Bicycle Toxin Conjugates to Target Solid Tumors

Steve Ludbrook, Director of Molecular Pharmacology

3rd ADC Target Selection Summit, Boston
Forward-looking statements

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Presentation Overview

- Background to *Bicycles* as fully synthetic and readily conjugated precision guided targeting systems

- EphA2 Targeting with a Bicycle Toxin Conjugate (*BTC™*) as a potential solution to an ADC-intractable target

- Opportunities in the target selection landscape for Bicycle Toxin conjugates
**Bicycles** are a new therapeutic modality – bicyclic peptides

- Robust patent protection
- Powerful/versatile platform
  - Immense combinatorial potential
  - Combines advantages of both small molecules and antibodies

**Scaffold**
- Chemical modification with scaffold

**Short linear peptide**

**Bicycle®**

**Diverse Bicycle® phage libraries (>10^20)**

**Multi-formats**
- Monomeric Bicycles
- Bicycle Toxin Conjugates (BTCs)
- Tumor-targeted immune cell agonists (Bicycle TICAs)

**Robust patent protection**
**Bicycles** are designed to combine the advantages of both small molecules and antibodies.

<table>
<thead>
<tr>
<th></th>
<th>Bicycle®</th>
<th>Small molecule</th>
<th>Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small size</td>
<td>Yes – 1.5-2kDa</td>
<td>Yes – &lt;0.8kDa</td>
<td>No – &gt;150kDa</td>
</tr>
<tr>
<td>Specificity</td>
<td>High</td>
<td>Low</td>
<td>Multiple</td>
</tr>
<tr>
<td>Chemical synthesis (NCEs)</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Rapid tissue penetration</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Complex protein targets</td>
<td>Yes</td>
<td>Limited</td>
<td>Yes</td>
</tr>
<tr>
<td>targets druggable</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Route of elimination</td>
<td>Renal</td>
<td>Liver</td>
<td>Liver</td>
</tr>
</tbody>
</table>
**Bicycle®** platform delivers a toolkit of building blocks to create novel medicines.

### Bicycle® Phage Display - Discovery
- Linear peptide
- Protein III
- Bicycles DNA Sequence
- Gene III
- Bicycles DNA Sequence
- Diverse Bicycle® phage libraries (>10^12)

### Peptide & Medicinal Chemistry
- Optimize Bicycle® monomers
- Non-natural Amino Acids
- Build and Optimize Therapeutic Bicycles
- Easy conjugation of Linkers and Payloads

### Potential Bicycle® Medicines
- Monomeric Bicycles
- Targeted Drug Conjugates
- Targeted/ Multi-specific Bicycles

- Tumor Targeting and Effector Bicycles

*Natural Amino Acids*
Chemical optimization of a high affinity EphA2 targeting Bicycle® with improved properties

2 scaffolds x 10 libraries, ➔ 8 peptide families

BCY6014 Ki = 16 nM – early Bicycle

ARDCPLVLNPCLHPGWTC

Chemistry Optimisation <40 analogs

BCY6099 Ki = 3 nM – newly optimised Bicycle

AhRDChyPLVLNPCLHPdWDhRCA

Modifications (*) to increase polarity, stability and/or potency

PET imaging of HT-1080 xenograft at 60 minutes

MMAE Delivery Using the Bicycle Toxin Conjugate BT5528

Gavin Bennett1, Amy Brown1, Gemma Mudie1, Philip Huxley1, Katerine Van Rietshelden2, Silva Pavan2, Liuhong Chen3, Sophie Wathlam3, Johanna Lundemans3, and Nicholas Keen7
Potential of Bicycles as precision guided therapeutics

Bicycles rapidly penetrate tumour, eliminated through renal route

Short systemic exposure & tumour retention

Activity at site of action with reduced body burden

Can be used to deliver key pharmacological activity for solid tumours:

