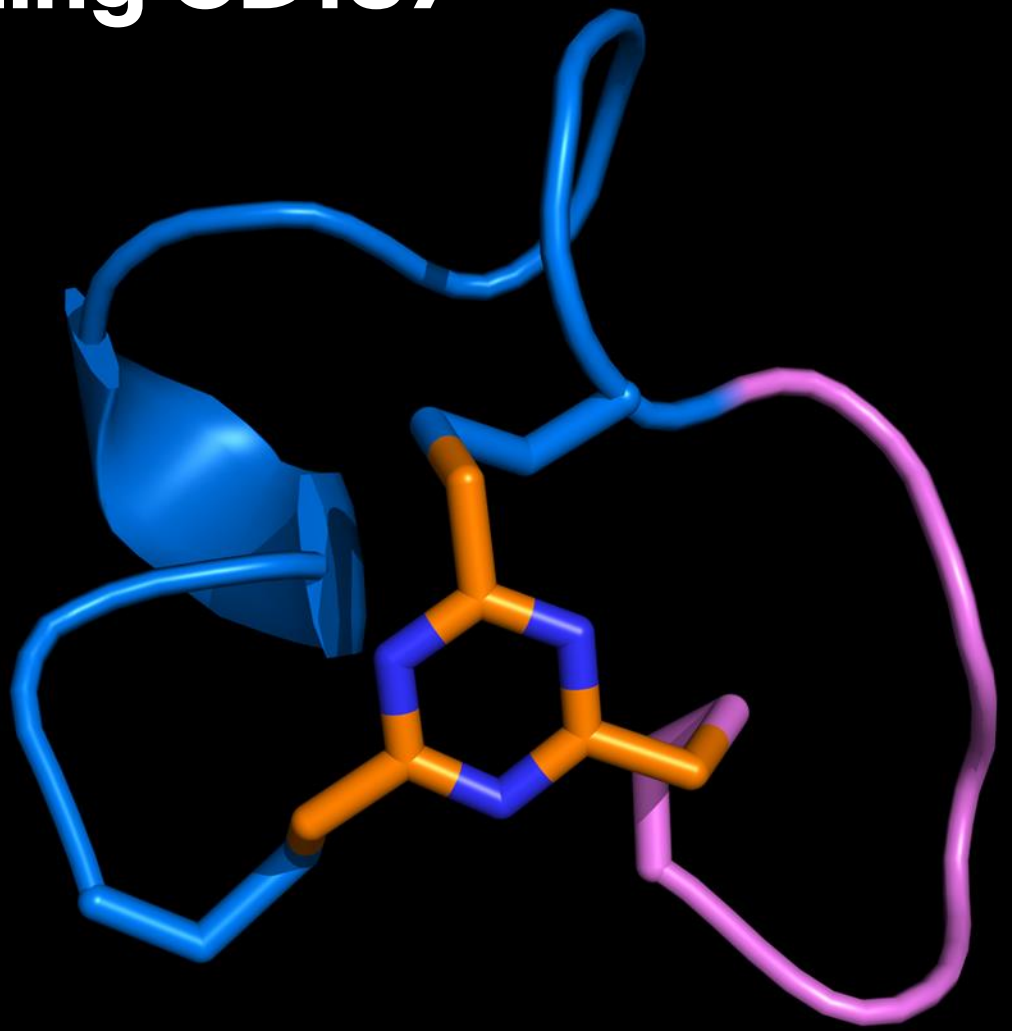
Key DMPK Attributes of BT7480, a *Bicycle* Tumor-targeted Immune Cell Agonist™ Targeting Nectin-4 and Agonizing CD137

Dian Su
Senior Director, DMPK

NEDMDG symposium, Boston
May 31st, 2023



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 business performance, conditions, plans, prospects, trends or strategies and other 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 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 and the risk that we will be unable to obtain and maintain regulatory approval 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 May 4, 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 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.

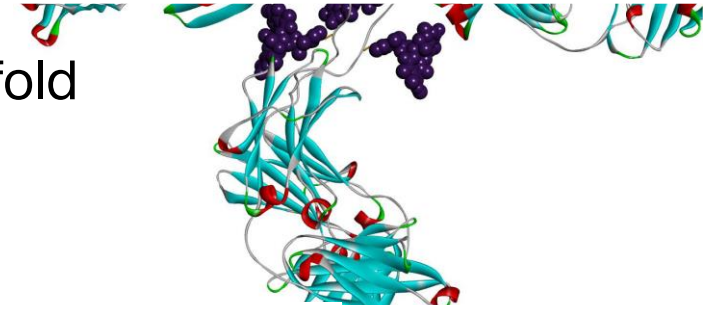
This presentation does not constitute an offer to sell or a solicitation of an offer to buy securities, nor shall there be any sale of any securities in any state or jurisdiction in which such offer, solicitation or sale would be unlawful prior to registration or qualification under the securities laws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

Bicycle therapeutics

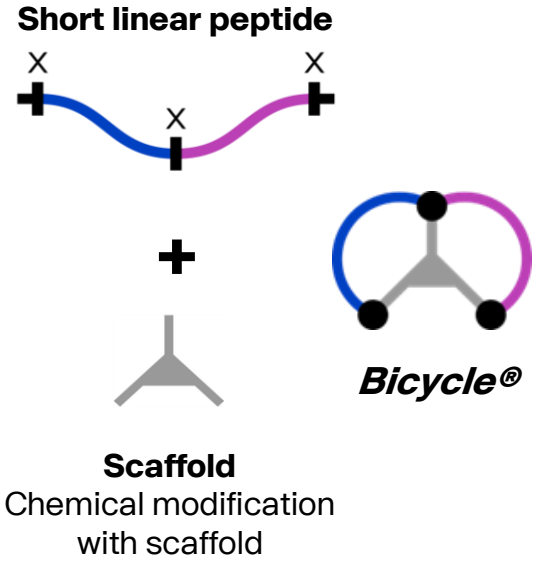
Founded by Sir Greg Winter & Prof. Christian Heinis

UK & US based (Cambridge, UK; Boston, USA)

2018 Nobel Prize in Chemistry
"for the phage display of peptides and antibodies"



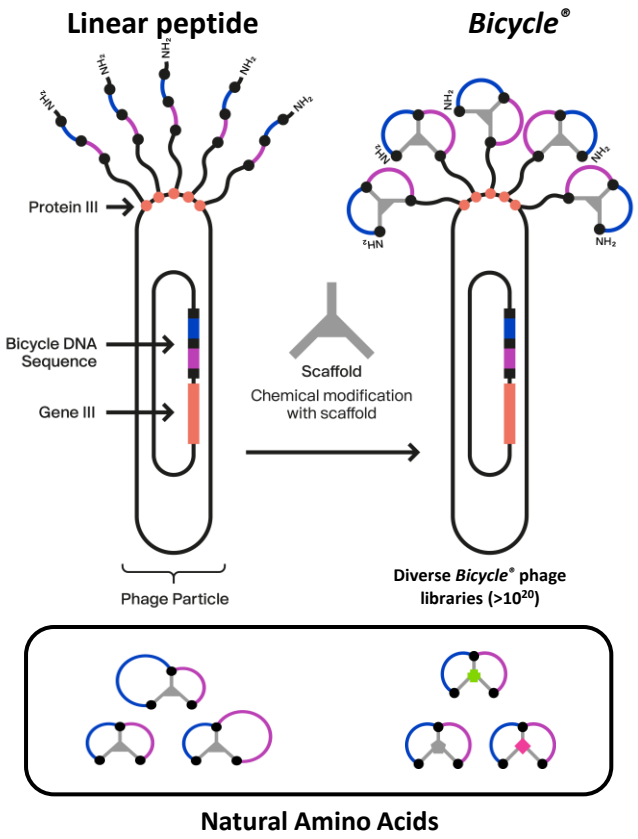
- Bicycles are short peptides chemically constrained with a central scaffold
- Bicycles combine the advantages of small molecules and antibodies



