Turning preclinical findings into clinic-ready biomarker assays to support BT7480 development

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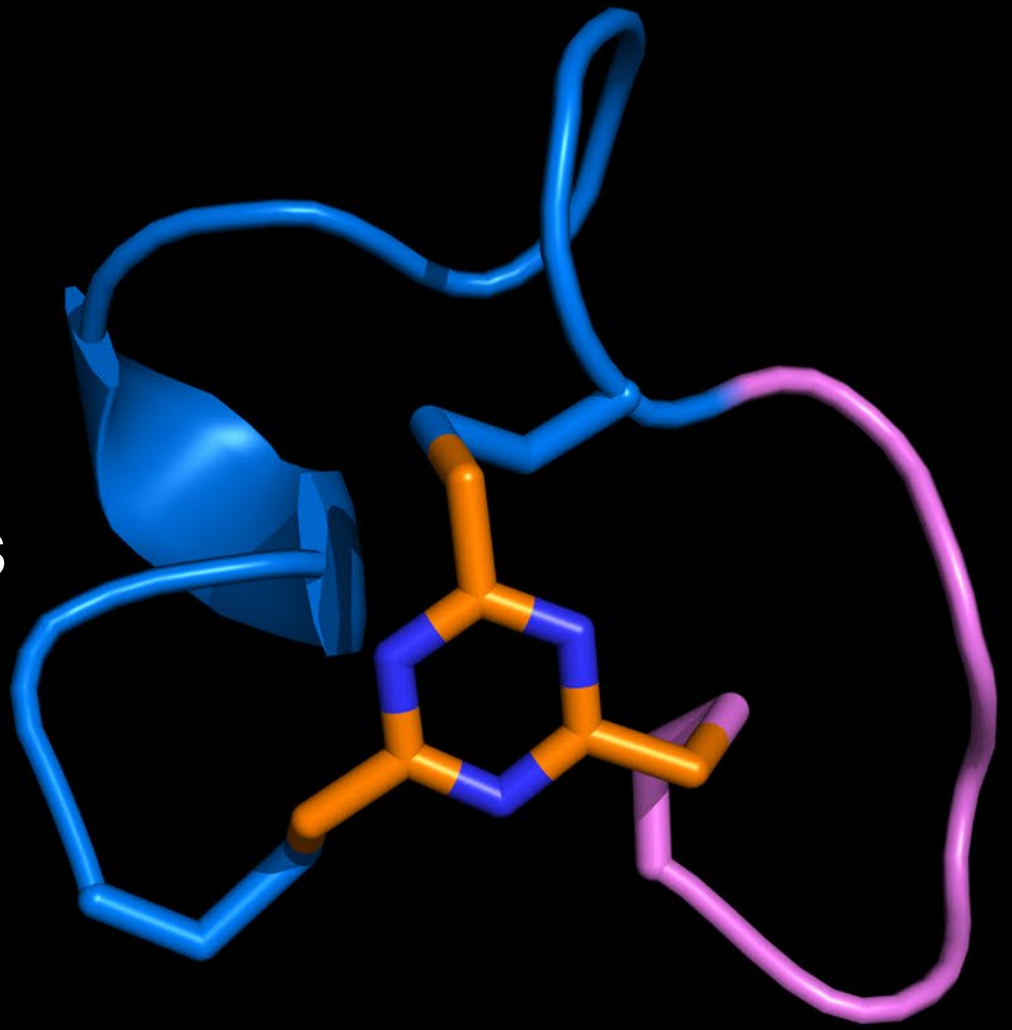

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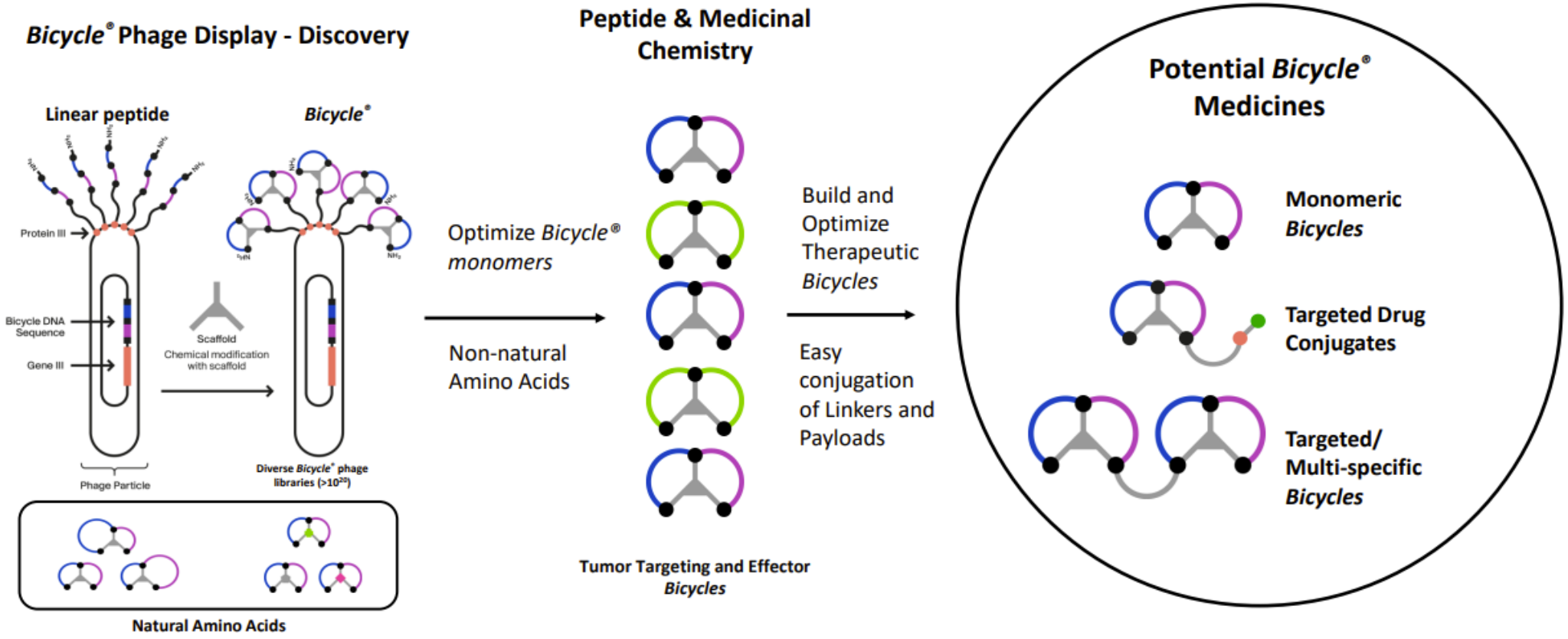
Forward-looking statement

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.

