A CD117-Amanitin Antibody Drug Conjugate (ADC) Effectively Depletes Human and Non-Human Primate Hematopoietic Stem and Progenitor Cells (HSPCs): Targeted Non-Genotoxic Conditioning for Bone Marrow Transplant

Bradley Pearse, Jennifer Proctor, Sean McDonough, Rajiv Panwar, Ganapathy Sarma, Lena Kien, Junia Dushime, Hillary Adams, Sharon Hyzy, Melissa Brooks, Rahul Palchaudhuri, Qing Li, Pranoti Sawant, Tahirih Lamothe, Nidhi Jain, Katia George, Charlotte McDonagh, Anthony Boitano, Michael Cooke

Magenta Therapeutics
Cambridge, MA
Financial Disclosures

Magenta Therapeutics
• Shareholder
• Inventor
• Salary
• Employment
Current Transplant Conditioning Agents are Non-Specific and Genotoxic

Acute toxicities can include:

• Neutrophil loss (infections)
• Platelet loss (bleeding)
• Anemia
• T-cell depletion (infection)
• Thymic damage (infection)
• Mucositis

Long-term toxicities can include:

• Secondary malignancies
• Organ damage
• Infertility
• Stunted growth
Targeted Conditioning: Patient Preparation for Transplant & Stem Cell Gene Therapy

**Opportunity:**
- Less toxic conditioning
- Potential for disease control
- Immune preservation
- Avoid secondary malignancy and infertility

**Applications in transplant:**
- Gene therapy
- Hematologic malignancies

**Target:**
- Selective depletion of HSCs

**Payload:**
- Potent killing of quiescent and dividing cells

**Engineering:**
- Fc silencing
- Rapid clearance following target cell depletion
CD117 (C-kit) is an Ideal Target for ADC-Mediated Conditioning

Restricted expression profile

- CD117 is overexpressed in >60% of AML and MDS patients
  
  *Ludwig et al. Haematologica 1997, 617-621*

A single dose of CD117 ADC enables robust engraftment in mice
Engineered Half-Life for Appropriate Clearance in Transplant

Non-human primate pharmacokinetics

<table>
<thead>
<tr>
<th>Group</th>
<th>Half Life (Hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Engineered half-life IgG</td>
<td>11</td>
</tr>
<tr>
<td>Wild-type IgG</td>
<td>60</td>
</tr>
</tbody>
</table>

Days Post Administration

Concentration (ng/mL)
Amanitin Conjugation to Effectively Deplete Target Cells Prior to Transplant

Full synthetic access by license from Heidelberg Pharma

<table>
<thead>
<tr>
<th>Amanitin</th>
<th>Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-DNA damaging inhibitor of RNA polymerase II</td>
<td>Avoid risks of secondary malignancy and infertility</td>
</tr>
<tr>
<td>Cytotoxic to quiescent and dividing cells</td>
<td>Deplete HSCs with anti-tumor activity</td>
</tr>
<tr>
<td>Potent cytotoxicity on low copy number cells</td>
<td>Allows for substantial elimination of CD117+ cells</td>
</tr>
<tr>
<td>Serum stable</td>
<td></td>
</tr>
<tr>
<td>Low membrane permeability</td>
<td>Low off-target toxicity</td>
</tr>
</tbody>
</table>
Anti-CD117 Amanitin ADC Demonstrates Potent Killing of AML and Human CD34+ Cells \textit{in vitro}

AML Cell Line (Kasumi-1)

![Graph showing the effect of different concentrations of Anti-CD117-AM on the AML cell line Kasumi-1. The IC$_{50}$ is 1 pM.]

