BT8009: A bicyclic peptide toxin conjugate targeting Nectin-4 (PVRL4) displays efficacy in preclinical tumour models

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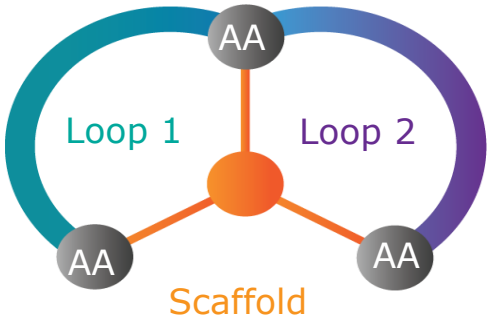
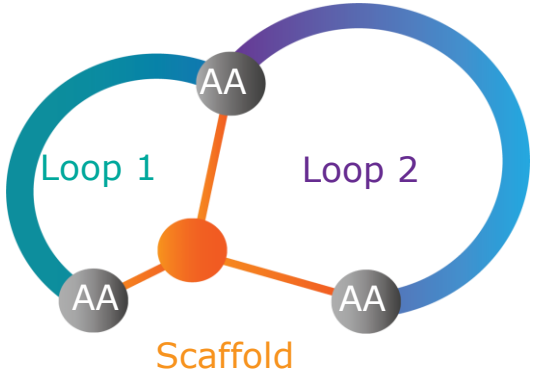
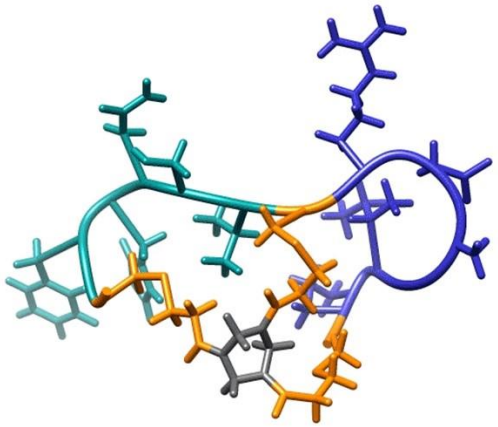
Bicycles®: a new therapeutic modality

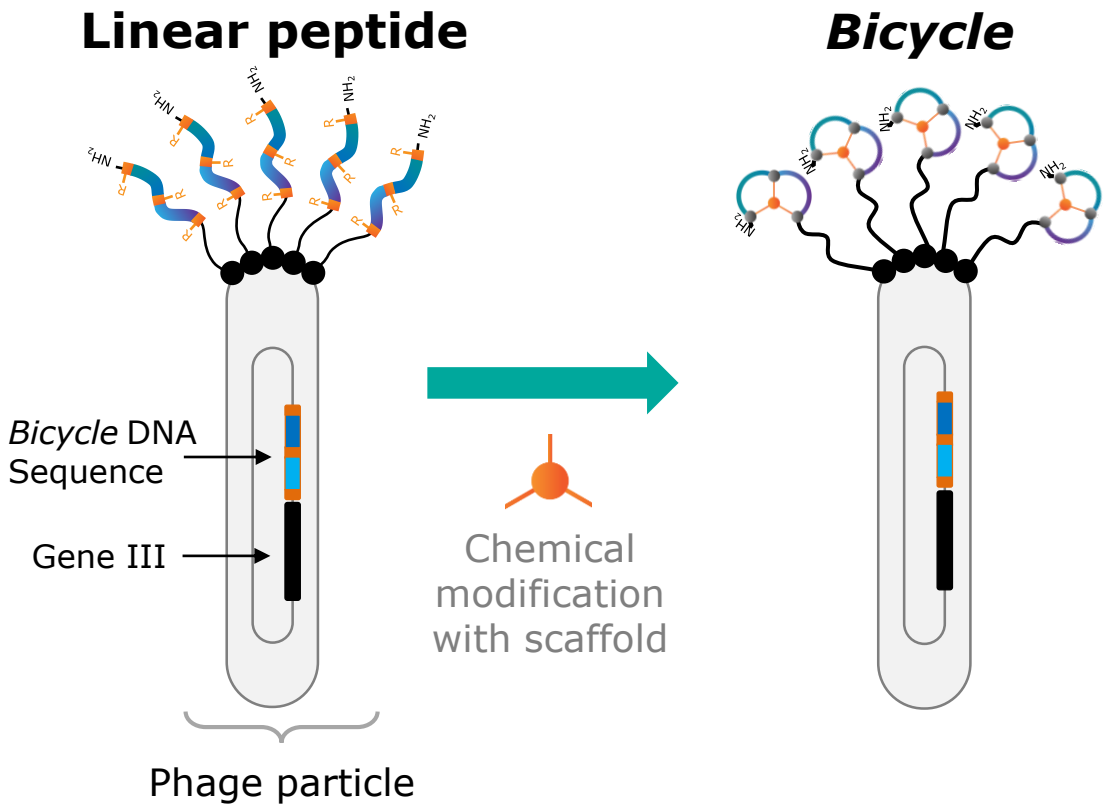
- *Bicycles*® are bicyclic peptides :
  - Highly constrained: sub-nM binding affinities
  - Large surface area and large binding footprint, so can disrupt protein-protein interactions
  - New Chemical Entity classification and fully synthetic molecules
  - Easily conjugated to deliver additional functionality; e.g. chelated radionucleotides, fluorescent dyes, affinity tags, PK extenders and cytotoxic agents
  - Mw of 1.5-2kDa compared with 150kDa for an antibody

