Bicycles® - An entirely new class of therapeutics

Paul Beswick
Bicycle Therapeutics
The challenges in treating cancer

- Tumours can be “silent”
- Can be hard to access
- Are difficult to differentiate from normal tissue
- Actively suppress the immune system
- Diverse set of diseases
- Heterogeneous and evolving
Overview

• Bicyclic peptides: A completely new, disruptive therapeutic modality

• Sir Greg Winter technology, platform derisked, industrialized, reduced to practice and validated

• Internal oncology pipeline, multiple therapeutic themes, BT1718 in Ph1: funded by CRUK. Partnered outside oncology

• UK/US presence, world class team & strong clinical / scientific collaborations

• >£65M Series B funded
**Bicycles®: a new therapeutic modality**

**Highly constrained:** high affinity, exquisite selectivity, excellent stability

**Large binding footprint:** disrupt protein-protein interactions

**Fully synthetic:** NCE classification and synthetic control

**Highly flexible modality:** modular building blocks retain pharmacology

**Adjustable PK:** excellent tissue penetration, renal elimination, tuneable $T_{1/2}$
## Comparison of therapeutic modalities

<table>
<thead>
<tr>
<th></th>
<th>Antibody</th>
<th>ScFv (fragment)</th>
<th>Bicycle</th>
<th>Small molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mw (kDa)</strong></td>
<td>150</td>
<td>28</td>
<td>1.5-2</td>
<td>&lt;0.8</td>
</tr>
<tr>
<td><strong>Volume of</strong></td>
<td>Low (vascular)</td>
<td>Intermediate</td>
<td>Whole body (typically whole body)</td>
<td></td>
</tr>
<tr>
<td><strong>distribution</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>t_{1/2}</strong></td>
<td>Days to weeks</td>
<td>Minutes to days</td>
<td>Min to hours (tunable). Days possible^2</td>
<td>Hours (tunable)</td>
</tr>
<tr>
<td><strong>Clearance</strong></td>
<td>hepatic</td>
<td>Renal, hepatic</td>
<td>Renal</td>
<td>Renal, hepatic</td>
</tr>
<tr>
<td><strong>Tumour penetration</strong></td>
<td>Low (outer rim only)</td>
<td>Low (poor exposure)</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td><strong>Target classes</strong></td>
<td>Many, small pockets restricted</td>
<td>Many, small pockets restricted</td>
<td>All tested successful, PPI trivial</td>
<td>Small pockets, PPI rare</td>
</tr>
<tr>
<td><strong>Selectivity</strong></td>
<td>Highly</td>
<td>Highly</td>
<td>Highly</td>
<td>Poor</td>
</tr>
<tr>
<td><strong>Modularity</strong></td>
<td>Low (bi-specifics)</td>
<td>Possible, difficult</td>
<td>Trivial (&quot;Lego like&quot;)</td>
<td>Low</td>
</tr>
<tr>
<td><strong>Synthesis</strong></td>
<td>Complex biologic</td>
<td>Complex biologic</td>
<td>Chemical, trivial</td>
<td>Chemical, trivial</td>
</tr>
<tr>
<td><strong>Immunogenicity</strong></td>
<td>Possible</td>
<td>Frequent</td>
<td>None detected</td>
<td>None</td>
</tr>
</tbody>
</table>

^2 Tunable clearance possible.
The Bicycle platform can deliver novel tumour targeting peptides

**Linear peptide**

**Bicycle**

**Diverse Bicycle phage libraries (\(>10^{15}\))**

**Evolution driven, informed selection**

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**Extremely large and diverse chemical library**

**Low synthetic burden**

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1. **Cyclise**
2. **Select**
3. **Amplify**

POC in 6 wk
Optimised lead in 9mnth

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**Protein III**

**Bicycle DNA Sequence**

**Gene III**

**Phage particle**

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**Chemical modification with scaffold**
Bicycles®: many shapes to drug many targets