	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

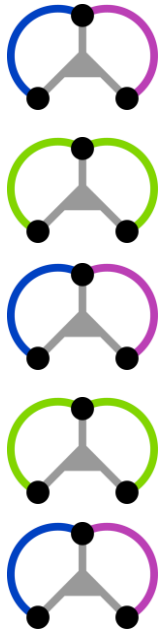
Bicycle® Phage Display - Discovery



Peptide & Medicinal Chemistry

Optimize *Bicycle*® *monomers*

Non-natural Amino Acids



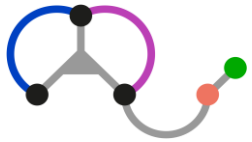
Tumor Targeting and Effector *Bicycles*

Build and Optimize Therapeutic *Bicycles*

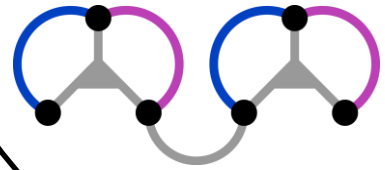
Easy conjugation of Linkers and Payloads

Potential *Bicycle*® Medicines

Monomeric *Bicycles*

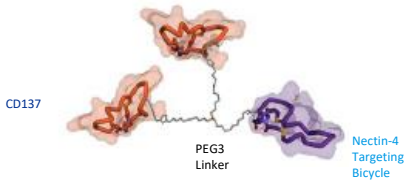


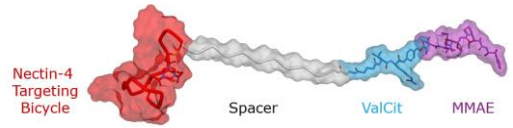
Targeted Drug Conjugates

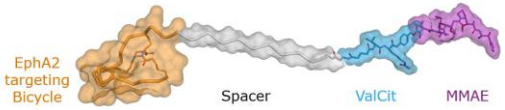


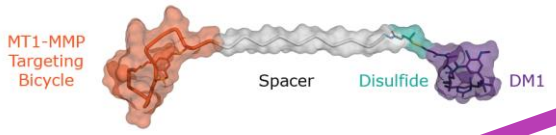
Targeted/ Multi-specific *Bicycles*

Diversifying the *Bicycle®* platform









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

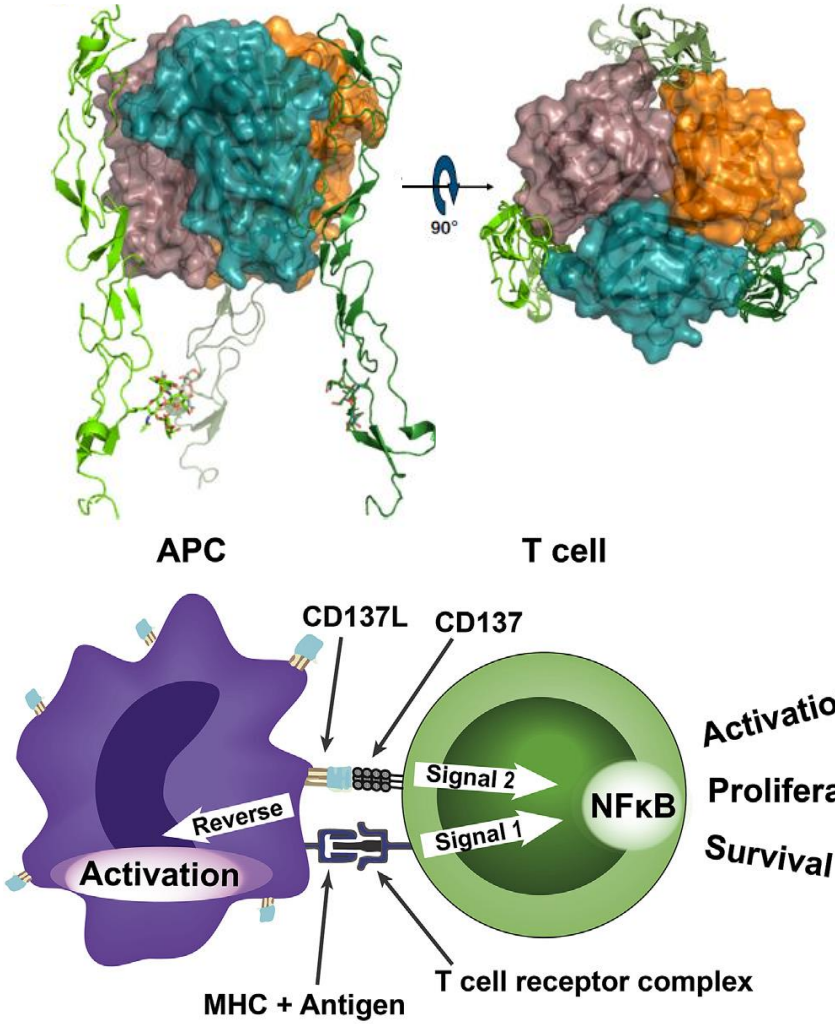
FIH 2018 2019 2020 2021 2023 and beyond

CD137 (4-1BB) is an immune co-stimulatory receptor with high therapeutic potential in cancer

- CD137 is expressed on activated immune cells – signaling enhanced function and survival, prevents anergy
- CD137 ligand expressed by APCs provides a co-stimulatory signal to T cells and NK cells – potential in anti-tumor immunity
- Sustained activation leads to exhaustion and AICD – transient, localized action may be the optimal approach
- Urelumab – anti-CD137 agonist mAb – some clinical activity but liver toxicity precluded development

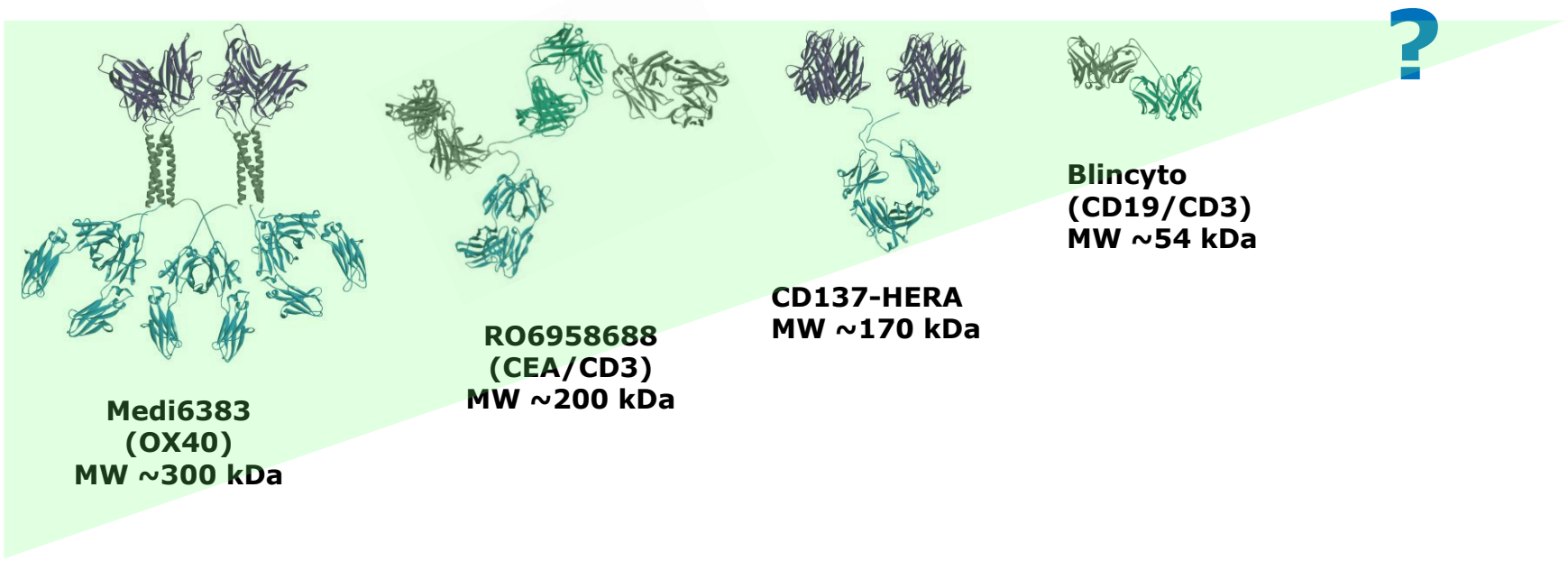
Many agents in development now – none meets design goals dictated by the biology – we sought to address this by using the *Bicycle®* platform:

