A New Site Specific Antibody Conjugation Using Bacterial Transglutaminase

ADC Summit, San Francisco
On 15th October, 2013
From Chemotherapy to Homogeneous ADCs
Improving the Therapeutic Index

**First Generation:**
Lysine or Cysteine conjugation
- Increased tumor delivery
- Decreased normal tissue exposure
- Heterogeneous PK

**Second Generation:**
engineered Cysteine conjugation
- Homogeneous PK
- Unstability questionmark
  - unpaired cystein
  - thiol-maleimide linkage
Next Generation ADCs
Homogeneous and Stable

Unnatural Amino Acid

- Expanded genetic code to incorporate orthogonal side chains
- POC (Axup et al., PNAS, 2012)
- Specific production system

Transglutaminase (TG)

- LLQG Tag to create conjugation site
- POC (Strop et al., Chem. Biol., 2013)
- Effector function preserved

- Aglycosylated mAb
- Conjugation on endogenous Q295 and possibly on N297Q
- Lower uptake by FcR+ cells might improve tumor-specific targeting and limit off-target toxicity
Bacterial Transglutaminase (BTG)
Site-specific and Stoichiometric Enzymatic Conjugation

• BTG catalyses reactions between glutamine and lysine

• BTG recognizes exclusively endogenous Q295 located in Fc region of aglycosylated IgG

• N297Q mutation provides 2 additional sites for conjugation

Jeger et al., Angew. Chem. Int. Ed., 2010
BTG Ligation Site in Fc Structure
Before and After Carbohydrate Removal

Q 295 is barely exposed and partially hidden by the carbohydrate

Degree of freedom is improved when carbohydrates are absent
BTG Coupling Reaction

BTG is calcium independent

Acylenzyme intermediate formation

Release of ammonia

K-substrate's attack of thioester bond

Isopeptide bond formation
One-step Approach
One-step Approach

Aglycosylated IgG
Single mutation
N297S or N297Q

BTG

NH₂-step1-

= toxin

x 2 for N297S
x 4 for N297Q
N297S coupling with \( \text{NH}_2\text{-step1a-vc-PAB-MMAE} \)

**LC/MS (ESI-qTOF)**

- 10 equivalents of toxin/site
- 24 hours
- M/Z shift = 2708
- Theoretical 2 x M Toxin = 2706

\[ \text{DAR} = 1.8 \]
N297Q coupling with NH$_2$-step1a-vc-PAB-MMAE

LC/MS (ESI-qTOF)

- m/z shift = 5415
- Theoretical 4x M Toxin = 5412
- DAR = 3.7
- 20 equivalents of toxin/site
- 24 hours
Two-step Approach
Two-step Approach

Aglycosylated IgG
Single mutation
N297S or N297Q

BTG

NH₂-step1-R

Z-step2-

● = toxin

x 2 for N297S
x 4 for N297Q
**Coupling with Azide Linker and DBCO Toxin**

**N297S-step1a-click-step2-vcMMAE**

- **m/z shift = 399**
- **Theoretical 2x M Linker = 402 Da**

- **10 eq. of linker NH$_2$-step1-N$_3$ per site**
- **24 hours**

- **m/z shift = 3775**
- **Theoretical 2x M Linker-toxin = 3774 Da**

- **1.5 eq. of DBCO-step2-vcMMAE per site**
- **4 hours**

**DAR=2.0**
Coupling with Azide Linker and DBCO Toxin

N297Q-step1a-click-step2-vcMMAE

- 10 eq. of linker NH$_2$-step1-N$_3$ per site
- 24 hours

- m/z shift = 801
- Theoretical 2x M Linker = 804 Da

- 1.5 eq. of DBCO-step2-vcMMAE per site
- 4 hours

- m/z shift = 7550
- Theoretical 2x M Linker-toxin = 7548 Da

DAR 4
DAR=4.0
Preclinical POC
Tools for POC

• Naked antibody
  o SGN30 (cAC10) targeting CD30
  o SGN30S or SGN30Q with 2 or 4 coupling sites

• Intermediates: various linkers
  o Structure of spacer (size, hydrophobicity):
    step1a, b or c
  o Reactive groups for click chemistry: -R, -R’, -R”

• BTG-ADCs
  o -vc-PAB-MMAE for all conjugates
  o One-step: NH2-step1-vcMMAE
  o Two-step: DBCO-step2-vcMMAE

• Comparator
  o ADCETRIS®, Brentuximab vedotin
Stability in Buffer and in Plasma
• Linker has significant impact on intermediates stability
• R’ reactive group abandoned
• All BTG-ADCs (except step1b) showed less soluble aggregates than ADCETRIS®

