Expanding the Potential of ADCs: Bicyclic Peptide (Bicycle®) Toxin Conjugates May Offer Advancements Over Traditional ADCs

Mark Frigerio, VP Chemistry
World ADC, Europe - 2023
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 plans, prospects, trends or strategies and other business matters; our current and prospective product candidates, planned clinical trials and preclinical activities, and the timing and success of our development of our anticipated product candidates.

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Bicycle Therapeutics

Founded by Sir Gregory Winter & Prof. Christian Heinis

UK & US based (Cambridge, UK; Boston, USA)

2018 Nobel Prize in Chemistry
“for the phage display of peptides and antibodies”
Bicycles are a new therapeutic modality – bicyclic peptides

Powerful/versatile platform
• Immense combinatorial potential
• Combines advantages of both small molecules and antibodies

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

Robust patent protection

Diverse Bicycle® phage libraries (>10^20)

World ADC Europe, 2023
Bicycle® platform delivers a toolkit of building blocks to create novel medicines.

Bicycle® Phage Display - Discovery

- Linear peptide
- Diverse Bicycle phage libraries (>10^2)
- Natural Amino Acids

Peptide & Medicinal Chemistry

- Build and Optimize Therapeutic Bicycles
- Easy conjugation of Linkers and Payloads
- Tumor Targeting and Effector Bicycles
- Optimize Bicycle® monomers
- Non-natural Amino Acids

Potential Bicycle® Medicines

- Monomeric Bicycles
- Targeted Drug Conjugates
- Targeted/Multi-specific Bicycles

World ADC Europe, 2023
Structural constraints create *Bicycle*® advantage

MT1-MMP affinity (FP competition)

<table>
<thead>
<tr>
<th></th>
<th>BCY00009863 (MT1-MMP)</th>
<th>BCY00009862 (MT1-MMP)</th>
<th>BCY00009520 (MT1-MMP)</th>
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</thead>
<tbody>
<tr>
<td>Ki (nM)</td>
<td>&gt;10000 (n=2)</td>
<td>115.1 ± 22.9 (n=2)</td>
<td>1.15 ± 0.07 (n=2)</td>
</tr>
</tbody>
</table>

Linear peptide

Cyclic peptide

Constrained Bicycle

Scaffold

Loop 1

Loop 2
Bicycles are chemically efficient, precision guided and fit for purpose delivery vehicles

**Bicycles**

- MWt 150kDa
- Poor/Slow extravasation
- Limited tissue penetration
- Hepatic/catabolic clearance
- Long half life

**Fab**

- MWt 50-60kDa
- Poor/Slow extravasation
- Limited tissue penetration
- Hepatic/catabolic/renal clearance
- Medium half life

<table>
<thead>
<tr>
<th></th>
<th>Bicycles</th>
<th>Fab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>2.3 kDa</td>
<td>48 kDa</td>
</tr>
<tr>
<td>Size</td>
<td>19 aa</td>
<td>445 aa</td>
</tr>
<tr>
<td>Binding residues</td>
<td>16 aa (85%)</td>
<td>24 aa (5%)</td>
</tr>
</tbody>
</table>

- MWt 10-15kDa
- Poor/Slow extravasation
- Limited tissue penetration
- Renal clearance
- Medium half life

- MWt 1-2kDa
- Rapid extravasation
- Extensive tissue penetration
- Renal elimination
- Tunable half life

**Antibodies**

- MWt 1-2kDa
- Rapid extravasation
- Extensive tissue penetration
- Renal elimination
- Tunable half life

**Fragments**

- MWt 10-15kDa
- Poor/Slow extravasation
- Limited tissue penetration
- Renal clearance
- Medium half life

**Domains**

- MWt 50-60kDa
- Poor/Slow extravasation
- Limited tissue penetration
- Hepatic/catabolic/renal clearance
- Medium half life

**Bicycles**

- MWt 150kDa
- Poor/Slow extravasation
- Limited tissue penetration
- Hepatic/catabolic clearance
- Long half life

**Fab**

- MWt 50-60kDa
- Poor/Slow extravasation
- Limited tissue penetration
- Hepatic/catabolic/renal clearance
- Medium half life

**EphA2-binding Bicycle**

**EphA2-binding Fab**
Bicycles are designed to combine the advantages of both small molecules and antibodies

<table>
<thead>
<tr>
<th>Characteristic</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 druggable</td>
<td>Yes</td>
<td>Limited</td>
<td>Yes</td>
</tr>
<tr>
<td>Route of elimination</td>
<td>Renal</td>
<td>Liver</td>
<td>Liver</td>
</tr>
</tbody>
</table>
Novel Format conjugates growth and impact