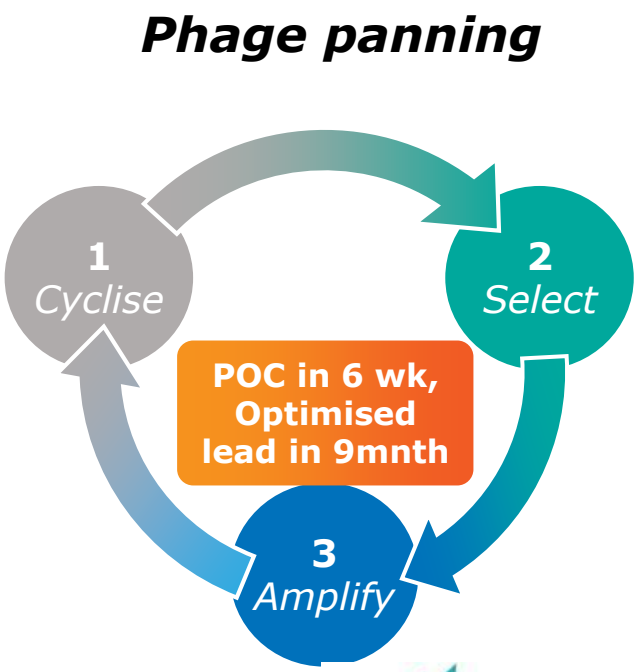


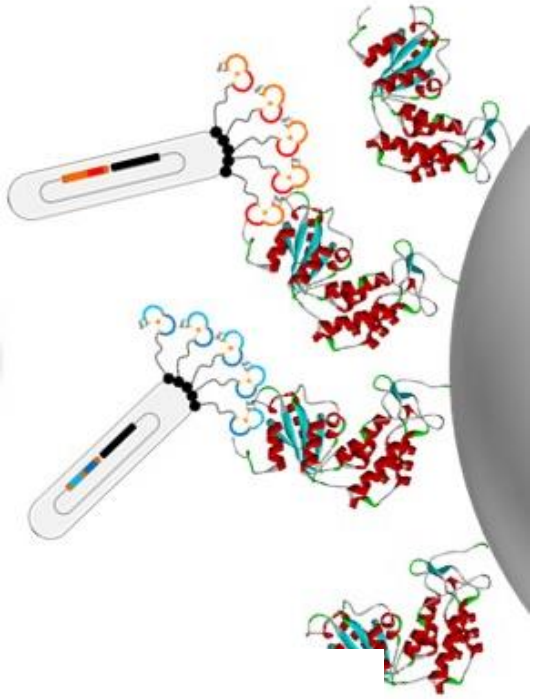


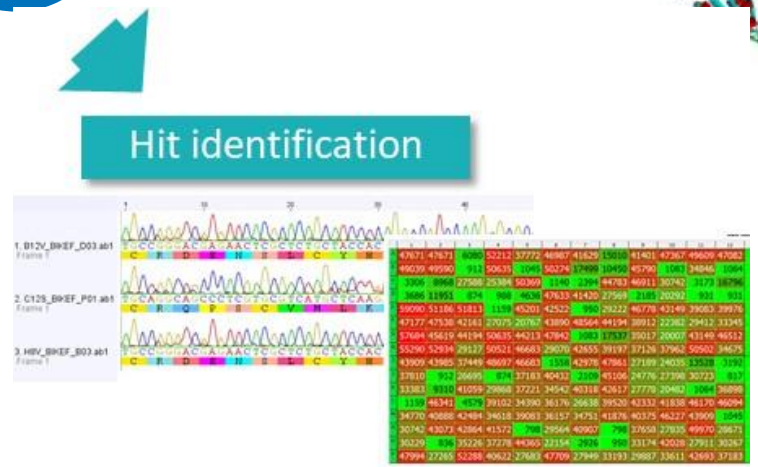


Bicycles®: phage display to derive hits





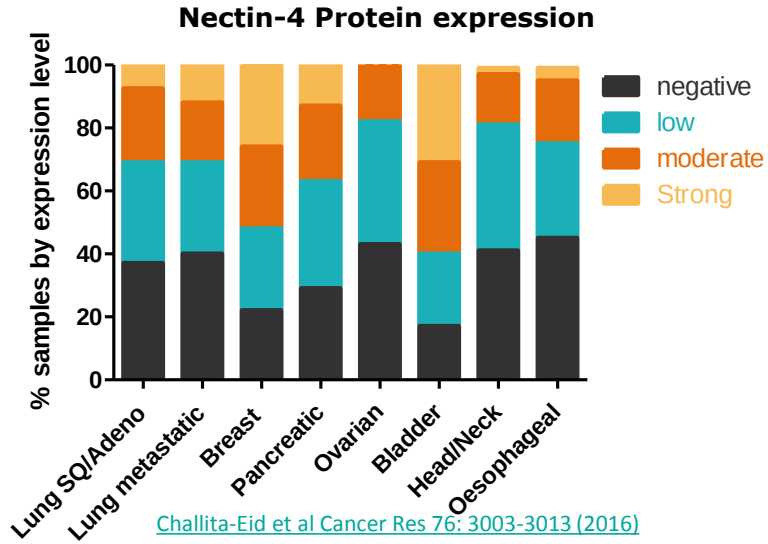




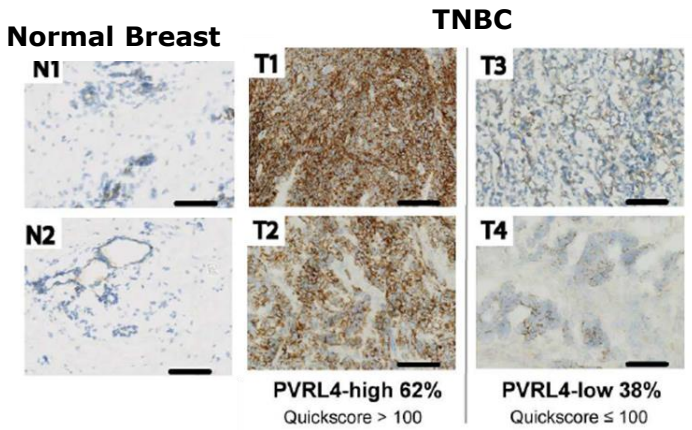
- Enormous diversity (upto 10^{17})
  - Sequence, loop size, symmetry, scaffolds
- Evolution driven
- Informed selection
- Low synthetic burden

