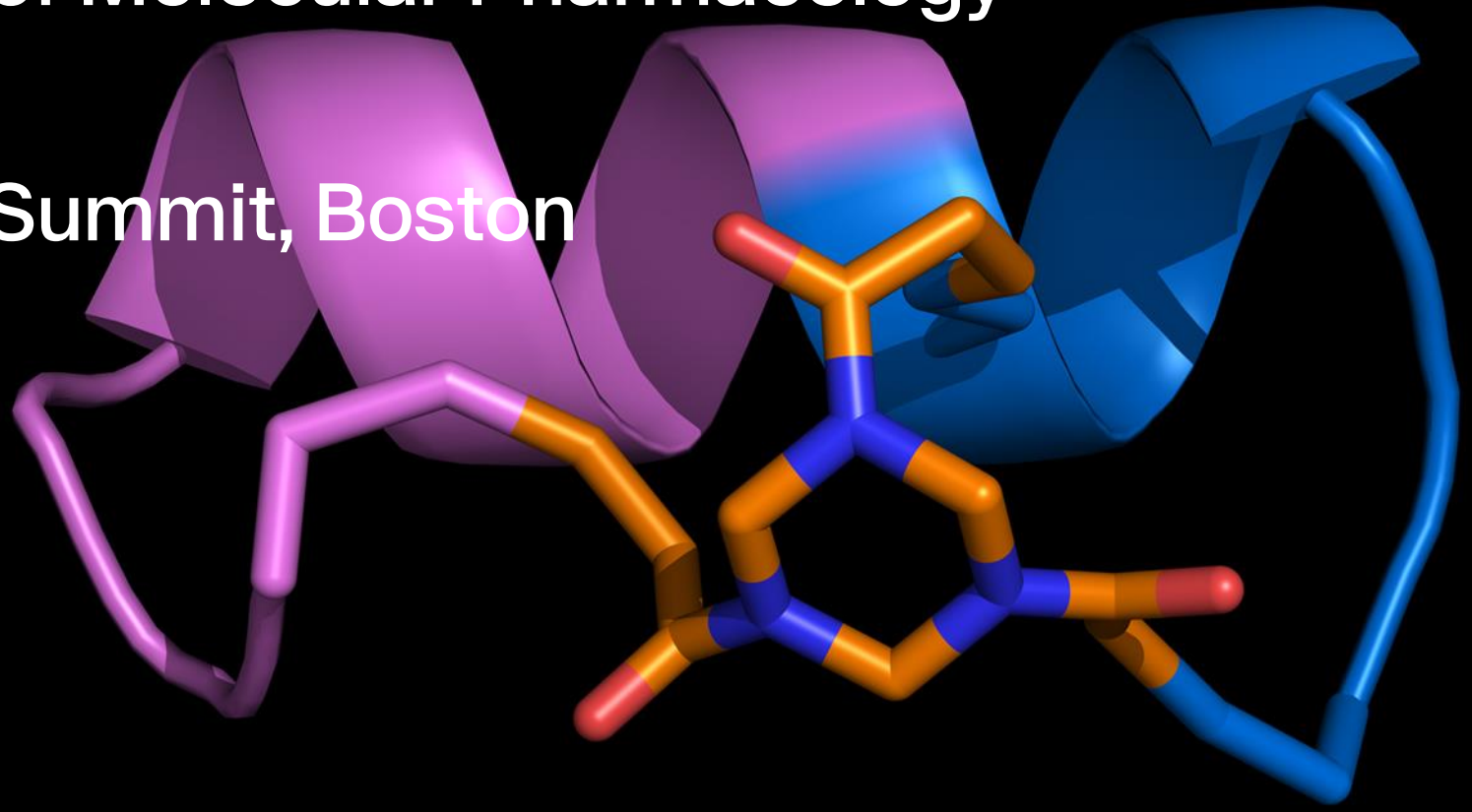
Bicycle Toxin Conjugates to Target Solid Tumors

Steve Ludbrook, Director of Molecular Pharmacology

3rd ADC Target Selection Summit, Boston



Bicycle®

Forward-looking statements

This presentation may contain forward-looking statements made pursuant to the safe harbor provisions of the Private Securities Litigation Reform Act of 1995. These statements may be identified by words such as “aims,” “anticipates,” “believes,” “could,” “estimates,” “expects,” “forecasts”, “goal,” “intends,” “may” “plans,” “possible,” “potential,” “seeks,” “will,” and variations of these words or similar expressions that are intended to identify forward-looking statements. All statements other than statements of historical facts contained in this presentation are forward-looking statements, including statements regarding: our future business performance, conditions, plans, prospects, trends or strategies and other business matters; our current and prospective product candidates, planned clinical trials and preclinical activities, the potential of Bicycle’s technology to improve on existing modalities and be used to develop therapeutics for targets where other modalities have failed or for new targets not accessed via existing modalities, and the timing and success of our development of our anticipated product candidates.

Forward-looking statements are neither historical facts nor assurances of future performance. Instead, they are based on our current beliefs, expectations and assumptions regarding the future of our business, future plans and strategies, our development plans, our preclinical and clinical results, our plans to initiate clinical trials and the designs of the planned trials and other future conditions, and are subject to a number of risks and uncertainties that could cause actual results to differ materially and adversely from those set forth in or implied by such forward-looking statements. These risks and uncertainties include, but are not limited to, the risk that any one or more of our product candidates will not be successfully developed or commercialized, the risk of cessation or delay of any ongoing or planned clinical trials, the risk that we may not realize the intended benefits of our technology, including that we may not identify and develop additional product candidates for our pipeline, the risk that our product candidates or procedures in connection with the administration thereof will not have the safety or efficacy profile that we anticipate, the risk that prior results will not be replicated or will not continue in ongoing or future studies or trials, the risk that we will be unable to obtain and maintain regulatory approval for our product candidates, the risk that the size and potential of the market for our product candidates will not materialize as expected, risks associated with our dependence on third-parties, risks regarding the accuracy of our estimates of expenses, risks relating to our capital requirements and needs for additional financing, and risks relating to our ability to obtain and maintain intellectual property protection for our product candidates. For a discussion of these and other risks and uncertainties, and other important factors, any of which could cause our actual results to differ from those contained in the forward-looking statements, see the section entitled “Risk Factors” in our Quarterly Report on Form 10-Q, filed with the Securities and Exchange Commission (the “SEC”) on November 2, 2023, as well as in other filings we may make with the SEC in the future, as well as discussions of potential risks, uncertainties and other important factors in our subsequent filings with the SEC. New risks and uncertainties may emerge from time to time, and it is not possible to predict all risks and uncertainties. Except as required by applicable law, we do not plan to publicly update or revise any forward-looking statements contained herein, whether as a result of any new information, future events, changed circumstances or otherwise.

This presentation does not constitute an offer to sell or a solicitation of an offer to buy securities, nor shall there be any sale of any securities in any state or jurisdiction in which such offer, solicitation or sale would be unlawful prior to registration or qualification under the securities laws of any such state or jurisdiction.

