Bicycle Toxin Conjugates (BTCs) targeting EphA2 for the treatment of solid tumours: Discovery and selection of BT5528

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ABSTRACT#
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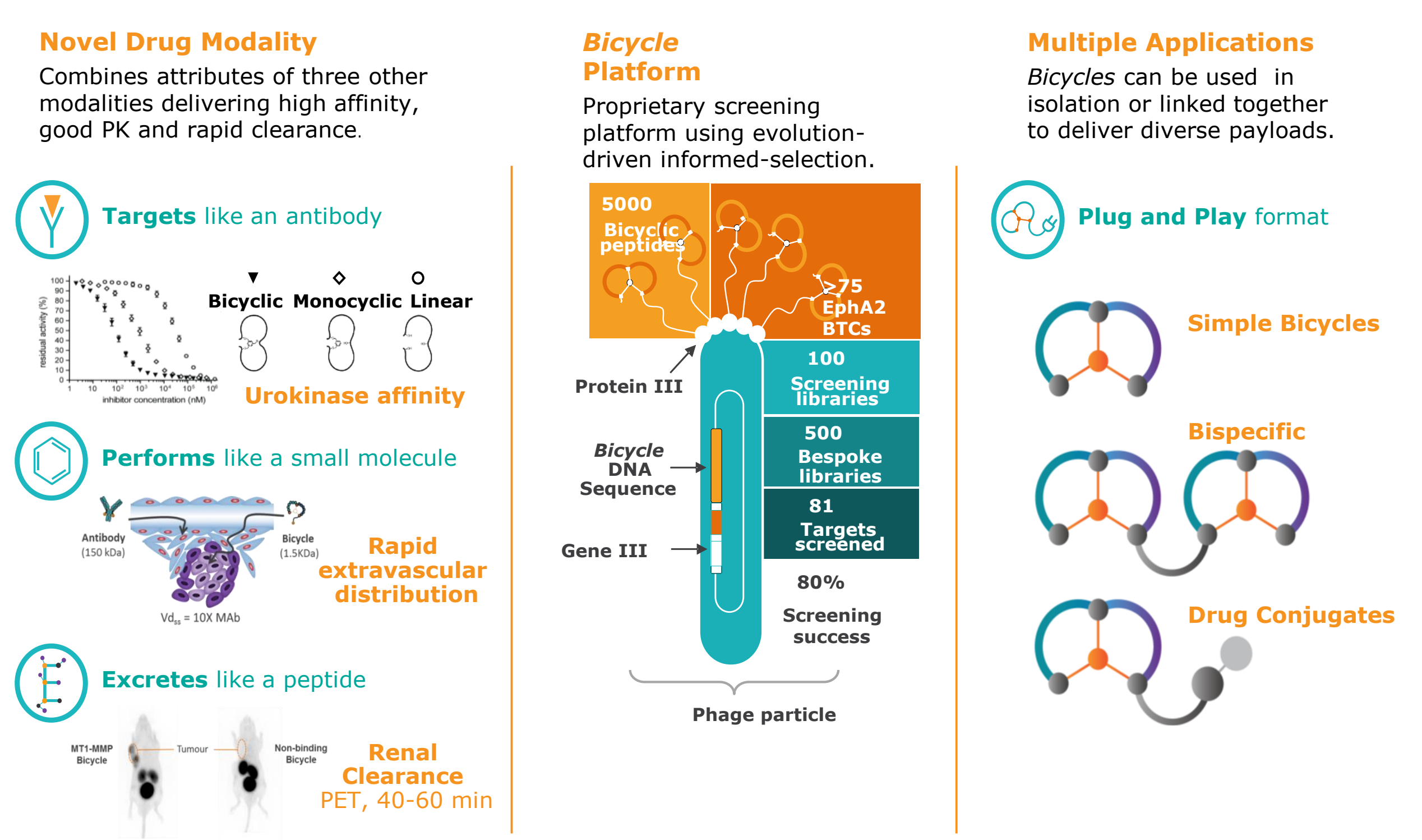
- Bicycle Therapeutics has developed a proprietary phage display platform technology allowing the selection of high affinity bicyclic peptide binding molecules (*Bicycles*®)
- *Bicycles* with high affinity for the EphA2 receptor were selected and optimized through phage screening and chemical modification, guided by X-ray crystallographic analysis of *Bicycle*-receptor binding
- A matrix of BTCs were produced by conjugating a range of *Bicycle* binders with multiple toxin-linker combinations. Screening of >75 BTCs allowed rapid differentiation by affinity, cytotoxicity, efficacy and toxicity.
- BT5528 was selected from the matrix of BTCs on the basis of high efficacy across a range of xenograft models, good tolerability and drug-like properties

