein			Binding affinity (Direct FP, using BCY6184, K_d nM)			Binding affinity (SPR, using BT5528, K_D nM)		
	Human	Mouse	Rat	Cyno	Human	Mouse	Rat	Human	Mouse	Rat
EphA1	54	91.5	92.1	96.6	>680			> @ 5uM		
EphA2	100	94.8	95.4	100	1.05	1.64	1.72	1.2	2.5	3
EphA3	58	99.4	99.4	100	>1600	>1600	>1600	> @ 5uM	> @ 20uM	> @ 20uM
EphA4	55	98.3	98.3	100	>1600	>1600		> @ 5uM	> @ 20uM	
EphA5	56	98.8	98.3	100	>680			> @ 25uM		
EphA6	56	96.5	94.8	100	>480			> @ 20uM		
EphA7	56	99.4	99.4	100	>1600			> @ 20uM		
EphA8	57	97.1	not found	99.2						
EphA9					not found in mammals					
EphA10	52	96.6	95.5	not found						
EphB1	49	100	100	100	>680		>1600	ND		> @ 20uM
EphB2	48	98.9	98.9	100						
EphB3	45	99.4	99.4	99.4						
EphB4	39	92.3	92.3	100	>1600			> @ 20uM		
EphB5					not found in mammals					
EphB6	36	90.7	91.2	97.4						

(b)

	$K_{b\,(app)}$ (nM)
BT5528	19.1 n=1
Non-binder BTC	>10000 n=1



(c)



	EC50 (nM)	Emax (% EphrinA1-Fc)
BT5528	41.9 ± 21.0 (n=4)	70.0 ± 14.7 (n=4)
EphrinA1-Fc	51.6 ± 6.8 (n=8)	100
MEDI-547	7.0 (n=1)	61 (n=1)

(d)

IC50 (nM)	HT-1080	MDA-MB-231	PC3	NCI-H1975
Bicycle Binder	>1000 (n=2)	ND	ND	ND
BT5528	29.1 ± 35.5 (n=3)	ND	5.2 (n=1)	21.6 (n=1)

Figure 1: *In vitro* profile of BT5528.

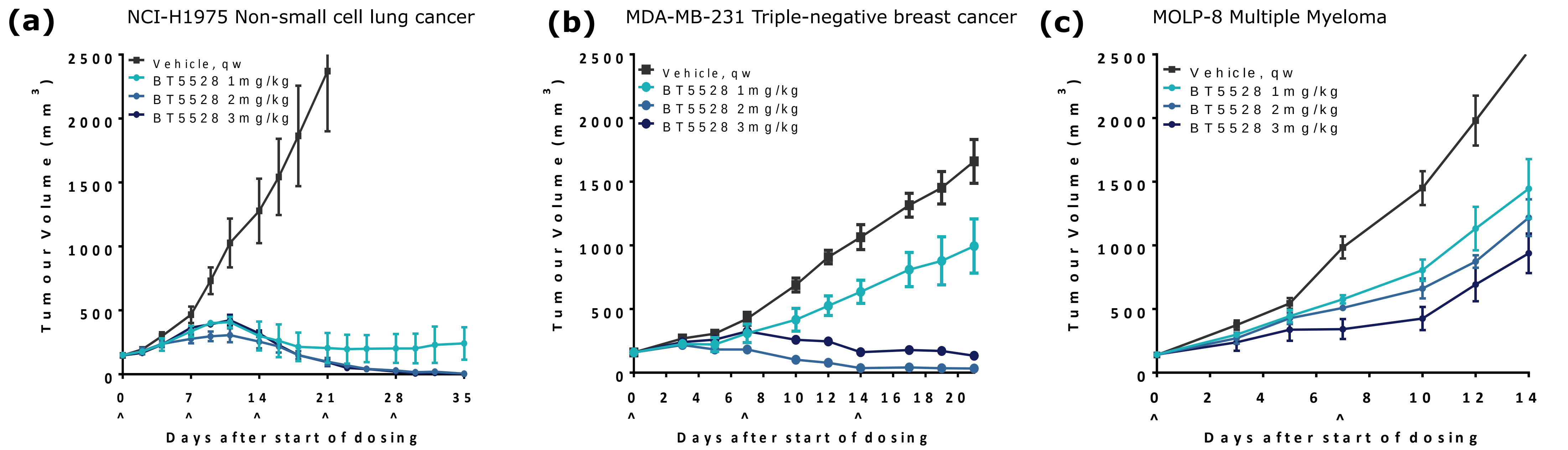


Figure 2: Efficacy of BT5528 across multiple cells lines. Significant efficacy is seen in NCI-H1975 and MDA-MB-231 xenograft models, with efficacy seen from 1mg/kg qw. Minimal efficacy was seen following administration of the same doses to the EphA2 low-expressing line MOLP-8

