Highly potent and selective tumour targeting bicyclic peptides - a novel approach to cancer therapy

Paul Beswick
Agenda

Bicycle therapeutics

The Bicycle platform

Applications of Bicycles®

BT1718 – Lead Bicycle Toxin Conjugate®
Bicycle Therapeutics

Co-founded 2009
• Sir Gregory Winter & Prof. Christian Heinis
• Laboratory of Molecular Biology (LMB - Cambridge UK)
• Two Sites
  • Cambridge, UK
  • Lexington, MA, USA

Oncology pipeline already delivering
• BT1718 phase 1 underway: fully funded by Cancer Research UK (CRUK)
• 2nd generation BDCs and immunomodulators in the pipeline

Multiple partner programs in therapeutic areas outside oncology

Leveraging the unique benefits of Bicycles®

October 2018
Peptide drug discovery – a growth area

- World-wide sales of pharmaceuticals > $1 trillion
  - Rising at ca. 4% per year
- Peptide sales $50 billion (2015)
  - Predicted growth ca. 9%


October 2018
Bicycle® Platform
Peptides therapies – the goldilocks zone

Linear peptide

Bicycle

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: Renal clearance with $T_{1/2}$ in vivo from minutes to days

October 2018
Proven platform using phage display

Linear peptide

Bicycle

[on cell screening option]

1 Cyclise

2 Select

POC in 6 wk Optimised lead in 9mnth

3 Amplify

Phage particle

- Enormous diversity (<$10^{15}$)
- Evolution driven
- Informed selection
- Low synthetic burden

October 2018
Bicycles®: many shapes to drug many targets

>90 diverse targets screened 80% success rate

Tractable target classes

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

October 2018
**Bicycles®** – large molecular footprint drives affinity and selectivity between close homologues

**Acetazolamide**

- CA IX \( K_i = 25 \text{ nM} \)
- CA XII \( K_i = 6 \text{ nM} \)

**Bicycle**

- CA IX \( K_i = 7.5 \text{ nM} \)
- CA XII \( K_i > 2000 \text{ nM} \)

<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>FactorXla ( K_i ) (nM)</th>
<th>FactorXlla ( 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>

October 2018
Tolerance to conjugation is built-in

**Bicycle**

1 nm

- Tag to specific target

**Bacteriophage**

900 nm x 7 nm

- Phage bulk readily replaced without compromising binding

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

**In vitro tools**

- Fluorescent probe

**In vivo tools/diagnostics**

- DOTA
- $^{68}$Ga

October 2018
Bicycle Toxin Conjugates® for Cancer Therapy

Case Study: MT1 Targeting BTC – BT1718
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

October 2018
MT1-MMP: target rationale

- MT1-MMP – also known as MMP-14
- Surface metalloproteinase that cleaves numerous extracellular matrix components and activates other MMPs
- Highly expressed in tumours of interest: TNBC, NSCLC, Sarcoma
  - Strong link with cell invasion, metastasis
  - Expression correlated to poor outcomes (Zhou et al., 2014 Oncology letters 7:1395)
  - Low expression in adult normal tissue
- In house diagnostic shows MT1-MMP expressed high level in target patient populations

<table>
<thead>
<tr>
<th>Tumour Type</th>
<th>% based of Tumour cell membranes</th>
<th>% based on Tumour cells membranes and Stroma</th>
<th>Number of individuals in group</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNBC</td>
<td>33</td>
<td>75</td>
<td>44</td>
</tr>
<tr>
<td>Squamous cell NSCLC</td>
<td>38</td>
<td>70</td>
<td>77</td>
</tr>
<tr>
<td>Adenocarcinoma NSCLC</td>
<td>13</td>
<td>50</td>
<td>78</td>
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</tbody>
</table>

NSCLC tumour cells  TNBC tumour cells

October 2018
Identification of a high affinity Bicycle targeting MT1-MMP

- Phage selections identified a 5 x 6 Bicycle
- Human/mouse MT1-MMP $K_d = 0.5 \text{ nM}$
- Selective to MT1-MMP, no binding to other MMPs.
- Binds and internalises in MT1-MMP expressing Cells

Confocal Microscopy with fluoresceinated MT1-MMP targeting Bicycle, using HT1080 cell line. 30 min exposure

October 2018
Bicycle metabolism - *in vivo* mouse

- *Bicycle* clears faster than renal filtration rate (mouse)
- LCMS analysis → Proteolytic Attack sites: Tyr 1 and Tyr 9
Proteolytic stability enhancement by chemical modification

1) Ala Substitution to identify
   - key energetic contributors
   - remove proteolytic recognition site(s)

<table>
<thead>
<tr>
<th>Alanine Substitution Position</th>
<th>Affinity ($K_d$, nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent</td>
<td>2.5</td>
</tr>
<tr>
<td>Ala1</td>
<td>3.8</td>
</tr>
<tr>
<td>Ala2</td>
<td>$&gt; 5000$</td>
</tr>
<tr>
<td>Ala3</td>
<td>5.1</td>
</tr>
<tr>
<td>Ala4</td>
<td>34</td>
</tr>
<tr>
<td>Ala5</td>
<td>$&gt; 5000$</td>
</tr>
<tr>
<td>Ala6</td>
<td>$&gt; 5000$</td>
</tr>
<tr>
<td>Ala7</td>
<td>$&gt; 5000$</td>
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<tr>
<td>Ala8</td>
<td>$&gt; 5000$</td>
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<td>Ala9</td>
<td>$&gt; 5000$</td>
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<td>Ala10</td>
<td>11</td>
</tr>
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<td>Ala11</td>
<td>8</td>
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</table>

2) D-amino acid substitution to stabilise backbone to proteolysis.