Nectin-4: Biological rationale

- Nectin-4; cell adhesion molecule
- Widely expressed during development
  - restricted expression in maturity – epithelial cells e.g. skin, airways, esophagus/stomach and bladder.
- Member of Nectin family and close relative to Nectin-like family
- Other family members more widely expressed through body
- Over expression in numerous tumours; highest frequency in bladder, breast, and pancreatic, but also in lung and esophageal cancers
- Nectin-4 targeting ADC, enfortumab vedotin, in Phase 2 - 3 trials, for metastatic urothelial carcinoma, with "Breakthrough Therapy Designation"



Challita-Eid et al Cancer Res 76: 3003-3013 (2016)



Rabet et al Annals of Oncology 28:769-776 (2017)

Bicycle optimization from lead to *Bicycle*® Toxin Conjugate; BT8009

Parent *Bicycle*

Ac C P F G C M K N W S W P I W C

Amino Acids Important For Target Engagement

Stabilised *Bicycle*

Ac C P F d C M hArg N W S W P I W C

Improve stability, hydrophilicity

Optimised *Bicycle*

Ac C P 1Nal d C M hArg D W S T P Hyp W C

+ affinity | + hydrophilicity | + affinity, hydrophilicity

Poor solubility, short half-life. AAs required for binding identified

Improve half-life and hydrophilicity, whilst retaining binding AAs

Increase affinity and improve hydrophilicity. Selected as candidate peptide binder for *Bicycle* Toxin conjugate, BT8009

Ki (nM)	cLogP	$T_{1/2}$ (plasma)
18.4	-6.98	1.3h

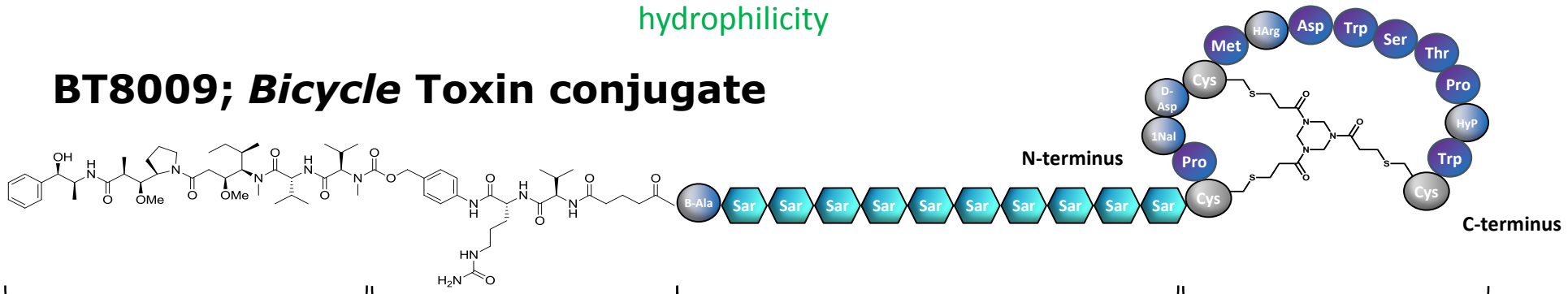
Ki (nM)	cLogP	$T_{1/2}$ (plasma)
13	-6.74	>24h