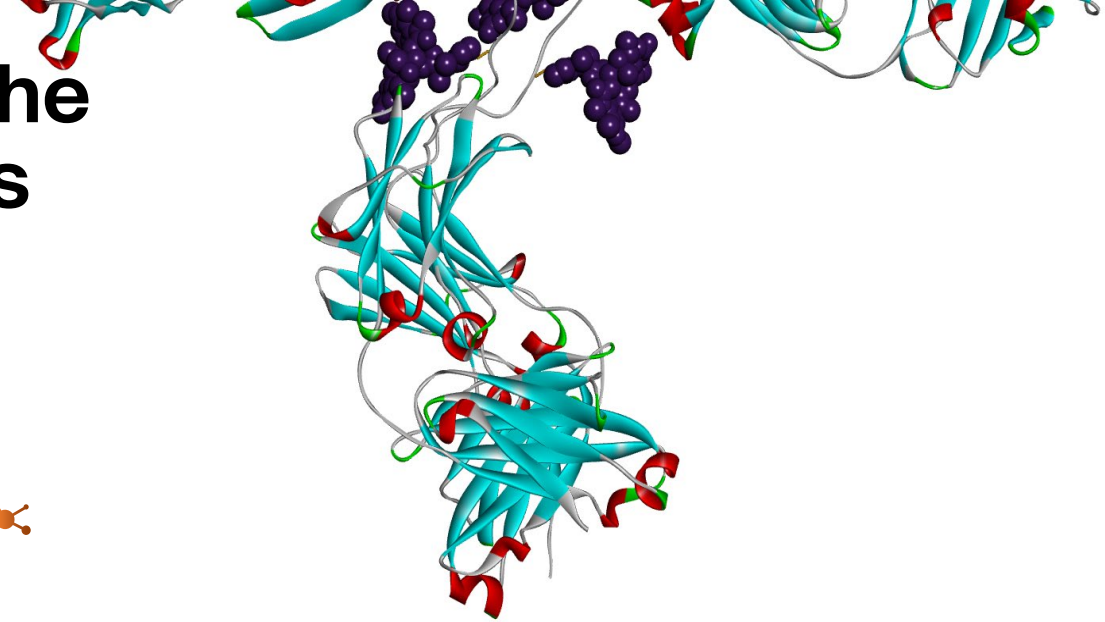
Forward-looking statements are neither historical facts nor assurances of future performance. Instead, they are based on our current beliefs, expectations and assumptions regarding the future of our business, future plans and strategies, our development plans, our preclinical and clinical results, our 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, risks related to the ongoing COVID-19 pandemic, 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, 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 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.

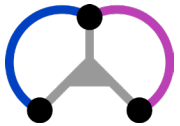
Bicycles are a new therapeutic modality

highly constrained, fully synthetic bicyclic peptides with antibody-like affinity and target selectivity



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



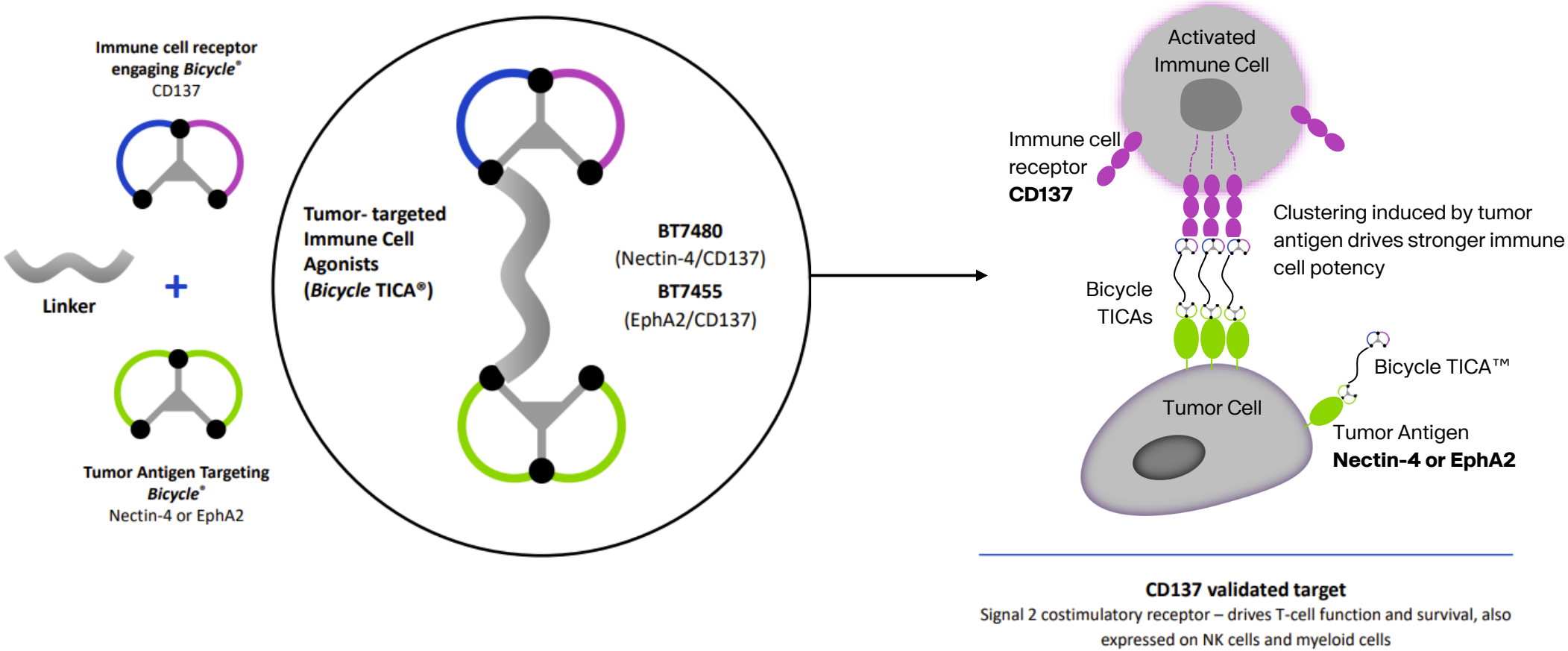


	Bicycle®	Small molecule	Antibody
Small size	**Yes 1.5 to 2 kDa**	Yes <0.8 kDa	No >150 kDa
Specificity	**High**	Low	Multiple
Chemical synthesis (NCEs)	**Yes**	Yes	No
Rapid tissue penetration	**Yes**	Yes	No
Complex protein targets druggable	**Yes**	Limited	Yes
Route of elimination	**Renal**	Liver	Liver