<table>
<thead>
<tr>
<th>D-Alanine Substitution Position</th>
<th>Affinity ($K_d$, nM)</th>
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<tbody>
<tr>
<td>Parent</td>
<td>2.5</td>
</tr>
<tr>
<td>D-Ala1</td>
<td>24</td>
</tr>
<tr>
<td>D-Ala2</td>
<td>$&gt; 5000$</td>
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<tr>
<td>D-Ala3</td>
<td>105</td>
</tr>
<tr>
<td>D-Ala4</td>
<td>$&gt; 5000$</td>
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<tr>
<td>D-Ala5</td>
<td>2.4</td>
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</table>

October 2018
Affinity enhancement by chemical modification

<table>
<thead>
<tr>
<th>Kd (nM)</th>
<th>MT1-MMP</th>
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<tbody>
<tr>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>0.75</td>
<td></td>
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<tr>
<td>0.36</td>
<td></td>
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<tr>
<td>630</td>
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<td>360</td>
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<tr>
<td>43</td>
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</table>

October 2018
Chemically optimised *Bicycle*® retains affinity and is proteolytically stable

**Parent Bicycle**
Kd: 0.5 nM

<table>
<thead>
<tr>
<th>Bicycle</th>
<th>$t_{1/2}$ (hrs) Human Plasma</th>
<th>$t_{1/2}$ (hrs) Mouse Plasma</th>
<th>$t_{1/2}$ (hrs) Rat Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent Bicycle</td>
<td>30</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Optimised Bicycle</td>
<td>&gt;36</td>
<td>&gt;36</td>
<td>&gt;36</td>
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</tbody>
</table>

**Optimised Bicycle**
Kd: 0.5 nM

- Tyr1 $\rightarrow$ D-Alanine 1
- Phe4 $\rightarrow$ 1-Naphthylalanine 4
- Gly5 $\rightarrow$ D-Alanine 5
- Ile11 $\rightarrow$ tert-Butylglycine 11

October 2018
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)

- **68Ga MT1-MMP Antibody**

High tumour retention:

Photoacoustic signal intensity (change from baseline):

- Bicycle show superior retention in tumours and lower background vs antibodies.

October 2018
BT1718: MT1-MMP targeting Bicycle Toxin Conjugate®

<table>
<thead>
<tr>
<th></th>
<th>2.6</th>
<th>1.8</th>
<th>&gt;10000</th>
<th>&gt;10000</th>
<th>&gt;2000</th>
<th>&gt;1000</th>
<th>&gt;1000</th>
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</thead>
<tbody>
<tr>
<td>huMMP14 (MT1-MMP) K_d (nM)</td>
<td></td>
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<tr>
<td>moMMP14 (MT1-MMP) K_d (nM)</td>
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<tr>
<td>huMMP15 (MT2-MMP) K_d (nM)</td>
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<tr>
<td>huMMP16 (MT3-MMP) K_d (nM)</td>
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<tr>
<td>huMMP24 (MT5-MMP) K_d (nM)</td>
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<tr>
<td>huMMP1 K_d (nM)</td>
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<tr>
<td>huMMP2 K_d (nM)</td>
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</tbody>
</table>
**Bicycle toxin conjugates® show profound efficacy**

**BT1718**: MT1-MMP targeting Bicycle Drug Conjugate

- **DM1 Toxin**
- **Cleavable linker**
- **Spacer**
- **Targeting Bicycle**

**Antigen mediated cell killing**

**Clears large tumours as quickly as small**

**Clears heterogenous PDXs**

**Cell-derived xenografts**

- **Vehicle, iv, biw**
- **BT1718, 1 mpk, biw**
- **BT1718, 3 mpk, biw**
- **BT1718, 10 mpk, biw**

**Patient-derived xenografts**

- **Vehicle, iv, biw**
- **Docetaxel 20mg/kg, qw**
- **BT1718 3mpk, biw**
- **BT1718 10mpk, biw**

**Large 1000mm³ PDX (Lu-01-0046)**

- **Vehicle**
- **BT1718 10mpk, biw**

**Days after start of dosing**

- **Vehicle day 14**
- **BT1718 day 28**

- **Vehicle day 7**
- **BT1718 day 28**

- **October 2018**
**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.

- **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

October 2018
Acknowledgements

• Team at Bicycle UK & US
Why Bicycles®

**Novel Drug Modality**
Combines attributes of three other modalities delivering high affinity, good PK and rapid clearance.

- **Targets** like an antibody
- **Perform**s like a small molecule
- **Excretes** like a peptide

**Bicycle Platform**
Proprietary screening platform using evolution-driven informed-selection.

- 5000 Bicyclic peptides
- 500 Bespoke libraries
- 50 Screening libraries
- 81 Targets screened
- 5000 Bespoke libraries
- 60 Bicycle conjugates
- 80% Screening success

**Multiple Applications**
Bicycles can be used in isolation or linked together to deliver diverse payloads.

- **Plug and Play** format

**Profound Efficacy**
Eradicates patient cell derived tumours rapidly and without re-growth.

PDX – Grade 3 primary tumour

- Fast in, fast out
- Novel kill mechanism
- Unique payload flexibility
- Well tolerated
- Broad in vivo activity

October 2018