- The wider conjugates landscape is continuing to expand, with novel formats at the front of this
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:**
- BTC plasma vs. Toxin tumor vs. Toxin plasma
- Time (h) vs. [Tissue] (μM)

**Images:**
- Tumor targeting via Bicycle® and Antibody

World ADC Europe, 2023
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 coagulopathy¹

- DS-8895a (Daiichi) antibody: limited efficacy in EphA2+ gastric and esophageal cancer, significant infusion reactions. Discontinued because of poor risk-benefit profile²

- MM-310 (Merrimack) antibody-targeted nanoliposome: terminated - “unable to reach optimal therapeutic index”³

1. Annunziata et al, Invest New Drugs. 2013 Feb;31(1):77-84
BT5528 is a first-in-class BTC-targeting EphA2

- BT5528 has potential to penetrate solid tumors; approximately 40X smaller than an ADC
- Toxin is released and retained in tumor cells, resulting in tumor cell death and bystander killing
- PK profile distinct from ADCs; renally eliminated, bypassing liver metabolism
- Recently completed dose escalation of Phase I clinical study
BT5528: Emerging relationship between EphA2 expression and response in ovarian and urothelial cancers

- Waterfall plot showing best response among urothelial and ovarian cancer patients in first in human study
- Immunohistochemistry data suggest EphA2 positive patients more likely to respond to BT5528
- Scan showing complete responder with ovarian cancer

Best response by RECIST in response evaluable patients

CT scans-abdomen. First in human dose escalation trial.
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></td>
<td>n=29</td>
<td>n=16</td>
<td>n=10</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></td>
<td>n=2</td>
<td>n=2</td>
<td>n=2</td>
<td>n=1</td>
</tr>
<tr>
<td>Cell binding by HCS ($K_b$ app, nM)</td>
<td>14.8 ± 10.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Membrane protein array: no binding of BT5528 @1μM to 5,527 other proteins
Chemical optimization of a high affinity EphA2 targeting *Bicycle®* with improved properties

2 scaffolds x 10 libraries, ➔ 8 peptide families

BCY6014

BCY6099

Chemistry Optimisation <40 analogs

4 non-natural amino acids

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

BCY6014 Ki = 16 nM – early *Bicycle*

```plaintext
ARDCLPVNPLCCLHPGWTC
```

BCY6099 Ki = 3 nM – newly optimised *Bicycle*

```plaintext
AhRDCLhyPLVNPLCLHPdDWhRC
```

PET imaging of HT-1080 xenograft at 60 minutes
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

Tumor Volume (mm³)

- Vehicle qw
- BT5528 3 mg/kg qw
- EphA2 ADC 3 mg/kg qw

^ time of administration

Tumor Volume (% of Day 0)

PDAC1

PDAC2

World ADC Europe, 2023
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 Mudd³, 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—tumoricidal potencies and toxicities 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:** [Image: http://mct.aacrjournals.org/content/molcanther/19/7/1385/F1.large.jpg]
BT5528: EphA2 targeted BTC™

Erythropoietin-producing hepatocellular A2 receptor: member of Eph subfamily of receptor tyrosine kinases

- Regulates cell migration, adhesion, proliferation and differentiation
- Overexpressed in human cancers and correlates with tumor progression
- Development of MEDI-547 (MedImmune) in ovarian cancer was halted following serious bleeding events in phase I

<table>
<thead>
<tr>
<th>Internal</th>
<th>Target</th>
<th>Modality</th>
<th>Pre-clinical</th>
<th>IND-enabling</th>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bicycle®</td>
<td>EphA2</td>
<td>Bicycle® Toxin Conjugate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- BT5528-100: Phase I/II multi-center first-in-human study in patients with advanced solid tumors associated with EphA2 expression
- NCT04180371 Study ongoing
- Clinical update presented by Dr Meredith McKean at AACR-NCI-EORTC Triple Meeting Oct 7 2021
- Topline data from escalation phase released Sep 2022
  - BT5528 demonstrates anti-tumor activity in highly refractory ovarian and urothelial cancer patients
  - Emerging safety profile distinguishes it from other EphA2-targeted molecules
  - No coagulopathy or bleeding seen related to BT5528 dosing
- Dosing at recommended Phase II dose of 6.5 mg/m² q2w in expansion cohorts is ongoing
- Further BT5528 update in 2023

BT8009: Nectin-4 targeted BTC™

Nectin-4:
A cell adhesion molecule and one of four members of the nectin family

All nectins share the same overall structure defined by three extracellular immunoglobulin domains, a single transmembrane helix and an intracellular domain

- Overexpressed in human cancers and correlated with tumor progression
- Solid tumors with high levels of Nectin-4 expression are urothelial, TNBC, ovarian, head & neck and NSCLC

