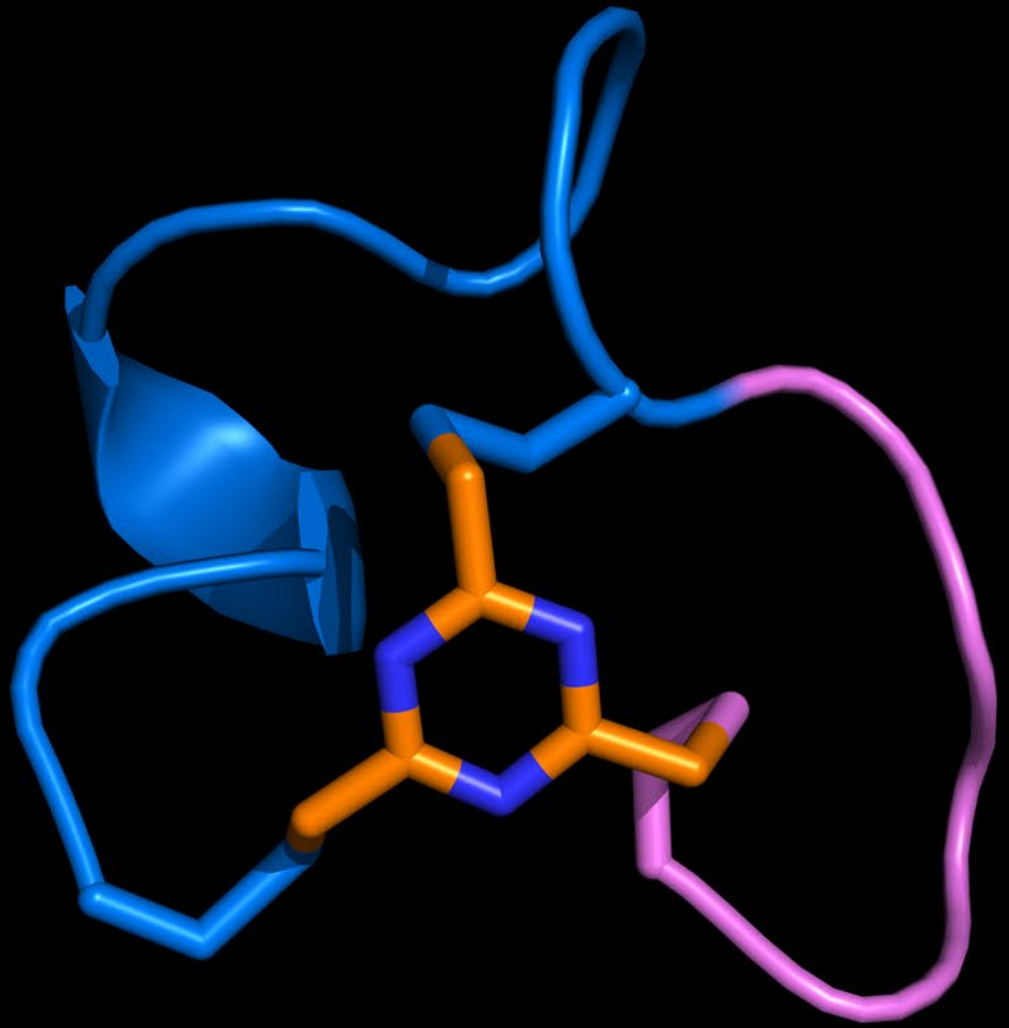
Bicycles for precision guided delivery

Kevin McDonnell
VP, Chemistry

Boulder Peptide Symposium
November 9th, 2022



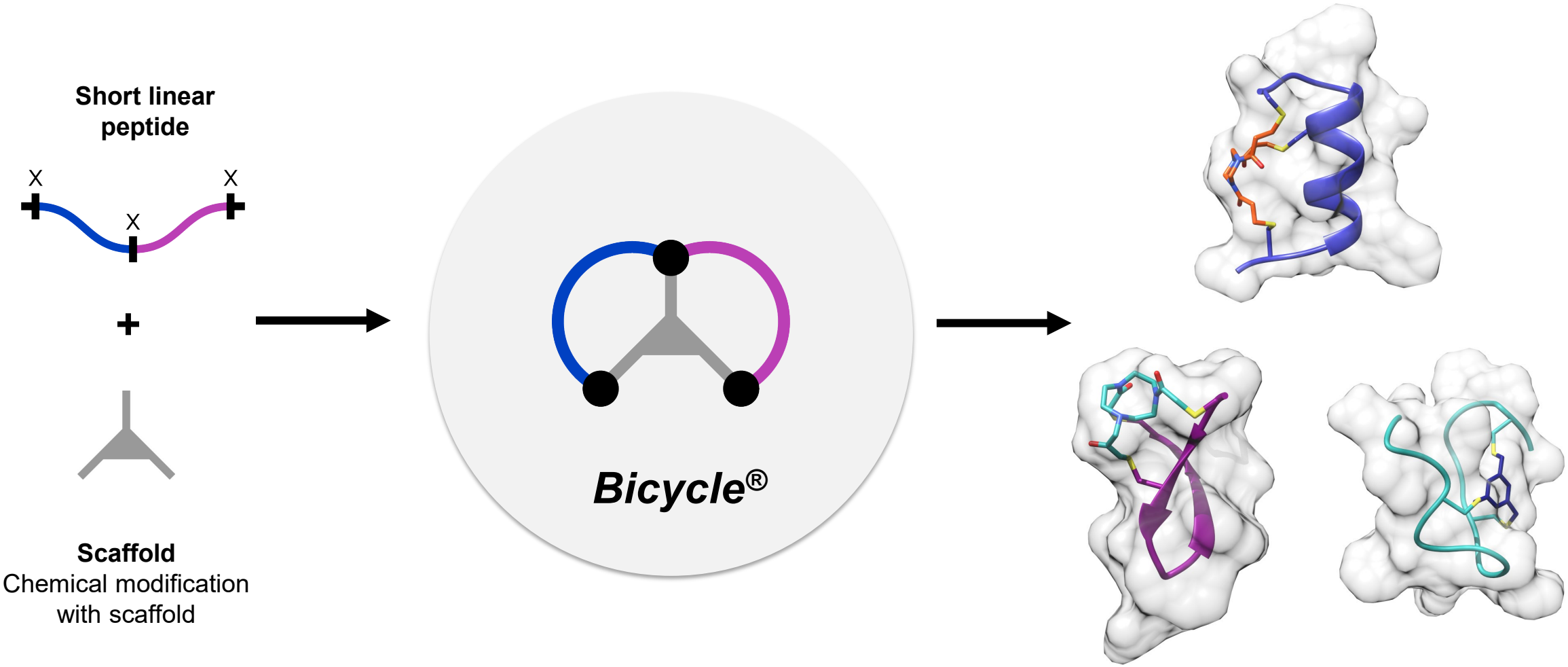
Bicycle®

Forward-looking statement

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 financial or business performance, conditions, plans, prospects, trends or strategies and other financial and business matters; our current and prospective product candidates, planned clinical trials and preclinical activities, current and prospective collaborations 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 we may not maintain our current collaborations or enter into new collaborations in the future, or that we may not realize the intended benefits of these collaborations, 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, 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 on November 3, 2022, 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 Securities and Exchange Commission. 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.

Bicycles are short peptides chemically constrained with a central scaffold that can induce diverse structures

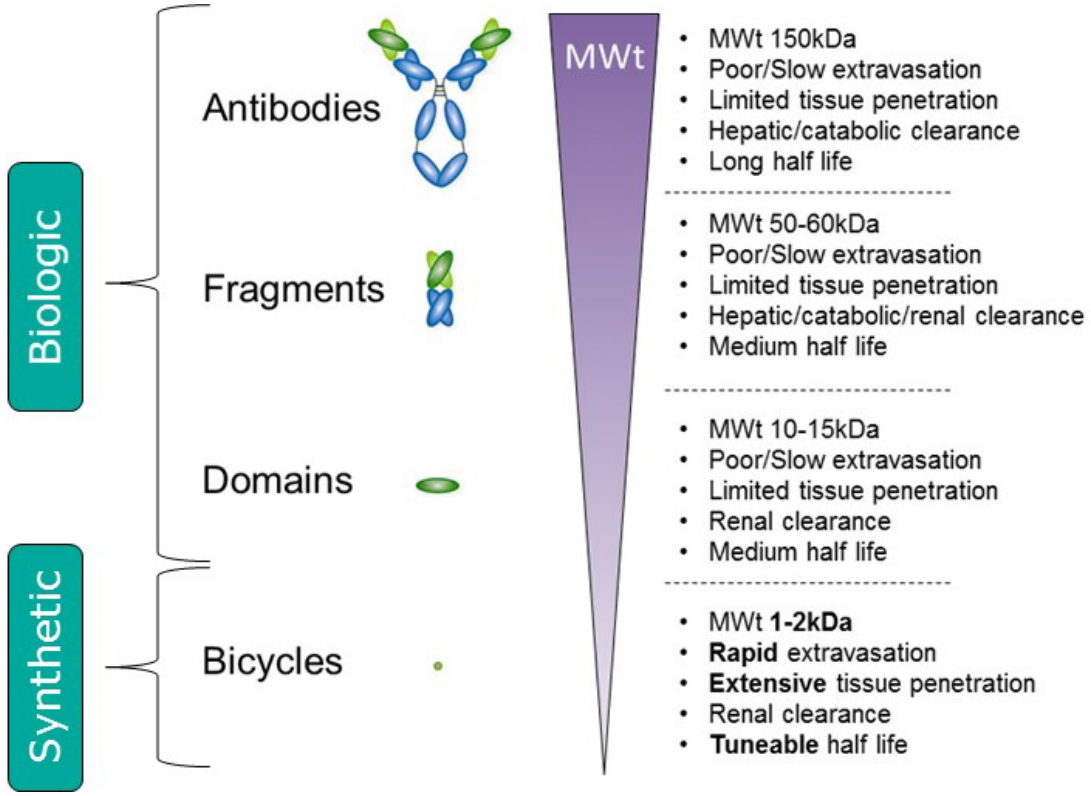


Drug delivery: Sometimes what you want is fast and efficient, not large and lumbering...

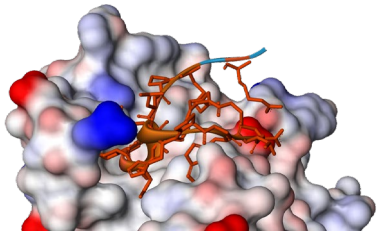
FOR:

OR

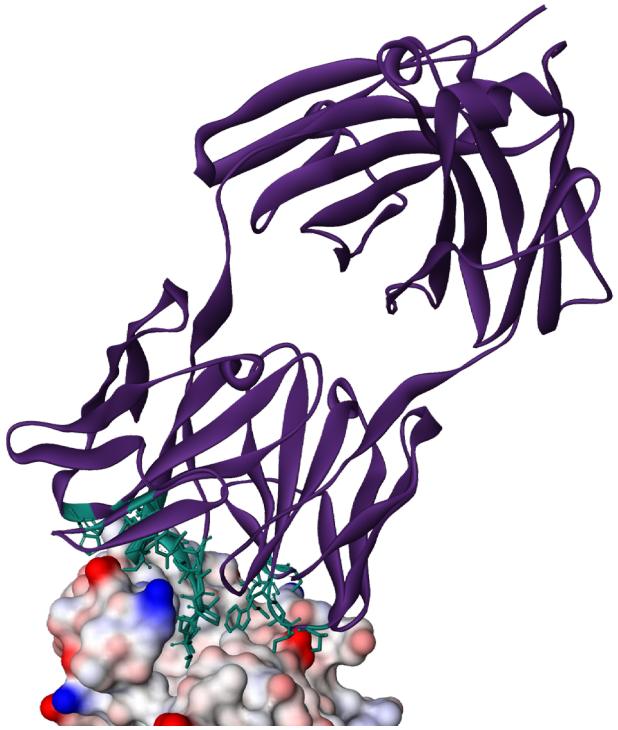
Bicycles are chemically efficient, precision guided and fit for purpose delivery vehicles



	Bicycles	Fab
Weight	2.3 kDa	48 kDa
Size	19 aa	445 aa
Binding residues	16 aa (85%)	24 aa (5%)