Solubility = 4.9 ug/ml, LogD = -0.7

Ki (nM)	cLogP	$T_{1/2}$ (plasma)
3.21	-13.32	>24h

Solubility = 420 ug/ml, LogD < -2.2

BT8009; *Bicycle* Toxin conjugate



Toxin MMAE | Cleavable linker | Sar10 Spacer | *Bicycle Binder*

BT8009; overall *in vitro* characteristics

BT8009 shows excellent selectivity over the close family members of Nectin and Nectin-like family

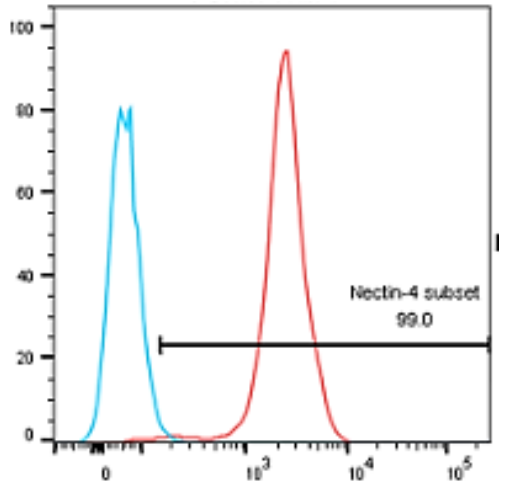
	Human Nectin KD (nM)				Human Nectin-like KD (nM)				
Subtype	1	2	3	4	1	2	3	4	5
BT8009	No binding	No binding	No binding	3.02	No binding	No binding	No binding	Weak @ 5 uM	No binding

BT8009 shows appropriate cross-species characteristics to enable safety studies

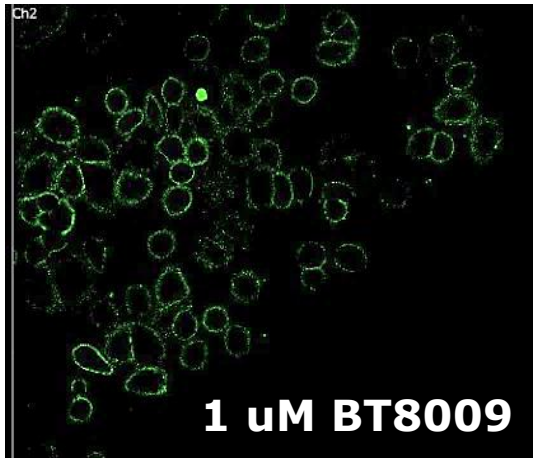
Parameter	Human	Cynomolgus	Rat	Mouse
Affinity for native Nectin-4 (K_D)	3.02	1.14	4.42	Not tested
PPB(% unbound)	20.7	20.7	19.4	11.8
Blood/plasma partition (recovery %)	0.64	0.37	0.65	Not tested
Plasma stability (T1/2 h)	>57.8	>57.8	60.7	4.4
Blood stability (T1/2 h)	26.1	28.3	8.5	5.0
Hepatocyte stability (T1/2 h)	>3.61	>3.61	>3.61	Not tested

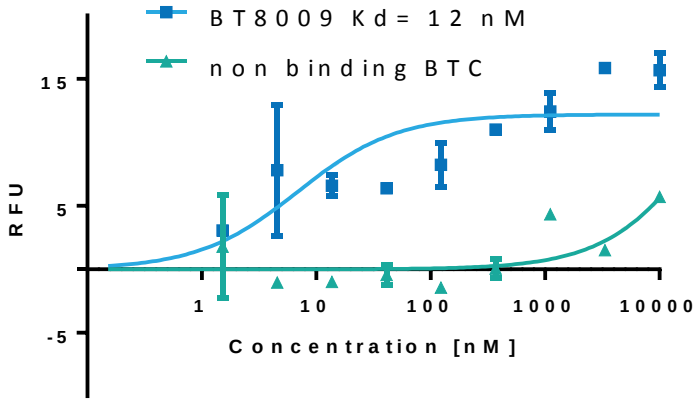
BT8009 binds Nectin-4 on MDA-MB-468 cells, and shows efficacy in xenograft model