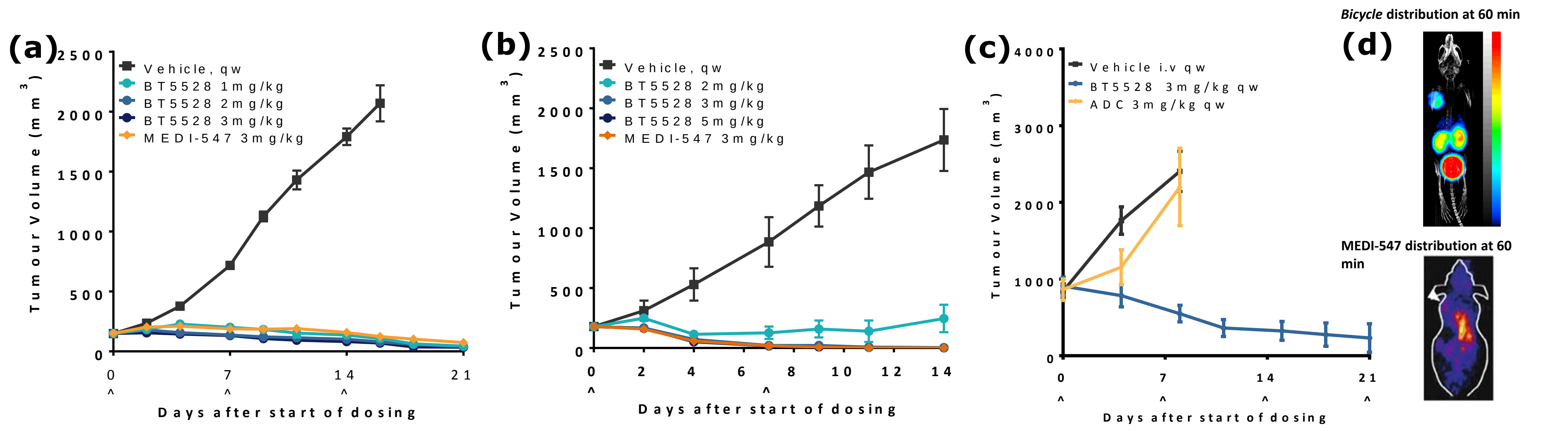


Figure 3: Efficacy of BT5528 and MEDI-547 in (a) PC-3 Prostate cancer model (b) HT-1080 fibrosarcoma model. Significant efficacy is seen, with the two agents showing similar efficacy at 3mg/kg qw. In an NSCLC PDX model (c), BT5528 maintains efficacy even in very large tumours, with significant regression at 3mg/kg qw. Lower efficacy is seen with MEDI-547 using the same dosing regime.

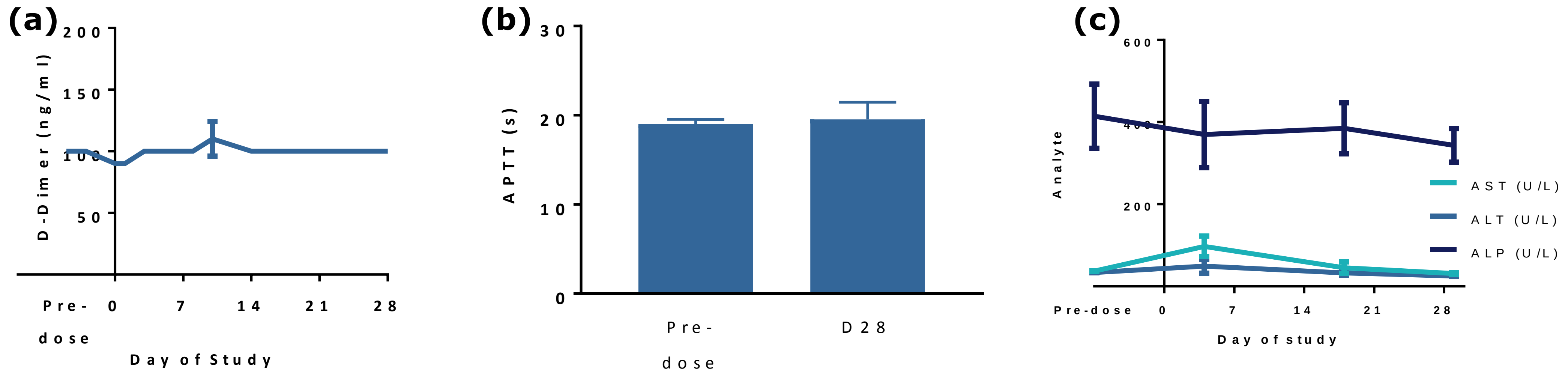


Figure 4: Toxicological differentiation from MEDI-547. In 28d exploratory toxicology studies, weekly dosing of BT5528 to NHPs at doses ~MTD showed no significant changes seen in D-Dimer, APTT or liver enzyme parameters.

CONCLUSION/SUMMARY

- BT5528 shows profound efficacy in a range of Epha2-expressing tumour models, comparable to that described for previous ADCs including MEDI-547
- Unlike MEDI-547, BT5528 maintains efficacy even in large (>1000mm3), heterogeneous PDX tumours, reflecting the rapid and complete penetration of *Bicycle* Toxin Conjugates into solid tumours
- BT5528 shows no bleeding or coagulation toxicity in preclinical species
- IND-enabling studies for BT5528 are currently underway

REFERENCES

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