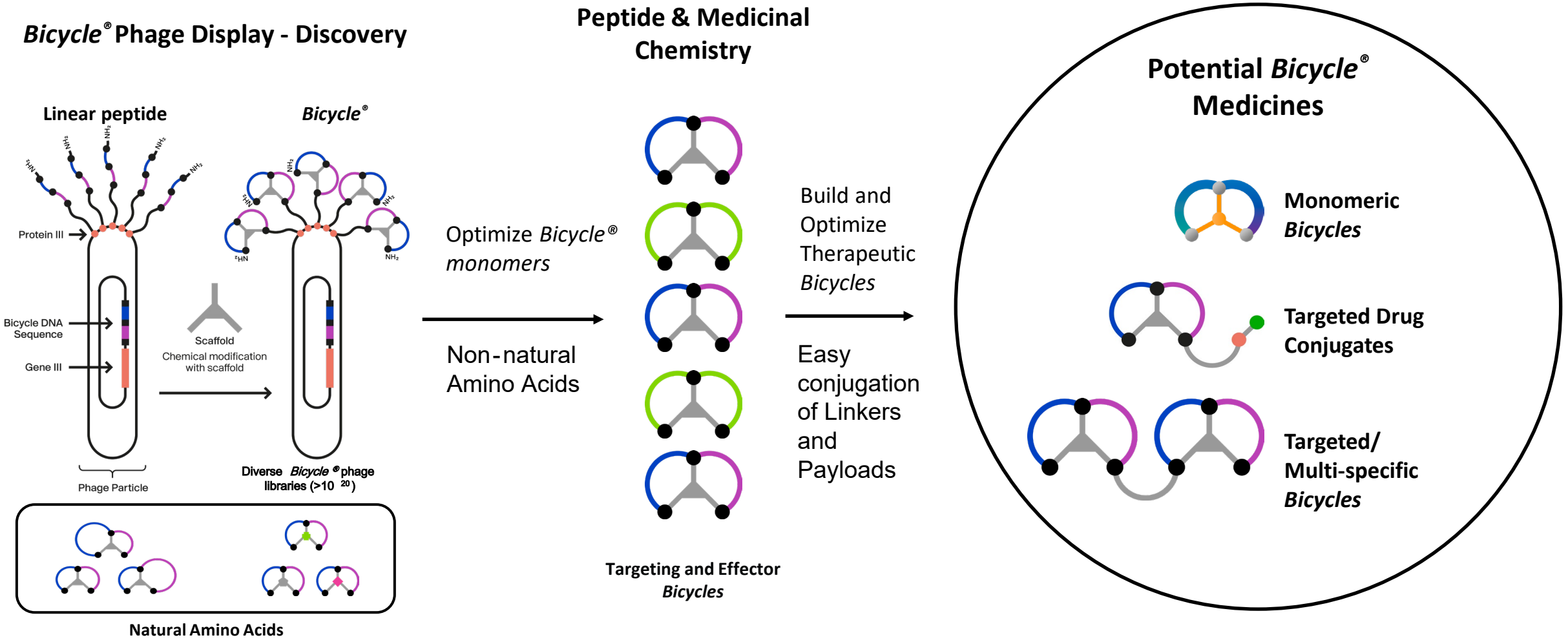


EphA2-binding *Bicycle®*



EphA2-binding **Fab**

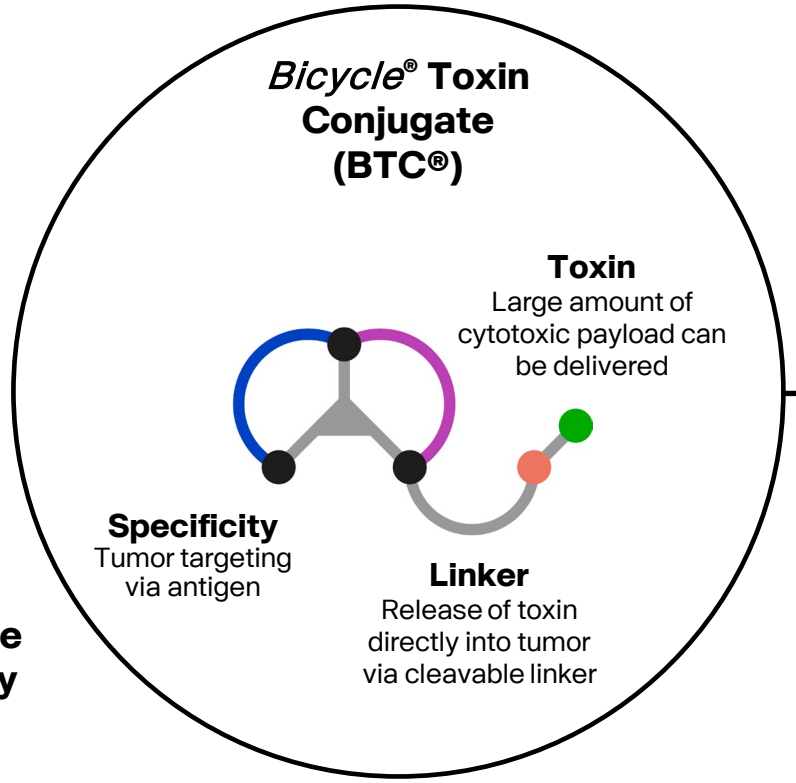
Bicycle® platform delivers a toolkit of building blocks to create novel precision guided medicines

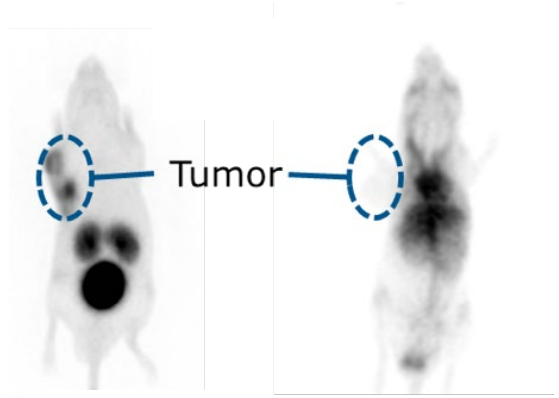


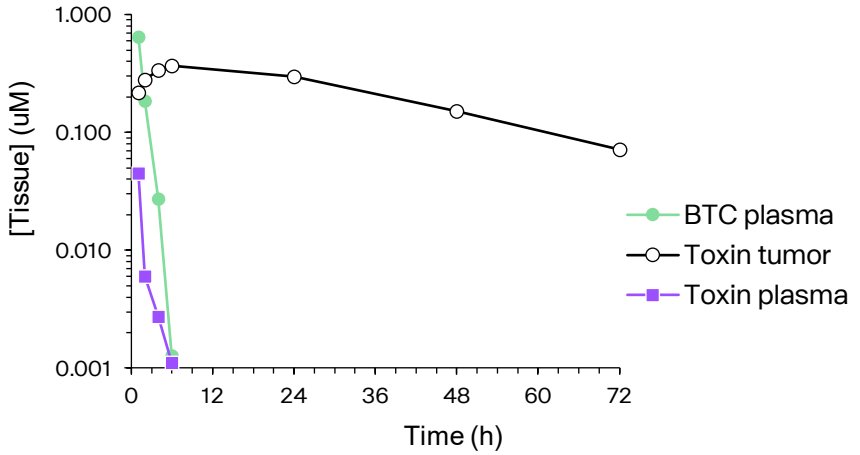
BTC® preclinical data – effective delivery of toxin payload to tumors leading to higher potency and specificity than ADCs

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

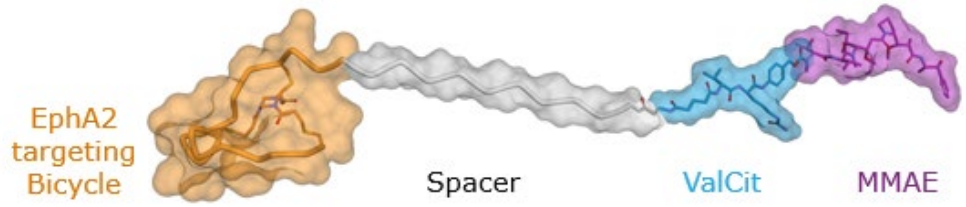
- **High selectivity**
- **Allows more potent toxin to be delivered directly to tumor**







BT5528 is a first-in-class BTC-targeting EphA2



- BT5528 has potential to penetrate solid tumors; approximately 40X smaller than an ADC
- Toxin is released and retained in tumor cells, resulting in tumor cell death and bystander killing
- PK profile distinct from ADCs; renally eliminated, bypassing liver metabolism
- Recently completed dose escalation of Phase I clinical study

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

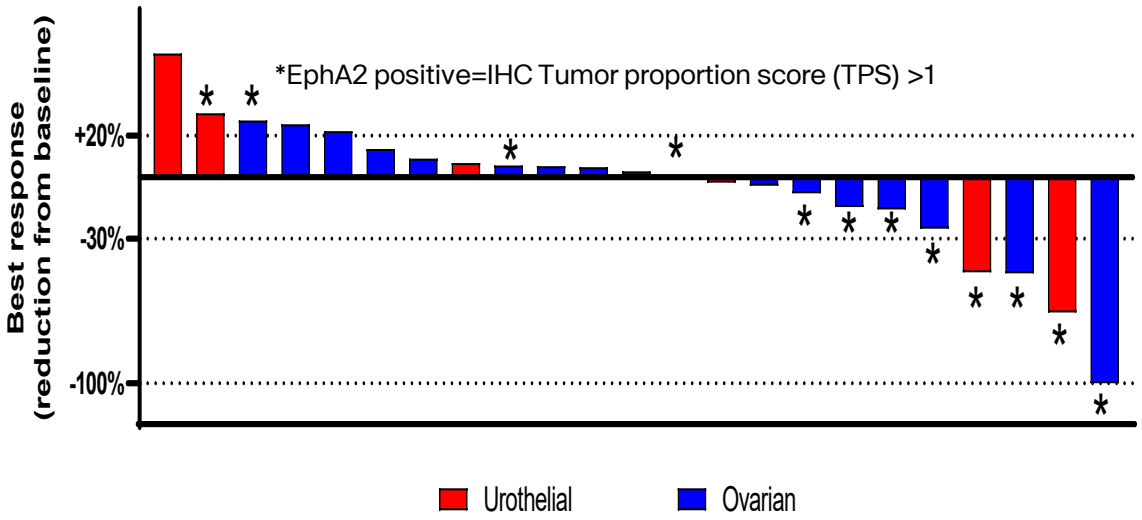
Published OnlineFirst May 12, 2020; DOI: 10.1158/1535-7163.MCT-19-1092
MOLECULAR CANCER THERAPEUTICS | SMALL MOLECULE THERAPEUTICS

MMAE Delivery Using the *Bicycle* Toxin Conjugate BT5528 AC

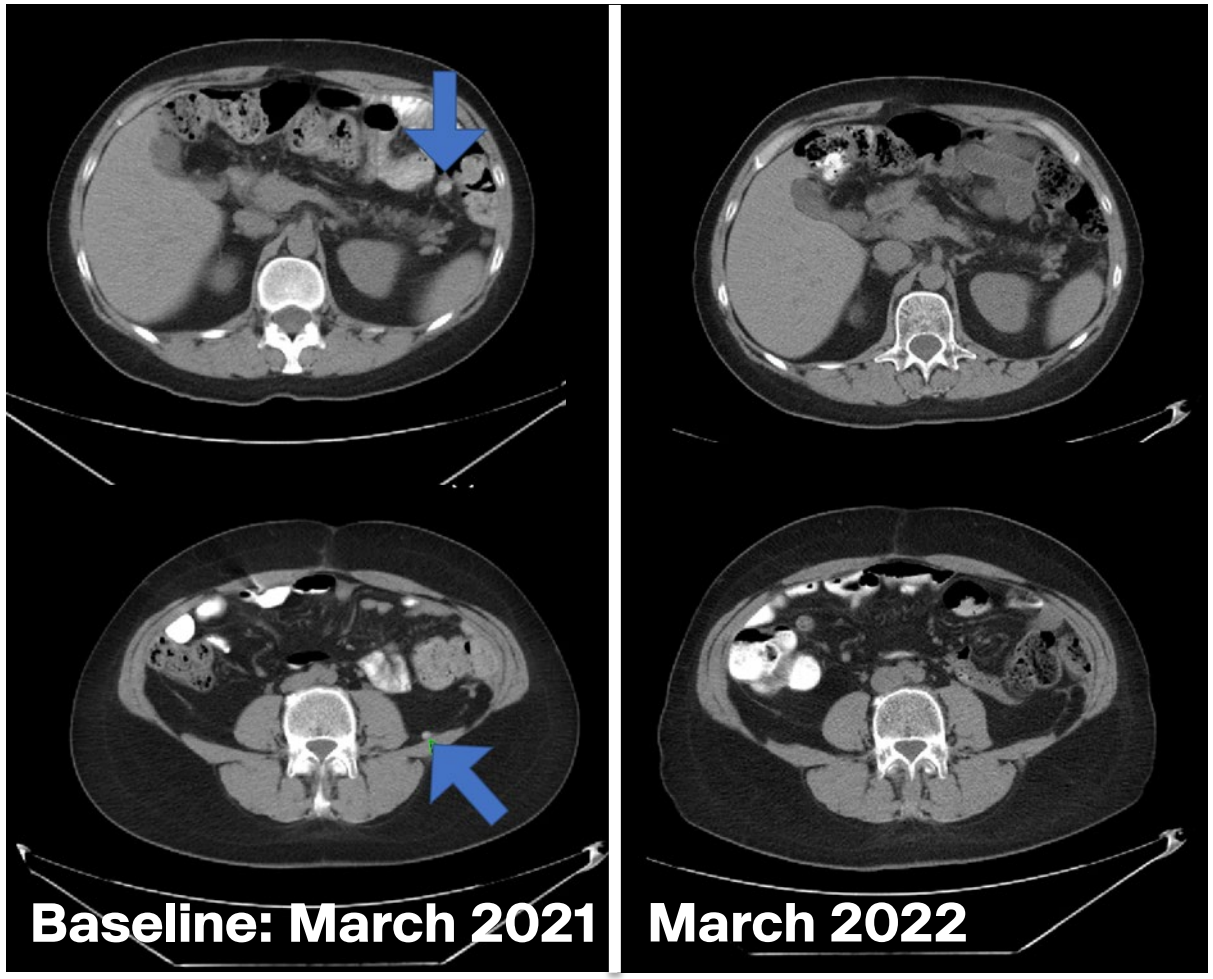
Gavin Bennett[1], Amy Brown[1], Gemma Mudd[1], Philip Huxley[1], Katerine Van Rietschoten[1], Silvia Pavan[2], Liuhong Chen[1], Sophie Watcham[3], Johanna Lahdenranta[4], and Nicholas Keen[4]