Presentation Overview

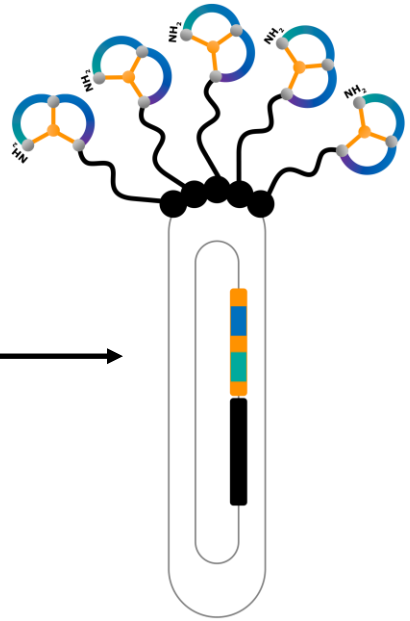
- Background to *Bicycles* as fully synthetic and readily conjugated precision guided targeting systems
- EphA2 Targeting with a Bicycle Toxin Conjugate (BTC™) as a potential solution to an ADC-intractable target
- Opportunities in the target selection landscape for Bicycle Toxin conjugates

Bicycles are a new therapeutic modality – bicyclic peptides

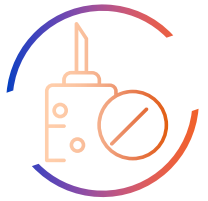
Short linear peptide

+

Scaffold
Chemical modification with scaffold



Diverse *Bicycle®* phage libraries (>10^{20})

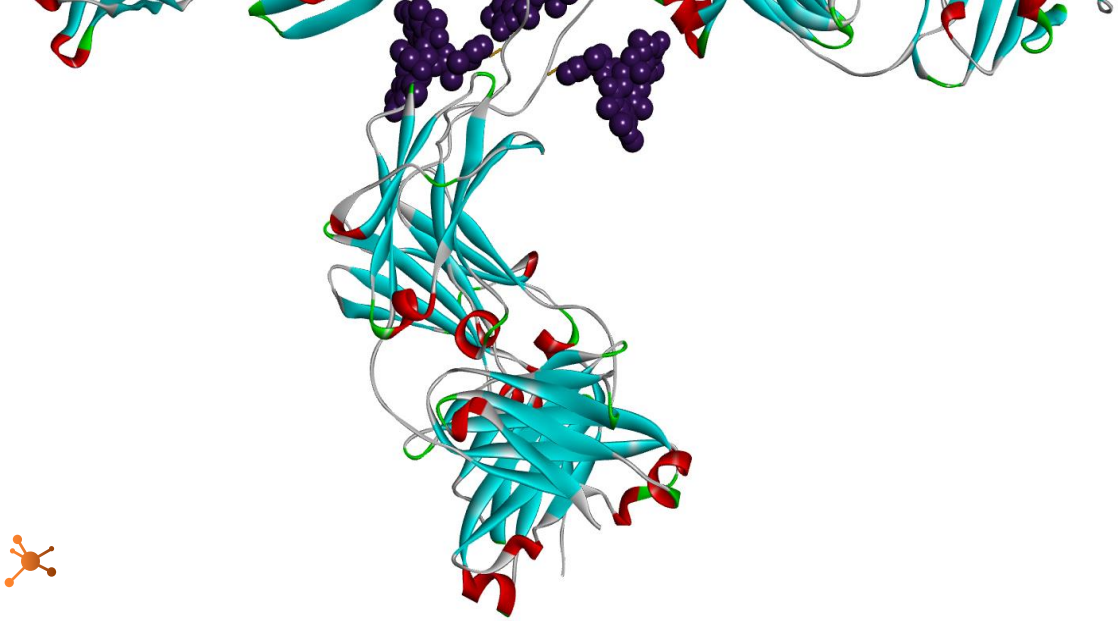


Powerful/versatile platform
- Immense combinatorial potential
- Combines advantages of both small molecules and antibodies

Multi-formats
- Monomeric Bicycles
- Bicycle Toxin Conjugates (BTCs)
- Tumor-targeted immune cell agonists (*Bicycle* TICAs)

Robust patent protection

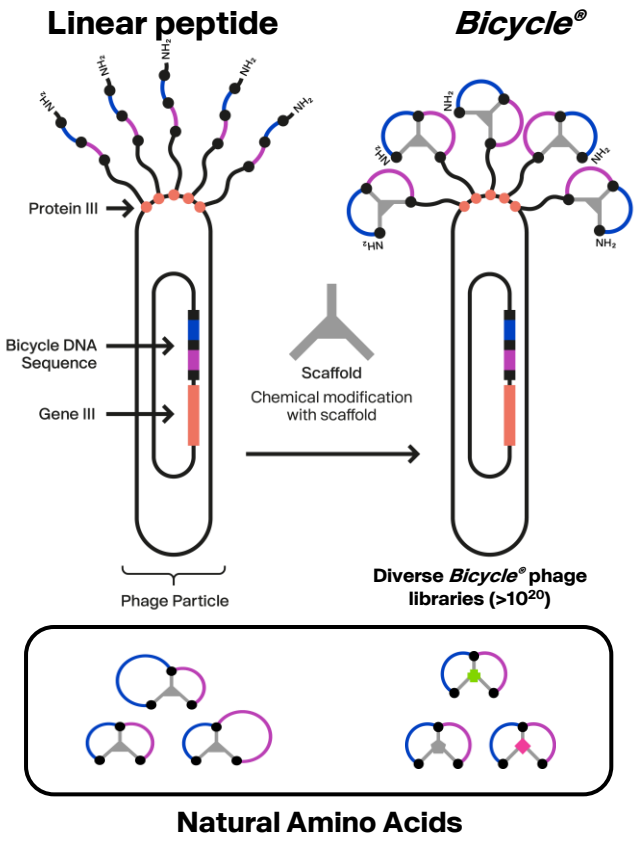
Bicycles are designed to combine the advantages of both small molecules and antibodies



	Bicycle®	Small molecule	Antibody
Small size	**Yes – 1.5-2kDa**	Yes – <0.8kDa	No – >150kDA
Specificity	**High**	Low	Multiple
Chemical synthesis (NCEs)	**Yes**	Yes	No
Rapid tissue penetration	**Yes**	Yes	No
Complex protein targets druggable	**Yes**	Limited	Yes
Route of elimination	**Renal**	Liver	Liver

Bicycle® platform delivers a toolkit of building blocks to create novel medicines

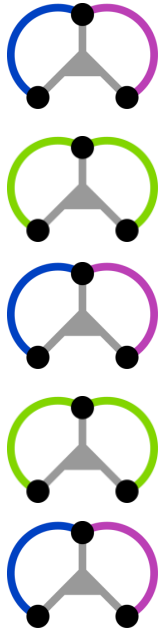
Bicycle® Phage Display - Discovery



Peptide & Medicinal Chemistry

Optimize *Bicycle*® *monomers*

Non-natural Amino Acids

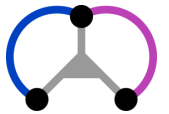


Tumor Targeting and Effector *Bicycles*

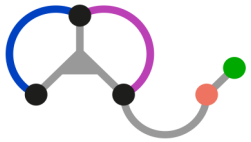
Build and Optimize Therapeutic *Bicycles*

Easy conjugation of Linkers and Payloads

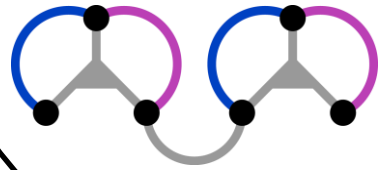
Potential *Bicycle*® Medicines



Monomeric *Bicycles*

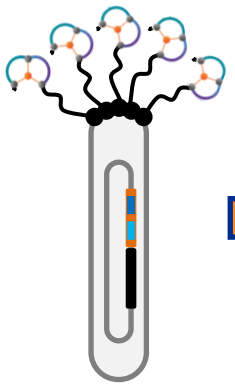


Targeted Drug Conjugates

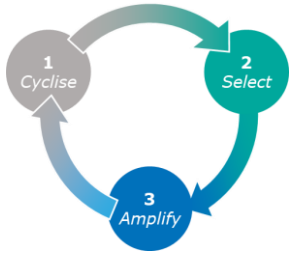


Targeted/ Multi-specific *Bicycles*

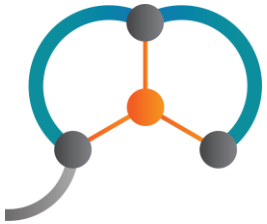
Chemical optimization of a high affinity EphA2 targeting *Bicycle*® with improved properties