WHY *BICYCLES*



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

Targets like an antibody

Urokinase affinity

Performs like a small molecule

Rapid extravascular distribution

Excretes like a peptide

Renal Clearance
PET, 40-60 min

***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.

Plug and Play format

Simple Bicycles

Bispecific

Drug Conjugates

Enormous (>10^{15}) & diverse libraries generate multiple chemical start points
Fully synthetic, faster, more versatile production than antibodies or ADCs

INTRODUCTION

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. *Bicycles* are novel therapeutic agents: bicyclic peptides constrained via a chemical scaffold, which confer structural stability leading to high affinity and selectivity comparable to antibodies. The small size of *Bicycles* (1.5-3 kDa) allows extensive tissue penetration, a short duration of systemic exposure and liver-sparing rapid renal elimination. *Bicycles* are fully synthetic, allowing simple conjugation to a wide range of toxins and linkers

METHODS

Bicycle binders were identified by proprietary phage display technology. *Bicycles* are synthesised by standard Fmoc solid phase synthesis and a proprietary cyclization step. A range of toxin-linkers were then conjugated to *Bicycles* (to either N- or C- termini) using standard conjugation chemistry, allowing rapid production of a matrix of BTCs.

The affinity of *Bicycles* and BTCs for EphA2 was evaluated by competition fluorescence polarization, using a *Bicycle* conjugated to fluorescein as a tracer. A typical competition binding curve is shown in Figure 2.

Bio-distribution was evaluated using a *Bicycle*-DOTA conjugate, incorporating the radioisotope Ga-68. Mice bearing HT-1080 tumour xenografts were administered the radiotracer and scanned using microPET for 60 minutes.

Efficacy was evaluated using a range of xenograft models in nude mice, with BTCs or vehicle dosed by intravenous bolus.

