Bicycle Therapeutics: Precision-guided immune agonism for the treatment of cancer

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Presentation outline

• Introduce the *Bicycle*® platform and its application to cancer therapy

• Discuss the discovery, development and MOA of precision-guided CD137 agonists including BT7480

• Discuss NK cell engagers and application of the *Bicycle*® technology in this area
**Bicycles** are short peptides chemically constrained with a central scaffold.

Based on the work of Greg Winter to define the minimal mAb pharmacophore:
- 2018 Nobel Prize in Chemistry
- Founded Bicycle Therapeutics

**Bicycle**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small size</td>
<td>Yes 1.5 to 2 kDa</td>
</tr>
<tr>
<td>Specificity</td>
<td>High</td>
</tr>
<tr>
<td>Chemical synthesis (NCEs)</td>
<td>Yes</td>
</tr>
<tr>
<td>Rapid tissue penetration</td>
<td>Yes</td>
</tr>
<tr>
<td>Complex protein targets druggable</td>
<td>Yes</td>
</tr>
<tr>
<td>Route of elimination</td>
<td>Renal</td>
</tr>
</tbody>
</table>
How *Bicycles* are discovered and why they work

Cyclization on the surface of the phage means we screen for the constrained 3D structure, not the sequence.

Having been discovered while attached to a phage particle, conjugation to a payload, including other *Bicycles*, rarely impairs binding to target.

Switching to chemical synthesis after screening introduces non-natural amino acids & leverages enormous proprietary data sets.
First application in cancer – delivery of cytotoxic agents maximizing tumor concentration while minimizing exposure in the periphery

<table>
<thead>
<tr>
<th>Property</th>
<th>BTC</th>
<th>ADC</th>
<th>Importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor penetration</td>
<td>✓</td>
<td>?</td>
<td>Access to site of action</td>
</tr>
<tr>
<td>Tumor retention</td>
<td>✓</td>
<td>?</td>
<td>Maintenance at site of action, lower total body burden</td>
</tr>
<tr>
<td>Short systemic exposure</td>
<td>✓</td>
<td>✗</td>
<td>Minimizes toxicity, enhances combinability</td>
</tr>
<tr>
<td>Reduced liver metabolism</td>
<td>✓</td>
<td>✗</td>
<td>Improved safety profile</td>
</tr>
<tr>
<td>Renal elimination</td>
<td>✓</td>
<td>✗</td>
<td>Improved safety profile</td>
</tr>
<tr>
<td>Flexible dosing</td>
<td>✓</td>
<td>✗</td>
<td>Tailored dosing regimen minimizing toxicity</td>
</tr>
</tbody>
</table>

Better efficacy

Better therapeutic index

Patient experience
The properties that make *Bicycles* great for toxin delivery also make them great for immune agonism, but for different reasons

- In the body, activating signals (agonists) are *local*, *rapid*, and then *stop*
  - Neurotransmitters
  - Cytokines
  - Stress hormones

- Sustained (pathologic) signaling leads to desensitization and dysregulation

*Bicycles* are precision-guided (local), distribute in the tissues quickly (rapid), and are cleared rapidly from the body (stop)
Elevating the platform

- **BT1718**: Target: MT1-MMP, DM1 payload, Disulfide linker
- **BT5528**: Target: EphA2, MMAE payload, Val-Cit linker
- **BT7480**: Targets: Nectin-4, CD137, Val-Cit linker
- **BT8009**: Target: Nectin-4, CD137, PEG3 linker
- **BT7455**: Targets: EphA2, CD137, PEG3 linker, Next Bicycle TICA™ scheduled for clinic
- **NK-TICA™**: Next-gen BTCs, Different targets, linkers and payloads

Validation

Radiopharm

FIH 2018 2019 2020 2021 2023 and beyond
Bicycle® precision-guided immune activation

Immune cell receptor = CD137
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 meet design goals dictated by the biology – we sought to address this 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 liver toxicity

CD137 and Nectin-4 *Bicycles*: discovery and optimization by phage display and chemistry

**CD137**
- **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**
- **Phage Hits**
  - ID sequence families
- **Phage Optimized**
  - Focused libraries
  - Optimize natural AAs
  - Scaffold/Loop symmetry
- **Chemistry Optimized**
  - Non natural amino acids
  - Tune affinity and stability
  - Balanced phys chem properties

K<sub>D</sub> values:
- CD137: 1400 nM, 67 nM, 5 nM
- Nectin-4: 508 nM, 80 nM, 1 nM
BT7480 functional activity is dependent on Nectin-4 in cell-based assays *in vitro*
BT7480 induces complete responses and memory in vivo – mouse syngeneic MC38 tumor model

MC38-Nectin-4 in huCD137-C57Bl/6

**Tumor Volume (mm³)**

**Day**

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

***p<0.001 Mixed effects analysis with Tukey’s post test, days 0–17

Re-challenge

Day 59

- CRs Vehicle (n=7)
- CRs Isotype CTR (n=7)
- CRs with CD8 depletion (n=10)

No tumor growth in Vehicle or Isotype CTR CR animals
Transcriptional analysis in mouse mc38 tumor model revealed an unanticipated, rapid burst of T cell chemotactic cytokine production.