Bicycle's robust proprietary and partnered pipeline

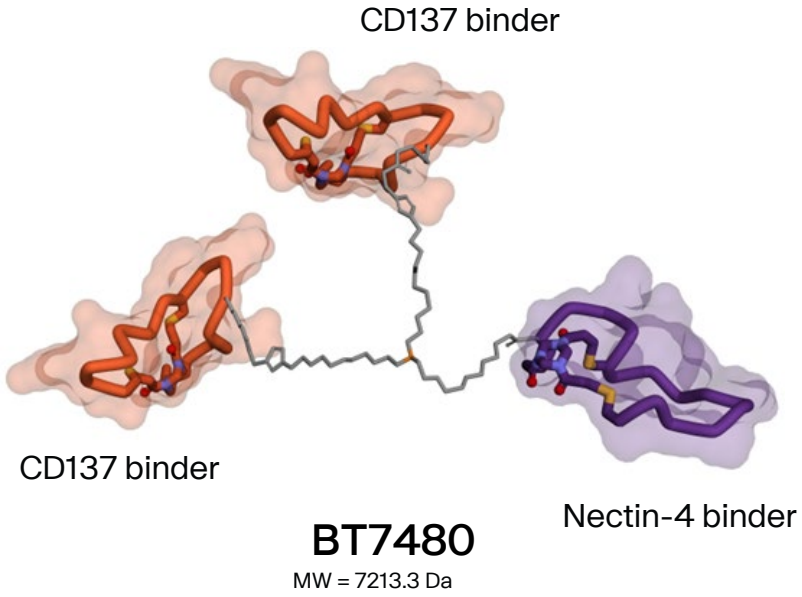
Target / Product	Partner / Sponsor	Indication	Modality	Preclinical	IND-enabling	Phase I	Expansion
Internal Programs							
BT5528 (EphA2)		Oncology	Bicycle® Toxin Conjugate				
BT8009 (Nectin-4)		Oncology	Bicycle® Toxin Conjugate				
BT7480 (Nectin-4/CD137)		Immuno-oncology	Bicycle TICA™				
BT7455 (EphA2/CD137)		Immuno-oncology	Bicycle TICA™				
Partnered Programs							
THR-149 (Kallikrein inhibitor)	OXURION	Ophthalmology					
BT1718 (MT1-MMP)	CANCER RESEARCH UK	Oncology	Bicycle® Toxin Conjugate				
BT7401 (multivalent CD137 system agonist)	CANCER RESEARCH UK	Immuno-oncology					
Undisclosed	Genentech A Member of the Roche Group	Immuno-oncology					
Multiple targets	AstraZeneca	Cardiovascular, metabolic, respiratory					
Novel anti-infectives	Innovate UK	Anti-infectives					
Novel CNS targets	Dementia Discovery Fund IONIS	CNS					
Novel neuromuscular targets	IONIS	Neuromuscular					

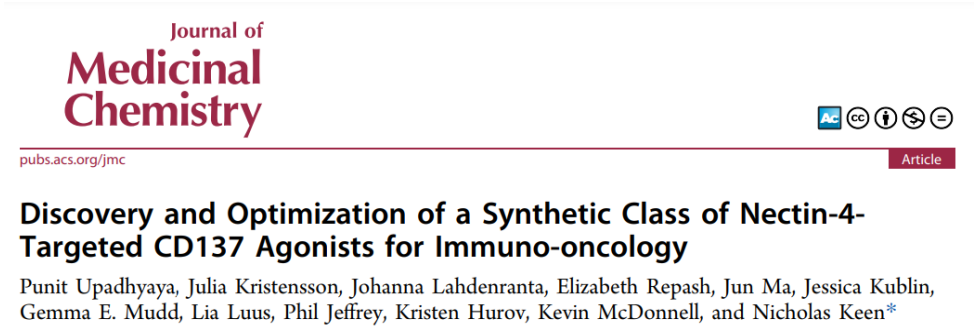
Bicycle TICA™– tumor-targeted immune cell agonists delivers immune agonism to tumors



First *Bicycle* TICA™ entered Phase 1 in Nov 2021 – BT7480

- **Immune activator effector arm = CD137 agonist**
  - Costimulatory receptor – drives T cell function and survival, also expressed on NK cells & myeloid cells
- **Tumor antigen binder arm = Nectin-4**
  - Highly expressed in a wide range of solid tumor indications including breast, bladder, head & neck, esophageal, ovarian, and lung cancer[1,2]
- **Many agents in development now in the field – none yet fully meet design goals dictated by the biology**
  - Immune activity localized to the tumor
  - Rapid onset & controllable duration of action
  - No Fc interactions to avoid liver toxicity
- **Potential first-in-class tumor-targeted CD137 therapeutic**

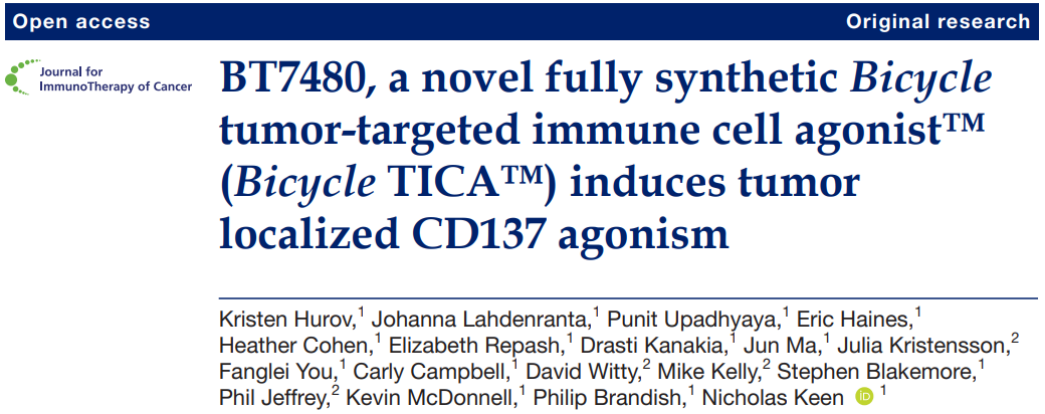



Journal of Medicinal Chemistry

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*


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]