FACS shows Nectin-4 surface expression

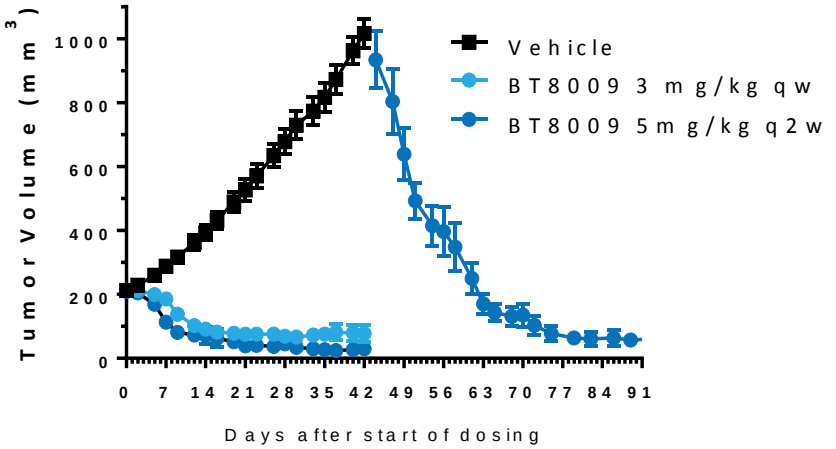


ICC of cells using anti-MMAE antibody. After preincubation with BT8009, a non-binding Bicycle Toxin Conjugate (BTC) or MMAE demonstrates only BT8009 is retained on cell surface





BT8009 shows excellent efficacy in MDA-MB-468 xenografts, even against larger tumours



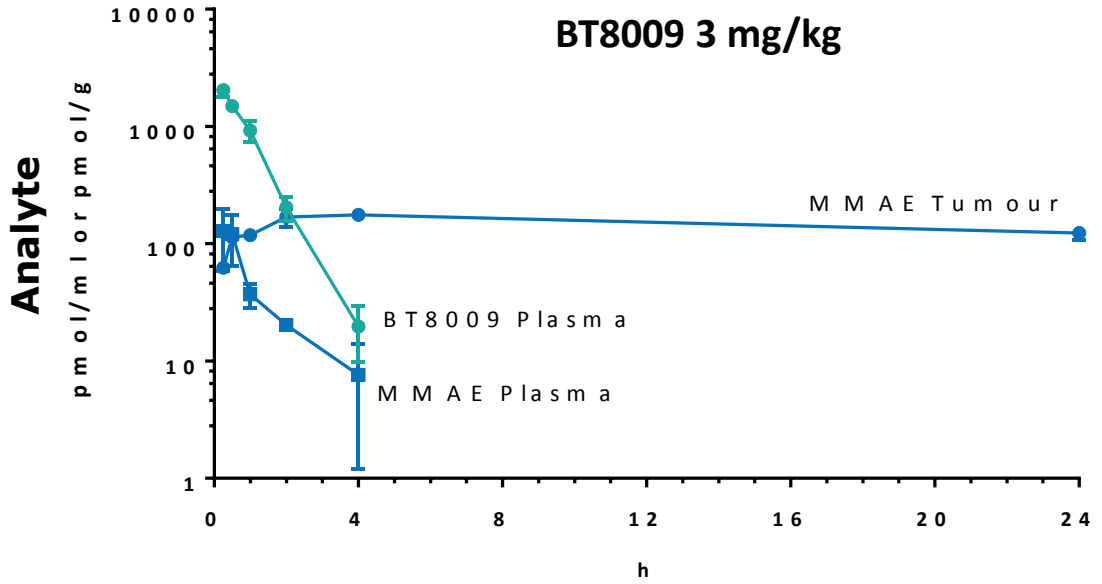
BT8009: *In vivo* PK

BT8009 shows high Cmax with a short plasma half-life, reflective of rapid clearance from systemic circulation, characteristic of Bicycles and unlike ADCs

