BT5528, an EphA2-targeting Bicycle® Toxin Conjugate

Nicholas Keen
World ADC congress 2019
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Bicycle Therapeutics

• Founded by Sir Gregory Winter & Prof. Christian Heinis
• UK & US based (Cambridge, UK; Boston, USA)
• Internal focus on Oncology
  • BT1718 – Phase 1/2a (Cancer Research UK)
  • 2nd Generation Bicycle Toxin Conjugates® in pre-clinical development
  • Bicycle® T-cell modulators and Bicycle® targeted innate immune activators in lead optimization
• Key strategic partnerships outside oncology

AstraZeneca

Innovate UK
Bicycles®: a new therapeutic modality

- Chemically synthesised, Low MWt (1.5-2kDa)
- Large binding footprint allowing targeting of protein-protein interactions
- Small molecule like PK and tumour penetration
- Renal elimination minimising bystander cell interactions in liver and gut
Proprietary screening platform: *Bicycles®* optimised using phage display and medicinal chemistry, informed by structural biology.

**Bicycle Phage Display**
- Linear peptide
- Phage particle
- Protein III, Bicycle DNA, Gene III
- Chemical modification with scaffold
- 1. Cyclise
- 2. Select
- 3. Amplify
- POC in 6 wk Optimised lead in 8month

**Bicycle**
- [on cell screening option]

**Structural Biology**
- Loop sizes
- Bicycle scaffolds

**Optimize binder & capture IP**

**Natural Amino Acids**
- Histidine
  - Ki = 11 nM

**Non-natural Amino Acids**
- 3,3-diphenylalanine (3,3-DPA)
  - Ki = 0.9 nM

**Dial in desired drug-like properties and PK profile**

**Peptide & Medicinal Chemistry**
Bicycles® can deliver distinct modes of action

Tractable target classes

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

>100 diverse targets screened
80% success rate
Bicycles® diversity drives hit rate & chemical optionality

- Amino acid content (n=20)
- Loop size (n=30)
- Loop symmetry (n=3)
- Scaffold (n>6)

Diversity per scaffold up to $10^{17}$

<table>
<thead>
<tr>
<th>Loop 1</th>
<th>Loop 2</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<td>8x2</td>
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<td>8x4</td>
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</tbody>
</table>

17 scaffold patents covering >200 proprietary scaffolds

TBMB EphA2 forms $\beta$-hairpin

TATA EphA2 forms $\alpha$-Helix
Bicycles® have built-in tolerance to conjugation

First Generation
Payload delivery

Second Generation
Receptor complexing

Third Generation
Dual Pharmacology

Bacteriophage
900 nm x 7 nm

Replace phage bulk with:
- Toxins
- Small molecule drugs
- Radionuclides
- Multimerised Bicycles
- PET ligand chelators
- PK extenders
- Fluorescent dyes
- Biotin and affinity tags

Tag to specific target

N-terminal extension
C-terminal extension

Bicycle

1 nm

World ADC Oct 2019
Tumour targeted *Bicycle* toxin conjugates

**Cell permeable**

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

**Bicycle selectively binds tumour**
- Targets tumour antigen
- Neutral binding site

**Tumour-selective Cleavable Linker**
- Negligible drug release outside tumour microenvironment
- Payload released extracellularly
Tumour antigen binding *Bicycles®* rapidly and specifically bind within tumours, and are renally eliminated.

MT1-MMP binding *Bicycle*

Non-binding *Bicycle*

MT1-MMP antibody

Identical sequence, all D-amino acids

PET imaging (40-60 mins)

15-20%* ID/g delivered into tumour


Rapid and target specific localization of a $^{68}$Ga conjugated MT1-MMP binding bicycle to an MT1-MMP expressing tumour was observed. A non targeting control bicycle comparator does not localize to the tumour. Free labelled bicycle is only observed in the kidney and bladder consistent with renal elimination. The antibody shows no tumour penetration, and significant non-MT1-MMP1 expressing tissue accumulation (mostly liver in this image)
Bicycle® toxin conjugates offer dramatically different ADME profile to antibodies and ADCs

<table>
<thead>
<tr>
<th>Molecule</th>
<th>$V_d^{ss}$ mL/kg</th>
<th>Cl mL/h/kg</th>
<th>$t_{1/2}$ h</th>
<th>AUC, dose-corrected h·ng/mL/(mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADC (Kadcyla$^a$)</td>
<td>57</td>
<td>0.67</td>
<td>58</td>
<td>504000</td>
</tr>
<tr>
<td>BTC (BT1718)</td>
<td>205</td>
<td>490</td>
<td>0.4</td>
<td>2070</td>
</tr>
</tbody>
</table>

NHP data, dose normalised

$^a$ Poon et al, Toxicol & Applied Pharmacol 2013

- Half-life 60-600x lower than antibodies
- AUC 100-1000x lower than antibodies
EphA2: Biological rationale

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

• Regulates cell migration, adhesion proliferation and differentiation

• Overexpression in human cancers, correlates with tumour progression

• Key area for pharma companies, multiple programs in discovery, and clinical stages but…
  
  • Development of MEDI-547 (MedImmune) in ovarian cancer was halted following on target bleeding events in phase I.

