BT5528: A Bicycle Toxin Conjugate Targeting EphA2 for the Treatment of Solid Tumours

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**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 $T_{1/2}$
Proven platform using phage display

**Linear peptide**

- Protein III
- Bicycle DNA Sequence
- Gene III

**Bicycle**

- Chemical modification with scaffold

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

- Evolution driven, informed selection

- >80 diverse targets screened; >80% success rate
- Antagonists, agonists and neutral binders

**Exremely large and diverse chemical library**

**Low synthetic burden**

1. Cyclise
2. Select
3. Amplify

POC in 6 wk
Optimised lead in 9mnth

Extremely large and diverse chemical library
Low synthetic burden
Bicycles® have built-in tolerance to conjugation

Replace phage bulk with:
- Toxins
- Small molecule drugs
- Radionuclides
- Multimerised Bicycles
- PET ligand chelators
- PK extenders
- Fluorescent dyes
- Biotin and affinity tags
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.”
Synthesis of a matrix of Bicycle Toxin Conjugates

**EphA2-Targeting Bicycle**

- Linear peptide
- Protein III
- Bicycle DNA
- Sequence
- Gene III
- Phage particle

**Molecular spacer**
- Sarcosine (Sar0-Sar10), PEG
- Acidic/basic group, saccharide
- Other

**Cleavable Linker**
- Peptidic: val-cit, val-lys, val-ala
- Plasmin-, legumain-, MMP-sensitive
- Disulphide: -SS-, -SSMe-
- Acidic: Hydrazone
- Other: eg glucuronide, “non-cleavable”

**Toxin**
- Auristatins: MMAE MMAF
- Maytansinoids: DM1, DM3, DM4
- Other derivatives
- Camptothecins, SN38
- PBDs
- Other: non-toxins
Profile of Bicycle conjugates

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 h EphA2</th>
<th>Binding affinity (SPR, using BT5528, K_D nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EphA1</td>
<td>Human</td>
<td>Human</td>
</tr>
<tr>
<td>EphA2</td>
<td>Human</td>
<td>&gt; 5uM</td>
</tr>
<tr>
<td>EphA3</td>
<td>Human</td>
<td>&gt; 5uM</td>
</tr>
<tr>
<td>EphA4</td>
<td>Human</td>
<td>&gt; 5uM</td>
</tr>
<tr>
<td>EphA5</td>
<td>Human</td>
<td>&gt; 25uM</td>
</tr>
<tr>
<td>EphA6</td>
<td>Human</td>
<td>&gt; 20uM</td>
</tr>
<tr>
<td>EphA7</td>
<td>Human</td>
<td>&gt; 20uM</td>
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<tr>
<td>EphB1</td>
<td>Rat</td>
<td>56</td>
</tr>
<tr>
<td>EphB4</td>
<td>Rat</td>
<td>&gt; 20uM</td>
</tr>
<tr>
<td>NTG</td>
<td>Rat</td>
<td>&gt; 20uM</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BT5528 affinity</th>
<th>Human</th>
<th>Mouse</th>
<th>Rat</th>
<th>NHP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FP comp (K_i, nM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BT5528</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><strong>SPR (K_D, nM)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>BT5528</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><strong>Cell binding by HCS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BT5528</td>
<td>14.8 ± 10.5</td>
<td></td>
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</tr>
</tbody>
</table>

Short half-life *in vivo*, rapid & selective binding to EphA2-expressing tumour, renal elimination.
Efficacy is EphA2-dependent across tumour types
Comparison to MEDI-547 (EphA2 targeting ADC)

- Efficacy reported with MEDI-547 in PC-3 xenograft
  - Regression at 3mg/kg qw, in line with published paper (Lee et al, 2009)
- BT5528 shows efficacy in PC-3 & HT1080 models
  - Complete regression seen from 1mg/kg qw (PC-3)
  - Efficacy with BTC and ADC are comparable at 3mg/kg qw
Extensive tumour penetration maintains efficacy even in very large PDX model

- BT5528 maintains efficacy seen in CDX models even in large PDX
  - Patient-derived xenograft
  - Lung adenocarcinoma
  - Heterogeneous tumour
  - 1000mm³ at dosing start

- Significant regression of tumour after 21d dosing 3mg/kg qw

- ADC (MEDI-547) shows no efficacy
  - Dosed 3mg/kg qw

- PET imaging shows rapid penetration of Bicycle conjugate into tumour
  - ADC data shows largely vascular distribution

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MEDI-547 toxicity

- MEDI-547 (ADC comprising 1c1 antibody conjugated to MMAF via a VC cleavable linker)
- Ph1 terminated following unexpected bleeding/coagulation events, study published
  - Annuziata et al, Invest New Drugs 2013 Feb 31(1) 77-84

- 5/6 patients dosed with MEDI-547 at a dose of 0.08 mg/kg once every 3 weeks experienced bleeding and coagulation events. Three patients had haemorrhage-related events, and 2 patients reported epistaxis. Three patients had bleeding/ coagulation AEs that were also SAEs.
  - Haemorrhage/ epistaxis occurred d3-9 post treatment. One further patient received a second cycle, after which SAEs (pain & liver disorder) occurred.

- Clinical events were “not unexpected based on the preclinical toxicology findings”
  - 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 and/or anus, whereas two patients experienced epistaxis and one patient experienced an oral cavity haemorrhage.
  - Preclinical toxicology studies in the monkey identified disseminated intravascular coagulation (DIC) as the DLT. The events observed in humans were considered to be consistent with the preclinical findings, in particular to the observation of DIC.
  - The dose used in the clinical study was 10-fold lower than the highest non-severely toxic dose predicted based on extrapolations from rat studies (MedImmune, LLC, data on file), and no evidence of drug accumulation after the administration of a second dose was apparent
BT5528 (EphA2 BTC) does not exhibit the primate toxicologies (clotting defects, hepatic effects) observed with the EphA2 ADC, MEDI-547.

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

Phases 1, open-label study of MEDI-547 in patients with relapsed or refractory solid tumors

Christina M. Amannstein - Eli Lilly
Patricia Lahtinen - Novo Nordisk
Robert L. Coleman - Merck Frosst
Gabriel Robin - Eli Lilly

Bleeding observed on days 3-8 following a single dose of MEDI-547

Animals dosed weekly, 1-10mg/kg ascending dose protocol

- Dosing: no bleeding events in primates at toxin equivalent doses >100 fold higher than the clinical 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 Bicycle Toxin Conjugate to target EphA2
  • High affinity binding
  • Short half-life and renal elimination
  • “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
  • Efficacy maintained even in large, heterogeneous PDX
  • No bleeding/ coagulation toxicity seen