BT5528: Emerging relationship between EphA2 expression and response in ovarian and urothelial cancers

Best response by RECIST in response evaluable patients

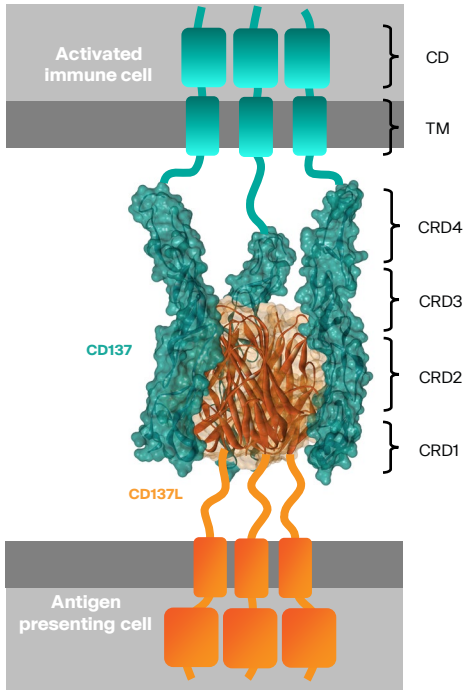


CT scans-abdomen. First in human dose escalation trial.

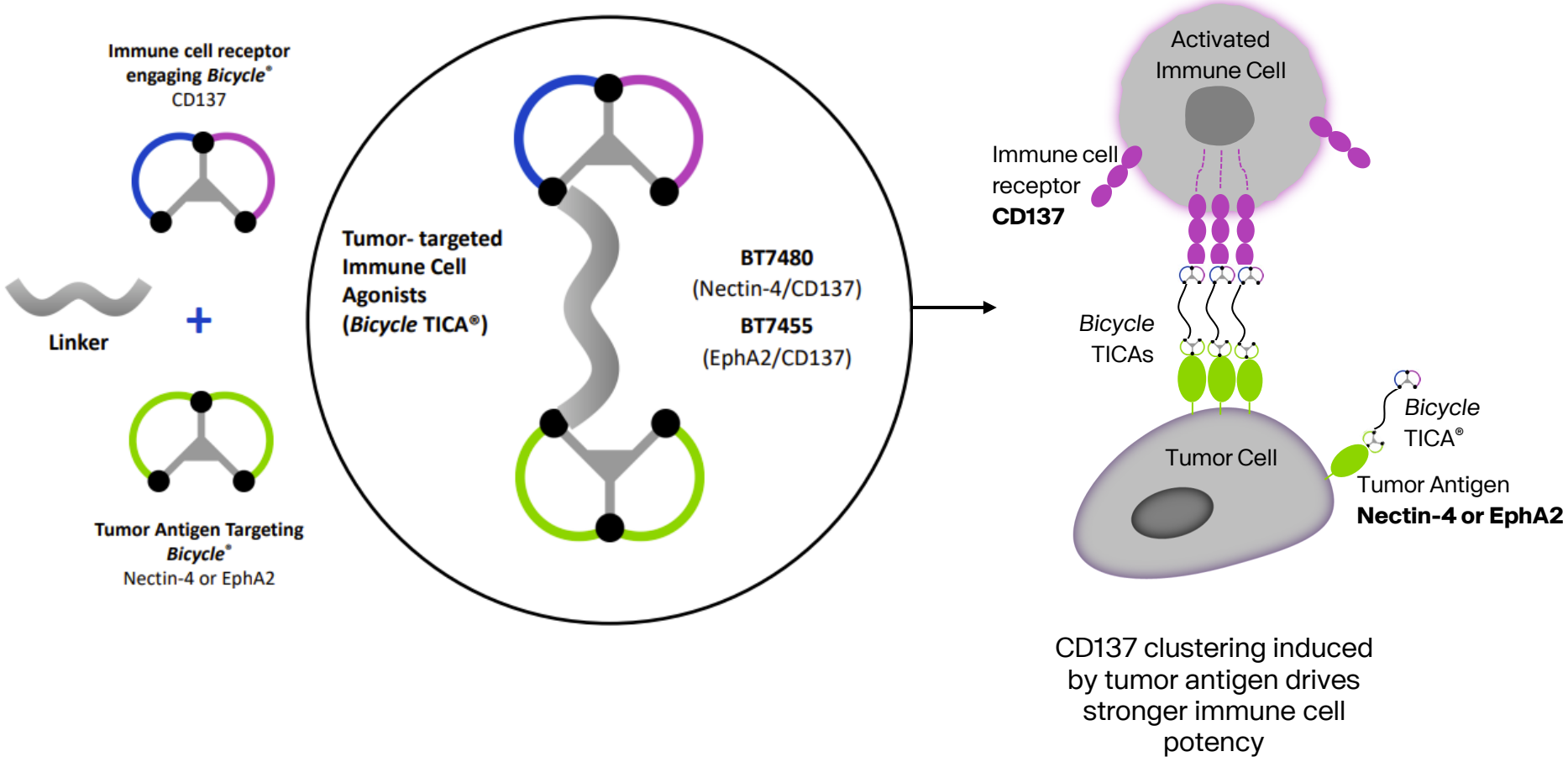


- Waterfall plot showing best response among urothelial and ovarian cancer patients in first in human study
- Immunohistochemistry data suggest positive patients more likely to respond to BT5528
- Scan showing complete responder with ovarian cancer

Bicycle TICA®– tumor-targeted immune cell agonists deliver immune agonism to tumors

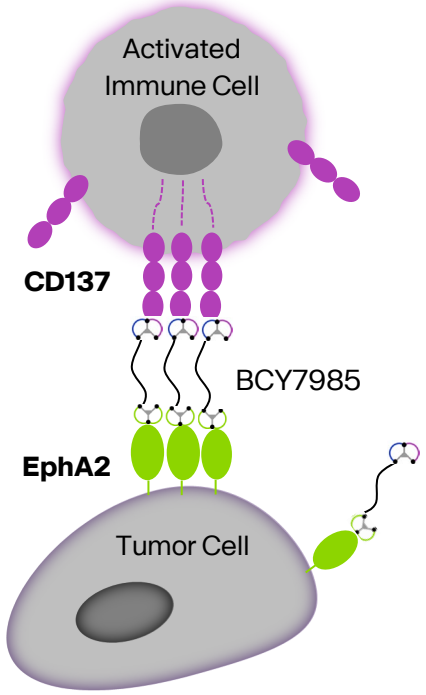


Activation induced by clustering of **CD137** by trimeric **CD137L**

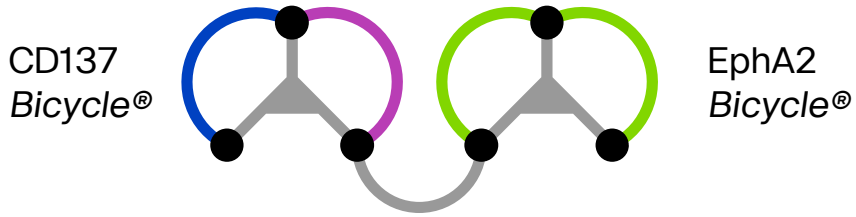


CD137 clustering induced by tumor antigen drives stronger immune cell potency

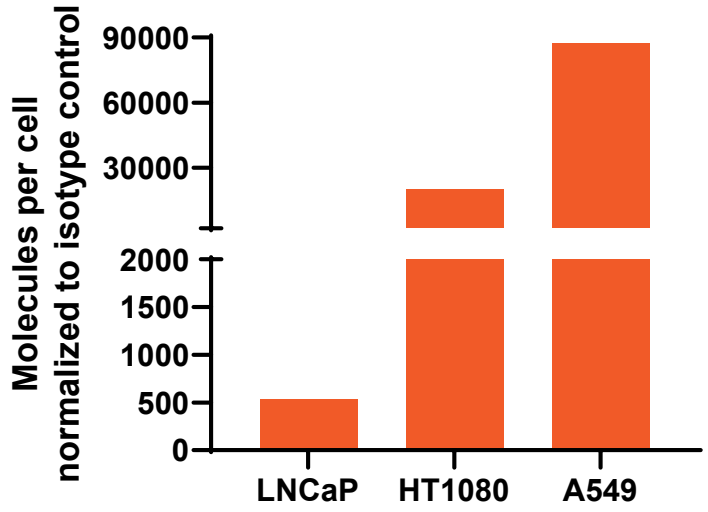
Preclinical in vitro proof of concept with the first EphA2/CD137 molecule



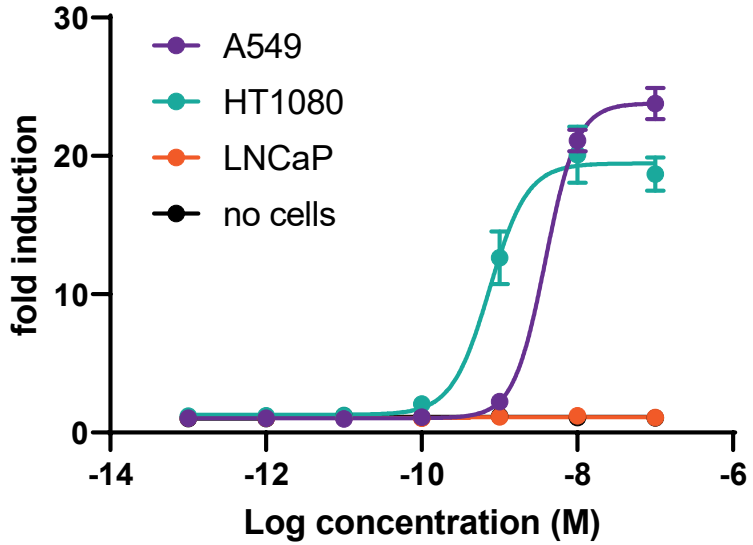
Cancer cells expressing high levels of EphA2



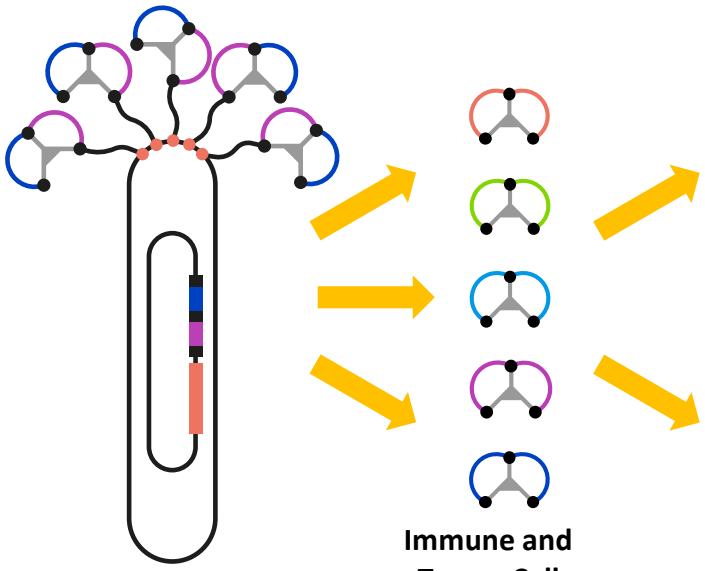
EphA2 expression



BCY7985: CD137 reporter assay in co-culture with EphA2 cells

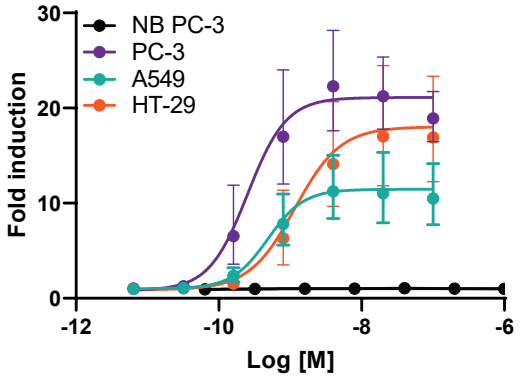


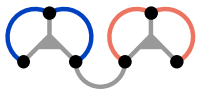
Bicycle TICA® is a generalizable concept