2 scaffolds x 10 libraries,
➔ 8 peptide families

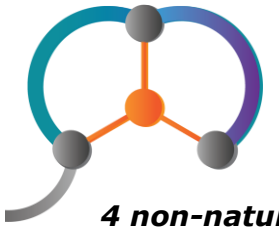


BCY6014

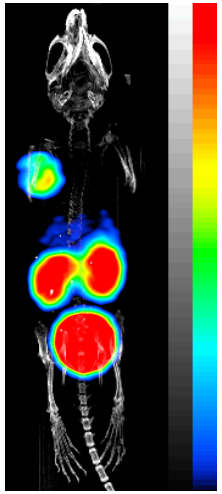


Chemistry Optimisation <40 analogs

BCY6099



4 non-natural amino acids



PET imaging of HT-1080 xenograft at 60 minutes

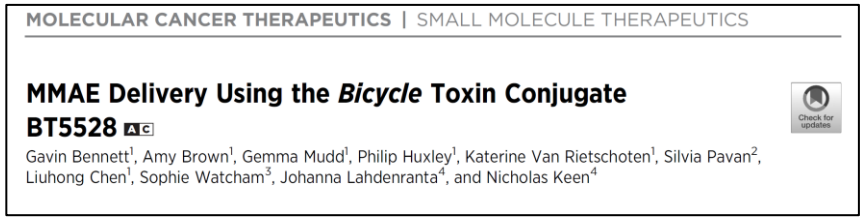
Modifications (*) to increase polarity, stability and/or potency

BCY6014 Ki = 16 nM – early *Bicycle*

A	R	D	C	P	L	V	N	P	L	C	L	H	P	G	W	T	C	A

BCY6099 Ki = 3 nM – newly optimised *Bicycle*

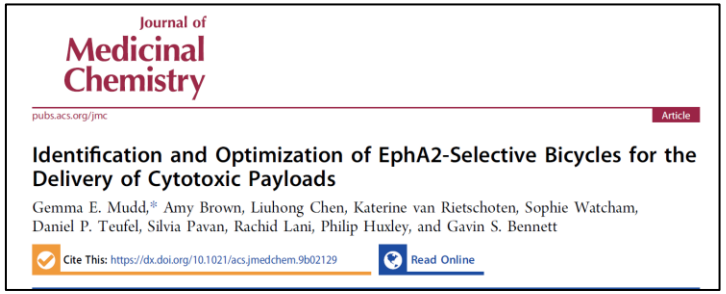
A	hR	D	C	hyP	L	V	N	P	L	C	L	H	P	dD	W	hR	C	A
	*			*										*		*		



MOLECULAR CANCER THERAPEUTICS | SMALL MOLECULE THERAPEUTICS

MMAE Delivery Using the *Bicycle* Toxin Conjugate BT5528 AC

Gavin Bennett[1], Amy Brown[1], Gemma Mudd[1], Philip Huxley[1], Katerine Van Rietschoten[1], Silvia Pavan[2], Liuhong Chen[1], Sophie Watcham[3], Johanna Lahdenranta[4], and Nicholas Keen[4]



Journal of Medicinal Chemistry

pubs.acs.org/jmc Article

Identification and Optimization of EphA2-Selective Bicycles for the Delivery of Cytotoxic Payloads

Gemma E. Mudd,* Amy Brown, Liuhong Chen, Katerine van Rietschoten, Sophie Watcham, Daniel P. Teufel, Silvia Pavan, Rachid Lani, Philip Huxley, and Gavin S. Bennett

Cite This: https://dx.doi.org/10.1021/acs.jmedchem.9b02129 Read Online

Potential of Bicycles as precision guided therapeutics

Bicycles rapidly penetrate tumour, eliminated through renal route

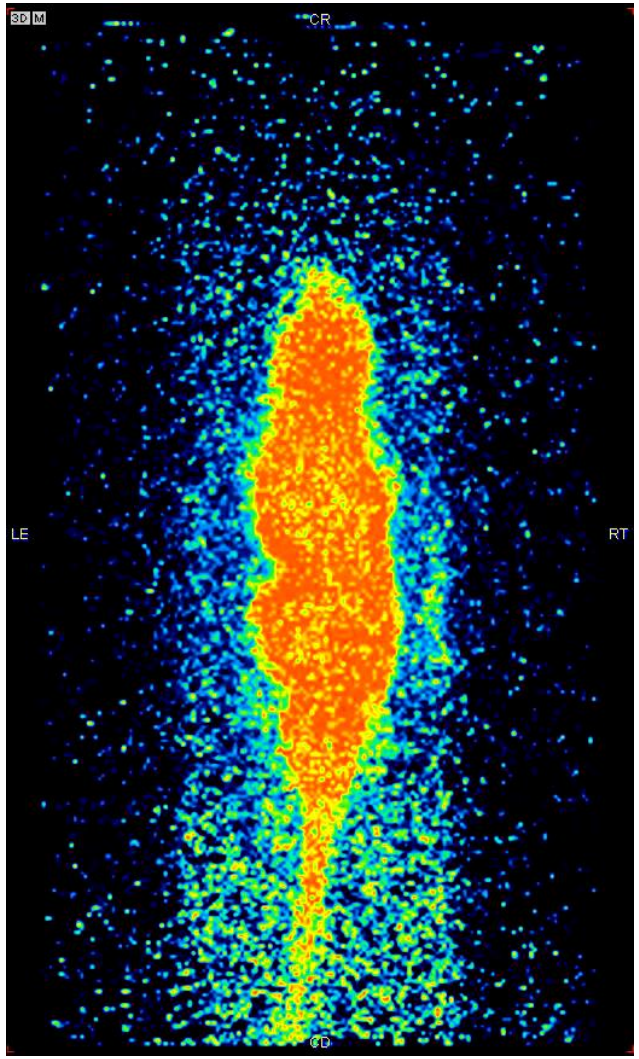
Short systemic exposure & tumour retention

Activity at site of action with reduced body burden

Can be used to deliver key pharmacological activity for solid tumours:

