Case Study

in vivo Evaluation of CRS induction with Bispecific Antibodies

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Mouse Model for *In Vivo* Assessment of Therapy-induced Cytokine Release Syndrome

- PBMC humanized NSG, NSG-SGM3 and NSG-MHC Class I/II KO mice
- 4-5 mice/arm
- Panel of compounds tested
  - OKT3
  - anti-CD28 mAb
  - TGN1412
  - Checkpoint inhibitors, bispecific antibodies
- Assessment of Cytokine Release Syndrome (CRS) by
  - Sera collection for cytokines concentrations by flow cytometry
  - Clinical scoring
Prescreen and Collect PBMC Donors with Different Cytokine Release Potential

IFN-γ

<table>
<thead>
<tr>
<th>Donor ID</th>
<th>PBS</th>
<th>OKT3 (0.25mg/kg)</th>
<th>Anti-CD28 (1mg/kg)</th>
<th>Keytruda (10mg/kg)</th>
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</thead>
<tbody>
<tr>
<td>8595</td>
<td>0</td>
<td>0</td>
<td>4000</td>
<td>100</td>
</tr>
<tr>
<td>8618</td>
<td>0</td>
<td>0</td>
<td>2000</td>
<td>50</td>
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<tr>
<td>8485</td>
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<td>0</td>
<td>500</td>
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IL-6

<table>
<thead>
<tr>
<th>Donor ID</th>
<th>PBS</th>
<th>OKT3 (0.25mg/kg)</th>
<th>Anti-CD28 (1mg/kg)</th>
<th>Keytruda (10mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8595</td>
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<td>90</td>
<td>10</td>
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<tr>
<td>8618</td>
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<td>0</td>
<td>60</td>
<td>5</td>
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<tr>
<td>8485</td>
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<td>0</td>
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<td>2</td>
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Blinatumomab

- The first-and-only FDA-approved CD19-directed CD3 bispecific T cell engager (BiTE®) immunotherapy

BLINCYTO® (blinatumomab) for injection, for intravenous use
Initial U.S. Approval: 2014

WARNING: CYTOKINE RELEASE SYNDROME and NEUROLOGICAL TOXICITIES
See full prescribing information for complete boxed warning.

- Cytokine Release Syndrome (CRS), which may be life-threatening or fatal, occurred in patients receiving BLINCYTO. Interrupt or discontinue BLINCYTO and treat with corticosteroids as recommended. (2.3, 5.1)
- Neurological toxicities, which may be severe, life-threatening, or fatal, occurred in patients receiving BLINCYTO. Interrupt or discontinue BLINCYTO as recommended. (2.3, 5.2)
CRS evaluation of CD19xCD3 Treatment
Raji-luc Tumor Cells in PBMC Humanized Mice
Cytokine release with CD19xCD3 Bispecific Donor Variability (TNF)

TNF in NSG Mice

(PBMC15M/Mouse; Raji 2M/Mouse)

- PBS
- Rituximab (5mpk)
- Anti-CD28 (1mpk)
- CD19xCD3 (0.25mpk)
- Rituximab+CD19xCD3

pg/ml

8485  8130  8259
Cytokine release with CD19xCD3 Bispecific Donor Variability (IFN-γ & IL-10)

**IFN-γ in NSG Mice**

- PBS
- Rituximab (5mpk)
- Anti-CD28 (1mpk)
- CD19xCD3 (0.25mpk)
- Rituximab+CD19xCD3

**IL-10 in NSG Mice**

- PBS
- Rituximab (5mpk)
- Anti-CD28 (1mpk)
- CD19xCD3 (0.25mpk)
- Rituximab+CD19xCD3
Cytokine release with CD19xCD3 Bispecific Donor Variability (IL-6 & IL-2)
Efficacy and CRS Combined in a single assay
Bispecific Treated Raji-luc disseminated model

**TNF**

- PBS
- anti-CD28
- Rituximab
- CD19xCD3
- CD19x CD3 +Rituximab

**Raji-Luc Xenogen Day 8**

- Total Flux [p/s]

<table>
<thead>
<tr>
<th>Condition</th>
<th>Total Flux [p/s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>3×10^7</td>
</tr>
<tr>
<td>Anti-CD28</td>
<td>5×10^7</td>
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<tr>
<td>Rituximab</td>
<td>4×10^7</td>
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<td>CD19 xCD3</td>
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<tr>
<td>CD19xCD3 +Rituximab</td>
<td>1×10^7</td>
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Summary

- Bispecific antibodies can induce donor-specific cytokines release in this preclinical humanized mouse model;

- Bispecific antibodies can have combination or synergy effect with other drug treatments using this humanized mouse model;

- We have developed a translational humanized mouse model for preclinical assessment of CRS adverse events to Bispecific antibodies therapeutics.