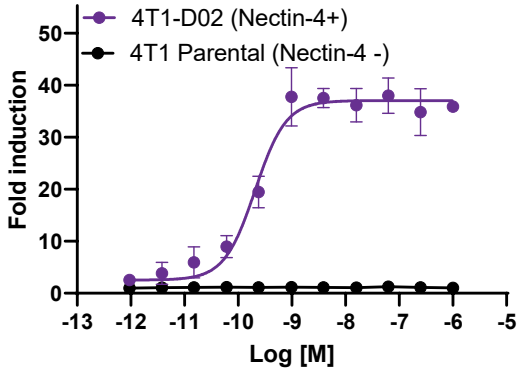
Diverse *Bicycle*® phage libraries (>10^{20})

Immune and Tumor Cell Targeting *Bicycles*

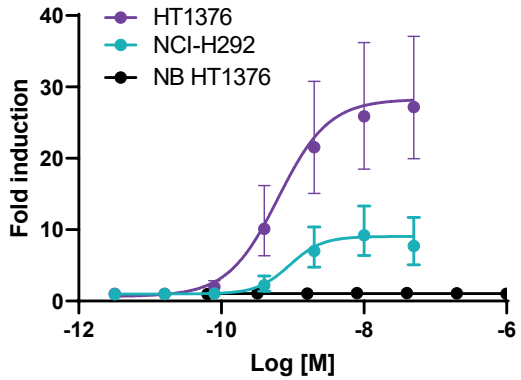




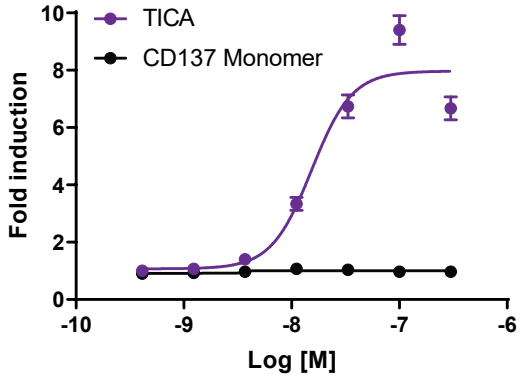
EphA2/CD137 1:1



Nectin-4/OX40 1:1



Nectin-4/CD137 1:1



PD-L1/CD137 1:1

Journal for ImmunoTherapy of Cancer

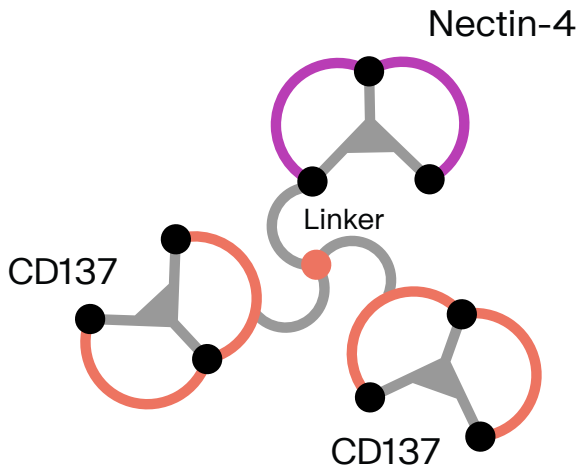
Anticancer immunity induced by a synthetic tumor-targeted CD137 agonist

Punit Upadhyaya,[1] Johanna Lahdenranta,[1] Kristen Hurov,[1] Sailaja Battula,[1] Rachel Dods,[2] Eric Haines,[1] Marianna Kleyman,[1] Julia Kristensson,[2] Jessica Kublin,[1] Rachid Lani,[2] Jun Ma,[1] Gemma Mudd,[2] Elizabeth Repash,[1] Katerine Van Rietschoten,[2] Tom Stephen,[1] Fanglei You,[1] Helen Harrison,[2] Liuhong Chen,[2] Kevin McDonnell,[1] Philip Brandish,[1] Nicholas Keen [1]

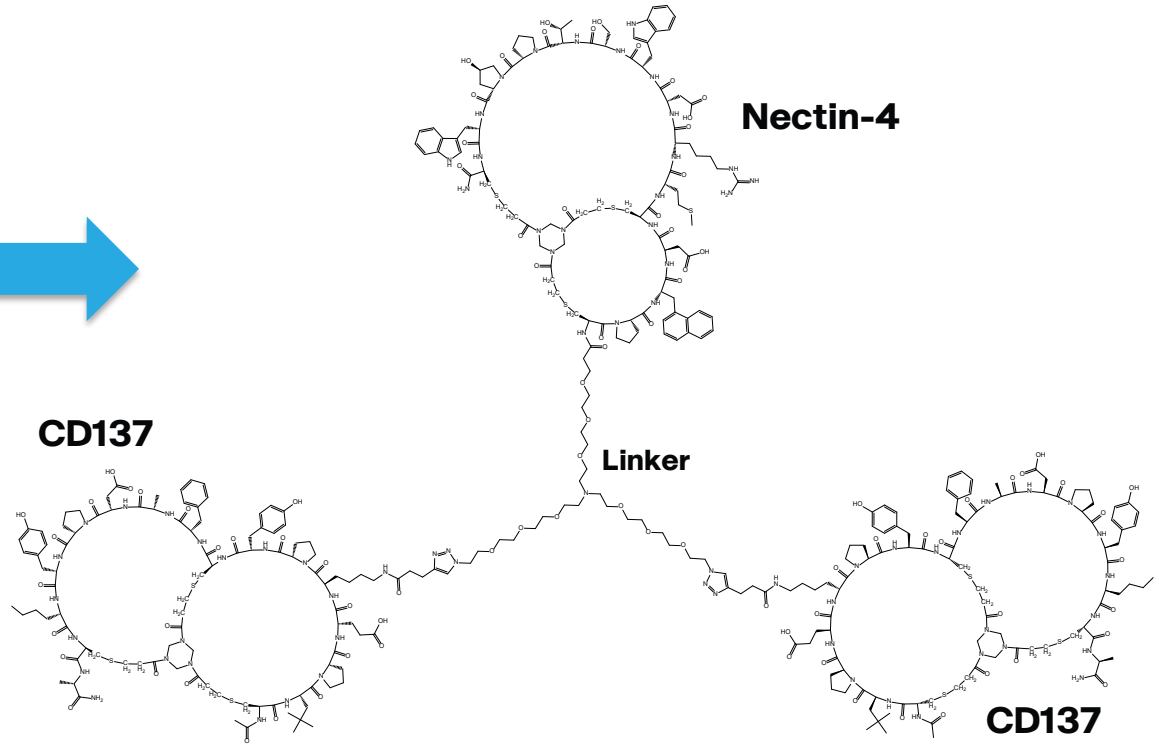
Immune effector and tumor targeting *Bicycles* can be combined in a modular fashion to construct a pipeline of *Bicycle*® tumor-targeted immune cell agonists

BT7480 is a fully synthetic, heterotrimeric conjugate with one Nectin-4 and two CD137 *Bicycles*

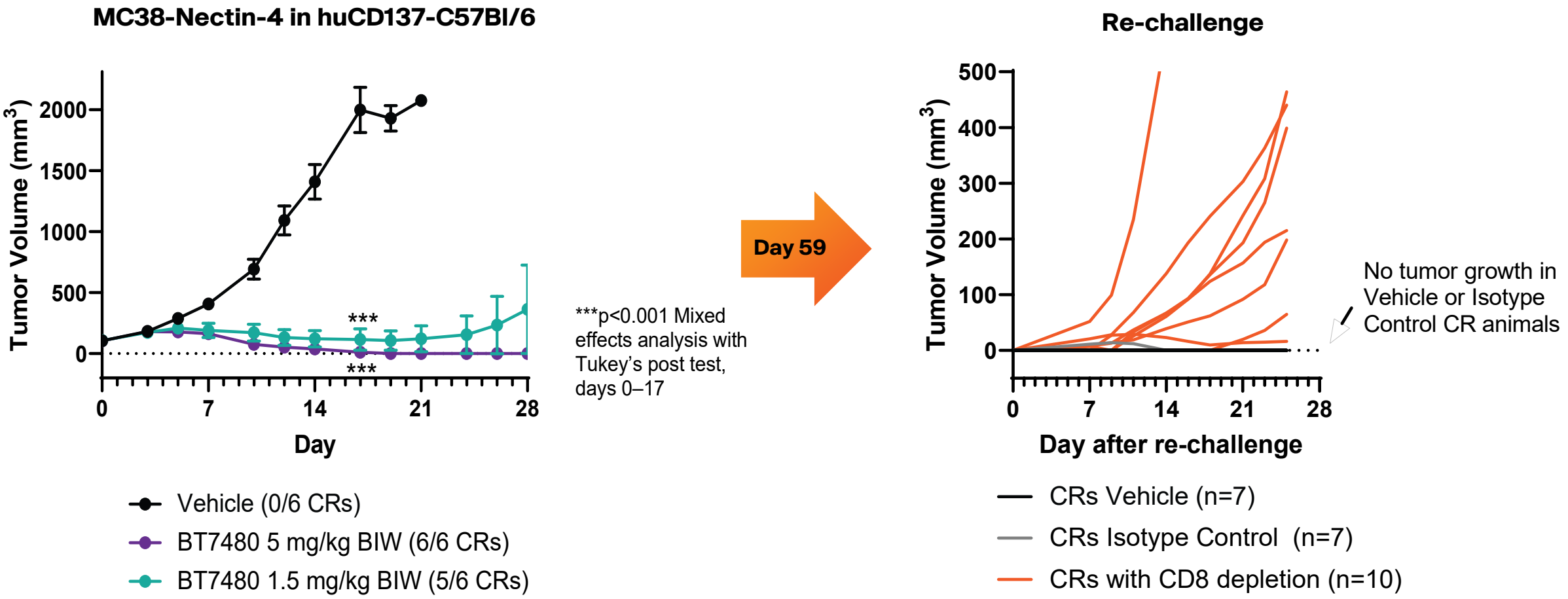
BT7480 selected as lead *Bicycle* TICA® candidate



Structure of BT7480
MW = 7.2 kDa



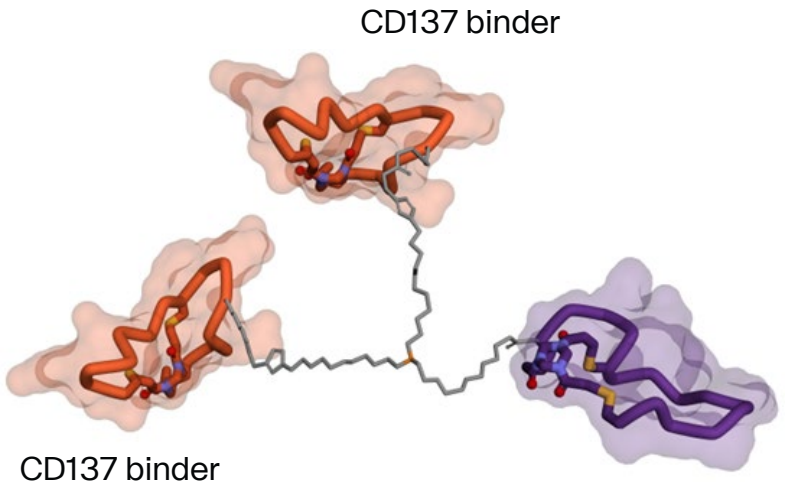
BT7480 induces complete responses and memory *in vivo* in a syngeneic mouse model