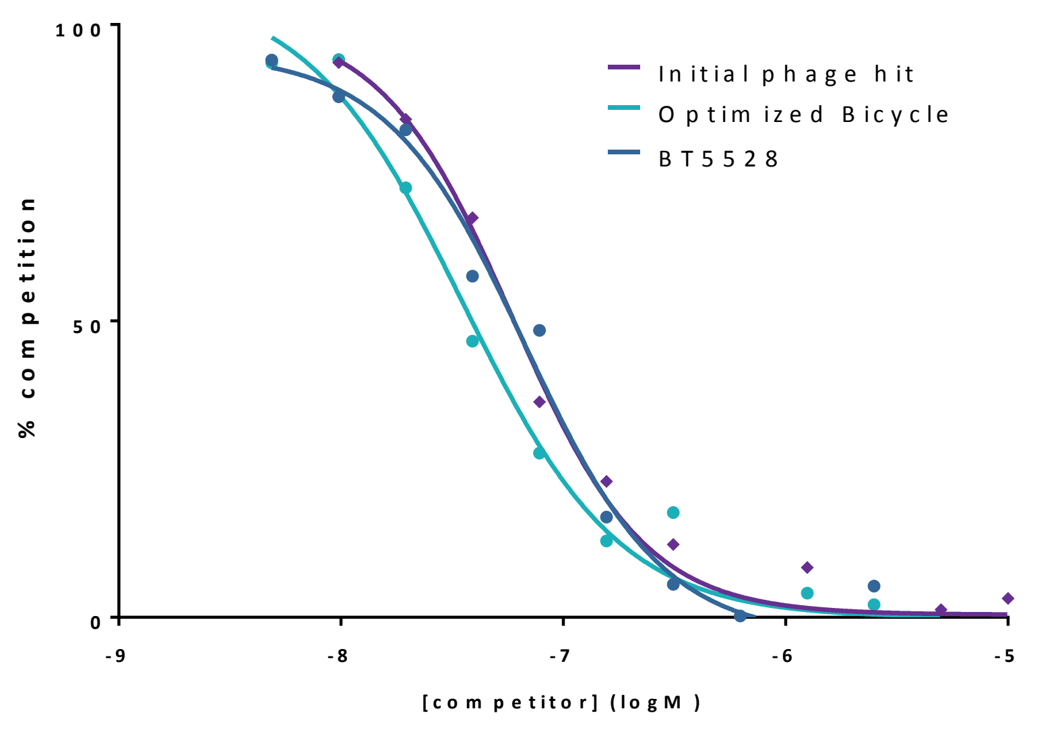
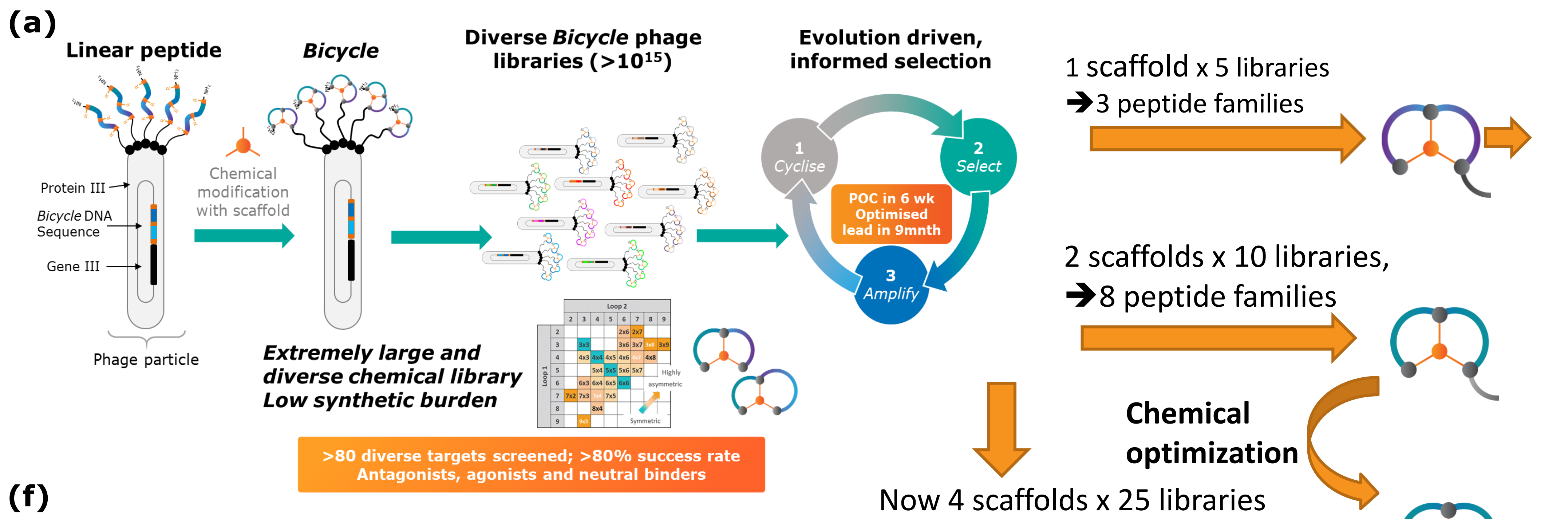


Figure 2: Representative competition binding curve for measurement of binding to EphA2 by *Bicycles* and BTCs