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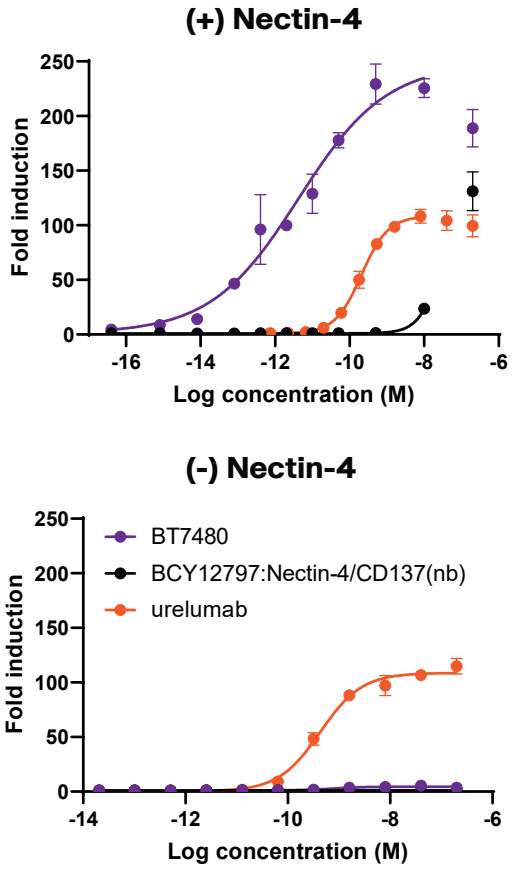
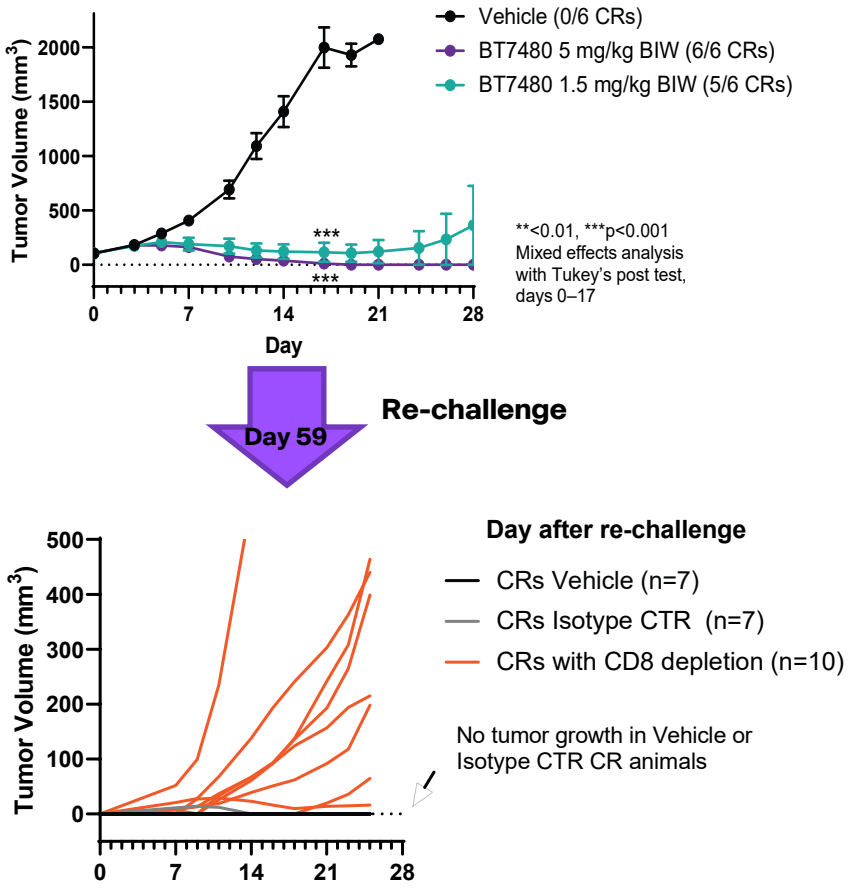
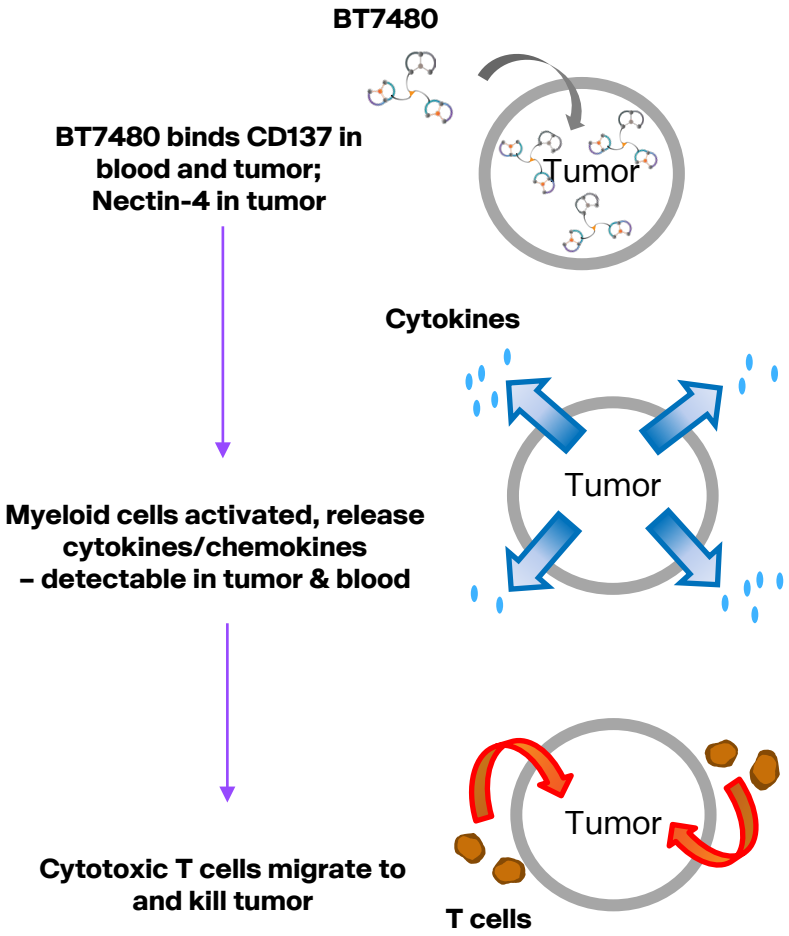
[1]Challita-Eid, et al. *Cancer Research.* 2016
[2] Campbell, et al. AACR. 2021
Hurov K, et al. *Journal for Immunotherapy of Cancer.* 2021
Upadhyaya, et al. *J Med Chem.* 2022

BT7480 activity is dependent on Nectin-4, induces complete responses & memory via differentiated MoA in pre-clinical studies







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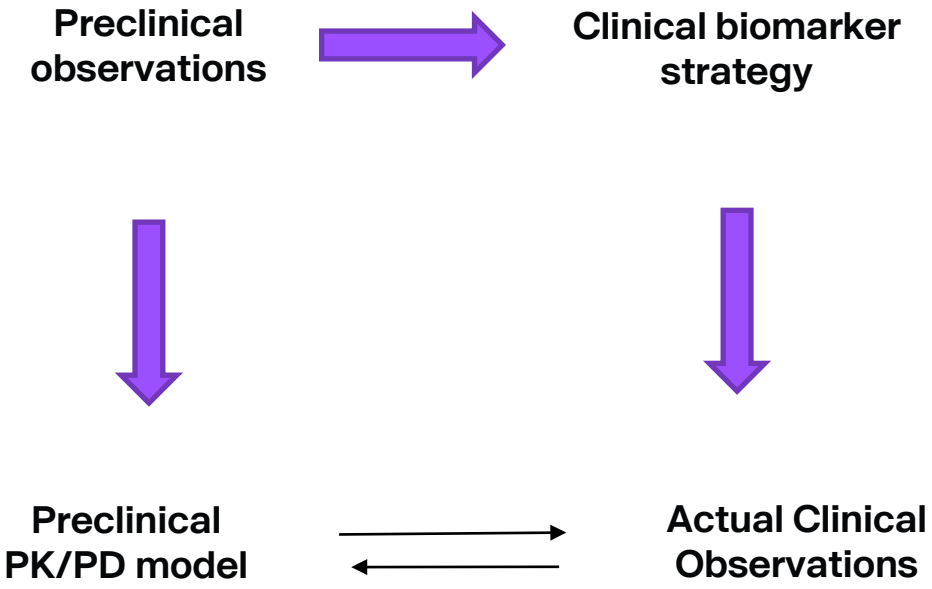
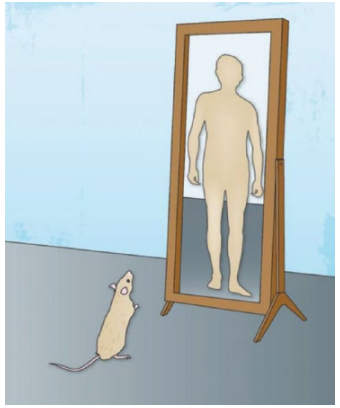
Hurov, K., et al. *Journal for ImmunoTherapy of Cancer.* 2021

Translating preclinical findings into meaningful biomarker strategies to inform clinical development

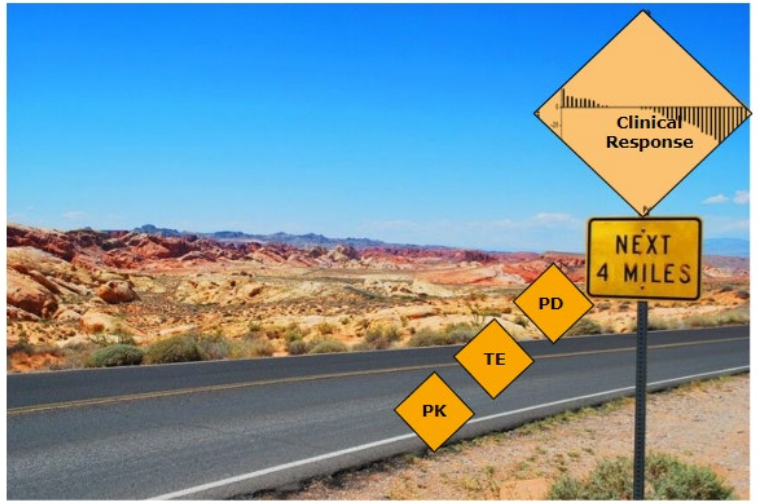
What is the most efficient route to clinical PoC?