Cytotoxic payloads

Immune-oncology



DKTK German Cancer Consortium

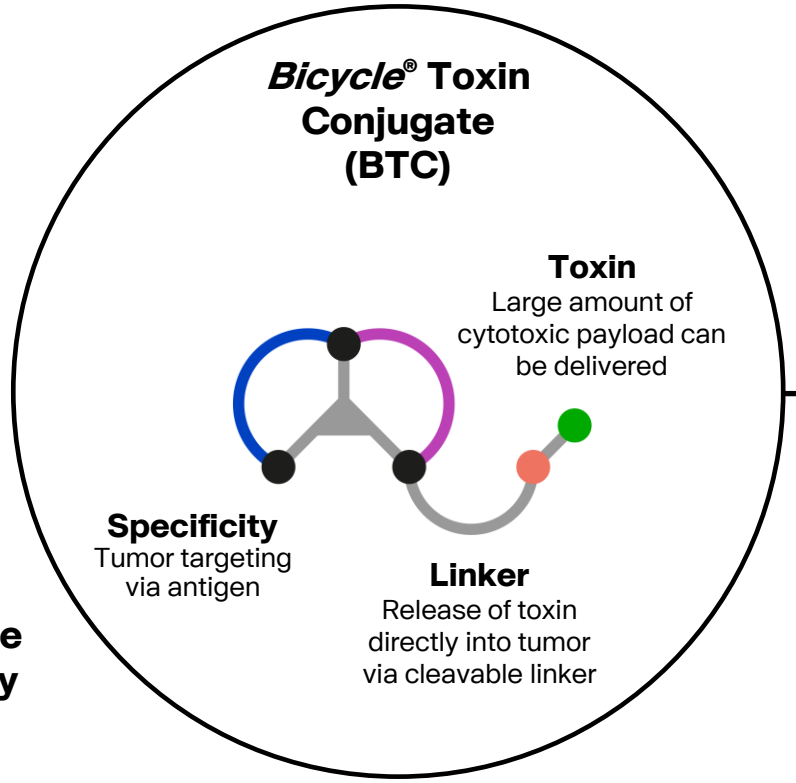
PET imaging of Bicycle-radioisotope conjugate, 0-60min post-injection

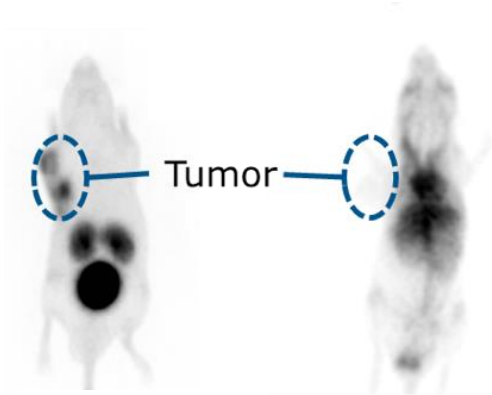
Imaging conducted in collaboration with
Prof. Dr. Matthias Eder
Dr. Ann-Christin Eder
Mohamed El Fakiri

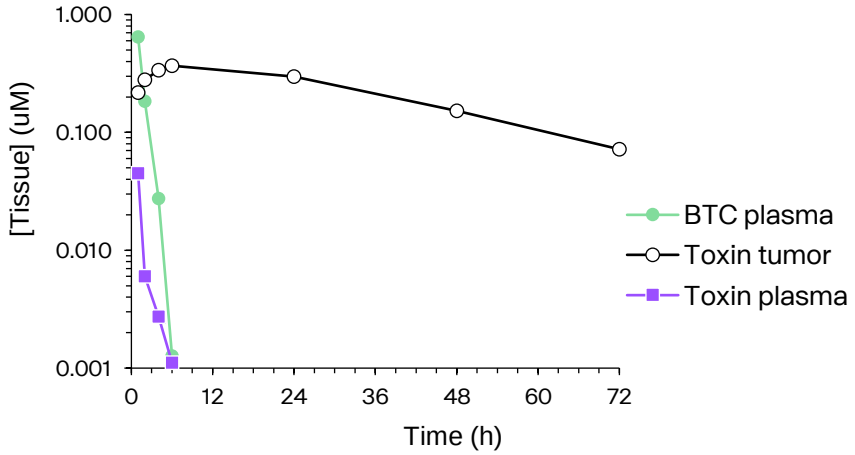
BTCs – preclinical data indicates higher potency and specificity with fewer side effects than ADCs

- **MW of 1.5-2kDa**
- **50-100x smaller than antibodies**

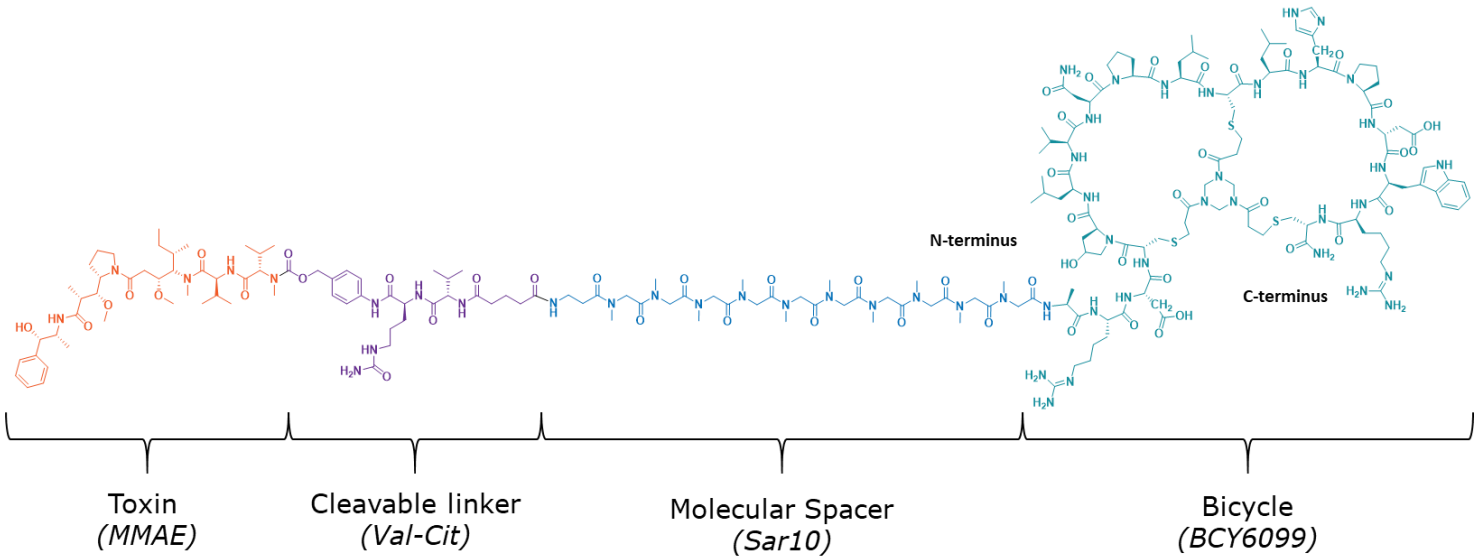
- **High selectivity**
- **Allows more potent toxin to be delivered directly to tumor**