RESULTS



Extremely large and diverse chemical library Low synthetic burden

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

1 scaffold x 5 libraries
→3 peptide families

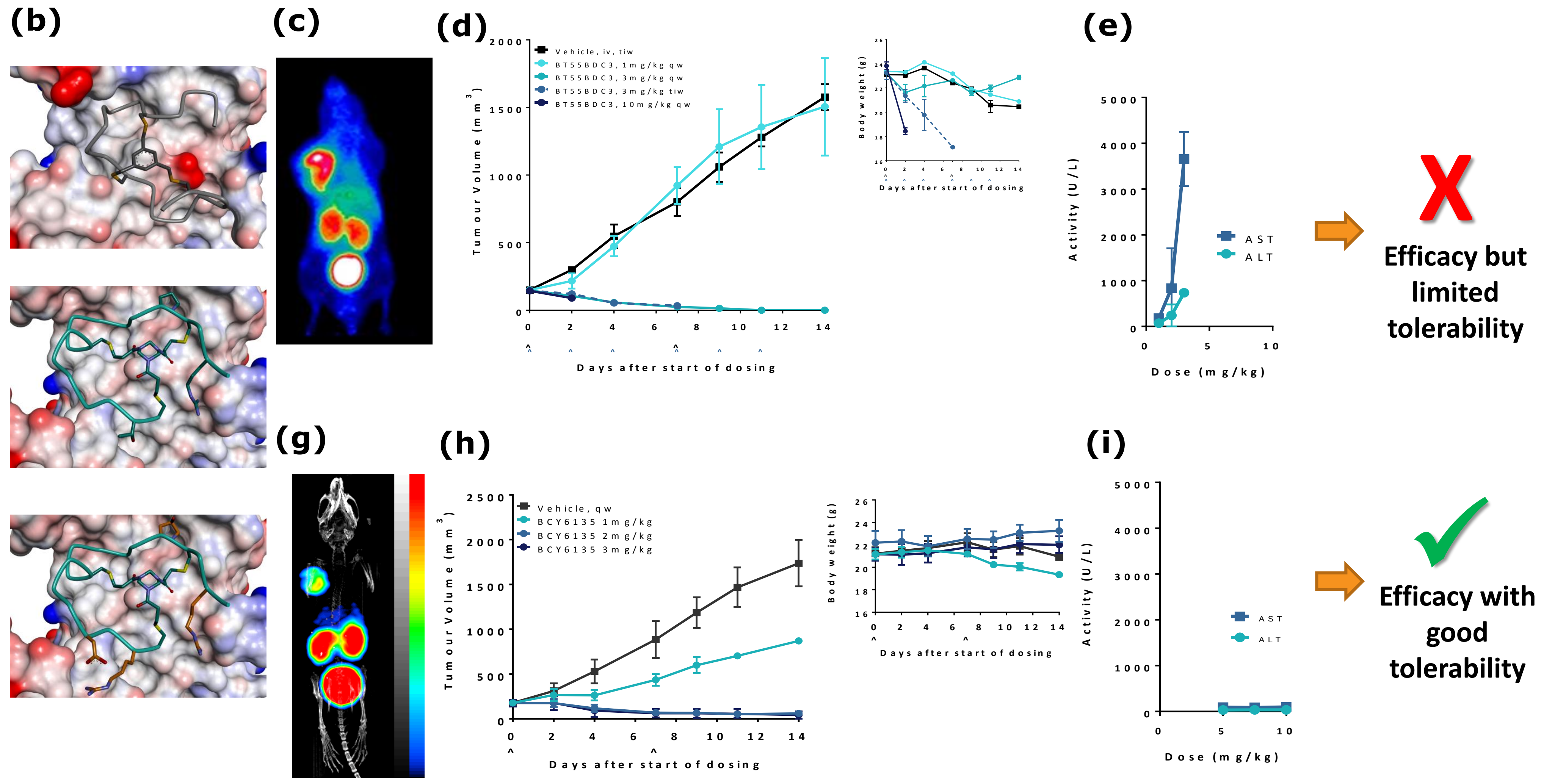
2 scaffolds x 10 libraries,
→8 peptide families

Chemical optimization

Now 4 scaffolds x 25 libraries

(f)

	Affinity (FP comp, Ki, nM) Human	Mouse	Rat	CHI Log P	PPB %
Initial phage screening hit	2.8 ± 0.6 (n=19)			1.79	>99.99%
Broader phage screening hit	17.3 ± 2.4 (n=26)	44.6 ± 8.5 (n=14)	22.5 ± 9.9 (n=8)		
Optimized *Bicycle* Binder	2.9 ± 2.1 (n=5)	6.0 ± 2.8 (n=7)	2.5 ± 0.9 (n=4)		
BT5528	**2.0 ± 0.6 (n=11)**	**6.1 ± 1.1 (n=12)**	**2.5 ± 0.8 (n=10)**	**0.89**	**67.6%**



Efficacy but limited tolerability

Efficacy with good tolerability

Figure 3: Optimization of *Bicycles* and BTCs. *Bicycles* were identified through phage screening (a). Initial hits were identified and conjugated to DOTA for imaging and to toxin-linker for efficacy studies. PET imaging shows clear visualization of tumour and clearance through kidney to bladder, but with evidence of binding in liver (c). Efficacy was seen in HT-1080 xenograft study, but with dose-limiting weight loss (d). Liver toxicity was seen in rat studies (as shown by elevated AST & ALT, (e)). Re-screening a more diverse library identified binders with improved physico-chemical properties (f) and different binding motifs (b). Structure-based modifications resulted in *Bicycles* with improved affinity and physico-chemical properties (f,b). Clear visualization of tumour was seen by PET, without liver binding (g). Efficacy of toxin conjugate was seen in HT-1080 model without affecting body weight (h). No effect is seen on liver enzymes in rat toxicology studies, even at far higher doses than for the previous conjugates (i).

