BT8009; a Nectin-4 targeting *Bicycle*® Toxin Conjugate for treatment of solid tumors.

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Supplementary Material

Supplementary Data

Supplementary Table 1: Binding affinities for BT8009 binding to extra-cellular domain of Nectin family members (determined by Surface Plasmon Resonance) and homologies with hNectin-4.

Family member	Human		NHP		Rat		Mouse	
	K_D (nM) Mean ±SD	hNectin-4 homology (%)	K_D (nM) Mean ±SD	hNectin-4 homology (%)	K_D (nM) Mean ±SD	hNectin-4 homology (%)	K_D (nM) Mean ±SD	hNectin-4 homology (%)
Nectin-4	2.5±1.3 (n=13)	100	6.3±1.3 (n=7)	99	6.0±1.2 (n=7)	92	2.9±1.1 (n=3)	91
Nectin-3	>20000	35	-	35	-	35	-	36
Nectin-2	>20000	28	-	28	-	26	-	26
Nectin-1	>20000	30	-	30	-	31	-	31
Necl-1	>20000	27	-	27	-	28	-	29
Necl-2	>20000	28	-	28	-	28	-	28
Necl-3	>20000	26	-	26	-	26.	-	26
Necl-4	>5000	27	-	27	-	26	-	26
Necl-5	>20000	30	-	30	-	28	-	29

Abbreviations: NHP=non-human primate. K_D = dissociation constant, SD = standard deviation

Supplementary Table 2: *In vitro* ADME properties of BT8009 in human, NHP, rat and mouse.

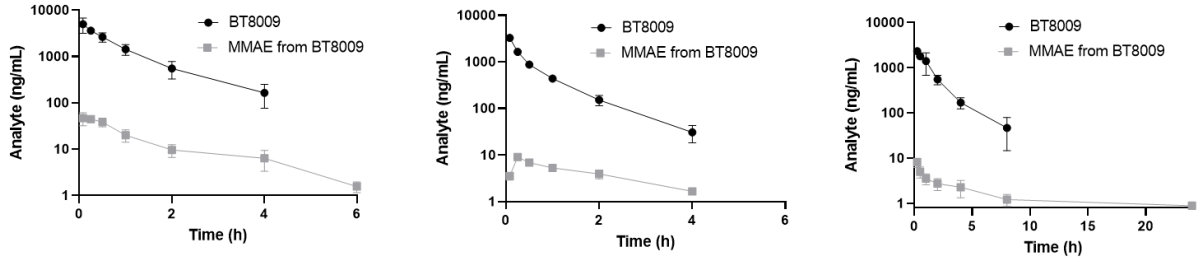
	Human	NHP	Rat	Mouse
Stability in plasma ($t_{1/2}$, h)	>57.8	>57.8	60.7	2.3-4.4
Stability in whole blood ($t_{1/2,}$ h)	26.1	28.3	8.5	5.0
Stability in hepatocytes (CL_{int}.mL/min/g liver)	<0.02	<0.02	<0.03	-
Stability in microsomes (CL_{int} mL/min/g liver)	<0.01	0.02	<0.02	-
Plasma Protein Binding (fu, p)	0.21	0.18	0.19	0.12

Abbreviations: CL_{int}=intrinsic clearance, C_{max}=maximum mean plasma concentration, fu, p=unbound fraction in plasma, MMAE=monomethyl auristatin E, NHP=non-human primate, $t_{1/2}$ = terminal half-life.