BT8009 1 mg/kg	CLp (ml/min/kg)	Vss (L/Kg)	T1/2 (h)	BT8009		MMAE	
				Cmax (uM)	AUC (uM. h)	Cmax (uM)	AUC (uM. h)
Mouse	3.5	0.25	0.98	1.401	1.131	0.065	0.103
Rat	9.4	0.44	0.86	1.114	0.432	0.013	0.022

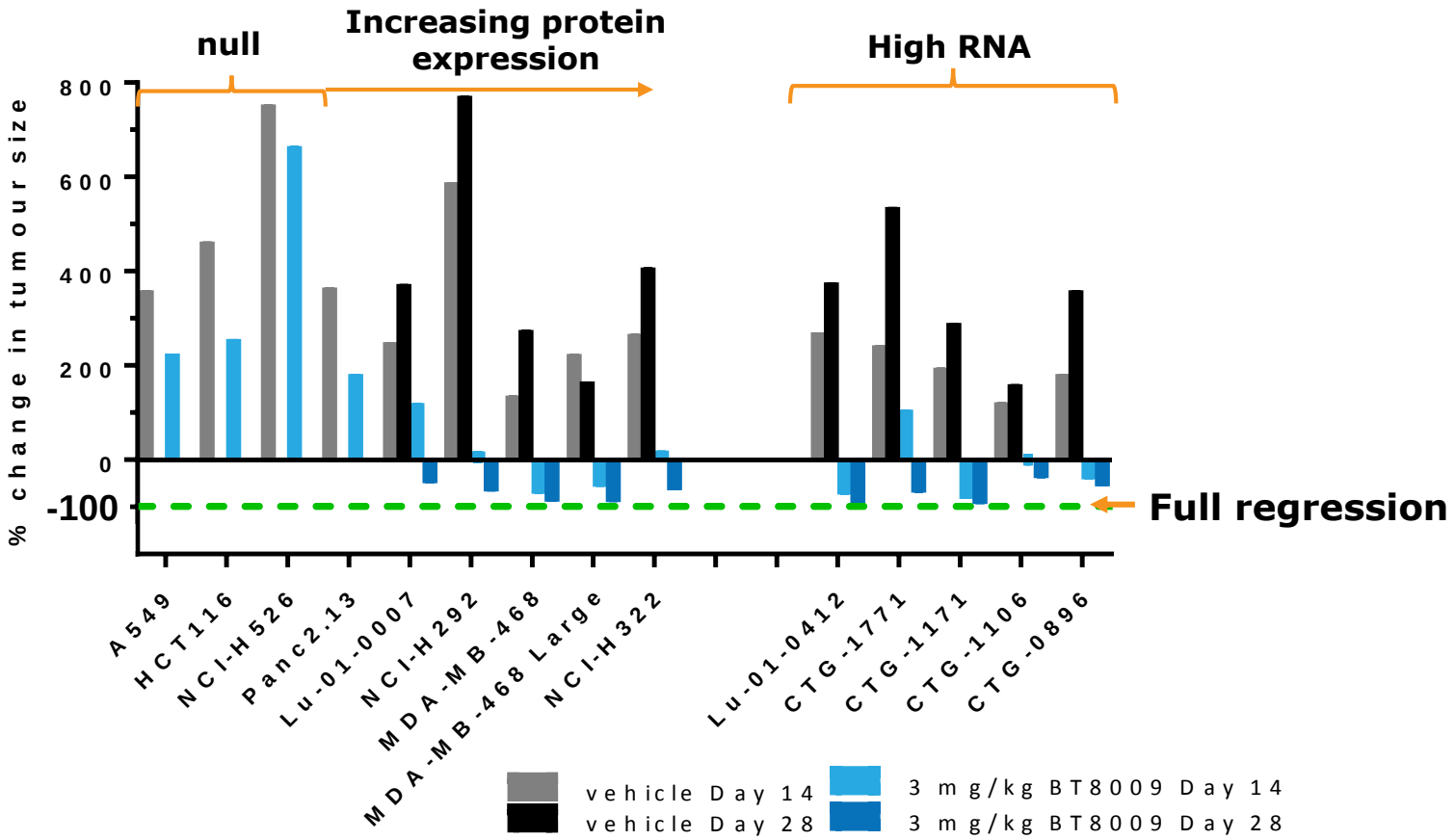
BT8009 affords rapid and long lasting MMAE retention in MDA-MB-468 tumour, with rapid plasma clearance of toxin and parent.