Primary Human CD34+CD90+

![Graph showing the effect of different concentrations of Anti-CD117-AM on primary human CD34+CD90+ cells. The IC$_{50}$ is 7 pM.]
Single Dose of Anti-CD117-Amanitin ADC Selectively Depletes Human CD34+ cells in Humanized Mice

Humanized mouse bone marrow

- Human CD34+ Cell
- Human CD45+ Cell
- Mouse CD45+ Cell

Single injection of Anti-CD117 ADC

Blood analysis at day 7, 14 and 21
Bone Marrow Analysis at Day 21

Graphs showing:
- Cells/femur (day 21)
- % of baseline
- % of baseline (Anti-CD117-AM)

N = 5 per group
A Single Dose of Anti-CD117-Amanitin ADC Extends Survival in AML Xenograft Models

Kasumi-1 AML xenograft

- Treatment
- Isotype-AM 1 mg/kg
- Anti-CD117-AM 1 mg/kg

AML PDX

- Treatment
- Isotype-AM 1 mg/kg
- Anti-CD117 1 mg/kg

N = 5 per group
The Engineered Anti-CD117-Amanitin ADC Effectively Depletes Target Cell Populations in Cynomolgus Monkeys

Bone marrow – Day 7

Vehicle

Treated

HSC

CD34+ CD90+ CD45RA-

Remaining cells (% of PBS control)

0 20 40 60 80 100

Reticulocytes

10⁹/ml

Days post-dose

0 7 14 21 28 35

CFU

5×10⁵

4×10⁵

3×10⁵

2×10⁵

1×10⁵

CFU/ml

PBS 0.1 0.3 0.6

Anti-CD117-AM

Anti-CD117

mg/kg

N = 3 per group

PBS

Anti-CD117-AM (0.1 mpk)

Anti-CD117-AM (0.3 mpk)

Anti-CD117 (0.6 mpk)
The Timing of ADC-Mediated Depletion and Clearance Provides a Window for Transplant Conditioning

No detectable ADC in sera

0.3 mg/kg ($t_{1/2}$: 19.6h)

N = 3 per group

Window for graft infusion

No target cell killing with ADC treated NHP sera

Mean plasma drug concentration (ng/ml)

Days post-dose
Engineered Anti-CD117-Amanitin ADC is Safe and Well-Tolerated in Non-Human Primates at Efficacious Doses

- **Lymphocytes**
  - Days post-dose vs. $10^3/\text{ml}$
  - Normal range shown

- **ALT**
  - Days post-dose vs. Units/L
  - Normal range shown
  - 1-3x, 3-5x, >5x

- **Tissue** (magnification)
  - Liver (10x)
  - Kidney (10x)

- **PBS (d35)**
- **Anti-CD117-AM 0.3 mg/kg (d35)**

No change from normal range for the additional parameters:

<table>
<thead>
<tr>
<th>GGT</th>
<th>Albumin</th>
<th>BUN</th>
<th>LDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP</td>
<td>Creatinine</td>
<td>Glucose</td>
<td>PT/PTT</td>
</tr>
</tbody>
</table>
Summary and Next Steps

- An ADC targeting CD117 may be a promising approach for conditioning in:
  - Autologous transplant for gene therapy
  - Allogeneic transplant for hematologic malignancies

- CD117-Amantin ADC effectively depletes hematopoietic stem and progenitor cells:
  - *In vitro*
  - in humanized mice
  - in non-human primates

- CD117-Amantin ADC extends survival in Acute Myeloid Leukemia xenograft models

- An engineered half-life ADC is well-tolerated in NHPs with appropriate pharmacokinetics and pharmacodynamics for the transplant setting

**Next Steps:**

- IND-enabling activities in 2019
- Evaluation of ADC-mediated conditioning in NHP transplant models

**Additional TCT abstracts on ADC conditioning:**

Jennifer Proctor
Poster 129
2/20

Sharon Hyzy
Poster 262
2/20

Rahul Palchaudhuri
2/21 5:30-5:45
Acknowledgements

Magenta Therapeutics

Michael Cooke
Tony Boitano
Jennifer Proctor
Sean McDonough
Lena Kien
Hillary Adams
Sharon Hyzy
Melissa Brooks
Tahirih Lamothe
Katia George

Charlotte McDonagh
Rajiv Panwar
Ganapathy Sarma
Junia Dushime
Rahul Palchaudhuri
Qing Li
Pranoti Sawant
Nidhi Jain

Heidelberg Pharma