- Activity localized to the tumor – potentiate immune activation
- Rapid onset of action and controllable duration of action
- No Fc interactions to avoid potential liver toxicity

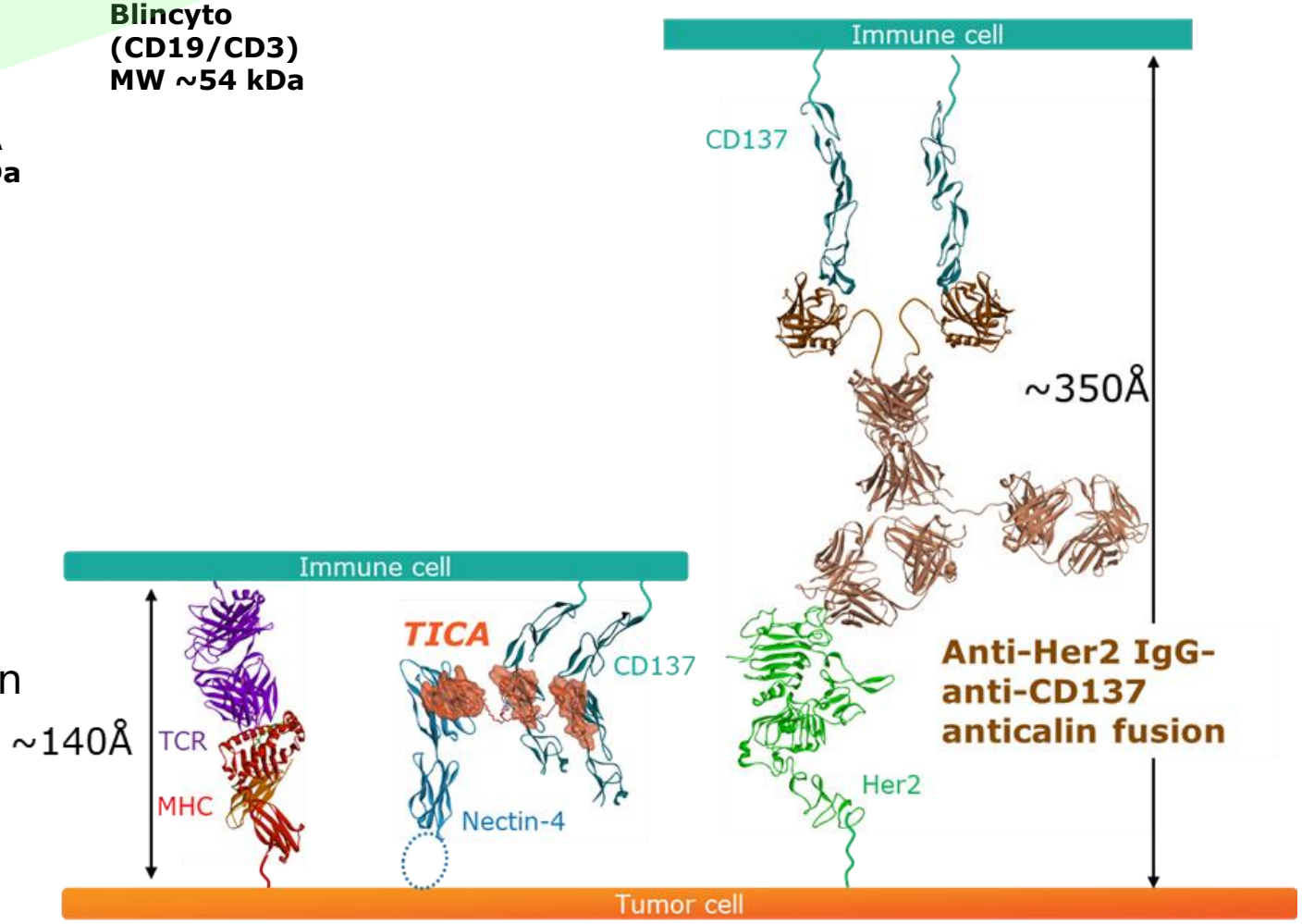


Yonezawa (2015); Melero (2008) TiPS 29, 383; Melero (2007) Nat. Immunol 3, 682; Wilcox (2004) Blood 103, 177; Wilcox (2002) J. Immunol. 169, 4230; Gomes-Silva (2017) Cell Rep. 21, 17; Segal (2016) Clin. Cancer Res. 23, 1929; Zheng – SITC2020 abstract 812; Chin (2018) Nat. Comm. 9, 4679; Soderstrom (2018) Atherosclerosis 272, 66

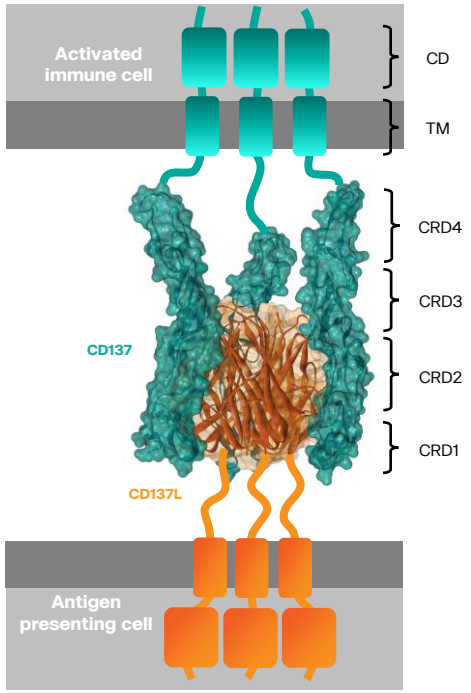
Biologics: not a perfect match for immune agonists



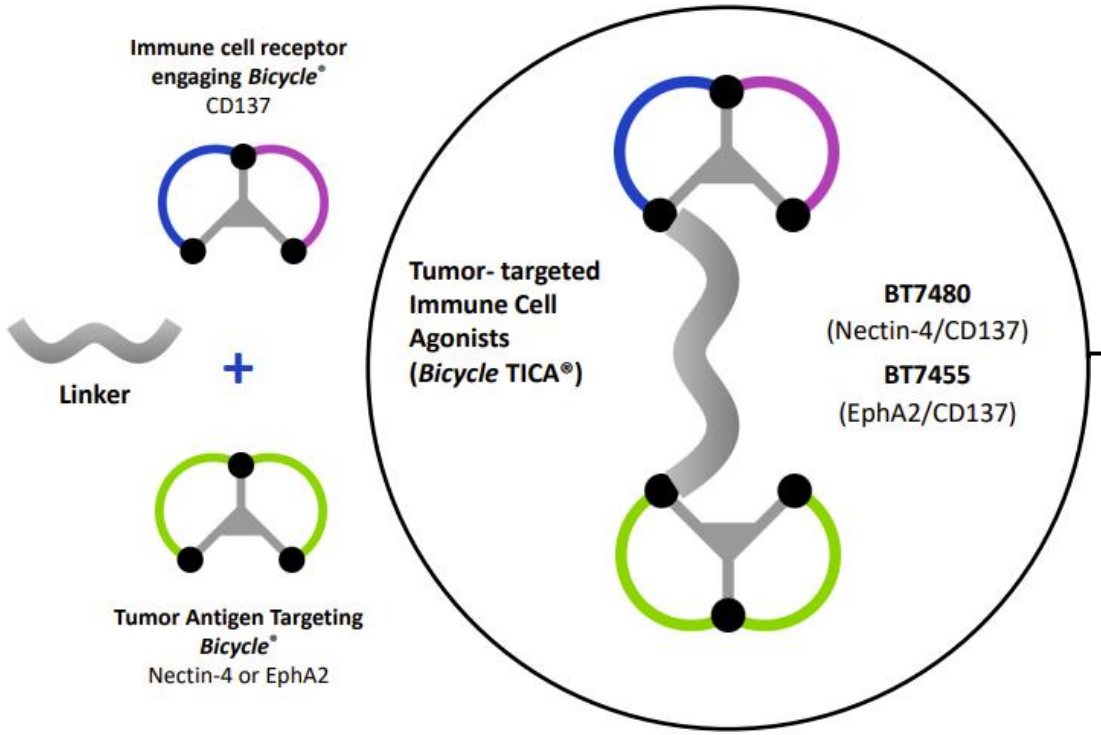
- High MW = low tissue penetration
- Inflexible architecture = difficult “med chem”
- Complex manufacturing = $$$
- Non–tunable PK = not ideal for immune activation (side effects / tox)
- Prone to immunogenicity