How to monitor biology in patients confidently?
Which types of samples/technologies?



On track as anticipated based on PK/PD model?



BT7480 now being evaluated in cancer patients in an innovative biomarker-enabled Phase 1 trial

Suite of custom built, fit-for-purpose assays to inform clinical decision making

19-plex *Bicycle* MultiOmyx™ Assay

Tumor Biomarkers
Nectin-4 Protein Expression
Genomic Profiling
Immune Cell Activation/Infiltration
Immune Cell/Tumor Spatial Proteomics
Immune Transcriptomic Profiling & Gene Expression Signatures

Collected at baseline for retrospective assessment of Nectin-4 and CD137 expression in patients treated with BT7480

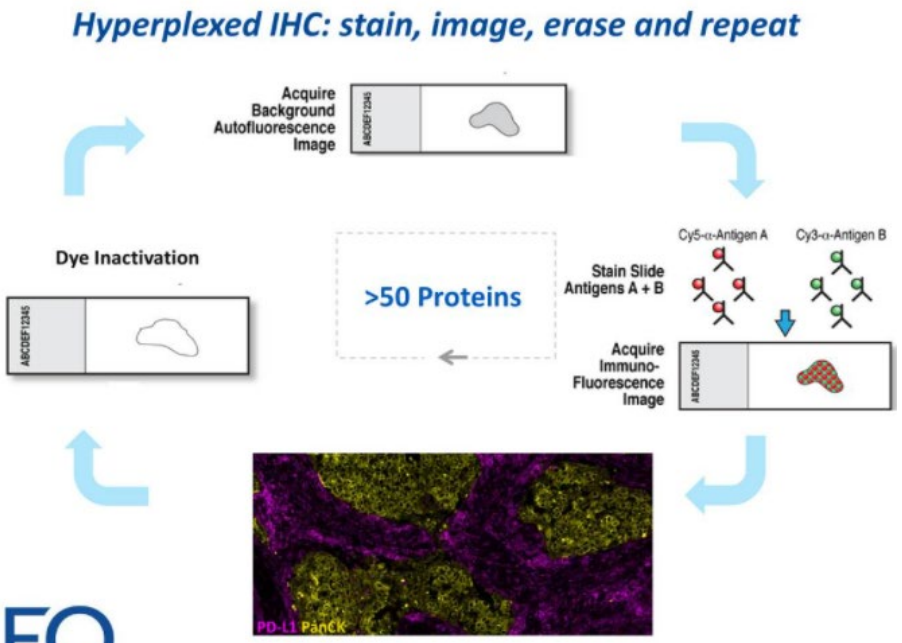
Blood Biomarkers
Pharmacokinetics
CD137 Receptor Occupancy
Immune Cell Activation (transcriptomic & proteomic)
Cytokines & Exploratory Mechanistic Soluble Proteins
ADA Incidence Monitoring
Molecular Changes

14-plex *Bicycle* CD137 RO Assay

Collected pre- and post-dose to assess PK, CD137 TE and immune cell PD profiling to support safety monitoring, study objectives and RP2D

Papadopoulos, K., et al. ASCO 2022.

Development of 19-plex spatial proteomic assay using proprietary Bicycle® Nectin-4 mAb and MultiOmyx™ technology



43 primary human tumors profiled using *Bicycle* MultiOmyx™ panel

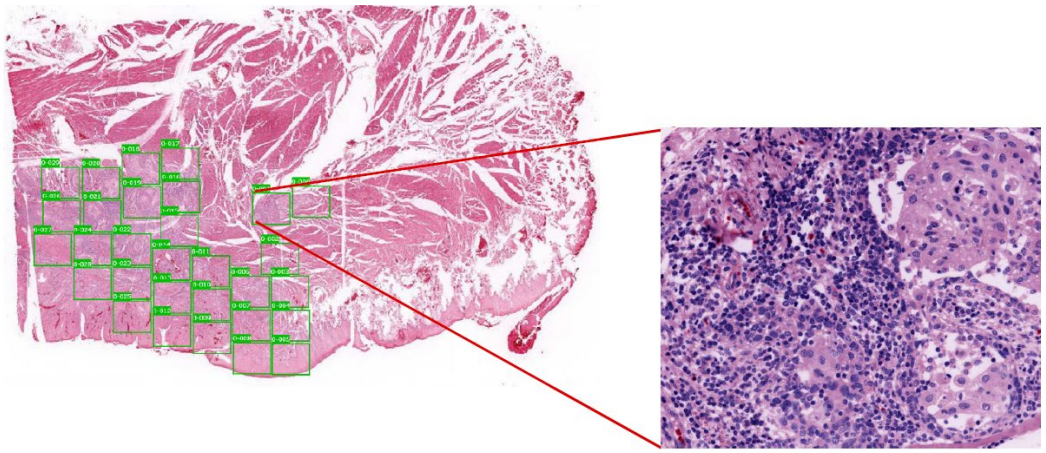
FFPE whole tissue slides
HNSCC (n=5), NSCLC (n=5) and bladder cancer (n=5)

