BT7480, a novel Nectin-4 dependent agonist of the immune cell costimulatory receptor CD137

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I have the following financial relationships to disclose:
Consultant for: HotSpot Therapeutics Inc, Kymera Therapeutics Inc.
Employee of: Bicycle Therapeutics

-and-

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**Bicycles are a new therapeutic modality for addressing intractable challenges**

- **Short linear peptide**
- **Scaffold**
- Chemical cyclization with scaffold

- **Chemical synthesis**
- **Rapid tissue distribution**
- **Complex protein targets druggable**
- **Route of elimination**

<table>
<thead>
<tr>
<th></th>
<th>Chemical synthesis</th>
<th>Rapid tissue distribution</th>
<th>Complex protein targets druggable</th>
<th>Route of elimination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small molecules</td>
<td>+++</td>
<td>+++</td>
<td>---</td>
<td>Liver</td>
</tr>
<tr>
<td>Antibodies</td>
<td>---</td>
<td>+</td>
<td>+++</td>
<td>Liver</td>
</tr>
<tr>
<td>Bicycles</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>Renal</td>
</tr>
</tbody>
</table>

**Built-in tolerance to conjugation**
- Generalizable approach
- Versatility to adopt multiple formats

**Phage-based screening platform**
- Nobel Prize-winning technology
- Rapid selection from >10^{17} potential candidates
Bicycle® platform delivers a toolkit of building blocks to create novel oncology medicines

**Bicycle Phage Display - Discovery**
- Linear peptide
- Bicycle DNA
- Protein III
- Gene III
- Chemical modification with scaffold
- Diverse Bicycle phage libraries (>10^15)
- Loop sizes
- Bicycle scaffolds

**Peptide & Medicinal Chemistry**
- Natural Amino Acids
- Non-natural Amino Acids
- Optimize Bicycle monomers
- Tumor Targeting and Effector Bicycles
- Build and Optimize Therapeutic Bicycles
- Payloads and Linkers

**Bicycle Medicines**
- Monomeric Bicycles
- Targeted Drug Conjugates
- Targeted/Multi-specific Bicycles

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

- CD137 is expressed on activated immune cells – signaling enhances function and survival, prevents anergy
- CD137 ligand expressed by APCs provides a co-stimulatory signal to T cells and NK cells – potential in antitumor 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 yet meet fully design goals dictated by the biology**
  - Activity localized to the tumor – potentiate immune activation
  - Rapid onset of action and controllable duration of action
  - No Fc interactions to avoid liver toxicity

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Chin (2018) Nat. Comm. 9, 4679
Soderstrom (2018) Atherosclerosis 272, 66
TICA™: Tumor Targeted Immune Cell Agonists join immune cell and tumor targeting *Bicycles*

- Activated immune cell
  - CD
  - TM
  - CRD 4
  - CRD 3
  - CRD 2
  - CRD 1
- CD137
- CD137L
- Antigen presenting cell

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

**CD137 Engaging Bicycle®**

**Linker**

**Tumor Targeting Bicycle**

**Tumor Target/CD137 TICA**

**CD137 clustering induced by tumor antigen**
Nectin-4 – targeting and scaffolding for a CD137 Bicycle®

- Cell adhesion molecule, widely expressed during development, restricted in adult normal tissue
- Highly expressed in a wide range of solid tumor indications including breast, bladder, head & neck, esophageal, ovarian, and lung cancer\(^1,2\)
- Nectin-4 and CD137 co-expressed in variety of human tumors

<table>
<thead>
<tr>
<th>Indication</th>
<th>Total cores (N)</th>
<th>% Nectin-4+ (H-score &gt; 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast (all)</td>
<td>225</td>
<td>80</td>
</tr>
<tr>
<td>TNBC</td>
<td>141</td>
<td>86</td>
</tr>
<tr>
<td>Bladder</td>
<td>142</td>
<td>78</td>
</tr>
<tr>
<td>Esophagus</td>
<td>140</td>
<td>55</td>
</tr>
<tr>
<td>Head &amp; Neck</td>
<td>69</td>
<td>58</td>
</tr>
<tr>
<td>Lung</td>
<td>157</td>
<td>39</td>
</tr>
<tr>
<td>Ovarian</td>
<td>89</td>
<td>45</td>
</tr>
<tr>
<td>Pancreas</td>
<td>96</td>
<td>19</td>
</tr>
<tr>
<td>Stomach</td>
<td>131</td>
<td>4</td>
</tr>
</tbody>
</table>

2 Campbell, et al. AACR. 2021. POSTER #1197
CD137 and Nectin-4 *Bicycles*: discovery and optimization by phage display and chemistry

**CD137**

- $K_D = 1400$ nM
- $K_D = 67$ nM
- $K_D = 5$ nM

**Phage Hits**

- ID sequence families
- Define initial pharmacophores

**Phage Optimized**

- Focused libraries
- Optimize natural AAs
- Scaffold/Loop symmetry

**Chemistry Optimized**

- Non natural amino acids
- Tune affinity and stability
- Balanced phys chem properties

**Nectin-4**

- $K_D = 508$ nM
- $K_D = 80$ nM
- $K_D = 1$ nM
Bicycle® scaffold nucleates secondary structure with CD137 and provides a pharmacophore.