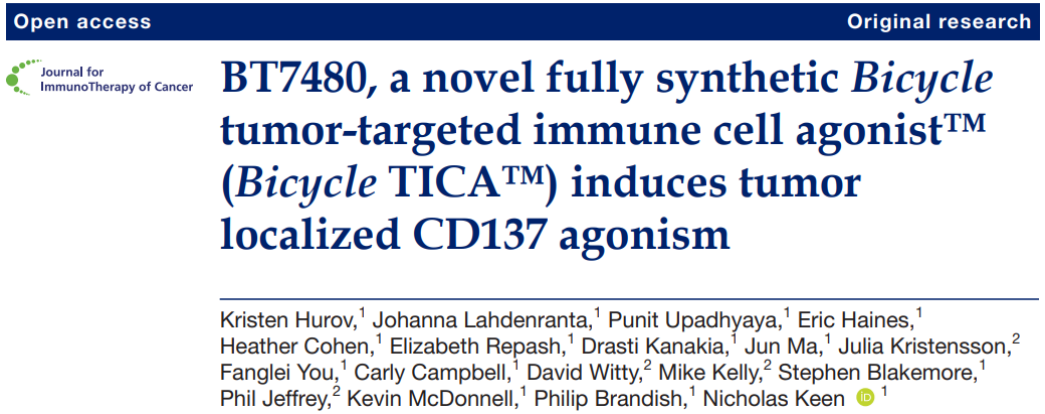
CRs=Complete Responders

BT7480 – the first chemically synthetic, conditionally active targeted CD137 activator

- Activity of the CD137 agonist arm is dependent on ligation of the Nectin-4 arm, leading to tumor specificity
- Causes complete regressions and anti-tumor activity with only intermittent dosing in syngeneic mouse models
- Causes an early increase in chemotactic cytokine production that precedes an increase in CD8+ T cell infiltration into the tumor
- Is well-tolerated in preclinical safety species
- Entered Phase I clinical testing in November 2021



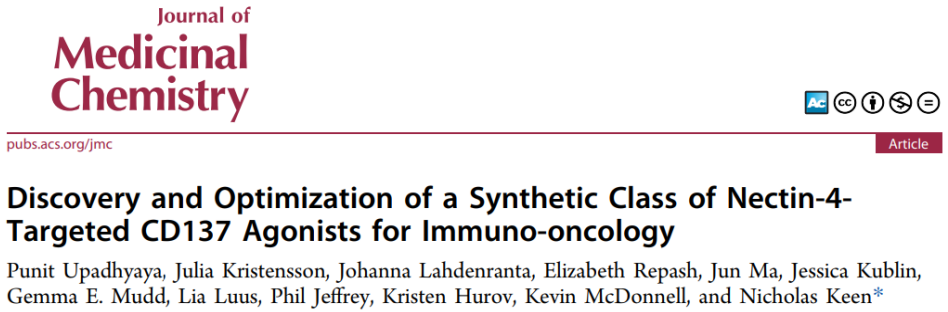
BT7480
MW = 7213.3 Da


Open access Original research

Journal for ImmunoTherapy of Cancer

BT7480, a novel fully synthetic *Bicycle* tumor-targeted immune cell agonist™ (*Bicycle* TICA™) induces tumor localized CD137 agonism

Kristen Hurov,[1] Johanna Lahdenranta,[1] Punit Upadhyaya,[1] Eric Haines,[1] Heather Cohen,[1] Elizabeth Repash,[1] Drasti Kanakia,[1] Jun Ma,[1] Julia Kristensson,[2] Fanglei You,[1] Carly Campbell,[1] David Witty,[2] Mike Kelly,[2] Stephen Blakemore,[1] Phil Jeffrey,[2] Kevin McDonnell,[1] Philip Brandish,[1] Nicholas Keen [1]


Journal of Medicinal Chemistry

pubs.acs.org/jmc Article

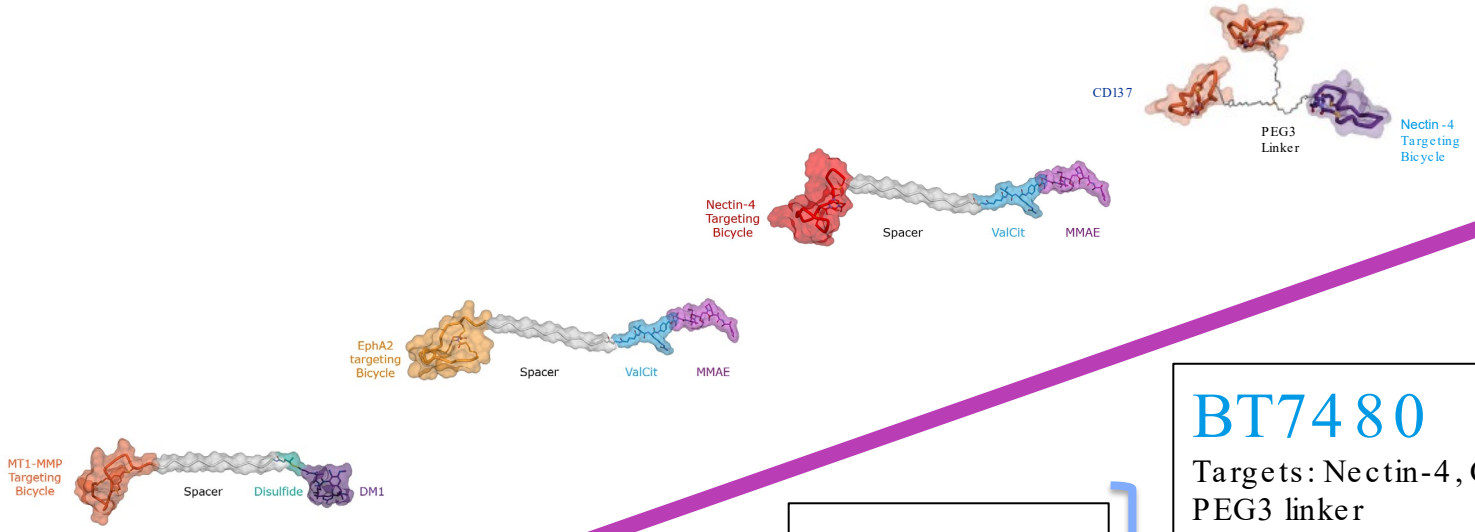
Discovery and Optimization of a Synthetic Class of Nectin-4-Targeted CD137 Agonists for Immuno-oncology

Punit Upadhyaya, Julia Kristensson, Johanna Lahdenranta, Elizabeth Repash, Jun Ma, Jessica Kublin, Gemma E. Mudd, Lia Luus, Phil Jeffrey, Kristen Hurov, Kevin McDonnell, and Nicholas Keen*

Hurov K, Lahdenranta J, et al., 2021, *J Immunother Cancer*, **9**(11):e002883; Upadhyaya, et al., 2022, *J Med Chem*, **65**(14):9858-72

Elevating the *Bicycle*® platform

Targeted ASO/SiRNA delivery



BT1718
Target: MT1-MMP
DM1 payload
Disulfide linker

BT5528
Target: EphA2
MMAE payload
Val-Cit linker

BT8009
Target: Nectin-4
MMAE payload
Val-Cit linker

BT7480
Targets: Nectin-4, CD137
PEG3 linker

Validation

BT7455
Targets: EphA2, CD137
PEG3 linker
Next Bicycle TICA®
scheduled for clinic

NK-TICA®

Validation

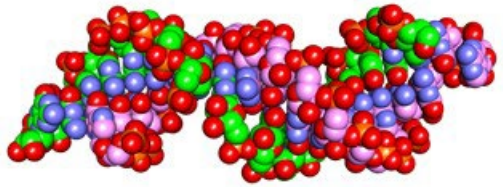
Next-gen BTCs
Different targets, linkers and payloads

Radiopharm

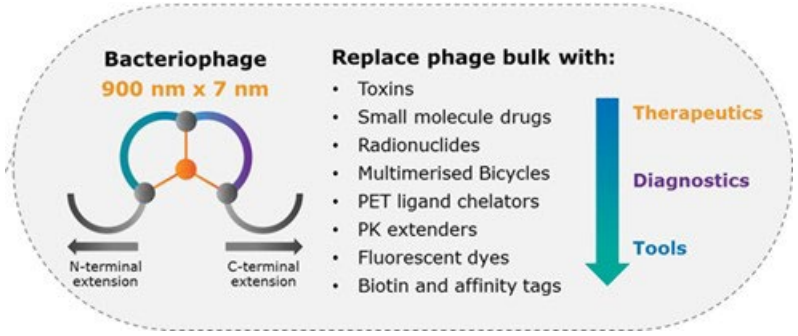
FIH 2018 2019 2020 2021 2023 and beyond

Could we apply the Bicycle technology to deliver antisense therapeutics to specific tissues to treat serious diseases?

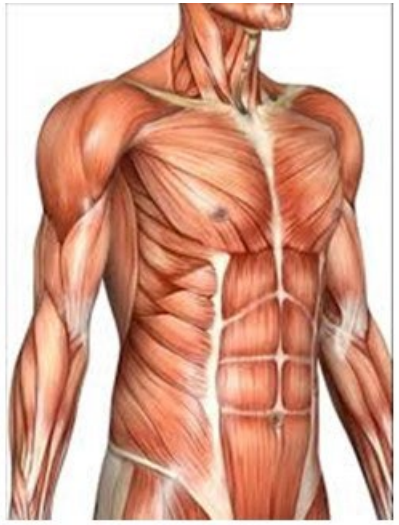
ASO (Mol Wt 5.5-7000 Da)



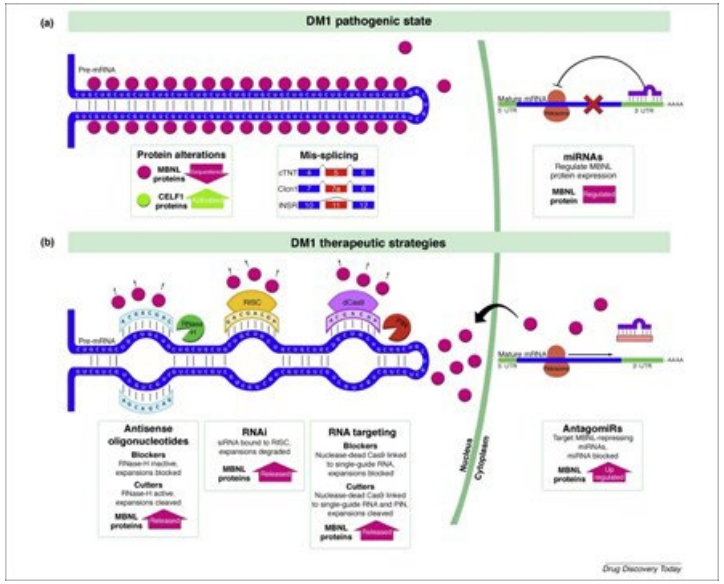
siRNA (Mol Wt 15,000 Da)



M13 phage (Mol Wt 1-2 million Da)