- BT7480 leads to an early increase in cytokine gene expression in tumor
- BT7480 leads to increase in CD8+ cell infiltration, cytotoxic and macrophage cell scores in tumor
- BT7480 induces significant changes in local immune cell populations
BT7480 now being tested in cancer patients in an innovative biomarker-enabled phase 1 trial

- Phase 1 safety & tolerability study, FIH Nov 2021
- ATD followed by 3+3 escalation, QW IV - tumor response assessed per RECIST every 8 weeks
- Sophisticated biomarker plan to monitor target engagement and immune responses in real time

- Immunophenotyping by flow cytometry on fresh blood drawn after dosing
- Proprietary fluorescent CD137 Bicycle as occupancy probe
- Custom clinical grade RO assay implemented -> guide dose escalation and inform RP2D

Cohen, H., et al. AACR 2022; Papadopoulos, K., et al. ASCO 2022
BT7480 – 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 1 clinical testing in November 2021
Bicycle® precision-guided NK cell activation

NK cell receptor = NKp46
Natural killer (NK) cells have emerged as important early drivers of the adaptive anti-tumor immune response

- Traditional understanding – NK cells kill tumor cells one by one through direct cytotoxic mechanisms
- New science: role for NK cells in orchestration of adaptive immunity -> catalysis (Bottcher, 2018)
- NK cell therapy emerging as an important new approach to cancer (Laskowski, 2022)
- NKp46 - an untapped target
  - Activating receptor constitutively expressed on NK cells
  - Not down-regulated in the TME like CD16 or NKG2D
  - Encouraging preclinical data reported (Gauthier, 2019)

Program hypothesis: Catalysis of adaptive immunity by NK cells has potential to enable tumor rejection and enhance the action of established therapeutics such as targeted toxins and immune checkpoint inhibitors
NKp46 Bicycles: discovery and optimization by phage display and chemistry

**Phage Hits**
- ID sequence families
- Define initial pharmacophores

**Phage Optimization**
- Focused libraries
- Optimize natural AAs
- Scaffold/Loop symmetry

**Chem Optimization**
- Non natural amino acids
- Tuned affinity and stability
- Balanced phys. chem properties
- Selective NK cell binding

**KD**
- NKp46: \(K_D \approx 250 \text{ nM}\)
- MT-1: \(K_D \approx 35 \text{ nM}\)
- EphA2: \(K_D < 5 \text{ nM}\)
- PD-L1: \(K_D = 5 \text{ nM}\)
- MT-1: \(K_D = 15 \text{ nM}\)
- EphA2: \(K_D = 1.7 \text{ nM}\)

NK-TICA®

Dufort et al., AACR 2022
Connecting NKp46 *Bicycles* to a tumor antigen *Bicycle*® quickly led to functionally active molecules.

- NK cells isolated from whole blood (negative selection)
- NK cells co-cultured with tumor cell lines in vitro

![Diagram of NK cells binding to tumor antigen](Images created with BioRender.com (2022))

**Functional Test - Degranulation**

- Binding of fluor-labeled Bicycle to PBMCs
- Functional test - degranulation

**Binding of fluor-labeled Bicycle to PBMCs**

**Diagram**

**MFI AF488 on CD56+ NK cells**

- BCY15663 (NKp46 Bicycle®)
- BCY15665 (Non-binding Bicycle®)

**Frequency of CD107a+ NK cells (%)**

- Dufort et al., SITC 2021 & May 2021 BCYC corporate presentation
NKp46/EphA2 Bicycle® conjugates enhanced the ability of primary human NK cells to kill target +ve tumor cells

- NK cells are co-cultured with HT1080 cells expressing luciferase in presence of NK-TICA™
- NK cells kill those tumor cells, measured by drop in luminescence

Images created with BioRender.com (2022)

Dufort et al., SITC 2021
EphA2/NKp46 *Bicycles* also cause cytokine production which has the potential to drive adaptive anti-tumor immunity.

Tumor target-dependent production of cytokines that can activate APCs is a key design goal.

Dufort et al., AACR 2022
NKp46 Bicycles work with multiple tumor antigen targets to drive potent tumor cell killing

- HT1080-luc cells in co-culture with primary human NK cells
- HT1080 cells express EphA2, MT-1 and PD-L1

Potential to create NK-TICAs to address multiple solid tumor indications

Dufort et al., AACR 2022
First series of chemically synthetic, conditionally active, targeted NKp46 activators

- Nanomolar biochemical potency
- Sub-nanomolar functional potency
- Directs NK cells to kill target +ve cells
- Drives cytokine secretion
- Activity is tumor antigen-dependent

Potential for activity as a monotherapy and as an adaptor molecule to combine with universal NK cell therapy
Bicycle Therapeutics – creating versatile new precision-guided medicines with potential to fill major gaps in cancer therapy

Bicycle Toxin Conjugates (BTCs)
- Precision delivery of MMAE - BT8009 & BT5528
- Emerging clinical data

Bicycle Tumor-Targeted Immune Cell Agonists (TICAs)
- Rapid, local and controlled immune agonism
- Pathfinder molecule for CD137 – BT7480 in phase 1
- Pathfinder molecule for NKp46 - preclinical