CD137 Bicycle binds to epitope common to CD137L.

Bicycle loop 2 forms third strand of β-sheet with CD137.

Loop 2 antiparallel β-sheet H-bonding.

Triazinane ring of TATA scaffold forms close packing interaction with Met101.
Bicycles are highly modular and TICAs are built and optimized using medicinal chemistry.

CD137 & Nectin-4 Bicycles

Linkers

Diverse Nectin-4/CD137 TICAs

Tunable formats = Tunable properties

Reporter cell assay data for 30 Nectin-4/CD137 TICAs

CD137 Jurkat reporter cells in co-culture with HT1376
BT7480 is a fully synthetic, heterotrimeric conjugate with 1 Nectin-4 and 2 CD137 Bicycles

BT7480 selected as lead TICA™ candidate

Structure of BT7480
MW = 7.2 kDa
Bicycle® TICAs are ~30x smaller than other targeted agonists

Bicycle
TICA™
BT7480

Anti-FAP
IgG-CD137L fusion
Roche
RG7826

Anti-Her2 IgG-
anti-CD137 anticalin fusion
Pieris PRS-343

7.2kDa
~185kDa
~190kDa
BT7480: a Nectin-4/CD137 TICA™ clinical development candidate

<table>
<thead>
<tr>
<th>Assay</th>
<th>Target Value</th>
<th>BT7480</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Immune Cell Assay (EC₅₀)¹</td>
<td>&lt; 2 nM</td>
<td>0.37±0.23 nM (IL-2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.22±0.12 nM (IFNγ)</td>
</tr>
<tr>
<td>CD137 dependent activity</td>
<td>Require CD137</td>
<td>CD137 dependent</td>
</tr>
<tr>
<td>Tumor antigen dependent activity</td>
<td>Require Nectin-4</td>
<td>Nectin-4 dependent</td>
</tr>
<tr>
<td>Immune target selectivity (Vs OX40/CD40)</td>
<td>&gt;10 fold</td>
<td>Selective²</td>
</tr>
<tr>
<td>Tumor target selectivity (Vs other Nectin family members)</td>
<td>&gt;10 fold</td>
<td>Selective</td>
</tr>
<tr>
<td>Define species selectivity (CD137)</td>
<td>Define for tox species</td>
<td>Human, NHP specific²</td>
</tr>
<tr>
<td>Define species selectivity (Nectin-4)</td>
<td>Define for tox species</td>
<td>Cross Reactive</td>
</tr>
<tr>
<td>POC in vivo activity (syngeneic or engraftment model)</td>
<td>Anti-tumor activity</td>
<td>Achieved</td>
</tr>
<tr>
<td>Rodent IV-PK (t½, h)</td>
<td>&gt;1</td>
<td>2.6 (mouse); 4.1 (rat)</td>
</tr>
<tr>
<td>NHP IV-PK (t½, h)³</td>
<td>&gt;5</td>
<td>5.3</td>
</tr>
<tr>
<td>Thermodynamic Solubility (mg/ml)</td>
<td>&gt;10</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Tractable synthesis / CMC Risk</td>
<td>Low Risk</td>
<td>Synthesis scaled to &gt;300g</td>
</tr>
<tr>
<td>Cytokine Release Syndrome Assay</td>
<td>Low Risk</td>
<td>Inactive⁴</td>
</tr>
<tr>
<td>Safety margin vs predicted human efficacious dose</td>
<td>&gt;50-fold margin</td>
<td>Achieved</td>
</tr>
</tbody>
</table>

¹Human PBMC/HT-1376 tumor cell co-culture; average of 13 donors
²Demonstrated using biotinylated version of BT7480
³Scaling consistent with desired human profile
⁴No cytokine release in unstimulated human whole blood

MW=7213.3 Da
BT7480 binds potently and specifically to its targets

- BT7480 binds to Nectin-4 (across species) and CD137 (human, NHP) with high affinity
- BT7480 binds both targets simultaneously by SPR assay
- Exquisite selectivity, no binding seen to >5000 other membrane proteins
- No interactions seen in CYP and hERG channel inhibition assays