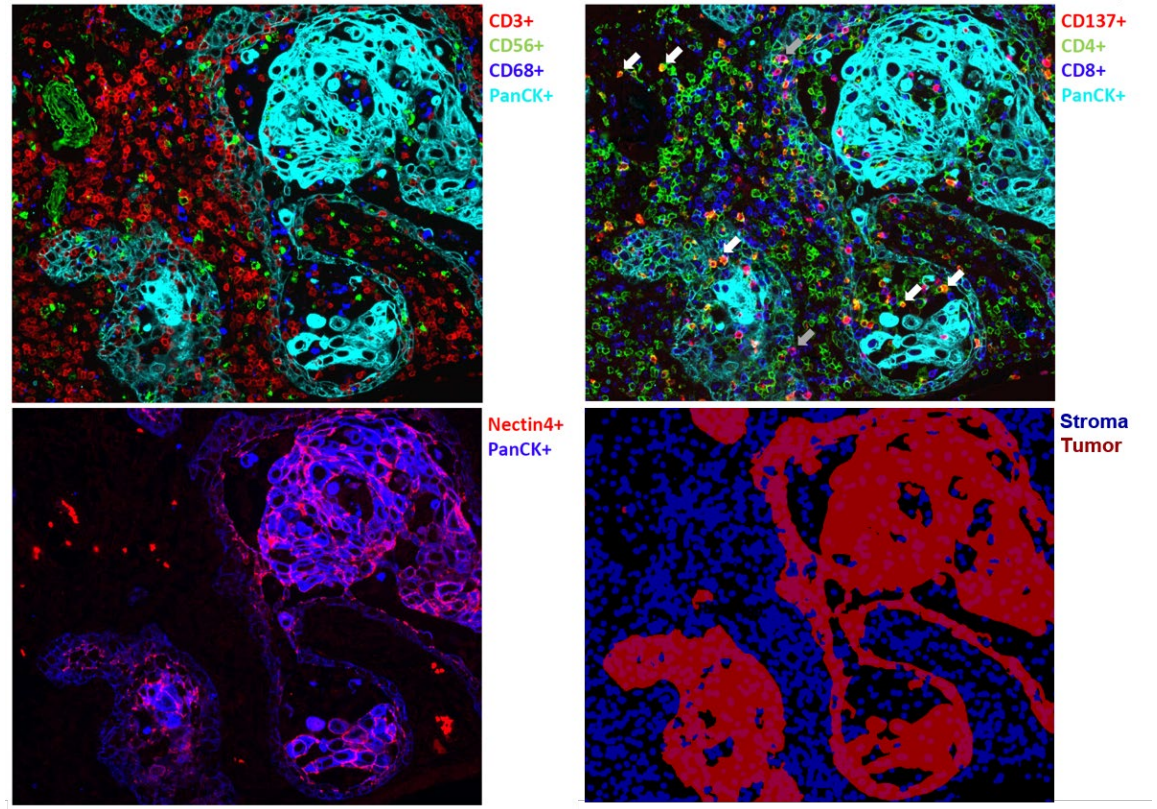
FFPE TMA cores
breast (n=14) and lung (n=14) cancers

Tumor samples analyzed procured from TriStar Technology Group

Allows for simultaneously quantification of Nectin-4+ and CD137+ cells, immune cell subsets of interest and their spatial topography in a single FFPE sample!

Spatial proteomic profiling of Nectin-4+ and CD137+ cells using MultiOmyx™ technology





A single ROI from a representative HNSCC sample is shown. Tumor and stroma regions were identified using a PanCK and DAPI mask respectively.

- Each FFPE slide was presented to a pathologist for tissue annotation and ROI selection
- Proprietary deep learning-based workflows were applied to identify stroma and tumor regions, individual cells and perform cell classification for phenotypes of interest

Tumor samples analyzed procured from TriStar Technology Group

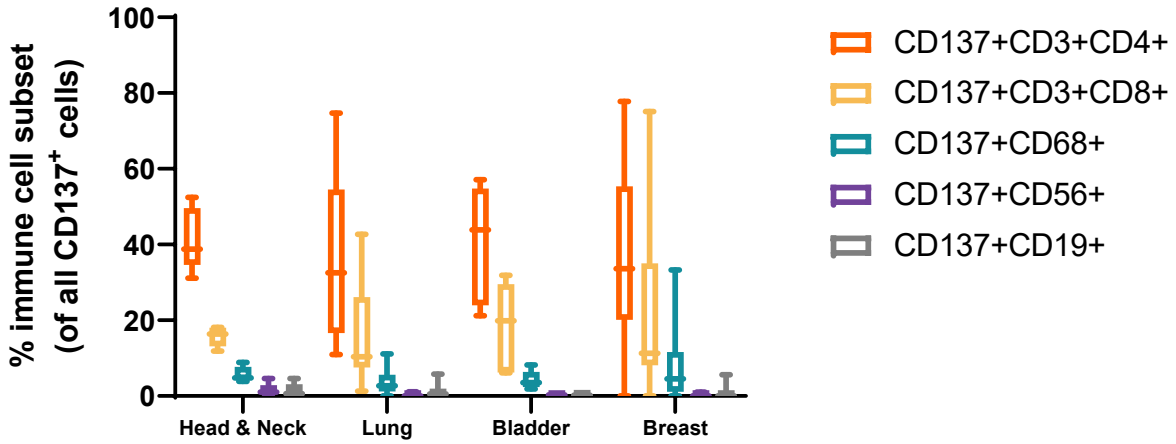
Co-expression of CD137 and Nectin-4 proteins detected in >50% cancer samples tested – good concordance with RNA

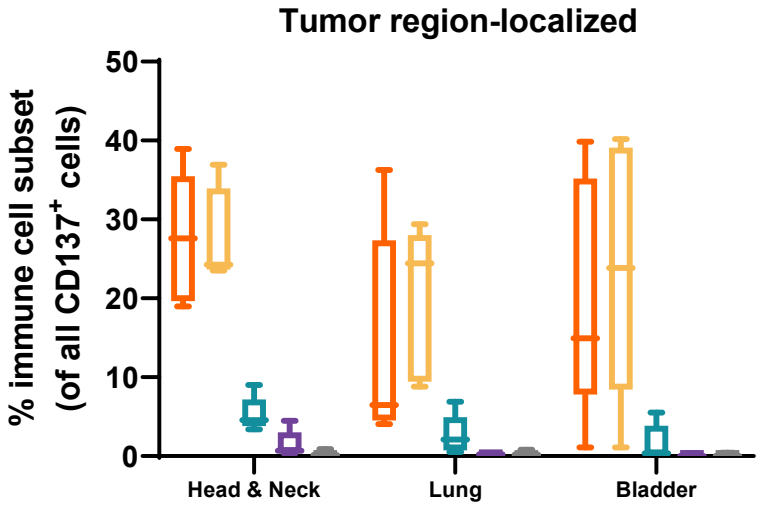
results help support prioritization of indications for clinical development

	transcriptomic		proteomic	
Indication	TCGA Total samples (N)	% Nectin-4/CD137+ (of samples with > average RNA expression)	MultiOmyx™ Total samples (N)	% Nectin-4/CD137+ (of samples with > 1% target+ cells)
Head & Neck	520	78.5	5	100
Lung (all)	1018	74.4	19	73.7
Lung adeno	517	75.5	8	75
Lung squam	501	73.3	10	70
Breast	1093	50.3	14	57.1

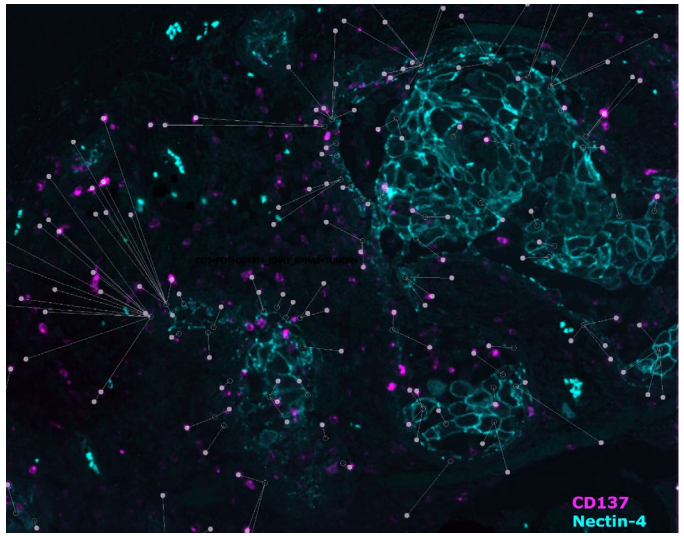
Frequency of samples co-expressing Nectin-4 and CD137 at the protein level (>1% positive cells) is shown
Tumor samples proteomically analyzed procured from TriStar Technology Group