>90 diverse targets screened
80% success rate

<table>
<thead>
<tr>
<th>Tractable target classes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymes</td>
</tr>
<tr>
<td>Serine proteases</td>
</tr>
<tr>
<td>Other proteases</td>
</tr>
<tr>
<td>Metalloenzymes</td>
</tr>
<tr>
<td><strong>Matrix metalloproteinases</strong></td>
</tr>
<tr>
<td>Coagulation factors</td>
</tr>
<tr>
<td>Other enzymes</td>
</tr>
<tr>
<td>Immune checkpoint</td>
</tr>
<tr>
<td><strong>TNFR superfamily members</strong></td>
</tr>
<tr>
<td><strong>IG domain receptors</strong></td>
</tr>
<tr>
<td><strong>Receptor Tyrosine kinases</strong></td>
</tr>
<tr>
<td>Interleukin receptors</td>
</tr>
<tr>
<td>Signalling</td>
</tr>
<tr>
<td><strong>Interleukins</strong></td>
</tr>
<tr>
<td><strong>Growth Factors</strong></td>
</tr>
<tr>
<td>Cytokines</td>
</tr>
<tr>
<td>Adhesion</td>
</tr>
<tr>
<td><strong>Integrins</strong></td>
</tr>
<tr>
<td>Other cell adhesion proteins</td>
</tr>
<tr>
<td>GPCRs</td>
</tr>
<tr>
<td>Chemokine receptors</td>
</tr>
<tr>
<td>Adrenergic receptors</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td><strong>Heat shock proteins</strong></td>
</tr>
<tr>
<td>Serum proteins</td>
</tr>
</tbody>
</table>

Bicycles®: many shapes to drug many targets

![Bicycles diagram](image)

Table of Tractable Target Classes

- **Enzymes**: Serine proteases, Other proteases, Metalloenzymes, **Matrix metalloproteinases**, Coagulation factors, Other enzymes
- **Immune checkpoint**: **TNFR superfamily members**, **IG domain receptors**, **Receptor Tyrosine kinases**, Interleukin receptors
- **Signalling**: Interleukins, **Growth Factors**, Cytokines
- **Adhesion**: **Integrins**, Other cell adhesion proteins
- **GPCRs**: Chemokine receptors, Adrenergic receptors
- **Other**: **Heat shock proteins**, Serum proteins

Bicycles®: many shapes to drug many targets

![Bicycles diagram](image)
Bicycle® – large molecular footprint drives affinity and selectivity between close homologues

CA IX  $K_i = 25$ nM
CA XII $K_i = 6$ nM

CA IX  $K_i = 7.5$ nM
CA XII $K_i > 2000$ nM

<table>
<thead>
<tr>
<th>Bicycle inhibitors</th>
<th>Human Kallikrein $K_i$ (nM)</th>
<th>Rat Kallikrein $K_i$ (nM)</th>
<th>Thrombin $K_i$ (nM)</th>
<th>Plasmin $K_i$ (nM)</th>
<th>FactorXIIa $K_i$ (nM)</th>
<th>FactorXIIa $K_i$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exemplar 1</td>
<td>0.8</td>
<td>17.6</td>
<td>&gt;10,000</td>
<td>&gt;15,000</td>
<td>&gt;50,000</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>Exemplar 2</td>
<td>0.2</td>
<td>3.7</td>
<td>&gt;10,000</td>
<td>&gt;35,000</td>
<td>15,000</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>Homologue active site sequence identity</td>
<td>85%</td>
<td>92%</td>
<td>100%</td>
<td>85%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Tolerance to conjugation is built-in

**Bicycle**

- Small molecule drugs
- Other *Bicycles* (tandems)
- Chelated radionuclides
- Fluorescent dyes
- Affinity tags
- PK extenders

**Bacteriophage**

900 nm x 7 nm

*Phage bulk readily replaced without compromising binding*

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**In vitro tools**

- Fluorescent probe

**In vivo tools/diagnostics**

- Fluorescent probe
- DOTA
- $^{68}$Ga

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30th RSC-BMCS symposium on Medicinal Chemistry in Eastern England
Bicycle® Toxin Conjugates (BTCs)

Cell permeable Cytotoxin
- Too potent to be dosed alone
- Not toxic once conjugated