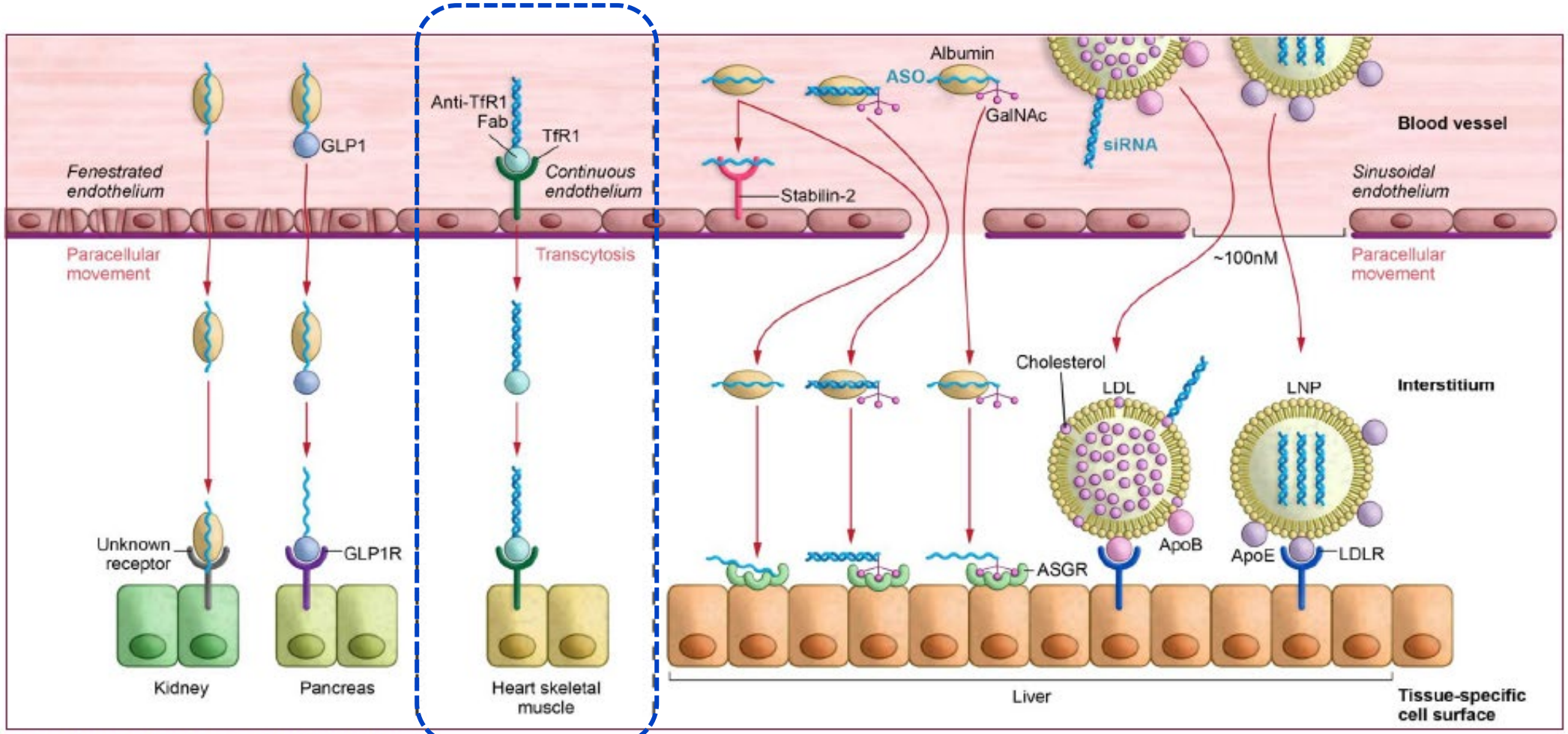


Myotonic Dystrophy



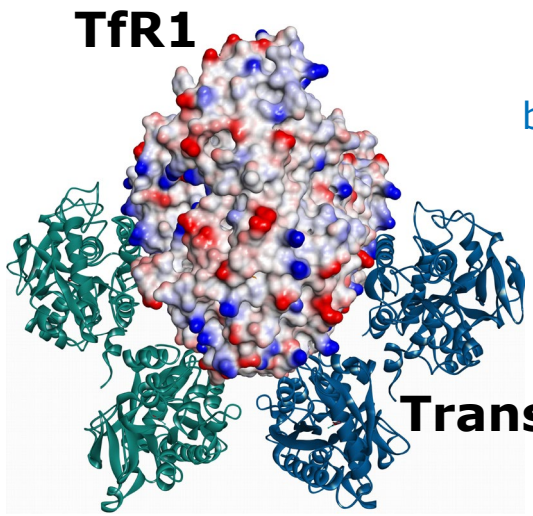
IONIS®

Delivery of many therapeutics to tissues is facilitated by specific receptor mediated uptake pathways

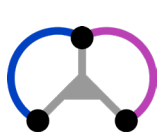


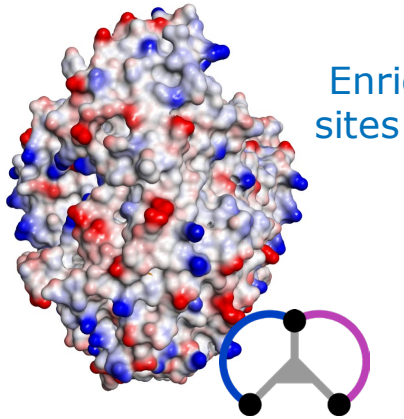
Seth et al, *J Clin Invest*. 2019,*129*, 915-925

Screening identifies *Bicycles* to alternate TfR1 epitopes

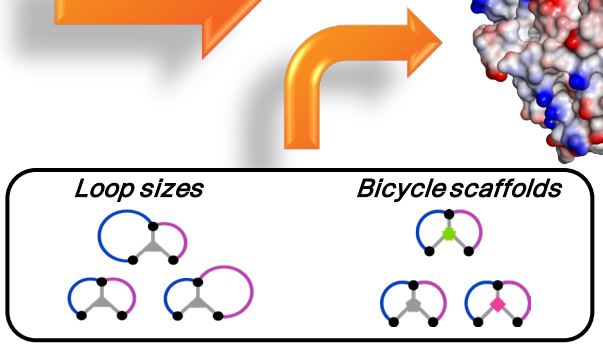


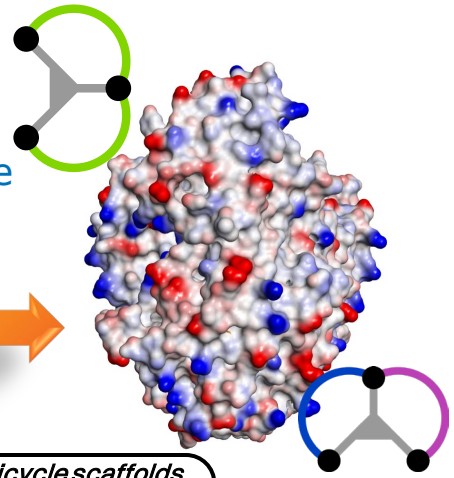
Blocking *Bicycle*® that binds the Transferrin site



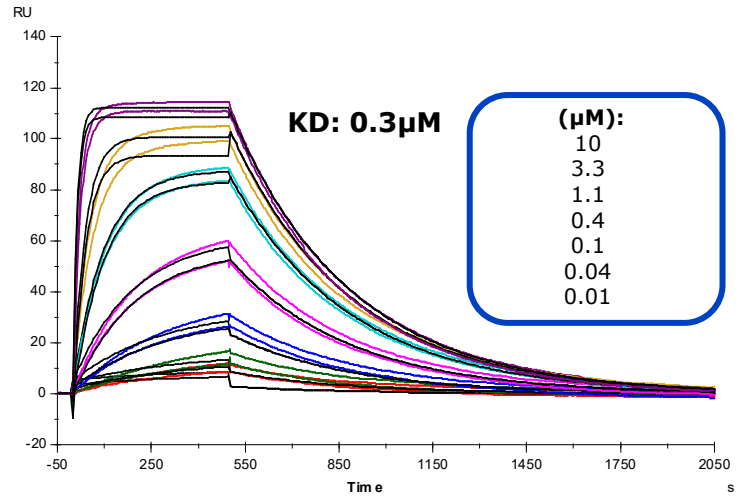


Enrich for binders to other sites outside transferrin site

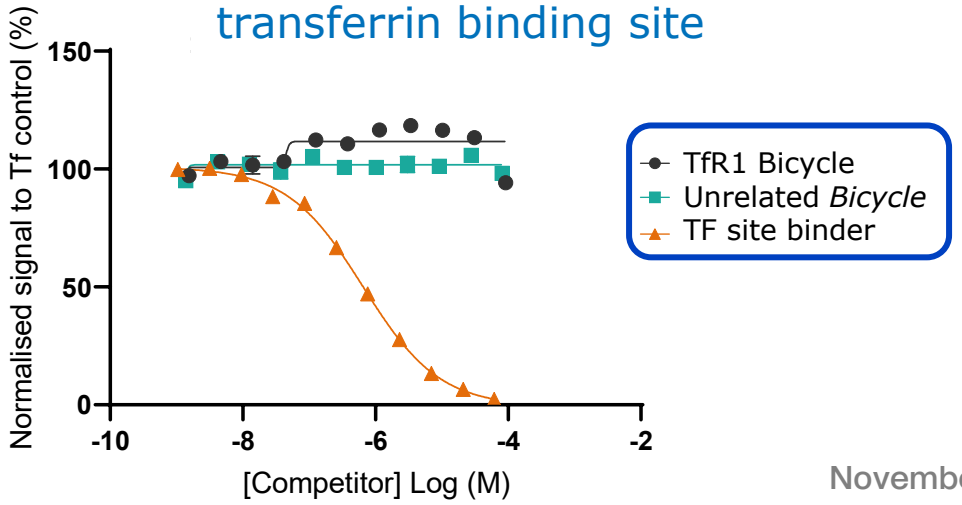




Bicycle® binds TfR1 with sub μM affinity

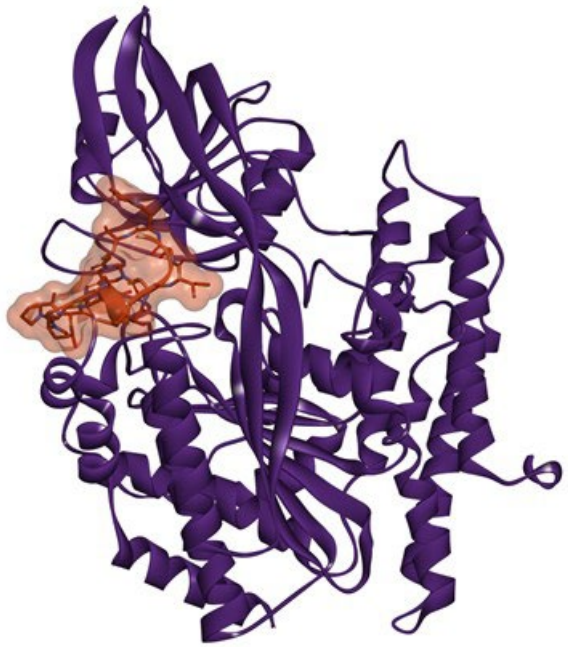


Bicycle® does not compete the transferrin binding site



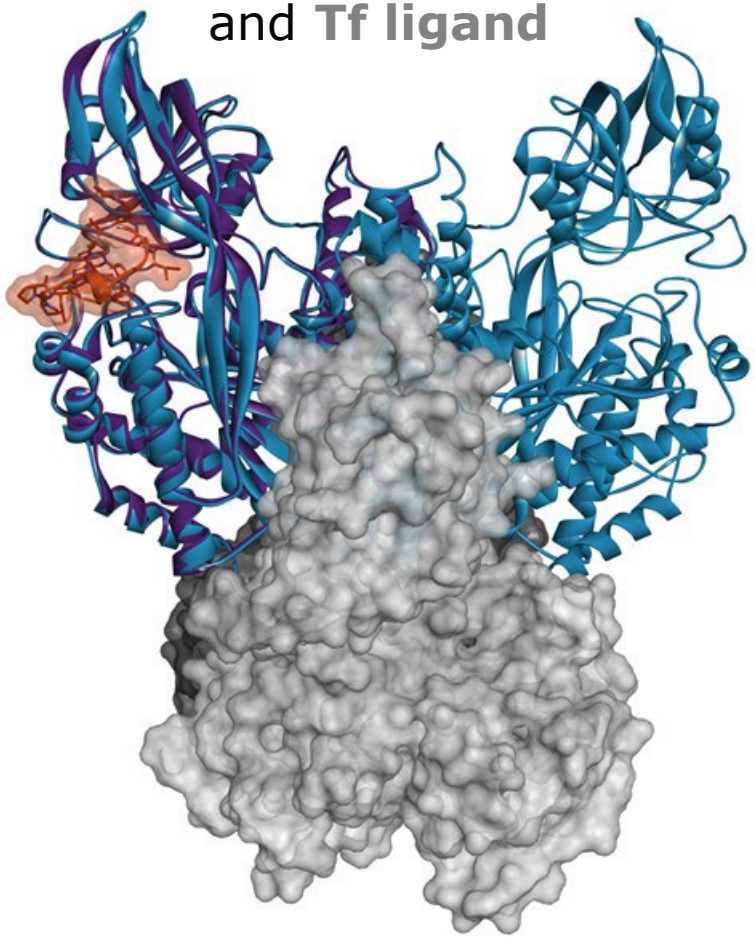
Crystal structure of *Bicycle*® bound into hTfR1