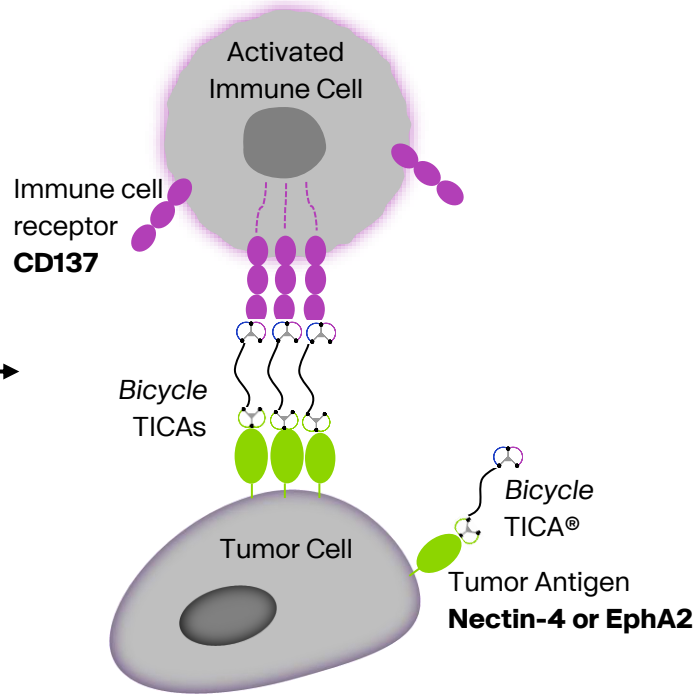


Bicycle TICA™– tumor-targeted immune cell agonists delivers immune agonism to tumors



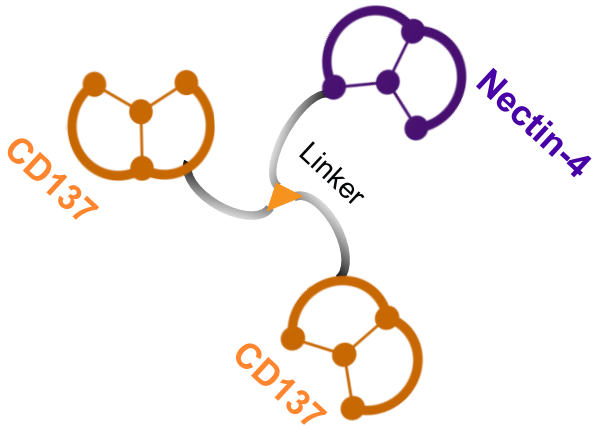
Activation induced by clustering of **CD137** by trimeric **CD137L**

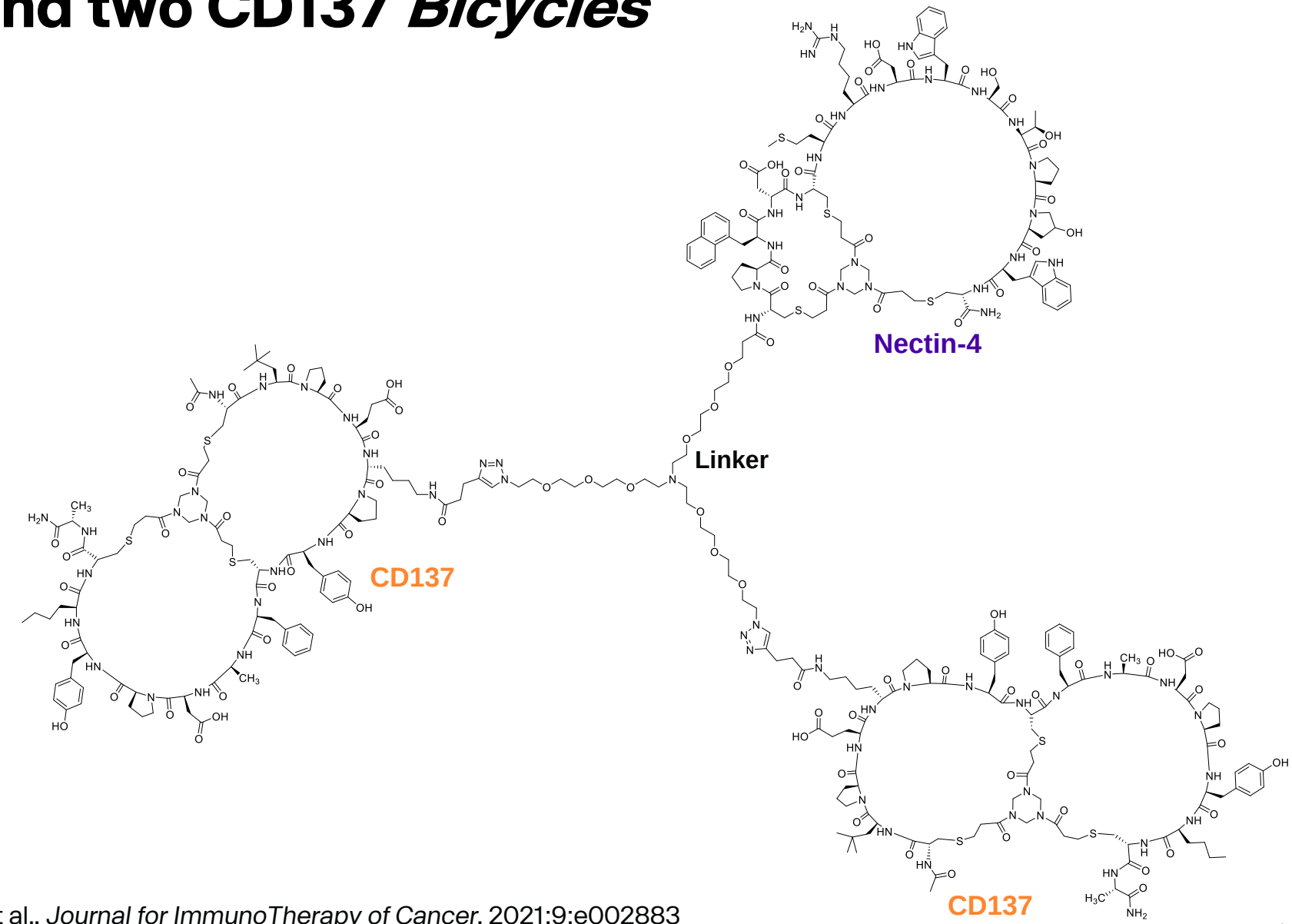




CD137 clustering induced by tumor antigen drives stronger immune cell potency

BT7480 (BCY11863) is a fully synthetic, heterotrimeric conjugate with one Nectin-4 and two CD137 *Bicycles*

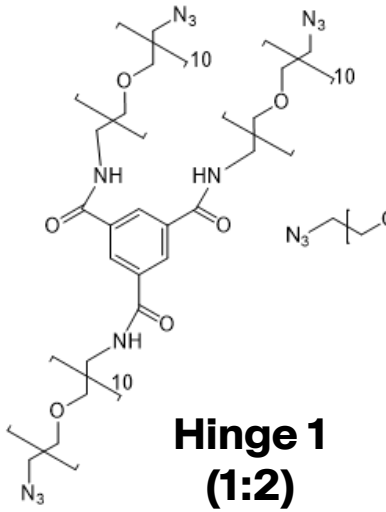




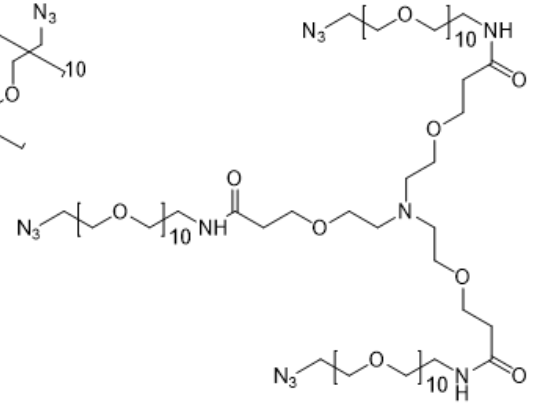
Hurov K. et al., *Journal for ImmunoTherapy of Cancer*, 2021;9:e002883