Bicycle selectively binds tumour

Tumour-selective Cleavable Linker
- Negligible drug release outside tumour microenvironment
- Payload released extracellularly

Bicycle therapeutics
Bicycles® are retained in tumours and rapidly cleared from systemic circulation

Ideal distribution for imaging

**68Ga MT1-MMP Bicycle**

- Coronal slices 0.8 mm ROI: tumor
- Maximum intensity projection (MIP)

40-60 min

**68Ga MT1-MMP Antibody**

- Tumour
- Heart
- Liver

40-60 min

High tumour retention

Photoacoustic signal intensity (change from baseline)

- Bicycle
- Antibody

Bicycle show superior retention in tumours and lower background vs antibodies
Case study: Nectin 4 targeting BTC – BT8009
Biological rationale for Nectin-4 as tumour target

- Nectin-4 cell adhesion molecule
- Wide expression during development,
  - restricted expression in maturity – epithelial cells e.g. skin, airways, eosophagus/stomach and bladder.
- Member of Nectin family and close relative to Nectin-like family
- Other family members more widespread through body
- Over expression in tumours, highest frequency in bladder, breast, and pancreatic, but also in lung, gastric ovary
- Immunoreactivity predominantly on cell membrane and/or cytoplasm of tumour cells
- Nectin-4 targeting ADC, enfortumab vedotin, in Phase 1 - 3 trials, for metastatic urothelial carcinoma, with “Breakthrough Therapy Designation”
**Bicycle® optimization**

**Parent Bicycle**

<table>
<thead>
<tr>
<th>Ac</th>
<th>C</th>
<th>P</th>
<th>F</th>
<th>G</th>
<th>C</th>
<th>M</th>
<th>K</th>
<th>N</th>
<th>W</th>
<th>S</th>
<th>W</th>
<th>P</th>
<th>I</th>
<th>W</th>
<th>C</th>
</tr>
</thead>
</table>

Amino Acids Important For Target Engagement

**Stabilised Bicycle**

<table>
<thead>
<tr>
<th>Ac</th>
<th>C</th>
<th>P</th>
<th>P</th>
<th>d</th>
<th>C</th>
<th>M</th>
<th>hArg</th>
<th>N</th>
<th>W</th>
<th>S</th>
<th>W</th>
<th>P</th>
<th>I</th>
<th>W</th>
<th>C</th>
</tr>
</thead>
</table>

Improve stability, hydrophilicity

**Optimised Bicycle**

<table>
<thead>
<tr>
<th>Ac</th>
<th>C</th>
<th>P</th>
<th>1Nal</th>
<th>d</th>
<th>C</th>
<th>M</th>
<th>hArg</th>
<th>D</th>
<th>W</th>
<th>S</th>
<th>T</th>
<th>P</th>
<th>Hyp</th>
<th>W</th>
<th>C</th>
</tr>
</thead>
</table>

+ affinity

+ hydrophilicity

+ affinity, hydrophilicity

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

<table>
<thead>
<tr>
<th>Ki (nM)</th>
<th>cLogP</th>
<th>T_{1/2} (plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.4</td>
<td>-6.98</td>
<td>1.3h</td>
</tr>
</tbody>
</table>

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

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

Solubility = 4.9 ug/ml, LogD = -0.7

**Optimised to increase affinity and improve hydrophilicity. Selected as candidate peptide binder for Bicycle Toxin conjugate, BT8009**

<table>
<thead>
<tr>
<th>Ki (nM)</th>
<th>cLogP</th>
<th>T_{1/2} (plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.21</td>
<td>-13.32</td>
<td>&gt;24h</td>
</tr>
</tbody>
</table>

Solubility = 420 ug/ml, LogD < -2.2
Bicycle® Toxin Conjugate, BT8009

Toxin MMAE

Cleavable linker

Sar10 Spacer

Bicycle Binder

N-terminus

C-terminus
BT8009 shows binding to MDA-MB-468 cells, and efficacy in xenograft model

FACS shows Nectin-4 surface expression

 ICC of cells using anti-MMAE antibody. After preincubation with BT8009, a non-binding Bicycle Toxin Conjugate (BTC) or MMAE demonstrates only BT8009 is retained on cell surface.