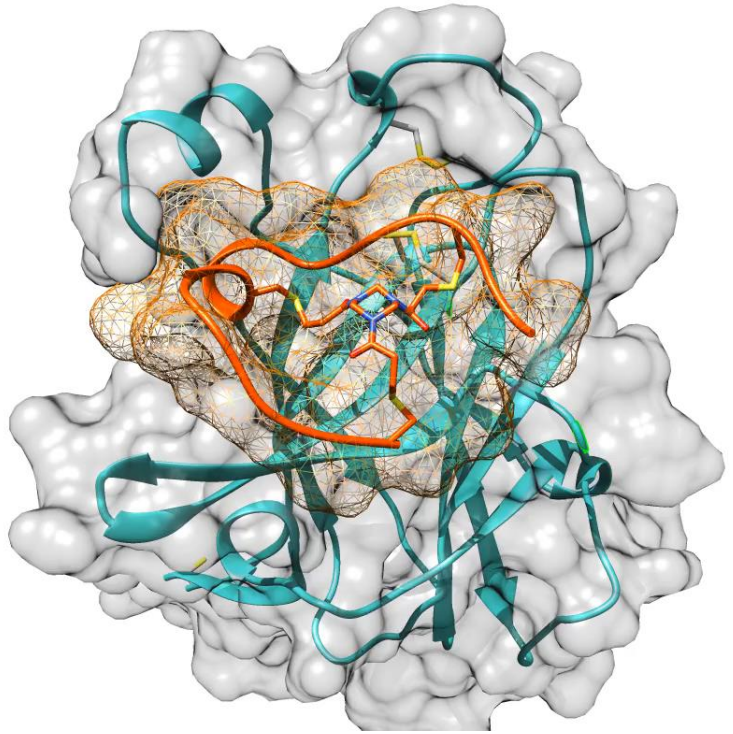




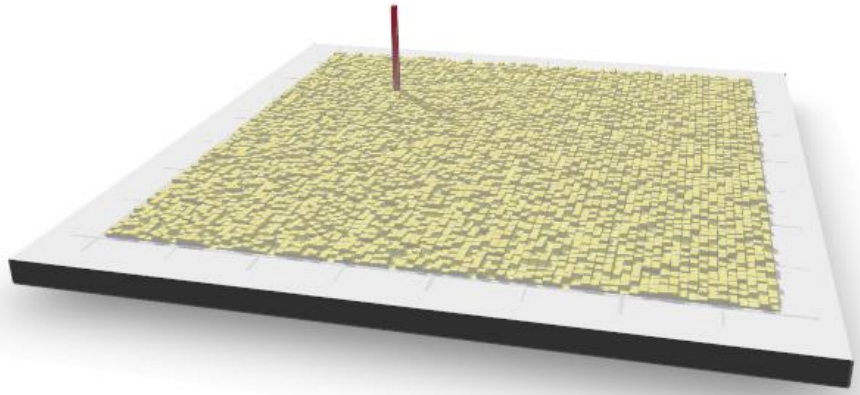


BT5528: structure and profile





BT5528 only binds EphA2



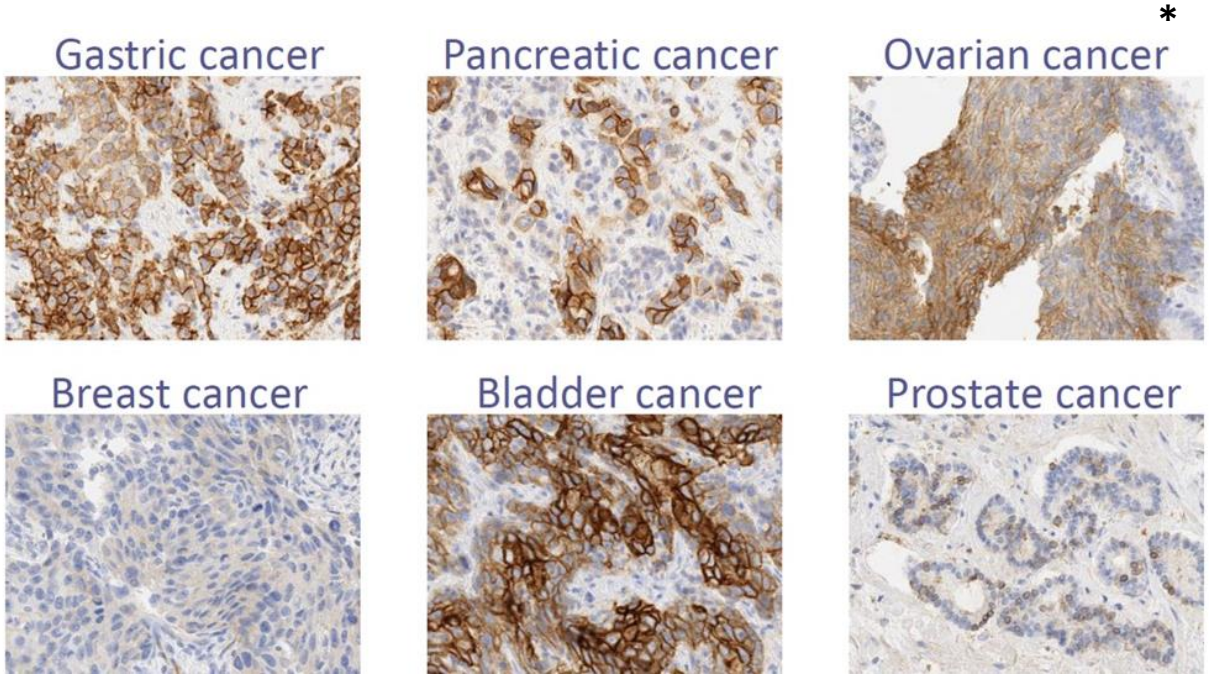
Membrane protein array: no binding of BT5528 @1µM to 5,527 other proteins

High affinity binding to EphA2 protein across species and 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			

EphA2 is a high value target for the treatment of cancer

- EphA2, a member of Eph subfamily of receptor tyrosine kinases
- Regulates cell migration, adhesion, proliferation and differentiation
- Highly expressed in many human cancers and correlates with tumor progression
  - Ovarian
  - Urothelial
  - NSCLC
  - Head & Neck
  - Gastric
  - TNBC



*

*Kamoun, et al, Nanoliposomal Targeting of Ephrin Receptor A2 (EphA2): Clinical Translation, Merrimack Pharmaceuticals

Multiple approaches targeting EphA2-expressing tumors have failed

- MEDI-547 (MedImmune) ADC: halted following first dose-cohort coagulopathy[1]
  - ATRC-301 ADC: stopped Nov22, bleeding in NHP tox
- DS-8895a (Daiichi) antibody: limited efficacy in EphA2+ gastric and esophageal cancer, significant infusion reactions. Discontinued because of poor risk-benefit profile & low tumor uptake, consistent with lack of substantial tumor inhibition[2]
- MM-310 (Merrimack) antibody-targeted nanoliposome: terminated - “unable to reach optimal therapeutic index”, due to cumulative peripheral neuropathy[3]

Invest New Drugs (2013) 31:77–84
DOI 10.1007/s10637-012-9801-2

PHASE I STUDIES

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

Investigational New Drugs
https://doi.org/10.1007/s10637-022-01237-3

PHASE I STUDIES

A phase 1 safety and bioimaging trial of antibody DS-8895a against EphA2 in patients with advanced or metastatic EphA2 positive cancers

Hui K. Gan[1,2,3] · Sagun Parakh[1,2,3] · F. T. Lee[1] · Niall C. Tebbutt[3] · Malaka Ameratunga[3] · Sze Ting Lee[1,2,4,5] · Graeme J. O'Keefe[1,4] · Sylvia J. Gong[1,4] · Christine Vanrenen[3] · Jaren Caine[3] · Mara Giovannetti[6] · Carmel Murone[1] · Fiona E. Scott[1,2] · Nancy Guo[1] · Ingrid J. G. Burvenich[1,2] · Cameron Paine[4] · Mary J. Macri[6] · Masakatsu Kotsuma[7] · Giorgio Senaldi[7] · Ralph Venhaus[6] · Andrew M. Scott[1,2,4,5]

Clinical Trial · J Immunother Cancer. 2019 Aug 14;7(1):219. doi: 10.1186/s40425-019-0679-9.