Effect of valency of Nectin-4/CD137 *Bicycles*:
Better activity with higher ratio of CD137 bicycles

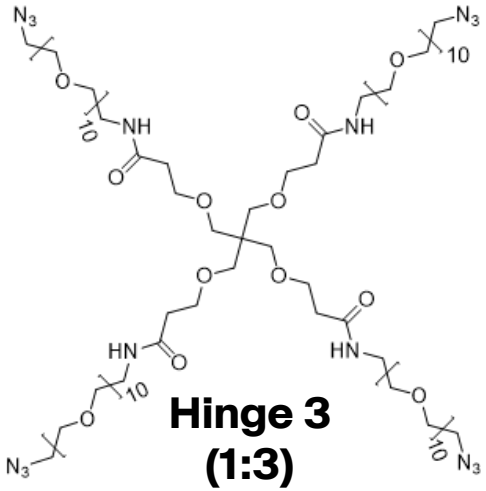
Different hinges synthesized for SAR exploration



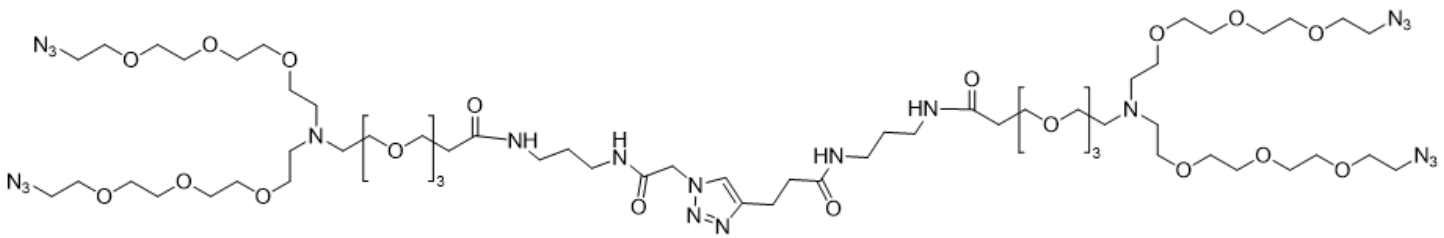
Hinge 1 (1:2)



Hinge 2 (1:2, 2:1)

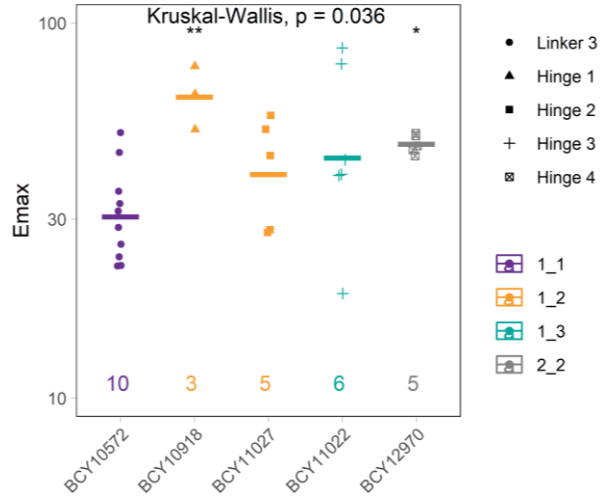


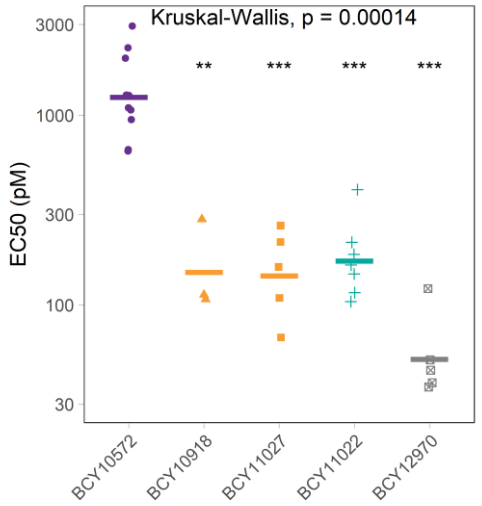
Hinge 3 (1:3)



Hinge 4 (2:2)