Cytotoxic payloads

Immune-oncology

PET imaging of Bicycle-radioisotope conjugate, 0-60min post-injection

Imaging conducted in collaboration with Prof. Dr. Matthias Eder

Dr. Ann-Christin Eder

Mohamed El Fakiri
BTCs – preclinical data indicates higher potency and specificity with fewer side effects than ADCs

- MW of 1.5-2kDa
- 50-100x smaller than antibodies
- High selectivity
- Allows more potent toxin to be delivered directly to tumor

**Bicycle® Toxin Conjugate (BTC)**

- **Toxin**
  - Large amount of cytotoxic payload can be delivered
- **Linker**
  - Release of toxin directly into tumor via cleavable linker
- **Specificity**
  - Tumor targeting via antigen

**Graph**

[Tissue] (μM) vs Time (h)
- BTC plasma
- Toxin plasma
- Toxin tumor

**Images**

- Bicycle®
- Antibody

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**BT5528: structure and profile**

**High affinity binding to EphA2 protein across species and on cells. Species cross-reactivity, high selectivity.**

<table>
<thead>
<tr>
<th>BT5528 affinity</th>
<th>Human</th>
<th>Mouse</th>
<th>Rat</th>
<th>NHP</th>
</tr>
</thead>
<tbody>
<tr>
<td>FP comp ($K_i$, nM)</td>
<td>1.9 ± 0.9</td>
<td>5.2 ± 1.9</td>
<td>1.9 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>n=29</td>
<td>n=16</td>
<td>n=10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPR ($K_D$, nM)</td>
<td>0.9 ± 0.4</td>
<td>2.0 ± 0.8</td>
<td>2.7 ± 0.4</td>
<td>1.0</td>
</tr>
<tr>
<td>n=2</td>
<td>n=2</td>
<td>n=2</td>
<td>n=1</td>
<td></td>
</tr>
<tr>
<td>Cell binding by HCS ($K_b$ app, nM)</td>
<td>14.8 ± 10.5</td>
<td></td>
<td></td>
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Membrane protein array: no binding of BT5528 @1µM to 5,527 other proteins
EphA2 is a high value target for the treatment of cancer

- EphA2, a member of Eph subfamily of receptor tyrosine kinases
- Regulates cell migration, adhesion, proliferation and differentiation
- Highly expressed in many human cancers and correlates with tumor progression
  - Ovarian
  - Urothelial
  - NSCLC
  - Head & Neck
  - Gastric
  - TNBC

*Kamoun, et al, Nanoliposomal Targeting of Ephrin Receptor A2 (EphA2): Clinical Translation, Merrimack Pharmaceuticals
Multiple approaches targeting EphA2-expressing tumors have failed

- MEDI-547 (MedImmune) ADC: halted following first dose-cohort coagulopathy
  - ATRC-301 ADC: stopped Nov22, bleeding in NHP tox

- DS-8895a (Daiichi) antibody: limited efficacy in EphA2+ gastric and esophageal cancer, significant infusion reactions. Discontinued because of poor risk-benefit profile & low tumor uptake, consistent with lack of substantial tumor inhibition

- MM-310 (Merrimack) antibody-targeted nanoliposome: terminated - “unable to reach optimal therapeutic index”, due to cumulative peripheral neuropathy

BT5528: activity in difficult-to-treat xenograft models

Superior activity to EphA2 ADC in large NSCLC xenograft models

Activity in pancreatic xenograft models

Bicycle distribution at 60 min

ADC distribution at 60 min

Days after start of dosing
BT5528 offers a differentiated approach to EphA2

EphA2 has been viewed as a "difficult" target

Clinical trial of EphA2-targeting ADC, MEDI-547, terminated after bleeding & liver effects seen at starting dose

BT5528 PK/PD profile offers pre-clinical anti-tumor activity without prolonged vascular exposure

BT5528 toxicology study

- No signs of coagulopathy or bleeding in preclinical species
- No evidence of abnormal liver function
  - Dosing to toxin equivalent doses >100x dose of MEDI-547 used in patients
MMAE Delivery Using the Bicycle Toxin Conjugate