BTG-ADCs Stability in Buffer
HMWP by SEC at +40°C

- Intermediates
- BTG-ADCs

- Linker has significant impact on intermediates stability
- R’ reactive group abandoned
- All BTG-ADCs (except step1b) showed less soluble aggregates than ADCETRIS®
BTG-ADCs Stability in Buffer
DAR at +40°C
Ex Vivo Plasma Stability

- ADCs spiked in plasma
- Plasma types: rat (Wistar), cynomolgus and human

**Affinity capture**

- Rodents
- Human and cynomolgus

- Anti-HumanCk (nanobody coupled to biotin)
- CD30 coupled to biotin
- Paramagnetic beads coated with streptavidin
- LC/MS (ESI-qTOF)
- DAR
BTG-ADCs ex vivo Stability in Wistar Rat Plasma

DAR over one week

No DAR variation observed over one week at 37°C
BTG-ADCs ex vivo Stability in NHP and Human Plasma

DAR over one week

Cynomolgus

- SGN30S-sp1a-click-sp2-vcMMAE
- SGN30S-sp1a-vcMMAE

Human

- SGN30Q-sp1a-click-sp2-vcMMAE
- SGN30Q-sp1a-vcMMAE
PK Study
BTG-ADCs PK in Wistar Rat

Conjugated antibody

Days

Average DAR

n=4

Total antibody

Days

Conjugated antibody

Unit | SGN30Q-sp1a-click-sp2-vcMMAE | SGN30S-sp1a-click-sp2-vcMMAE | SGN30S | ADCETRIS®
---|--------------------------|--------------------------|-------|----------
DAR | N/A | 4.0 | 2.0 | N/A | ~4
Half-Life | days | 8.5 | 12.0 | 9.6 | 8.5
Cl  | ml/h | 0.099 | 0.071 | 0.088 | 0.168
In Vitro and In Vivo Efficacy
BTG-ADCs in vitro Efficacy

**KARPAS 299**

**RAJI-CD30+**

<table>
<thead>
<tr>
<th>DAR</th>
<th>SGN30S-sp1a-click-sp2-vcMMAE</th>
<th>SGN30Q-sp1a-click-sp2-vcMMAE</th>
<th>SGN30S-sp1a-vcMMAE</th>
<th>SGN30Q-sp1a-vcMMAE</th>
<th>ADCETRIS®</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.0</td>
<td>4.0</td>
<td>2.0</td>
<td>4.0</td>
<td>~4</td>
</tr>
<tr>
<td><strong>EC&lt;sub&gt;50&lt;/sub&gt; RAJI-CD30+ (ng/ml)</strong></td>
<td>5.1</td>
<td>2.0</td>
<td>5.4</td>
<td>2.3</td>
<td>1.4</td>
</tr>
<tr>
<td><strong>EC&lt;sub&gt;50&lt;/sub&gt; KARPAS 299 (ng/ml)</strong></td>
<td>11.2</td>
<td>3.1</td>
<td>14.6</td>
<td>4.7</td>
<td>2.4</td>
</tr>
</tbody>
</table>
BTG-ADCs *in vivo* Efficacy

- Karpas 299 (S.C.) in SCID mice
- Dose 0.6mg/kg, I.V., q4d X4,
- Treatment started when tumor ~100mm³
- 9 mice per group
Summary

• ADCs with DAR of exactly 2.0 or 4.0 from minimally modified antibody scaffold, i.e. with a single point mutation

• Rapid and versatile process appropriate for testing various linkers and toxins in HTS

• BTG two-step process yields to quantitative coupling using only 1 to 2 molar excess of toxin per site, making it a cost-efficient and scalable process

• BTG-ADCs are stable ex vivo in human and cynomolgus plasma and in vivo in rat, without DAR variation. In addition, BTG-ADCs clearance is lower compared to Adcetris®

• BTG-ADCs with DAR=4.0 show equivalent in vitro and in vivo efficacy compared to Adcetris®
Acknowledgment

- **Innate Pharma**
  - Delphine Bregeon
  - Christian Belmant
  - Angélique Boedec
  - Hélène Rispaud
  - Sandra Savard-Chambard
  - Naouel Lovera
  - Agnès Represa
  - Mélody Sapet
  - Céline Delcambre
  - Sophie Ingoure
  - Sylvia Trichard
  - Stéphane Delahaye
  - Cécile Bonnafous
  - Nicolas Viaud
  - Mathieu Bléry
  - Stéphanie Zerbib
  - Benjamin Rossi

- **ETH/PSI (Zurich)**
  - Patrick Dennler
  - Aris Chiotellis
  - Eliane Fisher
  - Roger Schibli

- **PIT2 (Marseilles)**
  - Sega N'Diaye
  - Claude Villard
  - Daniel Lafitte