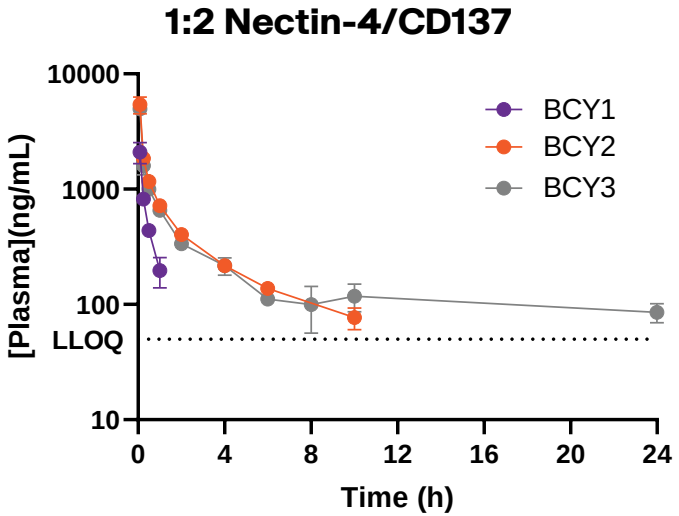
HT1376/CD137 reporter coculture Assay

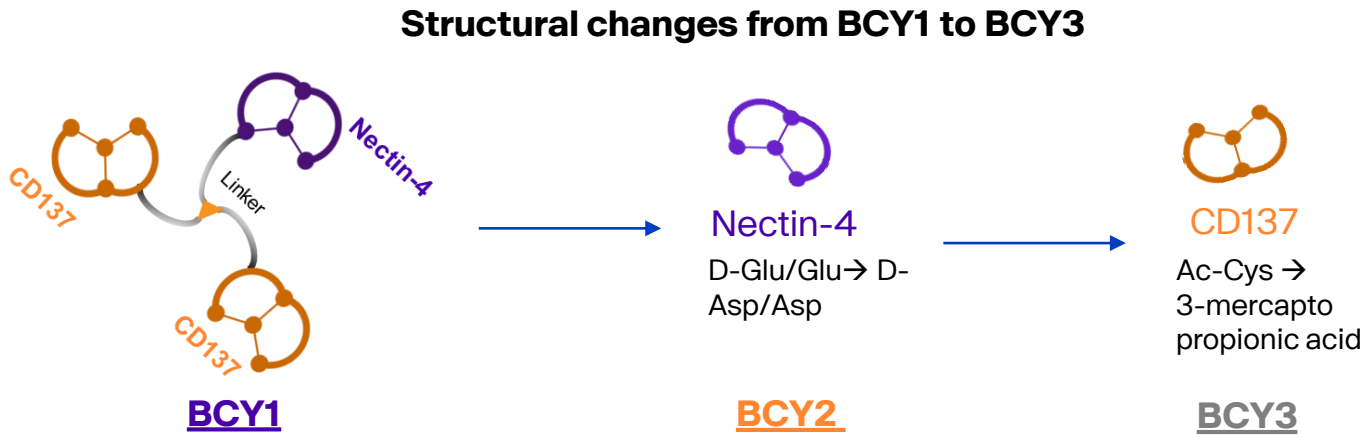


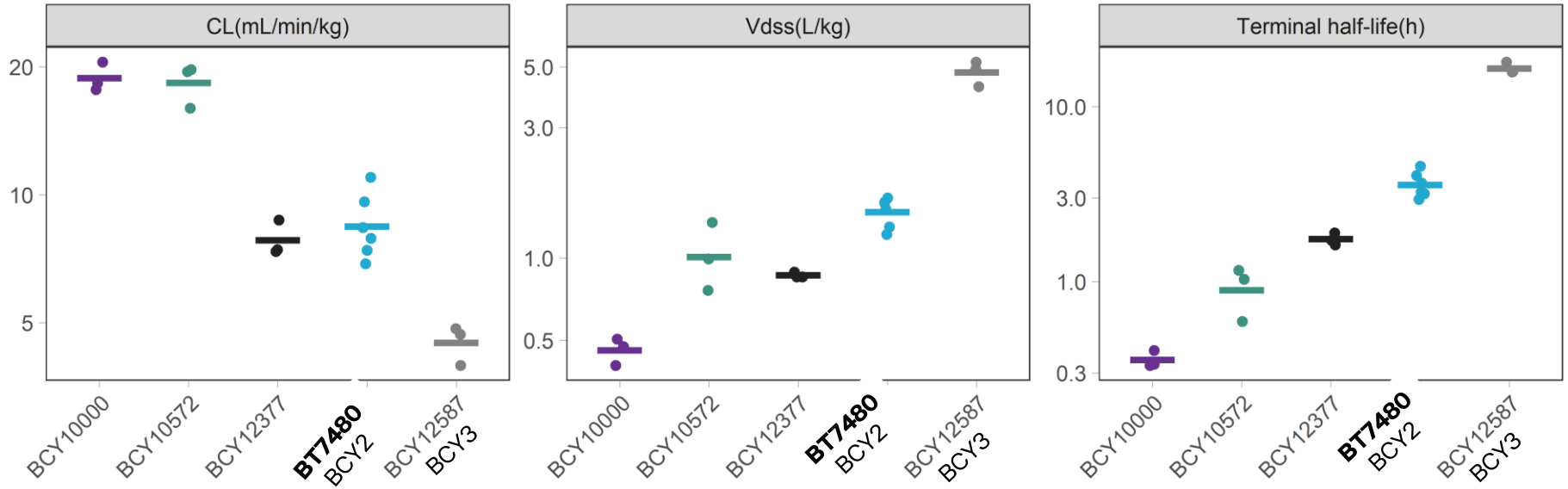


Upadhyaya P. et al., *J. Med. Chem. 2022, 65, 14, 9858–9872*

Optimization of pharmacokinetics of *Bicycle*® TICAs via minor structural changes



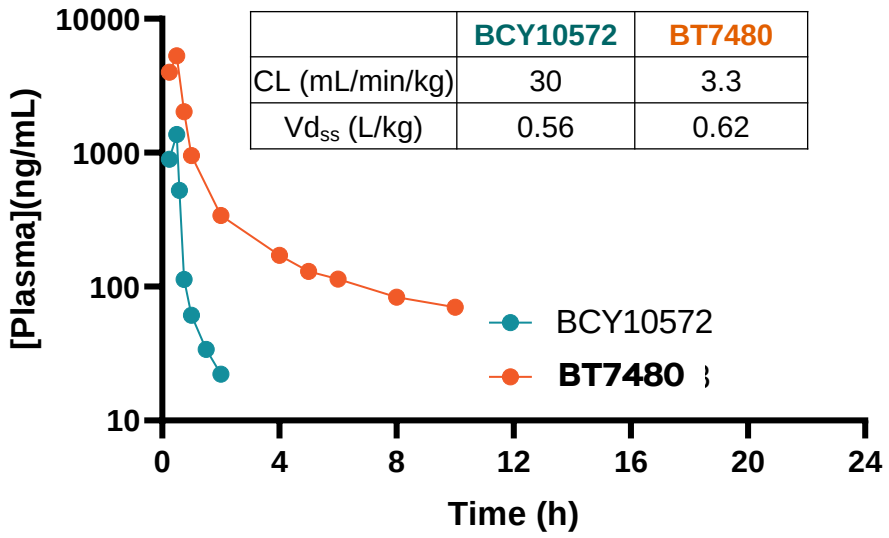




Upadhyaya P. et al., *J. Med. Chem. 2022, 65, 14, 9858–9872*

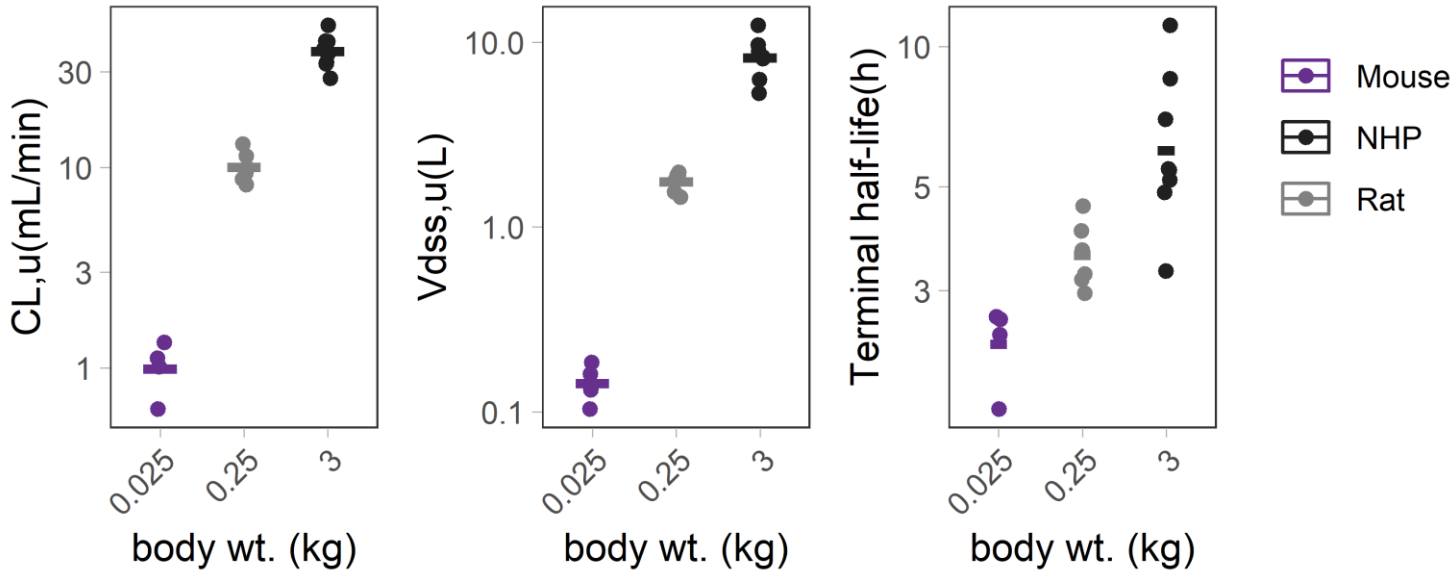
BT7480 pharmacokinetic profile is conserved across nonclinical species

NHP PK (BCY 10572 vs BT7480)



	BCY10572	BT7480
CL (mL/min/kg)	30	3.3
Vd_{ss} (L/kg)	0.56	0.62

BT7480 PK Parameters (CL, V, $t_{1/2}$) in preclinical species



Species	CLp (mL/min/kg)	Vss (L/kg)	Terminal half-life ($t_{1/2}$, h)
Mouse	16	2.3	2.3
Rat	8.5	1.5	3.6
NHP	4.0	0.88	6.2
Pred. Human	1.7	1.3	8.9

NHP: nonhuman primate

Upadhyaya P. et al., *J. Med. Chem. 2022, 65, 14, 9858–9872*

BT7480 demonstrated rapid tumor penetration and renal uptake

- ***In vitro* distribution:**
  - BT7480 does not distribute to red blood cells and is restricted to plasma.
  - BT7480 is subject to relatively low protein-binding.