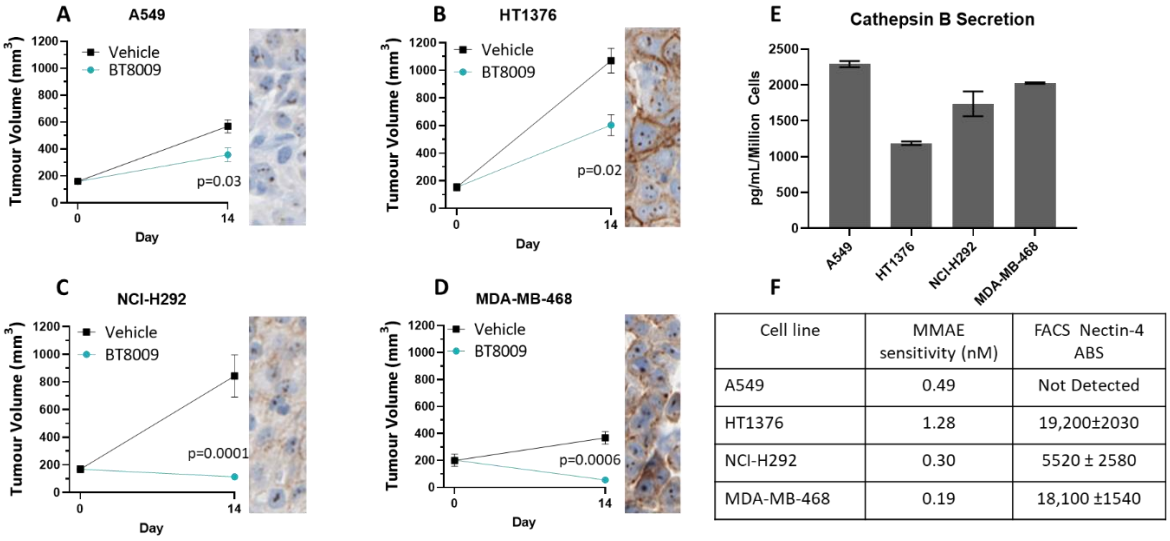
Supplementary Table 3: Calculated PK parameters for BT8009 and MMAE following IV dosing of BT8009 1 mg/kg in mouse, rat, and 1.25 mg/kg in NHP

Species	PK Parameters	BT8009	MMAE	EV
Mouse	C_{max} (ng/mL)	-	46.6	
	$t_{1/2}$ (h)	1.0	1.3	37[&]
	Vd_{ss} (L/kg)	0.25	--	0.08[&]
	CL (mL/min/kg)	3.5	--	0.02[&]
	$AUC_{0\text{-}last}$ (ng.h/mL)	4489	70.7	
	$AUC_{0\text{-}inf}$ (ng.h/mL)	4721	73.6	
Rat	C_{max} (ng/mL)	-	9.06	
	$t_{1/2}$ (h)	0.9	1.8	24-32
	Vd_{ss} (L/kg)	0.44	--	0.04-0.1
	CL (mL/min/kg)	9.4	--	0.03-0.05
	$AUC_{0\text{-}last}$ (ng.h/mL)	1779	15.6	
	$AUC_{0\text{-}inf}$ (ng.h/mL)	1804	20.5	
NHP	C_{max} (ng/mL)	5780*	8.3*	
	$t_{1/2}$ (h)	1.7*		41
	Vd_{ss} (L/kg)	0.39*	--	0.07-0.08
	CL (mL/min/kg)	4.1*	--	0.02
	$AUC_{0\text{-}last}$ (ng.h/mL)	4923*	23*	
	$AUC_{0\text{-}inf}$ (ng.h/mL)	5041*		

Abbreviations: AUC=area under the mean plasma concentration-time curve, CL=clearance, C_{max}=maximum mean plasma concentration, MMAE=monomethyl auristatin E, NHP=non-human primate, PK=pharmacokinetic, $t_{1/2}$ = terminal half-life, Vdss=volume of distribution at steady state. *NHP data obtained from TK studies. [&]In ICR SCID mouse. Enfortumab vedotin data from literature.