Safety, tolerability, pharmacokinetics, and pharmacodynamics of the afucosylated, humanized anti-EPHA2 antibody DS-8895a: a first-in-human phase I dose escalation and dose expansion study in patients with advanced solid tumors

Kohei Shitara [1], Taroh Satoh [2], Satoru Iwasa [3], Kensei Yamaguchi [4], Kei Muro [5], Yoshito Komatsu [6], Tomohiro Nishina [7], Taito Esaki [8], Jun Hasegawa [9], Yasuyuki Kakurai [9], Emi Kamiyama [9], Tomoko Nakata [9], Kota Nakamura [9], Hayato Sakaki [9], Ichinosuke Hyodo [10]

merrimack

Merrimack Discontinues Development of MM-310

April 4, 2019

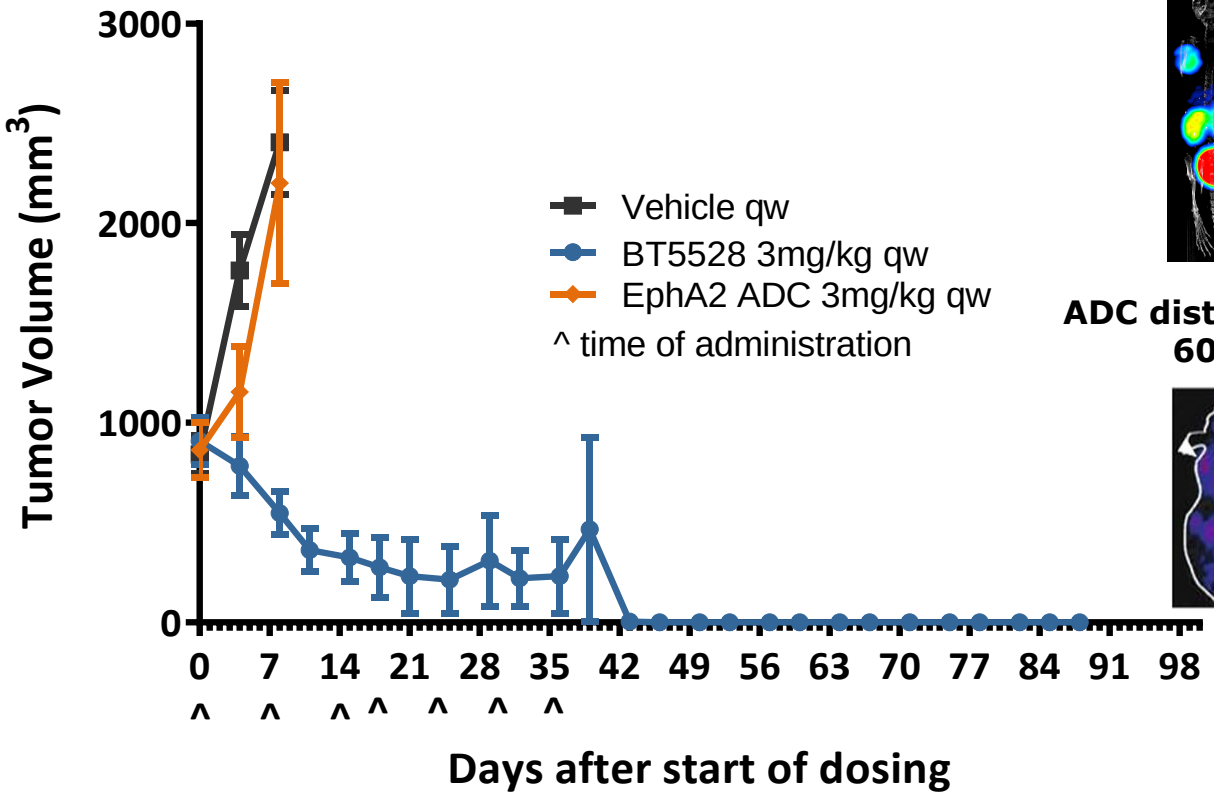
-- Safety update shows Phase 1 study unable to reach optimal therapeutic index for MM-310 due to continued observation of cumulative peripheral neuropathy --

-- Company expects to reduce workforce reflective of narrowed preclinical development pipeline; continues to prudently advance programs while completing the assessment of its strategic alternatives --

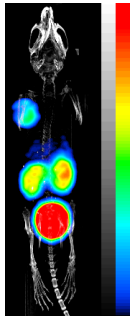
1. Annunziata et al, Invest New Drugs. 2013 Feb;31(1):77-84
2. Shitara et al, Journal for ImmunoTherapy of Cancer. 2019 7: 219-230 (Ph1 study); Gan et al, Invest New Drugs. 2022 40(4) 747-755
3. Merrimack Pharmaceuticals Inc., press release April 4, 2019

BT5528: activity in difficult-to-treat xenograft models

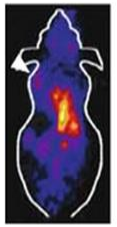
Superior activity to EphA2 ADC in large NSCLC xenograft models



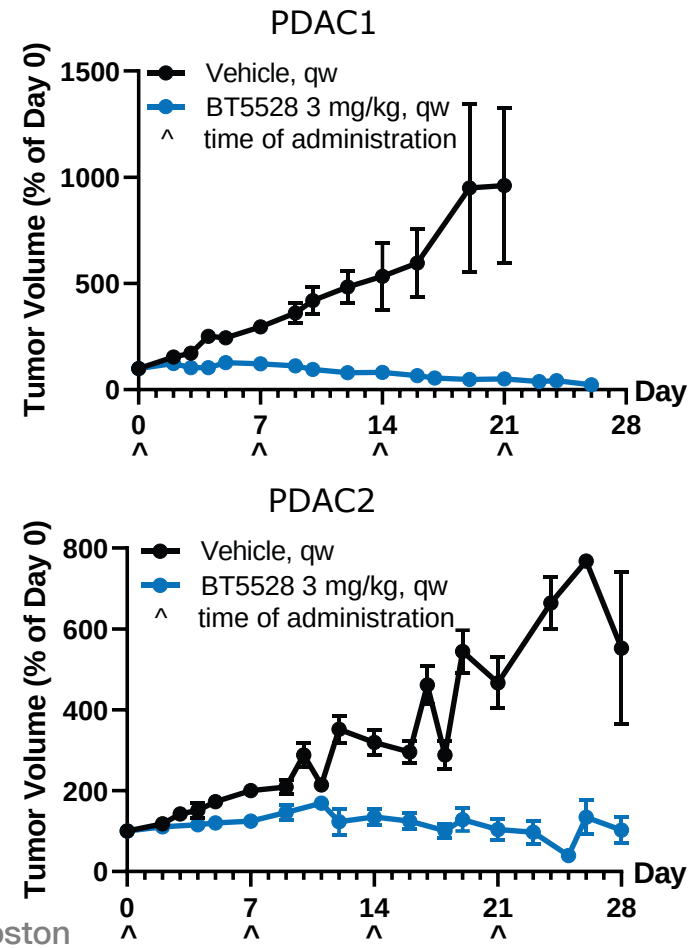
***Bicycle* distribution at 60 min**



ADC distribution at 60 min



Activity in pancreatic xenograft models



BT5528 offers a differentiated approach to EphA2

EphA2 has been viewed as a ”difficult” target

Clinical trial of EphA2-targeting ADC, MEDI-547, terminated after bleeding & liver effects seen at starting dose

BT5528 PK/PD profile offers pre-clinical anti-tumor activity without prolonged vascular exposure

Invest New Drugs (2013) 31:77–84
DOI 10.1007/s10637-012-9801-2

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Phase 1, open-label study of MEDI-547 in patients with relapsed or refractory solid tumors