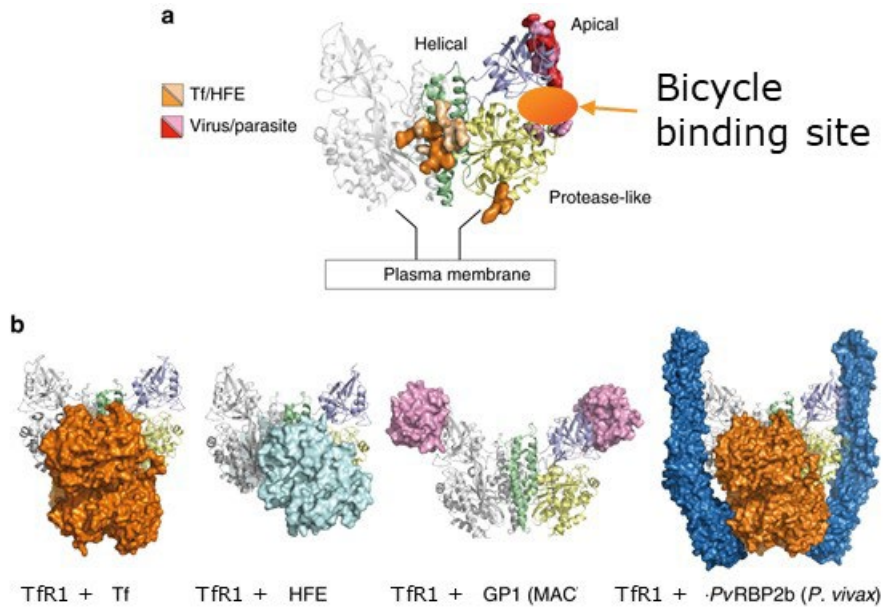
Bicycle
bound to **hTfR1**



(Only 1 TfR1 monomer shown)

Overlay with **TfR1 dimer**
and **Tf ligand**

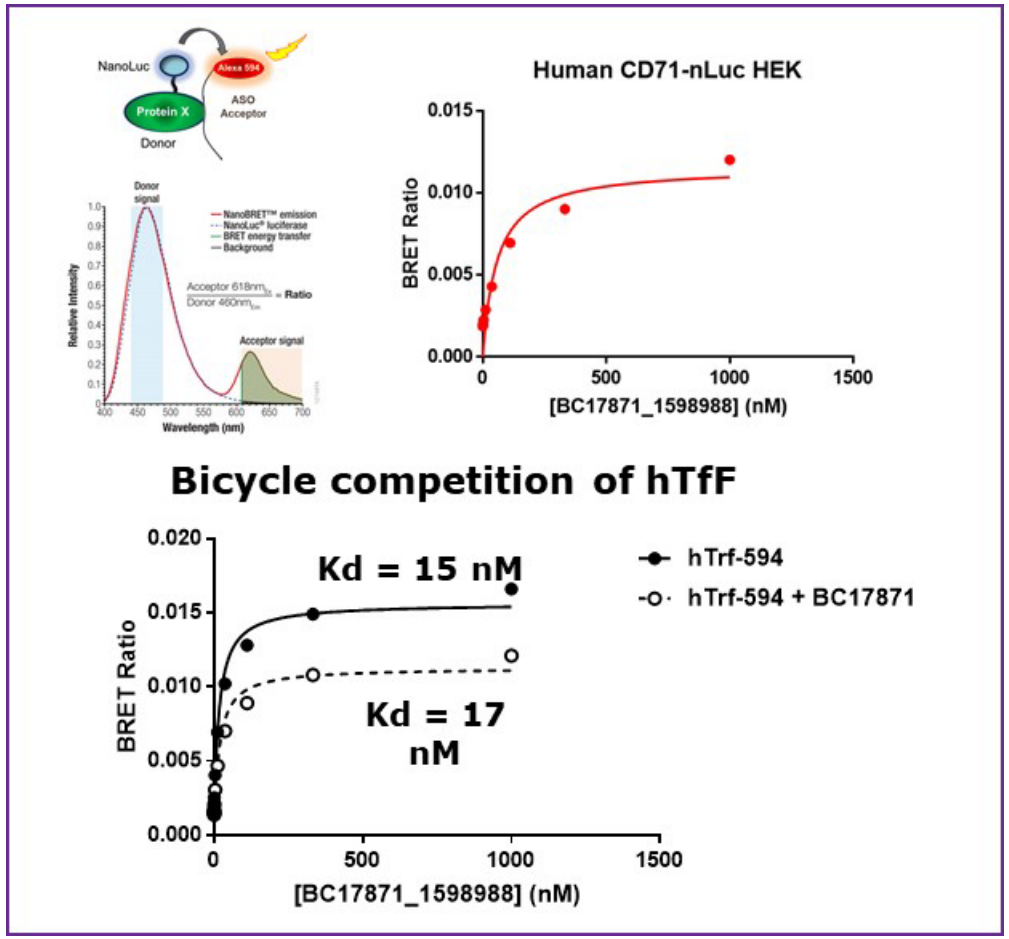


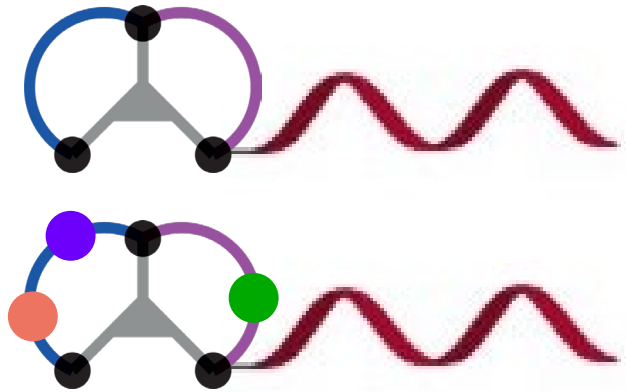


TfR1 with ligands and virus proteins

Bicycle **binds to a novel site between apical & protease-like domain, does not compete with transferrin ligand**

Binding to TfR1 is maintained following conjugation of an ASO and affinity can be tuned using medicinal chemistry





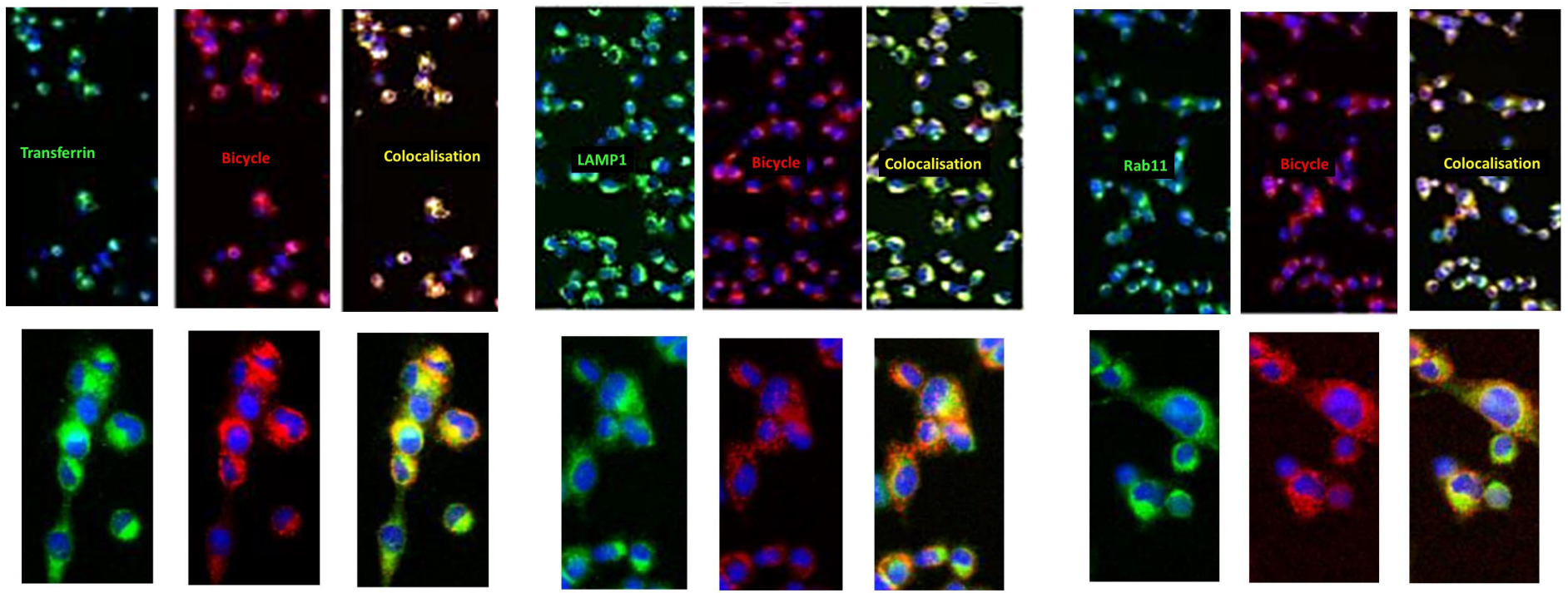
Conjugate	Ki (nM)
BCY82-ASO	55
BCY90-ASO	20
BCY92-ASO	11
BCY94-ASO	10
BCY96-ASO	2
BCY99-ASO	4
BCY01-ASO	1
BCY04-ASO	60
BCY06-ASO	22

Internalization and colocalization with endosomal markers - fully tunable pharmacology of TfR1 binding *Bicycles*

Colocalizes with Transferrin (& TfR1)

Colocalizes with LAMP1 (& inhibited by Dyngo 4a)

Colocalizes with Rab11



HT1080 cells incubated with labelled *Bicycle®*

K_D=12nM

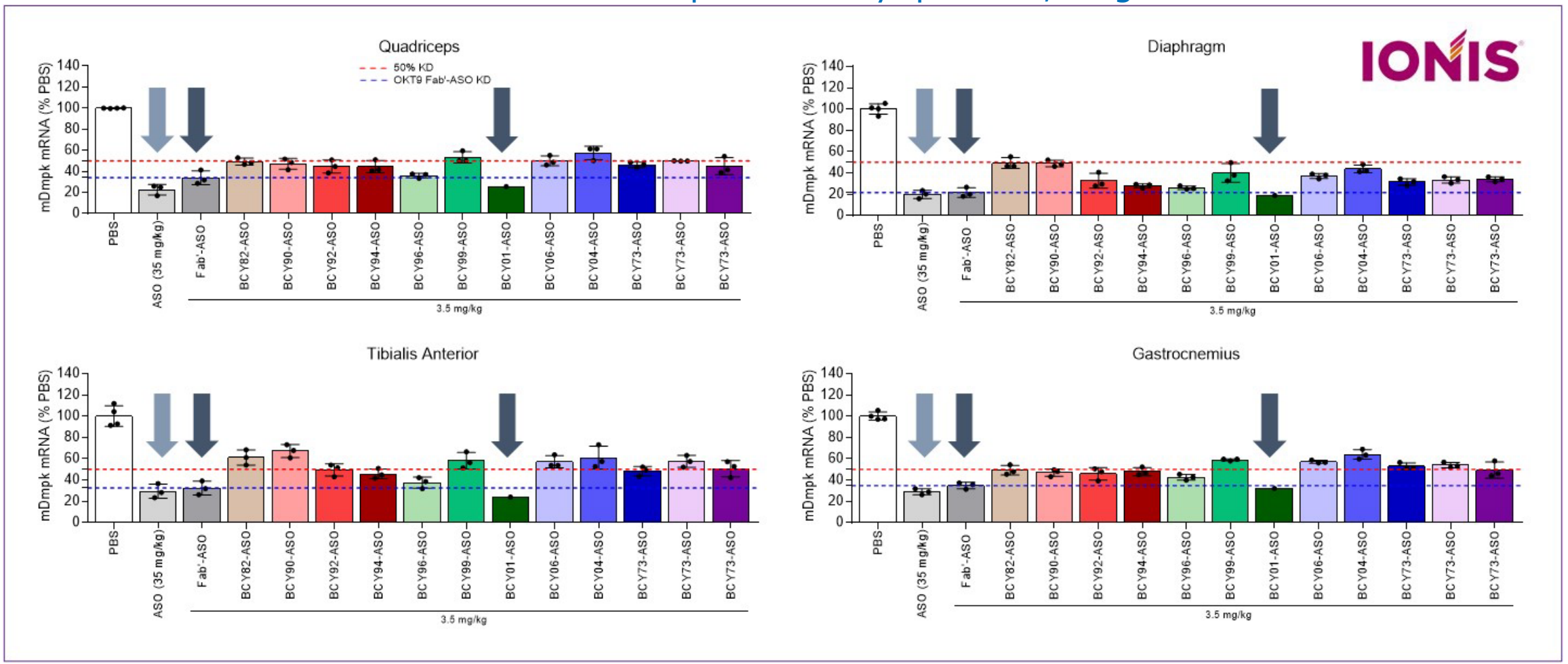
K_D=26nM

K_D=209nM

TfR1 binding *Bicycles* are internalized and colocalize with endocytic markers

Bicycles targeting hTfR1 enhance ASO activity in skeletal muscles in hTfR1$^{KI/+}$ mice

