CD117 antibody-drug conjugate-based conditioning allows for efficient engraftment of gene-modified CD34+ cells in a rhesus gene therapy model

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4. Translational Stem Cell Biology Branch, NHLBI, NIH, MD
Conflict of interest

Kellie Latimer, Rahul Palchaudhuri, Qing Li, Nick Yoder, Prashant Bhattarai, Kirk Bertelsen, and Lisa M Olson are or were employees of Magenta Therapeutics.
Hematopoietic stem cells (HSCs) from SCD patients

Corrected by β-globin gene transfer to CD34+ HSCs with lentiviral vectors

- Plerixafor mobilization
- Myeloablative busulfan conditioning
- High-efficiency transduction ex vivo

Autologous transplantation back to patient

Sickling of red blood cells

Phenotypic correction
Targeted conditioning with exploratory antibody-drug conjugate (ADC)

- Less toxic conditioning
- Immune preservation
- Avoid secondary malignancy and infertility
- Improve risk benefit profile to broaden access
Hypothesis: CD117 (c-kit) is an ideal target for HSC depletion?

Questions for CD117-ADC conditioning

- Sufficient for HSC ablation?
- Not immunosuppressive?
- Minimal systemic toxicity?

CD117 expression is limited to progenitor cells
CD117-ADC depletes both human and rhesus CD34+ cells

**Hematopoietic Stem Cell Killing Assay**

**Human CD34+ cell depletion in xenograft mouse BM**

% Viable CD34+

Concentration [M]

Rhesus IgG ADC

Rhesus EC_{50}=0.1pM

Human IgG ADC

Human EC_{50}=0.2pM

Day21

Number of CD34+ cells in BM

PBS  CD117 mAb  IgG ADC  CD117 ADC

p < 0.01

Tisdale JF. ASH. 2019
>99% depletion of non-human primate CD34+ CD90+ cells 7 days post-injection

Control (PBS)

>99% depletion
BM failure

CD117-ADC (0.1mg/kg)

CD117-ADC (0.2mg/kg)

Busulfan (5.5mg/kg x4)

CD34
CD90

CD34
CD90

CD34
CD90

CD34
CD90
Rapid clearance of CD117-ADC in rhesus plasma

Plasma ADC concentration (ng/ml)

Days post-ADC injection

- Wild Type CD117-ADC
- Engineered CD117-ADC

ADC in circulation

Lower limit of detection by ELISA

Effective concentration that is not cytotoxic to CD117 expressing cells

Safe to infuse graft

t1/2 = 10hrs

Modeled Pharmacokinetics
Preliminary data with durable and detectable gene marking after 0.2 mg/kg CD117-ADC conditioning

Single-dose CD117-ADC 0.2 mg/kg (N=2)

Rhesus macaques

CD34+ cells

β-globin vector

Transduction MOI 50

Mobilization

Conditioning

Transplantation

CD117-ADC

Vector copy number per cell (analyzed by qPCR)

In vitro VCN 5.5 ± 0.2

13U047

In vitro VCN 3.8 ± 0.0

12U032

VCN: Vector copy number per cell
MOI: Multiplicity of infection

Conditioning Regimen | Peripheral VCN
--- | ---
CD117-ADC | 0.01-0.05
Busulfan | 0.004-0.08

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Vector copy number per cell (analyzed by qPCR)

0.001 0.01 0.1 1 10

0 200 400 600

Granulocyte

Lymphocyte

VCN range with busulfan conditioning
CD117-ADC conditioning in a rhesus gene therapy model, compared to busulfan conditioning

**Single-dose CD117-ADC** 0.3 or 0.4 mg/kg \((N=4)\)

- **Rhesus macaques**
- Mobilization
- CD34\(^+\) cells
- Transduction \(\text{MOI 50}\)
- **CD117-ADC**
- Conditioning
- Transplantation

**Multi-dose busulfan** 5.5 mg/kg \(x\) 4 days \((N=2)\) Myeloablative regimen in clinical trials

- **Rhesus macaques**
- Mobilization
- CD34\(^+\) cells
- Transduction \(\text{MOI 50}\)
- **Busulfan**
- Conditioning
- Transplantation
Rapid and transient granulocyte suppression after CD117-ADC or busulfan conditioning

- **CD117-ADC (0.3mg/kg)**
  - ZL13
  - ZJ62

- **CD117-ADC (0.4mg/kg)**
  - H635
  - H96G

- **Busulfan (5.5mg/kg x4)**
  - 12U018
  - 12U020
  - 12U020

Granulocytes ($10^3$/μL)

Days post-transplant

**Legend:**
- Whole blood
- Platelet-rich plasma

**Transfusion**
Minimal lymphocyte suppression after CD117-ADC or busulfan conditioning

**CD117-ADC (0.3mg/kg)**
- ZL13

**CD117-ADC (0.4mg/kg)**
- H635

**Busulfan (5.5mg/kg x4)**
- 12U018

Lymphocytes ($10^3/\mu L$) vs. Days post-transplant

- Whole blood
- Platelet-rich plasma

Transfusion
Transient erythroid suppression after CD117-ADC or busulfan conditioning

**CD117-ADC (0.3mg/kg)**

- ZL13
- ZJ62

**CD117-ADC (0.4mg/kg)**

- H635
- H96G

**Busulfan (5.5mg/kg x4)**

- 12U018
- 12U020

Reticulocytes ($10^3/\mu L$)

Days post-transplant

Whole blood

Platelet-rich plasma

Transfusion
Transient platelet suppression after CD117-ADC conditioning, but not busulfan conditioning.