	Mouse	Rat	NHP	Human
B/P	0.61	0.53	0.33	0.50
PPB (% bound)	61.4	78.9	67.6	74.3

B/P: blood-to-plasma ratio; **PPB:** plasma protein binding; **NHP:** nonhuman primate

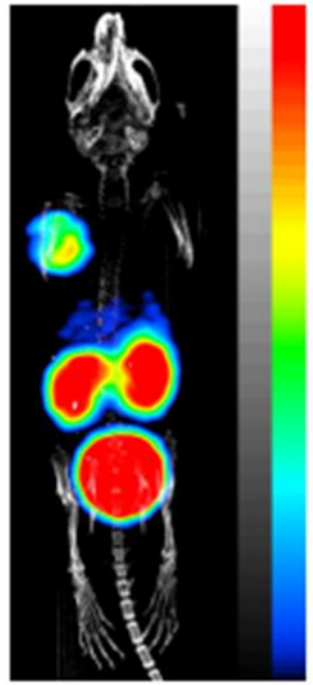
- ***In vivo* distribution:**
  - Rapid tumor penetration: T_{max} = 2 h
  - Colocalization in tumor: AUC ratio =10.6:1 (tumor: plasma)

BCY11863 Mean PK Parameters in Plasma and Tumors, BALB/c Mice (IV injection, 5 mg/kg)

PK Parameter	Plasma	Tumor
C_{max} (ng/mL)	3865	
C_{max} (ng/mL or ng/g)		4149
T_{max} (h)	---	2.0
$t_{1/2}$ (h)	1.65	13.4
T_{last} (h)	4	24
$AUC_{0\text{-last}}$ (ng.h/mL) or (ng.h/g)	3196	33775
$AUC_{0\text{-inf}}$ (ng.h/mL) or (ng.h/g)	3760	46568
AUC_{Extra} (%)	15.0	27.5
AUC Ratio	---	10.6

AUC: Area under curve; **AUC Ratio** = Tumor $AUC_{0\text{-last}}$ / Plasma $AUC_{0\text{-last}}$

- ***In vivo* disposition of EphA2 binding Bicycle in tumor, kidney and bladder**



PET imaging of HT-1080 xenograft @ 60 min

PET: positron emission tomography

Bicycle®

BT7480 underwent extensive extrahepatic metabolism and renal elimination

- Extensive **renal** metabolism and excretion
- No apparent to minimal hepatic metabolism and excretion, consistent with minimal metabolism in microsome/hepatocyte *in vitro*

BT7480 Metabolites in Plasma, Kidney, Urine and Bile from the bile duct cannulated rat (IV infusion for 3 h, 100 mg/kg/h)

ID	Proposed Transformation	Plasma	Kidney	Urine	Bile
BT7480	Parent compound	**√ (dominated)**	√	√	
BT7480 +18 amu	Hydrolytic ring opening	√	√		
> 3000 amu	Hydrolysis	√	**√ (dominated)**	**√**	
<1600 amu	Hydrolysis	√	√	**√ (dominated)**	

BT7480 *In Vitro* Stability

Stability	Mouse	Rat	NHP	Human
Whole blood (heparin) ($t_{1/2}$, h)	5.5	15.7	15.9	>57.8
Microsome (CL_{int}, mL/min/g liver)	< 0.43	< 0.43	< 0.43	< 0.43
Hepatocyte (CL_{int}, mL/min/g liver)	< 0.86	< 0.75	< 0.77	< 0.89

NHP: nonhuman primate

Low DDI risk is expected at clinically relevant concentrations

- **Low DDI RISK associated with BT7480 as the perpetrator of CYP inhibition, CYP induction, and transporter inhibition**
  - IC_{50} values > 50 µM for inhibition of CYPs 1A2, 2C9, 2C19, 2D6, and 3A4
  - No significant induction of CYPs 1A2, 2B6, or 3A4 up to 10 µM
  - Not an inhibitor of MDR1 or BCRP transporters up to 10 µM
  - No or low inhibition of MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3, and OCT2 transporters up to 10 µM

- **Low DDI potential as the victim of CYP enzymes**
  - Low substrate potential for CYP enzymes given the low Cl_{int} in microsomes and hepatocytes across species

IC50: half maximal inhibitory concentration

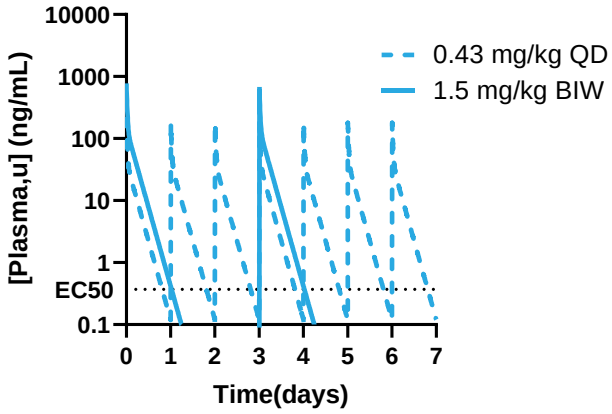
Continuous systemic exposure not required for maximal anti-tumor activity with *Bicycle*® TICAs

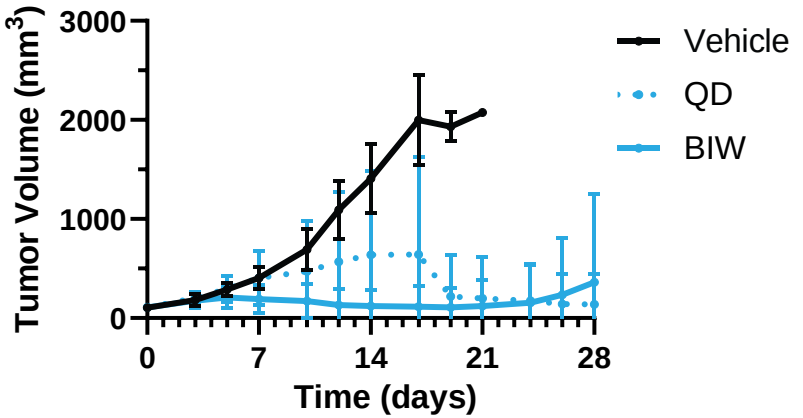
MC38-Nectin-4+ syngeneic mouse model in huCD137-C57Bl/6 mice (BT7480)