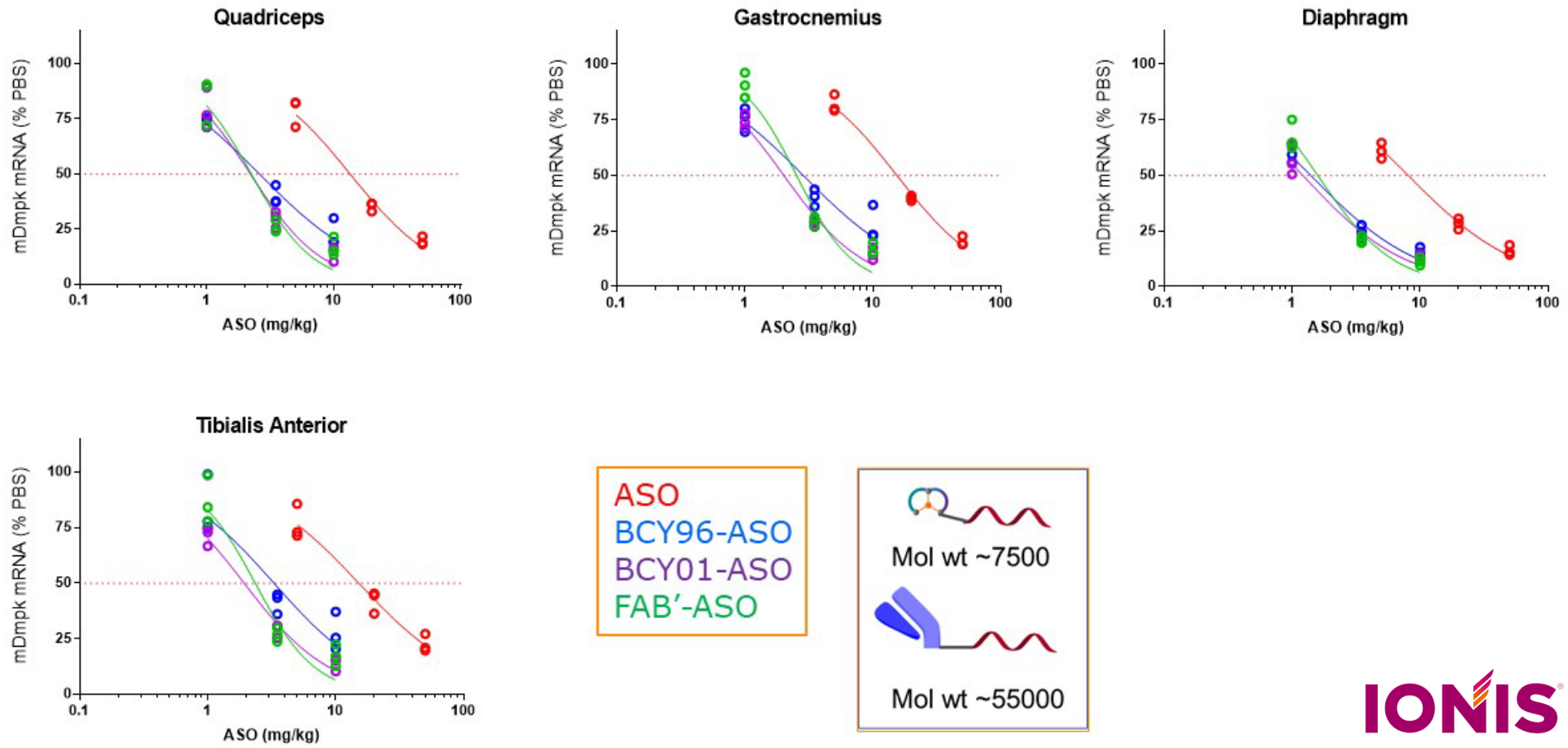
Reduction of DMPK mRNA quantified by qRT-PCR, single dose level



hTfR1$^{KI/+}$ mice were injected with 3.5 mg/kg/wk/3 wks of ASO-conjugates for 3 weeks.

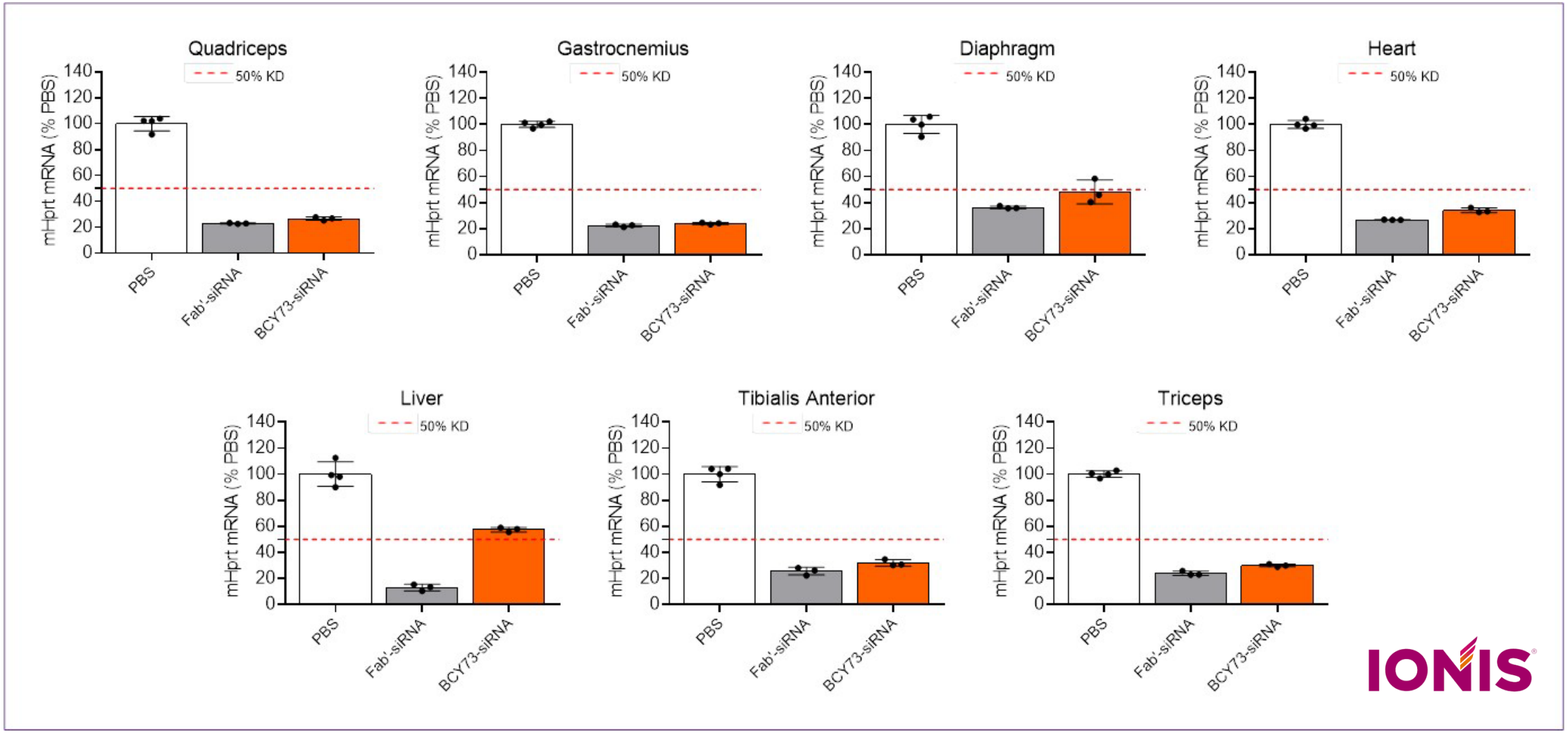
Bicycles targeting hTfR1 enhance ASO potency in striated muscles in hTfR1$^{KI/+}$ mice

Reduction of DMPK mRNA quantified by qRT-PCR, dose response



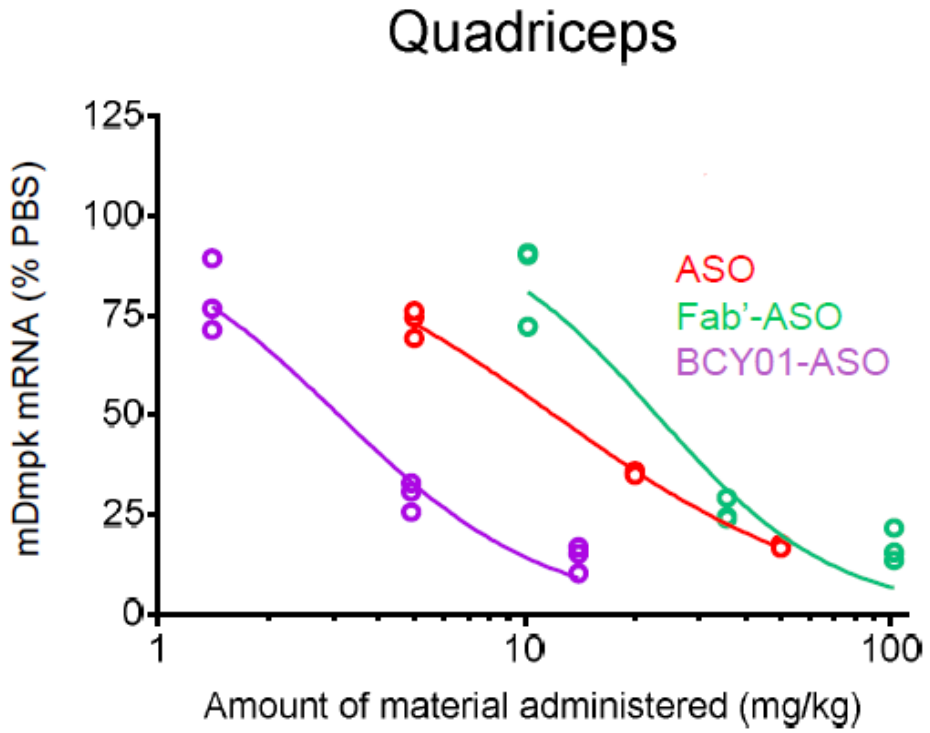
hTfR1$^{KI/+}$ mice injected with 3.5 mg/kg of ASO conjugates at days 0, 4 and 8 (day 12 data shown)

Bicycle® siRNA conjugate shows similar potency as FAB'-siRNA conjugate in hTfR1$^{KI/+}$ mice



hTfR1$^{KI/+}$ mice were injected with 3.5 mg/kg/wk/3 wks of siRNA-conjugates, 3-week study.

Bicycles are a size efficient and promising delivery system for oligonucleotide therapeutics



Dose required to deliver equivalent amounts of ASO are considerably higher with biologics

Compound	Mol wt (g/mol)	Conjugate dose (mg/kg)	Theoretical clinical dose (mg)
ASO	~5400	--	210
ASO-FAB	~55000	~33	2310
ASO-MAB	~155000	~93	6510
ASO-Bicycle	~7400	~4	280

(to deliver 3mg/kg ASO)

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Summary

- *Bicycles* are fully synthetic and readily conjugated precision guided targeting systems

- In oncology, *BTCs* and *Bicycle*® TICAs have demonstrated the utility of the modality in delivering potent toxin or immune effector cargos to solid tumors

- Prototype *Bicycle*® oligonucleotide conjugates further highlight the potential utility of Bicycles in a wide range of therapeutic applications

Acknowledgements

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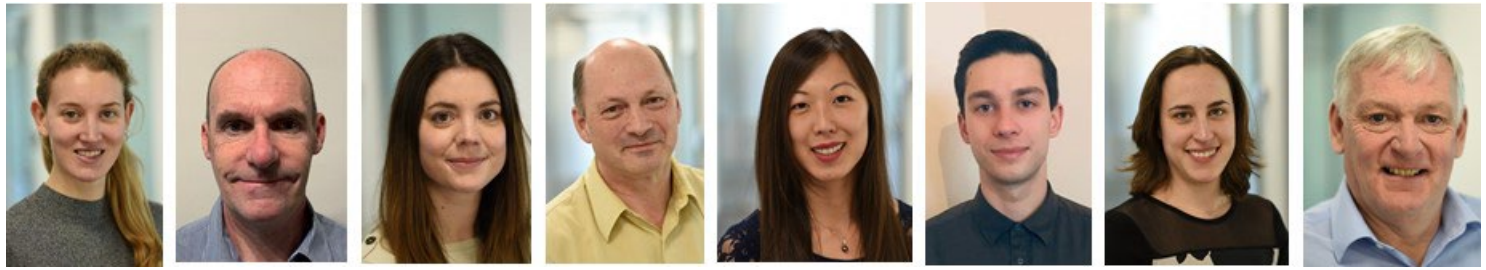

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Steve Stanway
Katerine Van Rietschoten
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Liudvikas Urbonas
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Michele Carrer
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Michael Tanowitz
Megan Afetian
Johnnatan Tamayo
Brooke Anderson
Hans Gaus
Ian Huggins
Paymaan Jafar-nejad Frank Rigo
Eric Swayze
Punit Seth (now @Alnylam)

Thank you

Bicycle®