 BT8009 shows excellent efficacy in MDA-MB-468 xenografts

<table>
<thead>
<tr>
<th>BT8009</th>
<th>Non-binding BTC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bmax</td>
<td>12.21</td>
</tr>
<tr>
<td>Kd</td>
<td>6.861</td>
</tr>
<tr>
<td></td>
<td>22.84</td>
</tr>
<tr>
<td></td>
<td>30624</td>
</tr>
</tbody>
</table>

Preincubated with 1μM BT8009

Preincubated with 1μM non-binding BTC

Preincubated with 1μM MMAE

Days after start of dosing

Tumor Volume (mm³)
BT8009: In vivo PK

BT8009 shows high Cmax with a short plasma half-life, reflective of rapid clearance from systemic circulation.

<table>
<thead>
<tr>
<th>BT8009</th>
<th>CLp (ml/min/kg)</th>
<th>Vss (L/Kg)</th>
<th>t 1/2 (h)</th>
<th>BT8009 Cmax (uM)</th>
<th>MMAE AUC (uM.h)</th>
<th>MMAE Cmax (uM)</th>
<th>MMAE AUC (uM.h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg/kg</td>
<td>3.5</td>
<td>0.25</td>
<td>0.98</td>
<td>1.401</td>
<td>1.131</td>
<td>0.065</td>
<td>0.103</td>
</tr>
<tr>
<td>Mouse</td>
<td>9.4</td>
<td>0.44</td>
<td>0.86</td>
<td>1.114</td>
<td>0.432</td>
<td>0.013</td>
<td>0.022</td>
</tr>
</tbody>
</table>

BT8009 affords long lasting MDA-MB-468 tumour retention of MMAE, with rapid plasma clearance of toxin and parent.

BT8009 efficacy in both “normal and large” MDA-MB-468 xenografts.

![Graph showing BT8009 3 mg/kg effect](image)

![Graph showing tumor volume changes](image)
BT8009 efficacy correlates with expression CDX/PDX xenografts

Xenografts with little/no Nectin-4 expression show reduced tumour growth rate. Xenografts expressing Nectin-4 show regressions of tumour

Lung adenocarcinoma PDX

Lung squamous carcinoma PDX

% change in tumour size

0 100 200 300 400 500 600 700 800


Full regression

null Increasing protein expression High RNA

vehicle D14 vehicle Day 28 3 mg/kg BT8009 D14 3 mg/kg BT8009 D28

30th RSC-BMCS symposium on Medicinal Chemistry in Eastern England
BT8009: A Nectin-4 targeting Bicycle® Toxin Conjugate, for the treatment of solid tumours

• Nectin-4 is highly expressed on tumour cell surface in a wide range of solid tumours

• BT8009 was developed as a Bicycle Toxin Conjugate to target Nectin-4
  • High affinity binding, selective for Nectin-4
  • Short half–life with renal elimination
  • Hit and run delivery of toxin

• BT8009 shows good efficacy in a range of PDX and CDX models, with rapid regression in small and large tumours
  • Efficacy correlates with expression of the Nectin-4 target
  • PK shows retention of toxin in tumour, well in excess of systemic clearance
  • Toxicology studies with BT8009 are progress
**Bicycles®** can meet many of the challenges in oncology

**Can be hard to access**
- Size and PK accesses tumours efficiently

**Tumours can be “silent”**
- Large toolkit of novel probes

**Are difficult to differentiate from normal tissue**
- Highly selective to tumour target
- Combine in bispecifics tandem etc.

**Actively suppress the immune system**
- Multimeric immune receptor agonists
- Targeted systemic delivery of innate immune activators

**Heterogeneous and evolving**
- Superior penetration & bystander effect kills whole tumour
- Extensive arson of different anti-cancer targeting agents

**Diverse set of diseases**
- Companion diagnostics to stratify patients
Acknowledgements

• Team at Bicycle UK & US