Lasting tumour retention also demonstrated in other models.

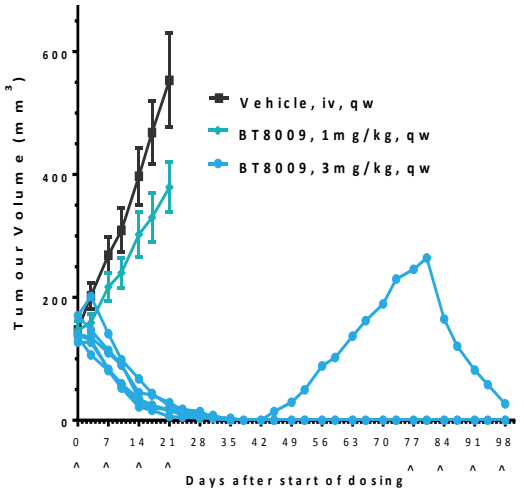


BT8009 efficacy correlates with expression in CDX/PDX xenografts

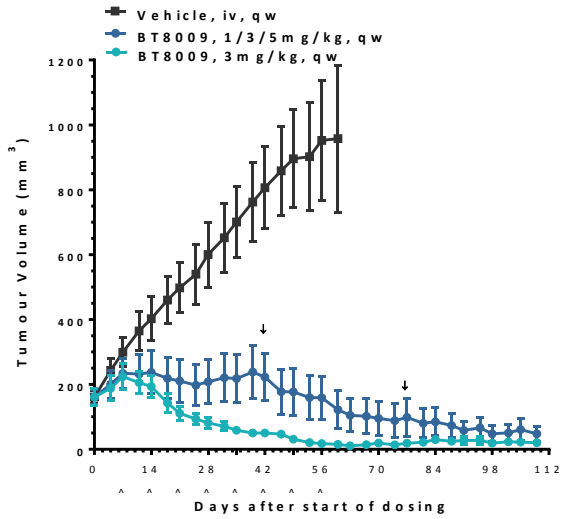
Xenografts with low/no Nectin-4 expression show reduced tumour growth. Xenografts expressing Nectin-4 show good tumour regression



Lung adenocarcinoma PDX



Lung squamous carcinoma PDX



BT8009: A Nectin-4 targeting Bicycle Toxin Conjugate, for the treatment of Solid Tumours

- Nectin-4 is highly expressed on tumour cell surface in a wide range of solid tumours

- BT8009 was developed as a *Bicycle* Toxin Conjugate to target Nectin-4
  - High affinity binding, highly selective for Nectin-4
  - Short plasma half–life, with renal elimination
  - Hit and run delivery of toxin to tumour cells, minimising systemic exposure

- BT8009 shows excellent efficacy in multiple PDX and CDX models, with rapid regression in small and large tumours
  - Efficacy in NSCLC, TNBC, Esophageal and bladder PDX models
  - Efficacy is dose related and correlates with expression of the Nectin-4 target
  - PK shows retention of toxin in tumour, well in excess of systemic clearance
  - IND enabling toxicology studies with BT8009 are ongoing