Translating preclinical findings into clinical biomarker assays to support the Phase I/II study of BT7480, a Bicycle tumor-targeted immune cell agonist®

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Director Translational Sciences
Forward-looking statement

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**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

<table>
<thead>
<tr>
<th>Feature</th>
<th>Bicycle®</th>
<th>Small molecule</th>
<th>Antibody</th>
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<tr>
<td>Small size</td>
<td>Yes 1.5 to 2 kDa</td>
<td>Yes &lt;0.8 kDa</td>
<td>No &gt;150 kDa</td>
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<tr>
<td>Specificity</td>
<td>High</td>
<td>Low</td>
<td>Multiple</td>
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<tr>
<td>Chemical synthesis (NCEs)</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Rapid tissue penetration</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Complex protein targets druggable</td>
<td>Yes</td>
<td>Limited</td>
<td>Yes</td>
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<tr>
<td>Route of elimination</td>
<td>Renal</td>
<td>Liver</td>
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### Bicycle's robust proprietary and partnered pipeline

<table>
<thead>
<tr>
<th>Target / Product</th>
<th>Partner / Sponsor</th>
<th>Indication</th>
<th>Modality</th>
<th>Preclinical</th>
<th>IND-enabling</th>
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<th>Expansion</th>
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<td><strong>Partnered Programs</strong></td>
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<td>THR-149 (Kallikrein inhibitor)</td>
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<td>Novel anti-infectives</td>
<td>InnovateUK</td>
<td>Anti-infectives</td>
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<td>Novel CNS targets</td>
<td>IONIS</td>
<td>CNS</td>
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<td>Novel neuromuscular targets</td>
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*September 2022*
Bicycle TICA®– tumor-targeted immune cell agonists delivers immune agonism to tumors

Immune cell receptor engaging Bicycle® CD137

Tumor-targeted Immune Cell Agonists (Bicycle TICA®)

BT7480 (Nectin-4/CD137)

BT7455 (EphA2/CD137)

Activated Immune Cell

Immune cell receptor CD137

Clustered induced by tumor antigen drives stronger immune cell potency

Bicycle TICAs

Tumor Antigen Nectin-4 or EphA2

Tumor Cell

CD137 validated target
Signal 2 costimulatory receptor – drives T-cell function and survival, also expressed on NK cells and myeloid cells

September 2022
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 cancer¹,²

- 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

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²Campbell, et al. AACR. 2021
BT7480 activity is dependent on Nectin-4, induces complete responses & memory via differentiated MoA in pre-clinical studies.

**Graphs and Figures:**
- **(+)** Nectin-4 induction profiles.
- **(-)** Nectin-4 induction profiles.

**Legend:**
- Vehicle (0/6 CRs)
- BT7480 5 mg/kg BIW (6/6 CRs)
- BT7480 1.5 mg/kg BIW (5/6 CRs)

- **Mixed effects analysis with Tukey’s post test, days 0–17**

**Table:**

<table>
<thead>
<tr>
<th>Day</th>
<th>Tumor Volume (mm³)</th>
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<tr>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td>14</td>
<td>200</td>
</tr>
<tr>
<td>21</td>
<td>500</td>
</tr>
<tr>
<td>28</td>
<td>1000</td>
</tr>
</tbody>
</table>

**Notes:**
- No tumor growth in Vehicle or Isotype CTR CR animals.
- Cytotoxic T cells migrate to and kill tumor.

**Re-challenge:**
- Day 59

**Cytokines:**

**References:**
Translating preclinical findings into meaningful biomarker strategies to inform clinical development

What is the most efficient route to clinical PoC?

Preclinical observations → Clinical biomarker strategy → Preclinical PK/PD model → Actual Clinical Observations

On track as anticipated based on PK/PD model?

How to monitor biology in patients confidently?
Which types of samples/technologies?
BT7480 now being tested in cancer patients in an innovative biomarker-enabled Phase 1 trial suite of custom built, fit-for-purpose assays to inform clinical decision making

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

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

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

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


September 2022
Development of 19-plex spatial proteomic assay using proprietary Bicycle Nectin-4 mAb and MultiOmyx™ technology

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

• 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

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

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

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
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

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

- 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

% TE = (1-(ΔTE post-dose/ΔTE pre-dose))*100
ΔTE = %CD137+Bicycle+ full stain panel - %CD137+Bicycle+ FMX panel
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) – innovative biomarker-enabled trial.

- 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

**Bicycle Therapeutics:** Carly Campbell, Cara Bray, Drasti Kanakia, Kristen Hurov, Johanna Lahdenranta, Punit Upadhyaya, Tara Gelb, Julia Kristensson, Kevin McDonnell, Phil Brandish, Sandra Hirschberg, Sebastien Hazard, Dominic Smethurst, Steve Blakemore, Nicholas Keen

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