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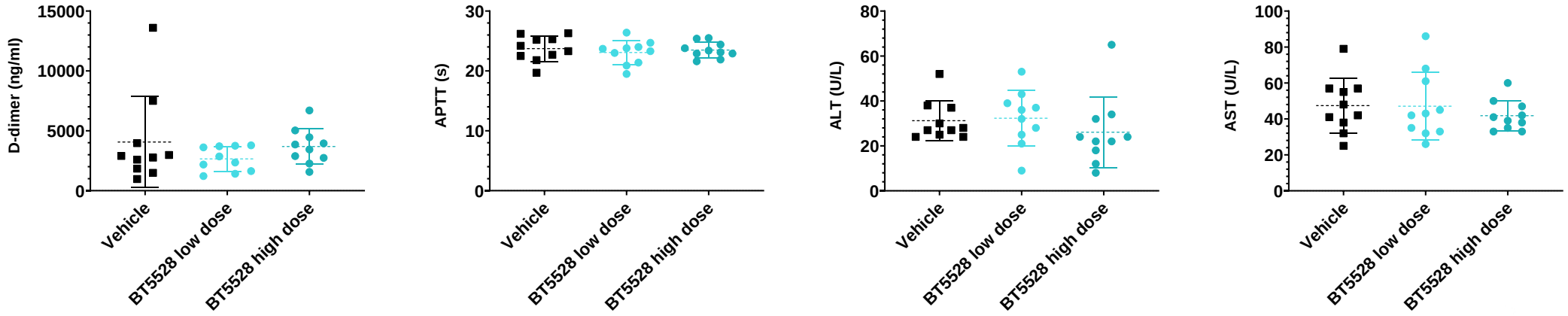
Treatment related adverse events	# events (% of patients) n of total
ALT increased	3 (50) 3/6
Haemorrhage	6 (83.3) 5/6

BT5528 toxicology study



- No signs of coagulopathy or bleeding in preclinical species
- No evidence of abnormal liver function
  - Dosing to toxin equivalent doses >100x dose of MEDI-547 used in patients

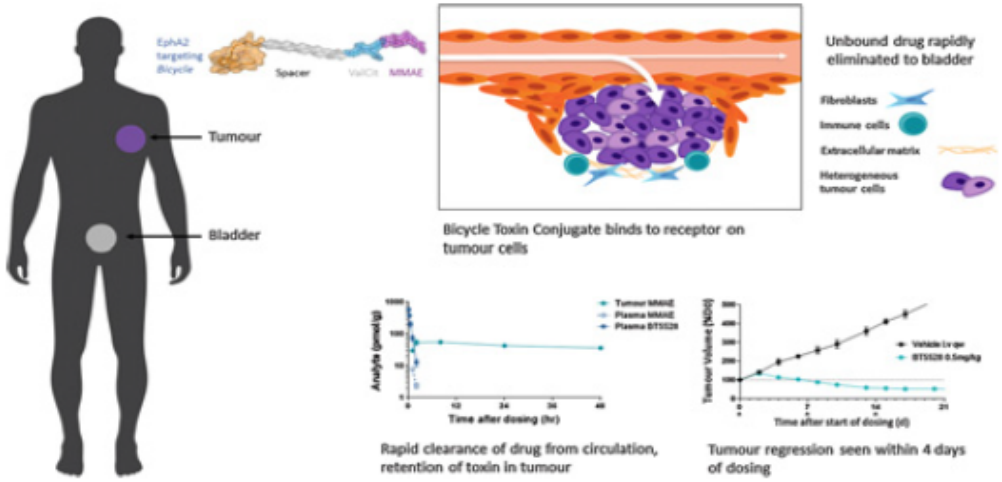
MMAE Delivery Using the *Bicycle* Toxin Conjugate BT5528 AC

Gavin Bennett[1], Amy Brown[1], Gemma Mudd[1], Philip Huxley[1], Katerine Van Rietschoten[1], Silvia Pavan[2], Liuhong Chen[1], Sophie Watcham[3], Johanna Lahdenranta[4], and Nicholas Keen[4]

ABSTRACT

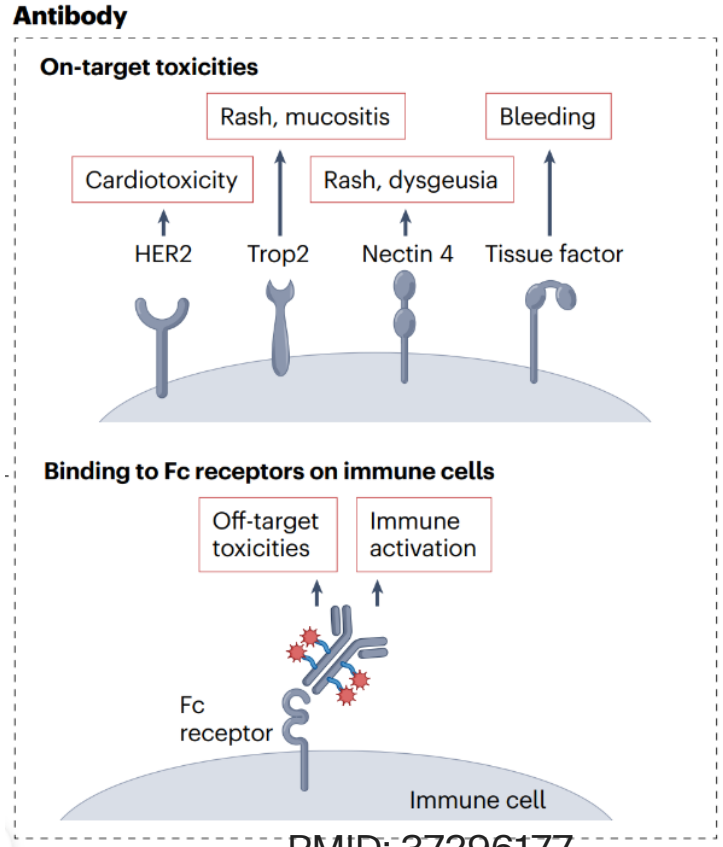
The EphA2 receptor is found at high levels in tumors and low levels in normal tissue and high EphA2 expression in biopsies is a predictor of poor outcome in patients. Drug discovery groups have therefore sought to develop EphA2-based therapies using small molecule, peptide, and nanoparticle-based approaches (1–3). However, until now only EphA2-targeting antibody–drug conjugates (ADC) have entered clinical development. For example, MEDI-547 is an EphA2-targeting ADC that displayed encouraging antitumor activity in preclinical models and progressed to phase I clinical testing in man. Here we describe the development of BT5528, a bicyclic peptide ("*Bicycle*") conjugated to the auristatin derivative maleimidocaproyl-monomethyl auristatin E to generate the *Bicycle* toxin conjugate BT5528. The report compares and contrasts the Pharmacokinetics (PK) characteristics of antibody and *Bicycle*-based targeting systems and discusses how the PK and payload characteristics of different delivery systems impact the efficacy—toxicity trade off which is key to the development of successful cancer therapies. We show that BT5528 gives rise to rapid update into tumors and fast renal elimination followed by persistent toxin levels in tumors without prolonged exposure of parent drug in the vasculature. This fast in, fast out kinetics gave rise to more favorable toxicology findings in rats and monkeys than were observed with MEDI-547 in preclinical and clinical studies.

Graphical Abstract: http://mct.aacrjournals.org/content/molcanther/19/7/1385/F1.large.jpg.