<table>
<thead>
<tr>
<th>Internal</th>
<th>Target</th>
<th>Modality</th>
<th>Pre-clinical</th>
<th>IND-enabling</th>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bicycle</td>
<td>Nectin-4</td>
<td>Bicycle® Toxin Conjugate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- BT8009-100: Phase I/II Study of the Safety, Pharmacokinetics, and Preliminary Clinical Activity of BT8009 in Patients With Nectin-4 Expressing Advanced Malignancies
- NCT04561362
- Study ongoing
- BT8009 Phase I trial results released at ASCO GU Feb 2023
- For more information, please visit www.bicycletx.com
Nectin-4 Bicycle® optimization from lead to BT8009

Parent Bicycle

Ac CPFGCMKNSWPIWC

Amino Acids Important For Target Engagement

Stabilised Bicycle

Ac CPFDCMhArgNWSWPIC

Improve stability, hydrophilicity

Optimised Bicycle

Ac CP1NaldCMhArgDWSTPHypWC

+ affinity

+ hydrophilicity

+ affinity, hydrophilicity

BT8009

Poor solubility, short half-life. AAs required for binding identified

Improvements made to half-life and hydrophilicity, whilst retaining binding AAs

AAs further optimised to increase affinity, improve hydrophilicity. Selected as candidate peptide binder

<table>
<thead>
<tr>
<th>Ki (nM)</th>
<th>cLogP</th>
<th>t(_{1/2}) (plasma)</th>
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</thead>
<tbody>
<tr>
<td>18.4</td>
<td>-6.98</td>
<td>1.3h</td>
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<table>
<thead>
<tr>
<th>Ki (nM)</th>
<th>cLogP</th>
<th>t(_{1/2}) (plasma)</th>
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<tbody>
<tr>
<td>13</td>
<td>-6.74</td>
<td>&gt;24h</td>
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</table>

<table>
<thead>
<tr>
<th>Ki (nM)</th>
<th>cLogP</th>
<th>t(_{1/2}) (plasma)</th>
</tr>
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<tbody>
<tr>
<td>3.2</td>
<td>-13.32</td>
<td>&gt;24h</td>
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</table>

MW=4173.8
**BT8009: Nectin-4 targeted BTC**

**Table: Human Nectin-4 KD plasma protein binding (%) and in vitro plasma stability**

<table>
<thead>
<tr>
<th>Human Nectin-4 KD (nM)</th>
<th>Plasma protein binding (%)</th>
<th>In vitro plasma stability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mouse</td>
<td>Human</td>
</tr>
<tr>
<td>2.50</td>
<td>88.2</td>
<td>79.3</td>
</tr>
</tbody>
</table>

**Mouse pharmacokinetics 3 mg/kg, IV (bolus)**

- **$T_{1/2}$ (h):** 1.0
- **$C_{lp}$ (mL/min/kg):** 3.5
- **Vss (L/kg):** 0.25

**Activity in breast adenocarcinoma (MDA-MB468) CDX model**

![Activity in breast adenocarcinoma (MDA-MB468) CDX model](image)
MT1-MMP targeting BRC shows far superior tumor uptake and contrast versus mAb in mouse model

MT1-MMP overexpressed in variety of cancers (non–small cell lung, gastric and breast)

Whole-body maximum intensity projections were obtained 40 to 60 minutes p.i. using uPET imaging.
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
Diversifying the Bicycle® platform

- **BT1718**
  - Target: MT1-MMP
  - DM1 payload
  - Disulfide linker

- **BT5528**
  - Target: EphA2
  - MMAE payload
  - Val-Cit linker

- **BT8009**
  - Target: Nectin-4
  - MMAE payload
  - PEG3 linker

- **BT7480**
  - Targets: Nectin-4, CD137
  - PEG3 linker

- **BT7455**
  - Targets: EphA2, CD137
  - PEG3 linker
  - Next Bicycle TICA™ scheduled for clinic

- **NK-TICA™**
  - Targeted ASO/SiRNA delivery

- **Next-gen BTCs**
  - Different targets, linkers and payloads

- **Radiopharm**

FIH:
- 2018
- 2019
- 2020
- 2021
- 2023 and beyond

World ADC Europe, 2023
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 TICAs show promise for targeted stimulation of immune cells in tumors
• BT7480 entered the clinic Q4 2021
• BT7455 (EphA2-CD137 Bicycle TICA™) in IND-enabling stage
• NK-TICA™ programs targeting natural killer (NK) cells identified and moving into lead optimization

Bicycle Toxin Conjugates® progressing in clinical studies
3 conjugates in clinical trials
• Clinical PK profile consistent with preclinical prediction
• Preliminary signs of anti-tumor activity seen
• Emerging safety profile supports potential of Bicycle platform