  “The bleeding and coagulation events observed in humans showed similarities to those evident in rats and monkeys. In all three species, increased activated partial thromboplastin time, increased fibrinogen/fibrin degradation product, and increased fibrin D-dimer were reported. Monkeys had red/blood discharge from the nose, mouth, gums.”
Identification of a high affinity Bicycle® targeting EphA2

- Early phage selection and chemical optimisation yielded a high affinity EphA2 Bicycle
- Good tumour targeting but phys-chem properties of Bicycle lead to unwanted liver distribution

**K_i = 2.8 nM**
**CHI LogP = 1.79**
**PPB = >99.9%**
Switching scaffolds improves physical properties and therapeutic index

\[ K_i = 17.3 \text{ nM} \]

\[ K_i = 2.9 \text{ nM} \]

\[ \text{CHI LogP} = 0.89 \]

\[ \text{PPB} = 67.6\% \]

- 2nd generation phage selection with more diverse library and chemical optimisation yielded a equally high affinity EphA2 Bicycle

- Improved phys-chem properties of Bicycle eliminated liver exposure while retaining strong tumour targeting
A diverse array of Bicycle conjugates were evaluated in vivo Vs CTX and PDX models to establish SAR and STR

Lead Optimisation of EphA2 BTC evaluated payload, linker and Bicycle components

World ADC Oct 2019
BT5528: structure & profile

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

<table>
<thead>
<tr>
<th>Ligand-binding domain</th>
<th>% identity to EphA2</th>
<th>Binding affinity (SPR $K_D$ nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EphA2</td>
<td>100</td>
<td>1.2</td>
</tr>
<tr>
<td>EphA1</td>
<td>54</td>
<td>&gt;5000</td>
</tr>
<tr>
<td>EphA3</td>
<td>58</td>
<td>&gt;5000</td>
</tr>
<tr>
<td>EphA4</td>
<td>55</td>
<td>&gt;5000</td>
</tr>
<tr>
<td>EphA5</td>
<td>56</td>
<td>&gt;5000</td>
</tr>
<tr>
<td>EphA6</td>
<td>56</td>
<td>&gt;5000</td>
</tr>
<tr>
<td>EphA7</td>
<td>56</td>
<td>&gt;5000</td>
</tr>
<tr>
<td>EphB4</td>
<td>39</td>
<td>&gt;5000</td>
</tr>
</tbody>
</table>

**BT5528 affinity**

<table>
<thead>
<tr>
<th></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>1.9 ± 1.3</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>Cell binding by HCS ($K_B$ app, nM)</td>
<td>14.8 ± 10.5</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
BT5528 delivers MMAE to tumour

Single dose of BT5528
- Produces high MMAE concentrations in tumour
  - Stable from 2h to >48h
  - Transient exposure of both BT5528 & MMAE in plasma

---

**BT5528 PK Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mouse</th>
<th>Rat</th>
<th>NHP</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>6321</td>
<td>4048</td>
<td>7643</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>0.4</td>
<td>0.3</td>
<td>~0.6h</td>
</tr>
<tr>
<td>V&lt;sub&gt;dss&lt;/sub&gt; (L/kg)</td>
<td>0.18</td>
<td>0.33</td>
<td>0.21</td>
</tr>
<tr>
<td>Cl (mL/min/kg)</td>
<td>6.2</td>
<td>15.5</td>
<td>4.9</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-last&lt;/sub&gt; (ng.h/mL)</td>
<td>2643</td>
<td>998</td>
<td>3516</td>
</tr>
</tbody>
</table>

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BT5528 PK Parameters

- Mouse
- Rat
- NHP

**BT5528 PK Parameters**

- C<sub>max</sub> (ng/mL): 6321, 4048, 7643
- T<sub>1/2</sub> (h): 0.4, 0.3, ~0.6h
- V<sub>dss</sub> (L/kg): 0.18, 0.33, 0.21
- Cl (mL/min/kg): 6.2, 15.5, 4.9
- AUC<sub>0-last</sub> (ng.h/mL): 2643, 998, 3516
BT5528 induces mitotic arrest in tumour