BT7480 only binds Nectin-4 and CD137

<table>
<thead>
<tr>
<th>Paralog screening</th>
<th>BT7480 (SPR, $K_D$ (nM))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nectin-1</td>
<td>&gt;200 (n=3)</td>
</tr>
<tr>
<td>Nectin-2</td>
<td>&gt;200 (n=3)</td>
</tr>
<tr>
<td>Nectin-3</td>
<td>&gt;200 (n=3)</td>
</tr>
<tr>
<td>Necl-1</td>
<td>&gt;200 (n=3)</td>
</tr>
<tr>
<td>Necl-2</td>
<td>&gt;200 (n=3)</td>
</tr>
<tr>
<td>Necl-3</td>
<td>&gt;200 (n=3)</td>
</tr>
<tr>
<td>Necl-4</td>
<td>&gt;5000 (n=3)</td>
</tr>
<tr>
<td>Necl-5</td>
<td>&gt;200 (n=3)</td>
</tr>
<tr>
<td>OX40*</td>
<td>&gt;100 (n=2)</td>
</tr>
<tr>
<td>CD40*</td>
<td>&gt;100 (n=2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BT7480 SPR affinity (nM)</th>
<th>Human</th>
<th>Mouse</th>
<th>Rat</th>
<th>NHP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nectin-4</td>
<td>5.6 ± 2.4 (n=11)</td>
<td>4.6 ± 2.1 (n=9)</td>
<td>15 ± 1 (n=6)</td>
<td>27 ± 15 (n=9)</td>
</tr>
<tr>
<td>Nectin-4 (simultaneous)</td>
<td>12 ± 2 (n=4)</td>
<td>6.7 ± 1.7 (n=3)</td>
<td>25 ± 2 (n=3)</td>
<td>28 ± 5 (n=3)</td>
</tr>
<tr>
<td>CD137</td>
<td>6.3 ± 0.7 (n=4)</td>
<td>&gt;100 (n=2)</td>
<td>&gt;100 (n=2)</td>
<td>18 ± 6 (n=3)</td>
</tr>
</tbody>
</table>

*Determined using biotinylated-BT7480
All data are mean ± SD and n= number of replicates

Retrogenix membrane protein array: no binding of biotinylated-BT7480 @1μM to 5,482 other proteins
BT7480 functional activity is dependent on Nectin-4 in cell-based assays *in vitro*

**(-) Nectin-4**

**(+ ) Nectin-4**

**Human PBMC**

**4T1 cells ± Nectin-4**

**HT1376 bladder tumor cells**

**EC50**

0.37 ± 0.23 nM (IL-2)  
0.22 ± 0.12 nM (IFNγ)
BT7480 induces complete responses and memory \textit{in vivo}.

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

**No tumor growth in Vehicle or Isotype CTR CR animals**

**CRs Vehicle (n=7)**

**CRs Isotype CTR (n=7)**

**CRs with CD8 depletion (n=10)**

**MC38-Nectin-4 in huCD137-C57Bl/6**

Day 59
PK and in vivo modeling projects target coverage and efficacy in humans with QW dosing

- Data suggest that continuous target coverage is not needed for robust efficacy
- Once weekly or less frequent dosing is predicted to be efficacious in humans

**Simulation of target coverage for human dose projection**

<table>
<thead>
<tr>
<th>Species</th>
<th>Terminal half-life (t₁/₂, h)</th>
<th>CLp (mL/min/kg)</th>
<th>Vss (L/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>2.3</td>
<td>16</td>
<td>2.3</td>
</tr>
<tr>
<td>Rat</td>
<td>3.6</td>
<td>8.5</td>
<td>1.5</td>
</tr>
<tr>
<td>NHP</td>
<td>6.2</td>
<td>4.0</td>
<td>0.88</td>
</tr>
<tr>
<td>Pred. Human</td>
<td>8.9</td>
<td>1.7</td>
<td>1.3</td>
</tr>
</tbody>
</table>
BT7480 has a unique and differentiated mechanism of action

Cytokines and Chemokines at 24 Hours

Myeloid cells activated, “shout for help”

Cytotoxic T cells migrate to and kill tumor

BT7480 binds Nectin-4 in tumor

Macrophage Score

Cell type score (linear)

Timepoint (h)

Days post BT7480 dosing

Vehicle

BT7480

NB-BCY

αCD137

Cytotoxic Cell Score

Ccl1-mRNA

Ccl17-mRNA

Ccl24-mRNA

Ccl21-mRNA

CD8+ T cells on Day 6

BT7480 is remarkably well-tolerated in preclinical species, with no evidence for liver effects.
What patients are most likely to benefit from BT7480?

Biomarker-driven approach to identifying indications that co-express Nectin-4 & CD137

Indications likely to benefit include:

>50% of breast, head & neck, ovarian and esophageal cancer patients
Summary

- BT7480 is a novel, fully synthetic, tumor antigen (Nectin-4) dependent CD137 agonist with high biological potency (ca. 300pM EC$_{50}$ in *in-vitro* assays)
- BT7480 is ca. 30x smaller than comparator biologics
- BT7480 induces robust anti-tumor responses to Nectin-4 expressing tumors in immune competent mouse models. BT7480 induces immunologic memory to those tumors
- BT7480 has a benign profile in preclinical safety evaluation with no liver effects observed
- BT7480 exhibits a unique mechanism in preclinical models
- Tumors that may benefit include >50% of breast, head & neck, ovarian and esophageal cancer patients
- BT7480 will be entering human trials in 2021
End