Bicycle Toxin Conjugate Target Selection Opportunities

- Improvements on existing validated ADC approaches by improvement of therapeutic index : rapid systemic clearance & rapid tumor penetration limits target dependent & independent toxicity while preserving anti-tumor activity in preclinical models



PMID: 37296177

- Short PK, coupled with rapid tumor penetration :
  - Lower target-independent toxin release in non-tumor tissues
  - Reduced target-dependent toxicity in non-tumor tissues (unbound target recycling or synthesis)
  - Retained tumor-directed activity via rapid penetration
- No Fc-dependent uptake
- Flexibility on phys chem properties (e.g. charge) to limit target-independent pinocytosis

Bicycle Toxin Conjugate Target Selection Opportunities

- Improvements on existing validated ADC targets by improvement of therapeutic index : rapid systemic clearance & rapid tumor penetration limits target dependent & independent toxicity while preserving anti-tumor activity in preclinical models

- Limitation of toxicity could enable:
  - Improvements on existing validated ADC approaches
  - Salvage of failed toxic ADC approaches
  - Novel target selection approaches outside the ADC space
  - Opportunities for alternative payloads for all approaches above

Bicycle Toxin Conjugate Target Selection Opportunities

- ADC Target Selection approaches widespread, often using RNA expression databases (e.g. TCGA & GTEX), coupled with cell surface protein expression validation (e.g. Protein atlas) & literature

Research Article

Data-Driven Discovery of Molecular Targets for Antibody-Drug Conjugates in Cancer Treatment PMID: 33490264

	Breast cancer	Carcinoid	Cervical cancer	Colorectal cance	Endometrial can	Glioma	Head and neck c	Liver cancer	Lung cancer	Lymphoma	Melanoma	Ovarian cancer	Pancreatic cance	Prostate cancer	Renal cancer	Skin cancer	Stomach cancer	Testis cancer	Thyroid cancer	Urothelial cance
ABCB1	0.0	75.0	0.0	141.7	0.0	41.7	0.0	160.0	25.0	8.3	33.3	0.0	54.5	0.0	16.7	0.0	41.7	27.3	0.0	41.7
ANO7	100.0	0.0	8.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	245.5	0.0	0.0	0.0	0.0	100.0	18.2
AQP5	25.0	0.0	25.0	0.0	216.7	0.0	0.0	33.3	45.5	0.0	8.3	66.7	162.5	0.0	0.0	0.0	33.3	0.0	0.0	9.1
ATP2B2	118.2	166.7	90.9	130.0	163.6	190.0	100.0	150.0	100.0	91.7	100.0	100.0	66.7	63.6	41.7	77.8	90.0	100.0	175.0	109.1
CD19	0.0	25.0	0.0	0.0	0.0	0.0	0.0	10.0	0.0	190.9	16.7	0.0	10.0	18.2	0.0	0.0	0.0	0.0	0.0	0.0
CD276	175.0	50.0	150.0	116.7	230.0	183.3	225.0	133.3	183.3	25.0	208.3	158.3	166.7	225.0	50.0	250.0	160.0	100.0	125.0	183.3
CD79B	54.5	0.0	41.7	18.2	81.8	0.0	100.0	16.7	8.3	216.7	25.0	45.5	91.7	141.7	8.3	0.0	54.5	16.7	25.0	8.3
CDCP1	83.3	100.0	83.3	191.7	150.0	16.7	125.0	83.3	54.5	0.0	36.4	133.3	150.0	63.6	50.0	81.8	136.4	0.0	100.0	127.3
CNR1	36.4	75.0	25.0	118.2	91.7	0.0	125.0	50.0	58.3	0.0	8.3	91.7	72.7	41.7	0.0	18.2	90.9	33.3	225.0	133.3
ERBB2	154.5	0.0	10.0	72.7	41.7	0.0	75.0	58.3	27.3	0.0	9.1	54.5	54.5	0.0	16.7	33.3	33.3	22.2	0.0	100.0
GPBAR1	145.5	0.0	58.3	9.1	50.0	0.0	33.3	0.0	50.0	0.0	0.0	66.7	122.2	0.0	0.0	18.2	41.7	0.0	175.0	122.2
HTR2B	250.0	100.0	127.3	163.6	125.0	0.0	125.0	50.0	125.0	0.0	0.0	108.3	190.0	36.4	36.4	8.3	154.5	25.0	75.0	166.7
ITGA3	83.3	100.0	175.0	141.7	200.0	41.7	175.0	127.3	200.0	0.0	141.7	227.3	245.5	30.0	208.3	225.0	191.7	25.0	275.0	258.3
MS4A1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.3	291.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MSLN	41.7	0.0	83.3	63.6	75.0	0.0	50.0	60.0	66.7	0.0	0.0	183.3	191.7	0.0	45.5	8.3	100.0	16.7	0.0	8.3
MUC16	10.0	0.0	58.3	0.0	141.7	0.0	0.0	12.5	63.6	0.0	9.1	227.3	41.7	0.0	0.0	0.0	27.3	0.0	50.0	0.0
NECTIN4	83.3	25.0	125.0	90.0	140.0	25.0	25.0	80.0	83.3	9.1	25.0	145.5	66.7	45.5	33.3	100.0	20.0	45.5	150.0	200.0
PCDH7	66.7	125.0	125.0	266.7	127.3	58.3	75.0	145.5	133.3	158.3	218.2	208.3	208.3	50.0	50.0	81.8	200.0	75.0	75.0	90.9
SIT1	8.3	0.0	33.3	125.0	16.7	9.1	0.0	81.8	72.7	218.2	63.6	50.0	145.5	0.0	45.5	0.0	150.0	0.0	66.7	0.0
SLC2A14	63.6	100.0	33.3	0.0	8.3	0.0	50.0	16.7	25.0	0.0	50.0	66.7	16.7	0.0	0.0	66.7	16.7	150.0	100.0	75.0
SLC39A10	258.3	0.0	83.3	70.0	100.0	0.0	75.0	75.0	191.7	0.0	0.0	183.3	208.3	20.0	16.7	16.7	127.3	45.5	275.0	220.0
SLC6A6	111.1	50.0	100.0	177.8	90.9	60.0	100.0	83.3	100.0	0.0	108.3	110.0	130.0	188.9	0.0	90.9	80.0	33.3	100.0	91.7
UPK1B	33.3	25.0	16.7	66.7	150.0	9.1	100.0	8.3	25.0	0.0	0.0	50.0	109.1	16.7	90.9	0.0	18.2	22.2	100.0	181.8

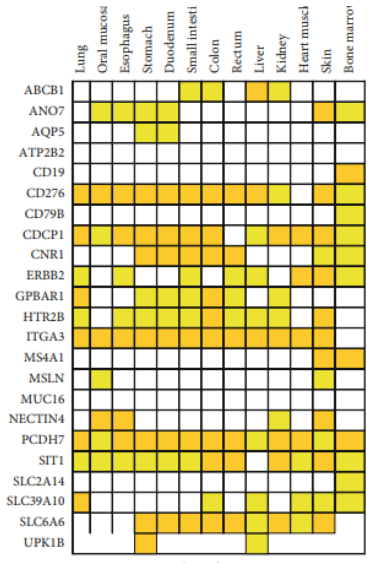


FIGURE 2: A heat map depicting the quasi *H*-score for candidate ADC targets across 20 tumor types.

Opportunities for Bicycle approach:

- Toleration of higher target expression in non-tumor tissue expands the list of potential tumor antigens significantly
- Such targets are likely not viable for ADC approaches due to toxicity via sustained delivery to recycled or neo-synthesized target

Summary

Bicycles offer a potential new modality for oncology therapeutics

- Antibody-like affinity and selectivity in a small molecule
- Chemically synthesized
- Rapid distribution to solid tumors, elimination via renal route
- Bicycle Toxin Conjugates progressing in clinical studies

Emerging safety and efficacy profile creates novel opportunities in oncology

- Improvements on existing validated ADC approaches
- Salvage of tumor targets through improved therapeutic index
- Potential novel opportunities for tumor antigens outside the ADC space due to improved therapeutic index

Thank you

Bicycle®