Simulated PK

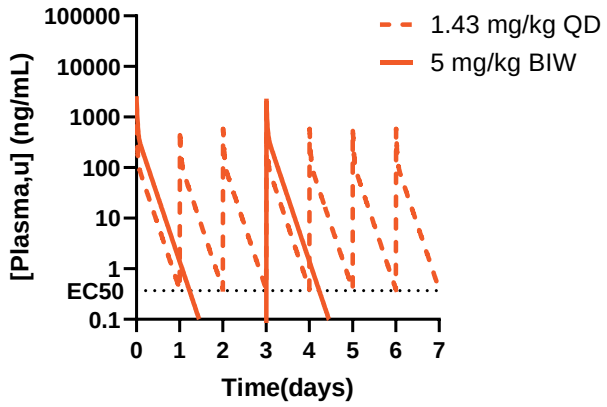
Anti-Tumor Activity

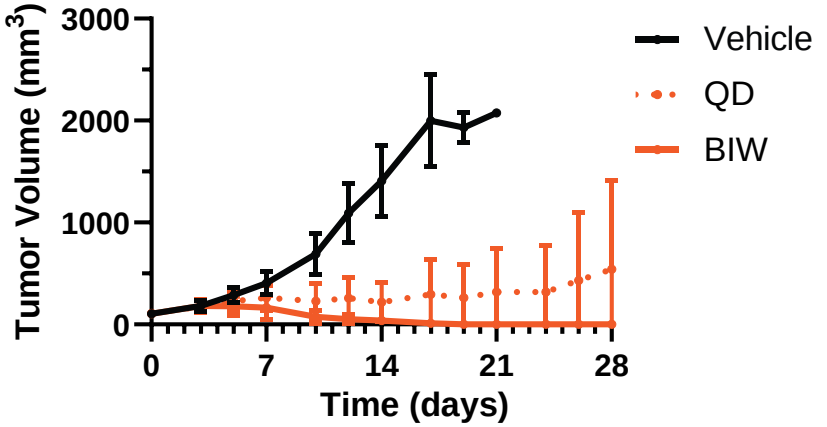
3 mg/kg/week





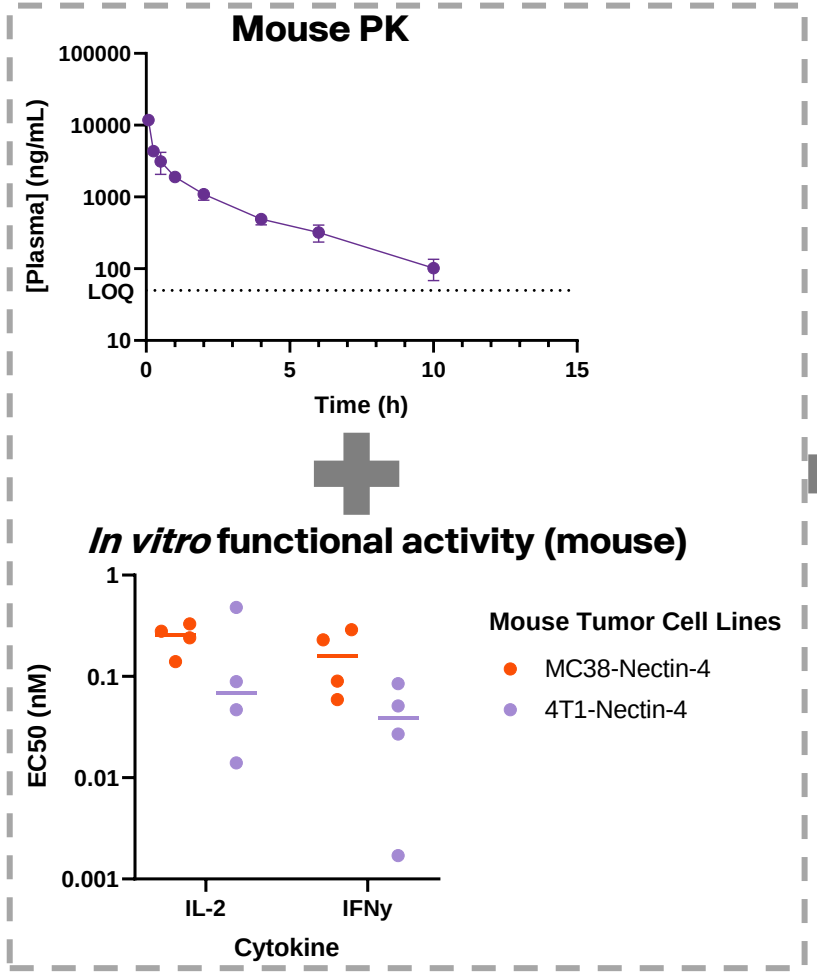
10 mg/kg/week

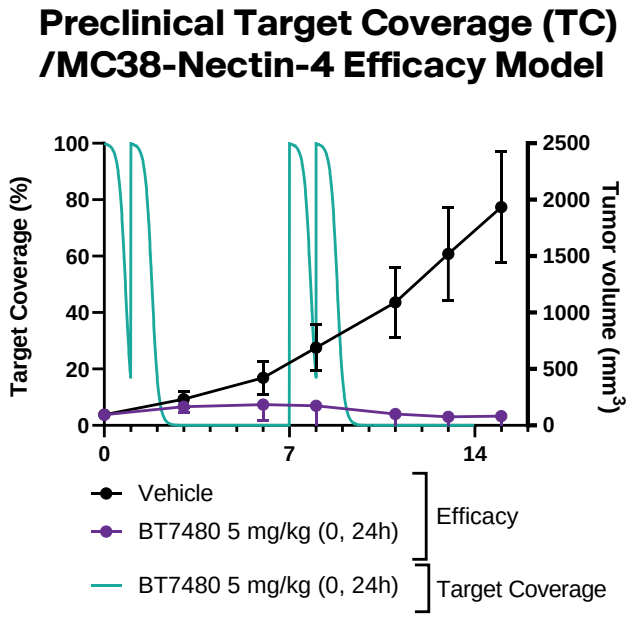




Preclinical PK/PD model projects target coverage at the efficacious dose in the mouse disease model

- A target coverage of 20% for 2 days over 7 days is required for robust anti-tumor activity, e.g., complete anti-tumor activity at 5 mg/kg (0, 24 h) (weekly dose of 10 mg/kg)





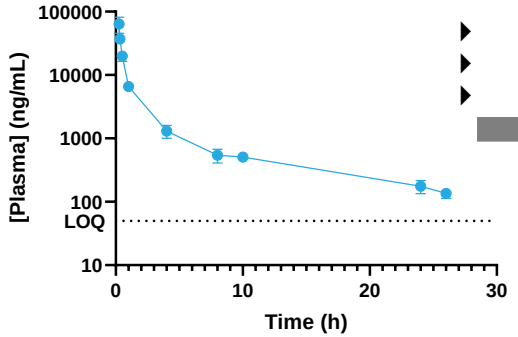
$$\%\,TC = \frac{100\ X\ [Cp,u]^H}{([EC50,u]^H + [Cp,u]^H}$$

Where [Cp,u] is unbound concentration of BT7480 in plasma, EC50,u is the unbound EC50 from the in vitro coculture assay and H is Hillslope.

Preclinical PK/PD model projects efficacy in humans with weekly dosing

- Human PK parameters was predicted using single species scaling from the NHP physiological parameters by the semi-PBPK modeling approach.
- A weekly human efficacious dose is projected to achieve the target coverage of 20% for 2 days.

NHP PK

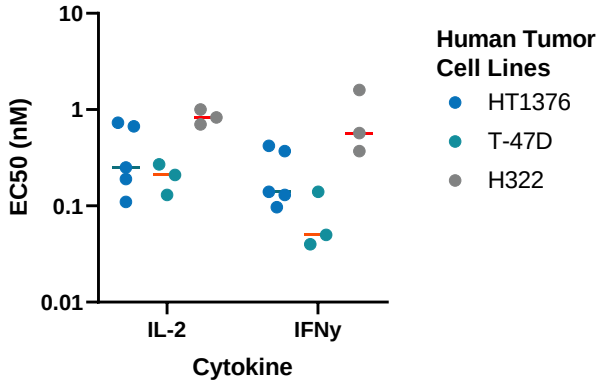


- Two-compartmental Phoenix model
- Allometric exponent of 0.75 (CL) and 1 (V)
- Corrected for protein binding

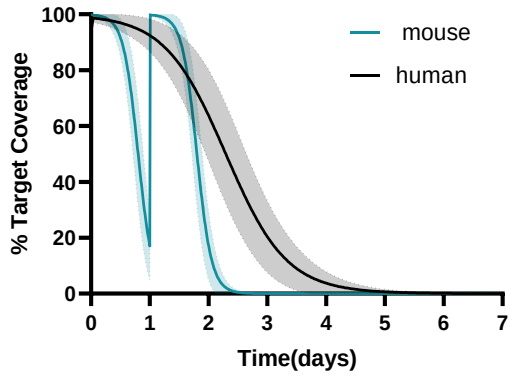
Predicted Human PK

(based on 1 hour zero-order IVinfusion)

***In vitro* functional activity (human)**



Projected target coverage in human vs mouse



$$\%\,TC = \frac{100\ X\ [Cp,u]^H}{([EC50,u]^H + [Cp,u]^H}$$

Where [Cp,u] is unbound concentration of BT7480 in plasma, EC50,u is the unbound EC50 from the in vitro coculture assay and H is Hillslope.

PBPK: Physiologically based pharmacokinetic

Summary

- **DMPK profiles** along with pharmacology and toxicology data **supports the clinical development of BT7480** for the treatment of solid tumors associated with Nectin-4 expression.
  - PK profiles is consistent across the nonclinical species.
  - Protein binding is relatively low.
  - Drug is localized in tumor vs plasma (drug concentration asymmetry in tumor/plasma).
  - The major metabolic pathway is extrahepatic (e.g., renal).
  - The major elimination pathway is renal.
- **Human dose projection** by the integrated PK/PD modeling approach suggested **weekly dosing**
- **Low DDI risk** is expected with BT7480 at clinically relevant concentrations based on existing *in vitro* CYP/transporter studies

Thank you

Johanna Lahdenranta
Heather Cohen
Phil Brandish
Mark Frigerio
Kristen Hurov
Hongmei Xu
Phil Jeffrey

Bicycle®