Single dose of BT5528
• Produces high MMAE concentrations in tumour
  • Stable from 2h to >48h
  • Transient exposure of both BT5528 & MMAE in plasma
• Induces mitotic arrest
  • Measurable by pHH3 IHC within 24h
BT5528 produces tumour regression

Weekly dosing of BT5528

- Produces high MMAE concentrations in tumour
  - Stable from 2h to >48h
  - Transient exposure of both BT5528 & MMAE in plasma
- Induces mitotic arrest
  - Measurable by pHH3 IHC within 24h
- Induces tumour cell death
  - Measurable regression by day 4
BT5528 efficacy: target-mediated, flexible dosing

- BT5528 shows target-dependent efficacy
  - Significant regression in a wide range of EphA2-positive tumours

- BT5528 shows equivalent efficacy with a wide range of dosing paradigms
  - Bolus, 1h infusion, 24h infusion

- BT5528 efficacious with intermittent dosing
  - Efficacy also shown dosing every 2 weeks

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**Graphs and Data**

- **Tumour Volume**
  - Days after start of dosing: 0, 7, 14, 21
  - Tumor Volume (mm$^3$)
  - Lines represent different dosing paradigms:
    - Vehicle
    - BT5528 1mg/kg (bolus)
    - BT5528 1mg/kg (1h infusion)
    - BT5528 1mg/kg (24h infusion)

- **EphA2 expression**
  - Target expression (by FACS, binding sites/cell)
  - Data points for different cell lines (e.g., MOLP-8, Lu-01-0486)

- **C$\text{max}$** (ng/mL) and **AUC** (ng.h/mL)
  - Values for different cell lines:
    - Lu-01-0486
    - Lu-01-0412
    - Lu-01-0451

---

**Table**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>C$\text{max}$ (ng/mL)</th>
<th>AUC (ng.h/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lu-01-0486</td>
<td>3120</td>
<td>1325</td>
</tr>
<tr>
<td>Lu-01-0451</td>
<td>1120</td>
<td>1325</td>
</tr>
<tr>
<td>Lu-01-0412</td>
<td>55</td>
<td>1325</td>
</tr>
</tbody>
</table>
BT5528: differentiation from ADC in complex PDX models

Moderate EphA2 expression

High EphA2 expression

Bicycle distribution at 60 min

ADC distribution at 60 min

World ADC Oct 2019
BT5528 is efficacious in treating metastatic disease in mouse

**Metastatic PC3 xenograft model**

- Intracardiac implantation of PC3-Luc tumour cells on D0
- High EphA2
- Grows in bone
- Weekly bioluminescence imaging during treatment
- Initiate treatment on D14 or D21 QWx4
- Monitor survival until D78

- PC3 metastatic lesions have the required enzymatic activity for payload release from BT5528 to yield significant anti-tumor activity
- 4 weekly BT5528 treatment cycles reduced the bone tumor cell burden significantly and extended the survival of the mice

**Total Bone Signal**

Day 63 is the last imaging day

**Survival**

* On D78, 1 mouse with no macroscopic disease
BT5528: differentiation from ADC in bleeding/coagulation & liver toxicology

Findings from MEDI-547 Phase I study

<table>
<thead>
<tr>
<th>Treatment related adverse events</th>
<th># events (% of patients) n of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT increased</td>
<td>3 (50) 3/6</td>
</tr>
<tr>
<td>Haemorrhage</td>
<td>6 (83.3) 5/6</td>
</tr>
</tbody>
</table>

Findings from BT5528 toxicology study

- No bleeding events seen in either species
- Dosing to toxin equivalent doses >100x dose of MEDI-547 used in patients
- No significant effect on clotting parameters
- No evidence of abnormal liver function
BT5528: a Bicycle Toxin Conjugate targeting EphA2 for the Treatment of Solid Tumours

• EphA2 is highly expressed on tumour cell surface in a wide range of solid tumours

• BT5528 was developed as a BTC to target EphA2
  • High affinity binding and tumour penetration
  • Engineered short systemic half-life and renal excretion
  • “Hit and run” delivery of toxin

• BT5528 shows profound efficacy in a wide range of tumour models
  • Efficacy correlates with EphA2 expression

• BT5528 shows clear differentiation from previous ADC approaches
  • Efficacy maintained even in large, heterogeneous PDX
  • No bleeding/ coagulation toxicity observed in preclinical models