Majority of CD137+ immune cells in Nectin-4-expressing tumors are T cells and macrophages





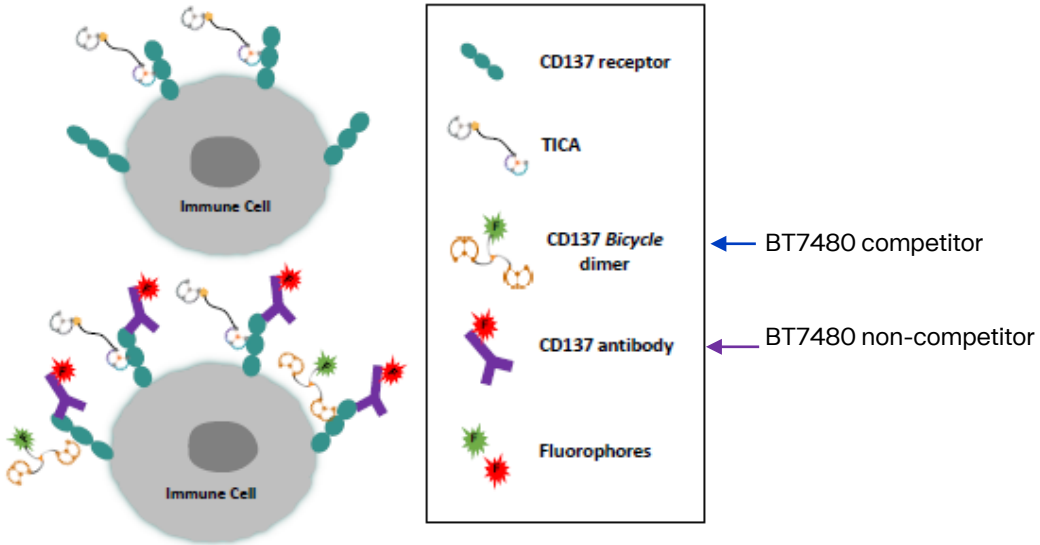
- A subset of CD137+ immune cells are deeply tumor penetrant
- Nearest neighbor analysis indicates CD137+ immune cells were detected within 150 microns of Nectin-4+ tumor cells across indications analyzed



Tumor samples analyzed procured from TriStar Technology Group

Development of a 14-plex CD137 RO flow cytometry assay to monitor target engagement in patients' blood

- **Receptor occupancy = on-cell competition binding assay to detect drug bound to target, associated with PD and efficacy signals**
- **Challenges in building a CD137 RO assay**
  - CD137 is dynamically expressed on small subset of circulating immune cells
  - Limited commercial CD137 reagents available
  - Clinical sample matrix and processing may impact drug binding/target expression
- **Solution? Use *Bicycles* as reagents to build clinical assay!**
- **Proprietary assay, differentiator among other CD137 agonists in the clinic**
- **Allows us to monitor target engagement and characterize immune cell types in a single blood sample**



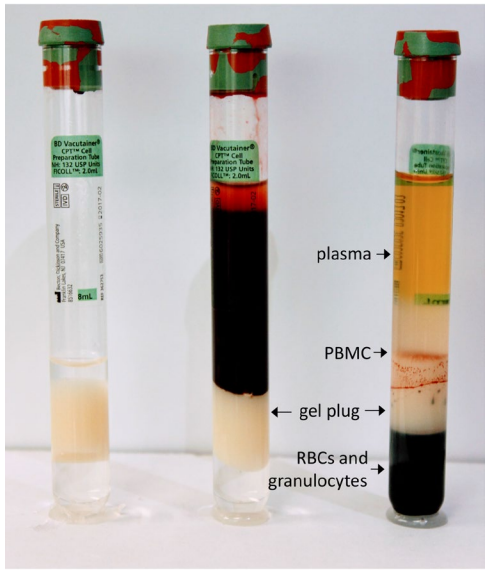
Bicycle® CD137 RO flow cytometry panel testing across clinically-relevant sample matrices

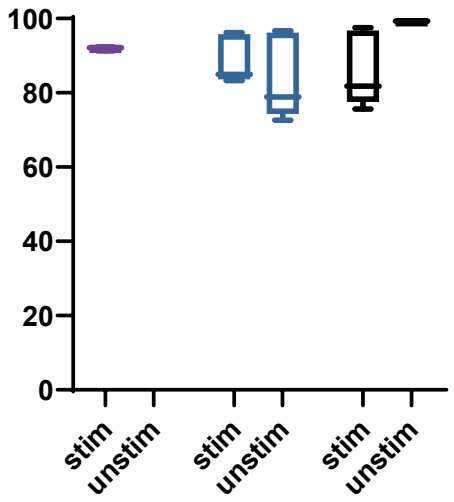
Sample stability, viability, batch-ability, customs suitability, bicycle interaction, antigen stability – differ among sample matrices