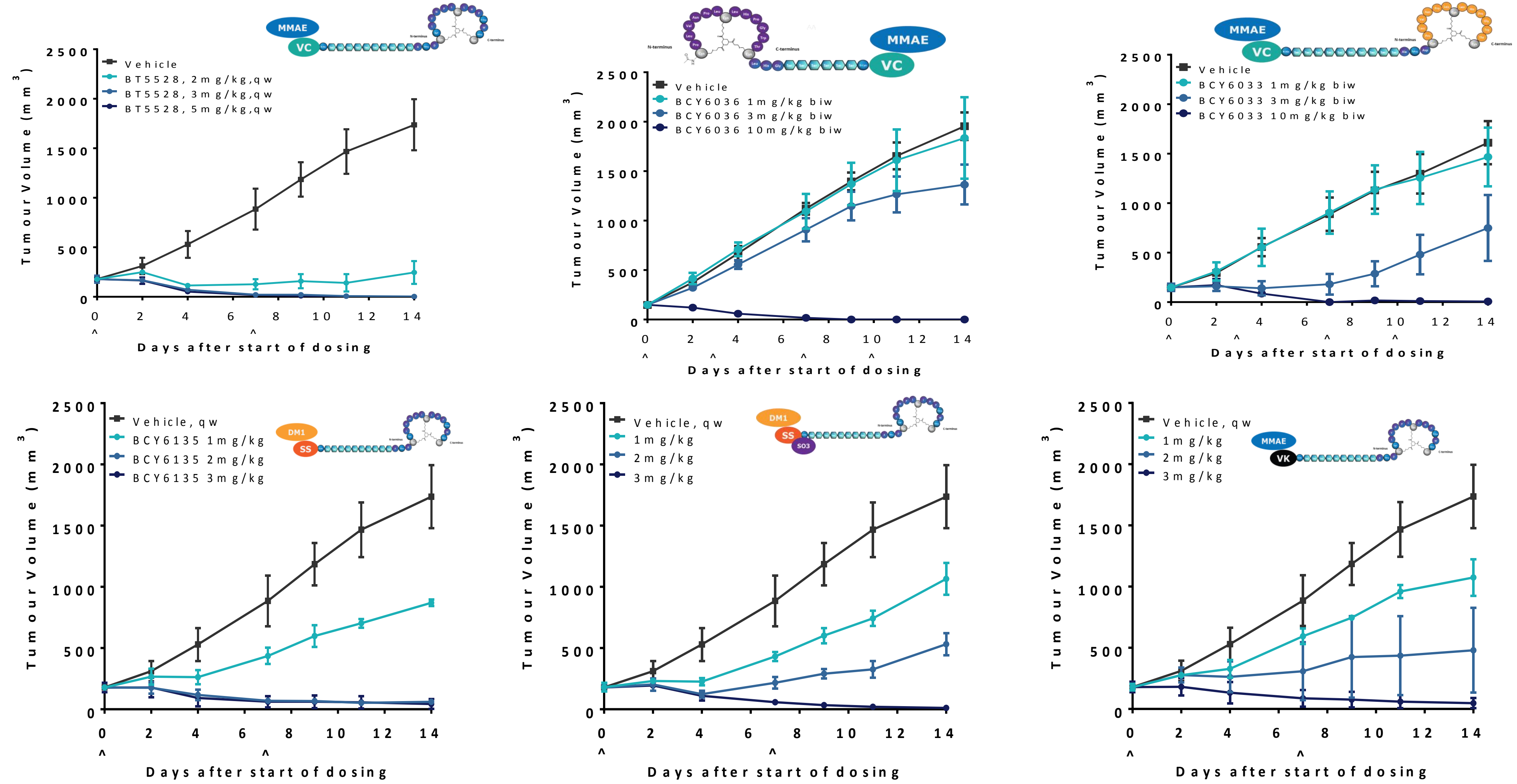


Figure 4: Efficacy of *Bicycle* Toxin Conjugates with different linker-toxin constructs in HT-1080 xenograft model. BTCs containing hindered disulphide-DM1 (-SS-DM1) or peptide linker-MMAE (vcMMAE= Val-Cit-PABC-MMAE; vkMMAE=Val-Lys-PABC-MMAE) show marked efficacy, producing complete regression of tumours within 14d.

CONCLUSION/SUMMARY

- *Bicycles* with high affinity for EphA2 were identified by phage display.
- Early BTCs using these *Bicycles* showed good efficacy in *in vivo* xenograft models, but with poor physicochemical properties, and poor tolerability profiles consistent with liver toxicity
- Screening a more diverse *Bicycle* library identified alternative *Bicycle* binders with a different mode of binding to EphA2 and a better physicochemical profile
- Binders were further optimized and conjugated to produce a matrix of >75 BTCs which were evaluated for efficacy and tolerability.
- The vcMMAE conjugate BT5528 showed the most optimal profile in efficacy and tox experiments

REFERENCES

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