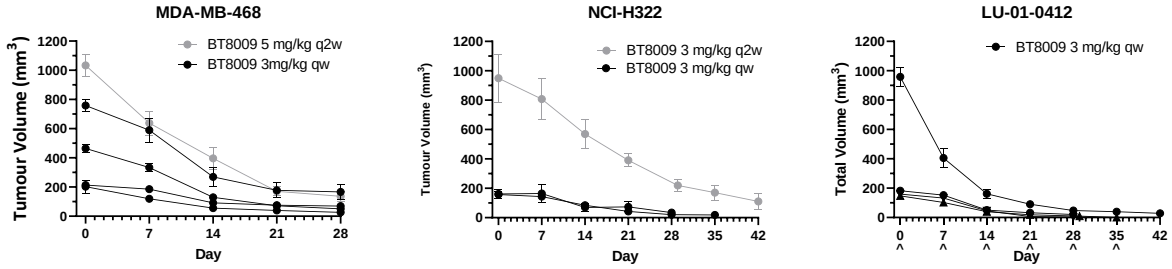
Supplementary Figure 1: Plasma concentration-time curves for BT8009 and MMAE following IV dosing of BT8009: (L-R) 1 mg/kg in mouse and rat (n=3, PK data), and 1.25 mg/kg in NHP (n=5, TK data), errors signify standard deviation. Note higher MMAE levels in mouse likely due to Ces1c activity.



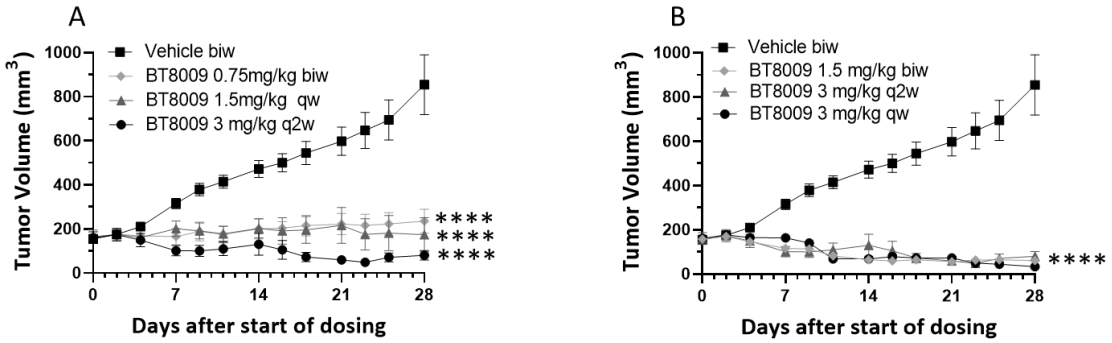
Cell line	MMAE sensitivity (nM)	FACS Nectin-4 ABS
A549	0.49	Not Detected
HT1376	1.28	19,200±2030
NCI-H292	0.30	5520 ± 2580
MDA-MB-468	0.19	18,100 ±1540

Supplementary Figure 2; **A-D)** Relationship between BT8009 efficacy in CDX models and Nectin-4 expression assessed by IHC, Nectin-4 expression shown by brown staining predominately on cell membrane; **E)** Cathepsin B secretion from cell lines *in vitro*; **F)** *In vitro* sensitivity to MMAE and extracellular Nectin-4 expression (ABS -antigen binding sites/cell, assessed by FACS) in these cell lines.

Animals were dosed with 3 mg/kg BT8009 or vehicle qw. Error bars indicate SEM of n=3-5 for xenograft studies. Day 14 data was analyzed by unpaired t-test with Welch's correction, comparing drug with vehicle treatment.



Supplementary Figure 3; Tumor regression curves in response to BT8009 from multiple studies. BT8009 delivers rapid tumor regression irrespective of the starting tumor size, indicative of good penetration throughout the tumor. Error bars indicate SEM of n=3-5.



Supplementary Figure 4; BT8009 shows efficacy in a range of dosing regimens in NCI-H322 xenografts, **A)** Dose equivalences of 0.75 mg/kg biw, **B)** Dose response to 1.5 and 3 mg/kg with different dosing intervals show equivalent efficacy. Tumor volumes are shown as mean +/- standard error of the mean (n=3-5) and statistical analysis performed with Ordinary one-way ANOVA with Tukey's post hoc test for multiple comparisons **** $p<0.0001$. There were no significant differences between drug treated groups.