EDTA vacutainer

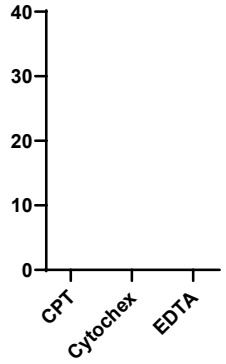
Cyto-Chex® vacutainer

CPT (Cell Preparation Tube) vacutainer

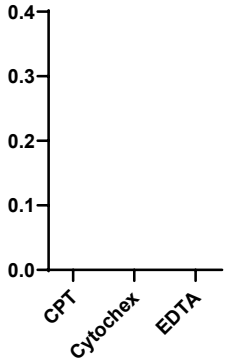




Stim blood



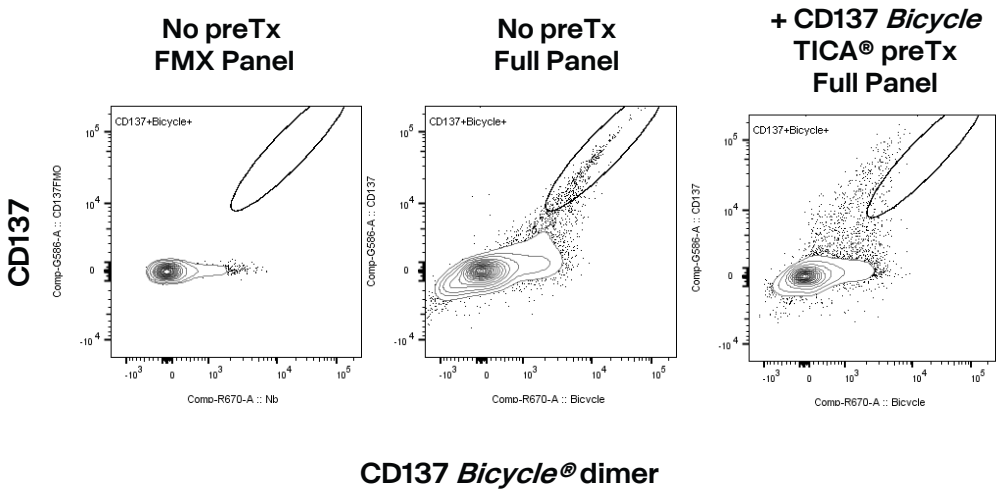
Unstim blood

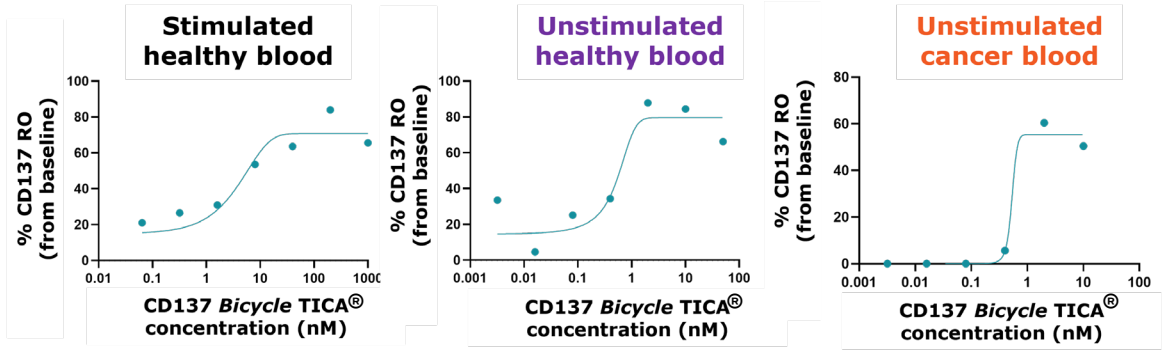


Which will give best quality data for *Bicycle* TICA™?
CPT selected as most optimal sample matrix
least amount of background, least sample variability, highest viability, & detection of CD137+ cells

Blood samples analyzed sourced from healthy volunteers

Bicycle® CD137 RO assay is functional in human blood, suitable for clinical testing purposes





% TE = (1-(DTE post-dose/DTE pre-dose))*100
DTE = %CD137+*Bicycle*+ full stain panel - %CD137+*Bicycle*+ FMX panel

- **Ex vivo RO assessments in healthy human blood collected in CPT demonstrated dose-dependent detection of CD137 RO by CD137 *Bicycle* TICA™**
- **pretreated with 10nM CD137 *Bicycle* TICA™ shown**

- **Method optimization resulted in consistent detection of CD137 RO by CD137 *Bicycle* TICA™ and >1000 CD137+ cells with >70% viability in unstimulated healthy and cancer blood samples**
- **pretreated with 10nM CD137 *Bicycle* TICA™ shown**

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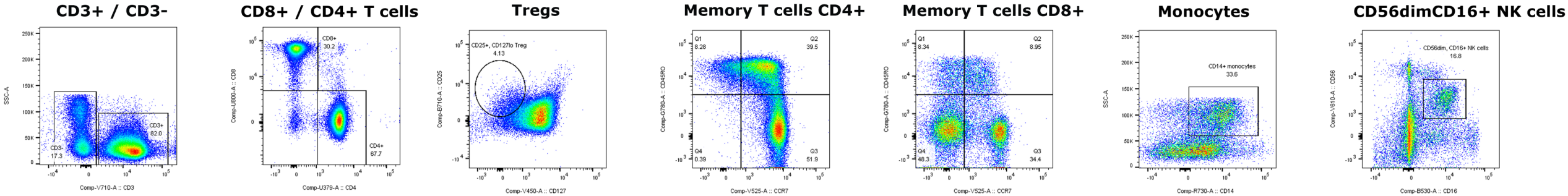
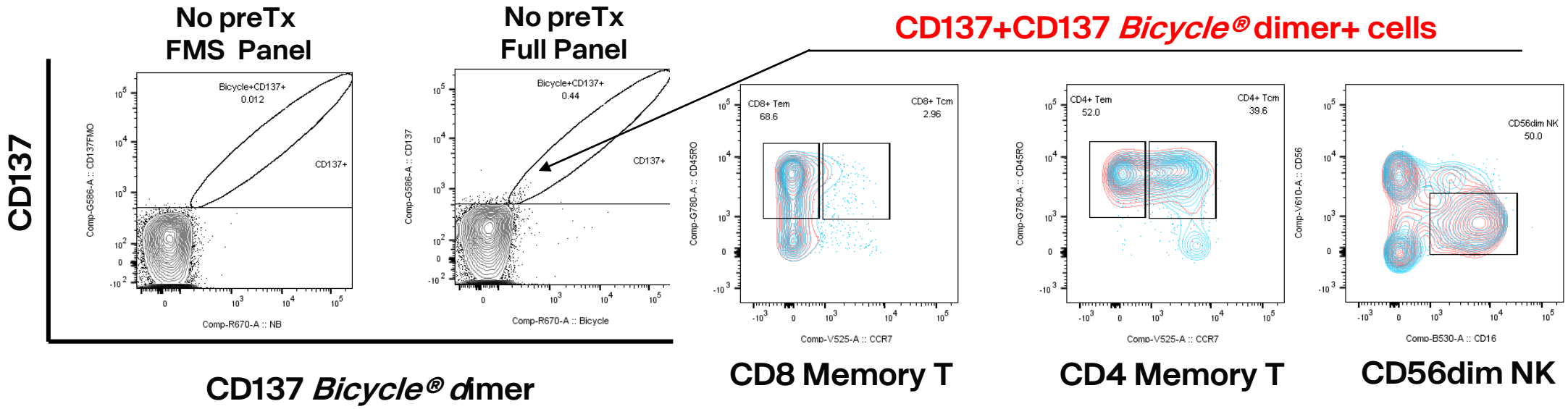
Blood samples analyzed sourced from healthy or non-clinical cancer patient volunteers

CD137 *Bicycle*® dimer detects CD137+ cells that are largely memory T cells in human blood





Blood samples analyzed sourced from healthy volunteers

BT7480 biomarker assay development summary

- BT7480 is a Nectin-4 dependent CD137 agonist with high biological potency and differentiated MoA leading to robust and durable anti-tumor responses in preclinical mouse models
- BT7480 Ph1/2 trial initiated in Q4-2021 and is currently active (NCT05163041)
- Assay development studies support the utility of the Bicycle® MultiOmyx™ assay to monitor Nectin-4 and CD137 protein expression and potentially demonstrate proof-of-mechanism in patient tumors
- Results demonstrate the first clinical flow cytometry assay using fluorescently labelled *Bicycle®* reagents and supports the utility of the Bicycle® CD137 RO assay to monitor target engagement in the BT7480 first-in-human clinical trial

Lowering barriers to assay translatability to the clinic

- Robust clinical biomarker strategies critically rely on reliable preclinical data packages
- Testing across sample matrices, tumor/sample types and ability to generate novel reagents enables ability to build clinically relevant biomarker assays
- Precious samples – prioritize readouts with clear hypotheses and clinically-experienced sample processing methods
- Regularly survey new approaches that yield high amount of data with low sample input & limited burden to patients
- Strong collaborations with preclinical, clinical operations/development, 3rd party labs needed for success

Thank you

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Flowmetric, Inc.: Chintan Jobaliya, Adam Cotty

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