BT5528

Gavin Bennett¹, Amy Brown¹, Gemma Mudd¹, Philip Huxley¹, Katerine Van Rietschoten¹, Silvia Pavan², Liuhong Chen¹, Sophie Watcham³, Johanna Lahdenranta⁴, and Nicholas Keen⁴

ABSTRACT

The EphA2 receptor is found at high levels in tumors and low levels in normal tissue and high EphA2 expression in biopsies is a predictor of poor outcome in patients. Drug discovery groups have therefore sought to develop EphA2-based therapies using small molecule, peptide, and nanoparticle-based approaches (1–3). However, until now only EphA2-targeting antibody–drug conjugates (ADC) have entered clinical development. For example, MEDI-547 is an EphA2-targeting ADC that displayed encouraging antitumor activity in preclinical models and progressed to phase I clinical testing in man. Here we describe the development of BT5528, a bicyclic peptide (“Bicycle”) conjugated to the auristatin derivative maleimidocaproyl-monomethyl auristatin E to generate the Bicycle toxin conjugate BT5528. The report compares and contrasts the Pharmacokinetics (PK) characteristics of antibody and Bicycle-based targeting systems and discusses how the PK and payload characteristics of different delivery systems impact the efficacy—toxicity trade off which is key to the development of successful cancer therapies. We show that BT5528 gives rise to rapid update into tumors and fast renal elimination followed by persistent toxin levels in tumors without prolonged exposure of parent drug in the vasculature. This fast in, fast out kinetics gave rise to more favorable toxicology findings in rats and monkeys than were observed with MEDI-547 in preclinical and clinical studies.

Graphical Abstract: http://mct.aacrjournals.org/content/molcanther/19/7/1385/F1.large.jpg.
Bicycle Toxin Conjugate Target Selection Opportunities

Improvements on existing validated ADC approaches by improvement of therapeutic index: rapid systemic clearance & rapid tumor penetration limits target dependent & independent toxicity while preserving anti-tumor activity in preclinical models.

- Short PK, coupled with rapid tumor penetration:
  - Lower target-independent toxin release in non-tumor tissues
  - Reduced target-dependent toxicity in non-tumor tissues (unbound target recycling or synthesis)
  - Retained tumor-directed activity via rapid penetration
  - No Fc-dependent uptake
  - Flexibility on phys chem properties (e.g. charge) to limit target-independent pinocytosis

PMID: 37296177
Bicycle Toxin Conjugate Target Selection Opportunities

- Improvements on existing validated ADC targets by improvement of therapeutic index: rapid systemic clearance & rapid tumor penetration limits target dependent & independent toxicity while preserving anti-tumor activity in preclinical models

- Limitation of toxicity could enable:
  - Improvements on existing validated ADC approaches
  - Salvage of failed toxic ADC approaches
  - Novel target selection approaches outside the ADC space
  - Opportunities for alternative payloads for all approaches above
Bicycle Toxin Conjugate Target Selection Opportunities

- ADC Target Selection approaches widespread, often using RNA expression databases (e.g. TCGA & GTEX), coupled with cell surface protein expression validation (e.g. Protein atlas) & literature

**Research Article**

Data-Driven Discovery of Molecular Targets for Antibody-Drug Conjugates in Cancer Treatment

PMID: 33490264

**Opportunities for Bicycle approach:**

- Toleration of higher target expression in non-tumor tissue expands the list of potential tumor antigens significantly
- Such targets are likely not viable for ADC approaches due to toxicity via sustained delivery to recycled or neo-synthesized target

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Summary

Bicycles offer a potential new modality for oncology therapeutics
• Antibody-like affinity and selectivity in a small molecule
• Chemically synthesized
• Rapid distribution to solid tumors, elimination via renal route
• Bicycle Toxin Conjugates progressing in clinical studies

Emerging safety and efficacy profile creates novel opportunities in oncology
• Improvements on existing validated ADC approaches
• Salvage of tumor targets through improved therapeutic index
• Potential novel opportunities for tumor antigens outside the ADC space due to improved therapeutic index
Thank you

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