- **CD117-ADC (0.3mg/kg)**: ZL13, ZJ62
- **CD117-ADC (0.4mg/kg)**: H635, H96G
- **Busulfan (5.5mg/kg x4)**: 12U018, 12U020

**Platelets** (10^3/μL) over **Days post-transplant**:
- Whole blood
- Platelet-rich plasma

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Whole blood
Platelet-rich plasma

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Transfusion
Efficient lentiviral gene marking *in vivo*

**CD117-ADC (0.3mg/kg)**
- ZL13
  - In vitro VCN 6.4 ± 0.1

**CD117-ADC (0.4mg/kg)**
- H635
  - In vitro VCN 15.3 ± 0.2
- H96G
  - In vitro VCN 8.4 ± 0.1

**Busulfan (5.5mg/kg x4)**
- 12U018
  - In vitro VCN 15.3 ± 0.4
- 12U020
  - In vitro VCN 5.0 ± 0.0

Vector copy number per cell (analyzed by qPCR)

Days post-transplant

Granulocyte and Lymphocyte
Vector-mediated fetal hemoglobin (HbF) induction

CD117-ADC (0.3mg/kg)
- ZL13
- ZJ62

CD117-ADC (0.4mg/kg)
- H635
- H96G

Busulfan (5.5mg/kg x4)
- 12U018
- 12U020

% HbF-positive cells (analyzed by flow cytometry)

Days post-transplant
HbF induction confirmed HPLC-quantitated HbF amounts

- **CD117-ADC (0.3mg/kg)**
  - ZL13
  - ZJ62

- **CD117-ADC (0.4mg/kg)**
  - H635
  - H96G

- **Busulfan (5.5mg/kg x4)**
  - 12U018
  - 12U020

% γ-globin protein amounts (analyzed by RP-HPLC)

Days post-transplant
Minimal and transient elevation of liver enzymes after CD117-ADC or busulfan conditioning

Liver enzyme (U/L)

Days post-transplant

CD117-ADC (0.3mg/kg)
ZL13

CD117-ADC (0.4mg/kg)
H635

Busulfan (5.5mg/kg x4)
12U018

ZJ62
H96G
12U020

Normal ranges
AST: 22-56 U/L
ALT: 19-64 U/L
Undetectable change in kidney function after CD117-ADC or busulfan conditioning

**Normal ranges**
- BUN: 9-22 mg/dL
- CREA: 0.4-1.1 mg/dL
Minimal toxicities after CD117-ADC conditioning, unlike busulfan conditioning

<table>
<thead>
<tr>
<th>Busulfan side effects</th>
<th>Outcomes with CD117-ADC</th>
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<tbody>
<tr>
<td>Emesis</td>
<td>Not observed</td>
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<tr>
<td>Diarrhea</td>
<td>Not observed</td>
</tr>
<tr>
<td>Mucositis</td>
<td>Not observed</td>
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<tr>
<td>Wasting syndrome</td>
<td>Not observed</td>
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<tr>
<td>Seizures</td>
<td>Not observed</td>
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<tr>
<td>Veno-occlusive disease</td>
<td>Not observed</td>
</tr>
<tr>
<td>Pulmonary fibrosis</td>
<td>Not observed</td>
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</tbody>
</table>
1. We developed a CD117-ADC targeting both human and rhesus cells, that depleted more than 99% of CD34+CD90+ cells after a single dose with minimal toxicities in rhesus macaques.

2. A single dose of CD117-ADC enabled efficient engraftment of gene-modified CD34+ HSCs in a rhesus gene therapy model, achieving a similar level as myeloablative busulfan conditioning.

3. Robust HbF induction was also confirmed at the protein level in this rhesus gene therapy model following CD117-ADC conditioning.

4. This targeted approach for safer conditioning could improve the risk benefit profile in HSC gene therapy.

### Summary

<table>
<thead>
<tr>
<th></th>
<th>CD117-ADC</th>
<th>Busulfan</th>
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</thead>
<tbody>
<tr>
<td><strong>Vector Copy Number</strong></td>
<td>0.28 ± 0.16*</td>
<td>0.44 ± 0.17</td>
</tr>
<tr>
<td>(VCN) in Granulocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>F-cell Percent</strong></td>
<td>8.5 ± 1.8*</td>
<td>13.7 ± 5.8</td>
</tr>
<tr>
<td><strong>HbF Percent</strong></td>
<td>8.0 ± 2.9*</td>
<td>11.1 ± 5.2</td>
</tr>
</tbody>
</table>

Mean +/- SD, * no significance (one-tailed t-test) vs myeloablative Busulfan (5.5 mg/kg x 4 days)
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