Non-genotoxic antibody-based conditioning paired with multi-plex base edited HSCs for the potential treatment of sickle cell disease

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Sickle cell disease (SCD) is a monogenetic disease caused by a single-letter mutation in the HBB gene.

The β-globin gene

- Adult β-globin gene
  - Glutamine at 6th amino acid (HbA)
  - Normal red blood cells

The T-to-A mutation causes sickling

- Sickle β-globin gene
  - Valine at 6th amino acid (HbS)
  - Sickling red blood cells

There are approximately 100,000 sickle cell disease patients in the US.
Long term strategy to potentially cure SCD and build a transformative platform within hematology

**Editing (HSCT + busulfan)**
- Highly efficient and specific correction of genotype
- Non-cutting, non-viral

**Editing + Conditioning (HSCT + novel)**
- Less toxic conditioning to eliminate old cells and make room for edited graft

**Editing + Delivery (infusion)**
- Transition from ex vivo editing (transplant) to in vivo editing (infusion)

**Goals**
- Potentially addressable patients
- Required technologies
- Programs

**Potentially addressable patients**
- Base editing (ex vivo)

**Required technologies**
- Multi-plex base editing
- Antibody interference with conditioning target

**Programs**
- BEAM-101 (HPFH)
- BEAM-102 (MKSR)
- ESCAPE-1 (HPFH+CD117)
- ESCAPE-2 (MKSR+CD117)
- TBD

**Goals**
- Highly efficient and specific correction of genotype
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Bone marrow niche harbors HSCs, the cells that reconstitute the hematopoietic system

- Hematopoietic stem cells (HSCs) are harbored in the bone marrow microenvironment
- Stromal cells secrete stem cell factor (SCF), that signals through CD117 on HSCs and support survival and renewal of HSCs
- Differentiation of HSCs both in bone marrow and the periphery are responsible for reconstitution of the hematopoietic system
- Genome-edited long-term HSCs are potentially curative for sickle cell disease
Autologous transplantation of engineered HSCs currently requires conditioning with toxic alkylating antineoplastic agents such as busulfan.

1. Mobilize & collect HSCs from patient
2. Electroporate cells with editor
3. Freeze engineered HSCs (eHSCs)
4. Conditioning clears bone marrow niche
5. Thaw & infuse eHSCs after conditioning
Next generation of conditioning agent are designed to enable graft cells to “ESCAPE” the conditioning agent

- **Alkylation agent (e.g., Busulfan)**
  - Conditioning
  - HSCs
  - Stroma

- **CD117 monoclonal antibody**
  - Conditioning
  - HSCs
  - Stroma
  - Desired attribute: Myeloablative ✓, HSC-specific X, Non-genotoxic ✓, Low cancer risk ✓, Preserve fertility ✓, Long half-life X, Re-dosable ✓

- **Edit escapes mAb**
  - Conditioning
  - HSCs
  - Stroma
  - Desired attribute: Myeloablative ❔, HSC-specific ✗, Non-genotoxic ✓, Low cancer risk ✓, Preserve fertility ✓, Long half-life X, Re-dosable ✓
Adenine base editors (ABEs) chemically modify target bases, permanently and predictably

ABEs conduct chemistry on the genome

A-to-G base editor ("ABE")

engineered TadA*

ABEs are highly efficient in primary cells such as HSCs

HSCs mobilized from patient or donor

Electroporation of disease-specific gRNA and mRNA encoding ABE

ABE performs gRNA-dependent base editing to create engineered HSCs

CD117 is expressed on HSCs and is critical for their self-renewal, survival, & differentiation.

• High expression in the long-term and short-term HSCs make CD117 an attractive target for immunologic conditioning.

• CD117 is an optimal target for conditioning with expression on short- and long-term HSCs.
CD117 requires SCF binding and autophosphorylation to enable downstream signaling and HSC cell proliferation.

Feng et al, Diabetologia, 2015
Many CD117 epitopes evaluated and targeted with ABE

**Goal:** identify a mutation in CD117 that disallows mAb binding

**Gene Editing Requirements:**

- CD117 gene edit uses an ABE editor previously validated for sickle cell disease correction
  - Enables multi-plex capability
- CD117 DNA targets contain an NGG PAM
  - Adenine nucleobase appropriately placed in the window
- ABE edit causes an amino acid substitution in a residue of CD117 that is solvent exposed for potential orthogonality in mAb binding relative to wild-type.

**ESCAPE:** Engineered Stem Cell Antibody Paired Evasion
Base edited CD117 epitope enables eHSCs to selectively ESCAPE mAb binding

Unedited CD34 cell
No CD117 mAb
Normal signaling

Unedited CD34 cell
CD117 mAb displaces SCF
CD117 signaling blocked

Edited CD34 cell
Escapes CD117 mAb
Normal signaling

ESCAPE: Engineered Stem Cell Antibody Paired Evasion
ABE compatible CD117 antigen engineering and antibody screening summary

**Engineered antigen screening funnel:**

- **189 gRNA**
- **72 gRNA**

**Antibody screening funnel:**

- **188 Abs**

188 fully human clones identified from CD117 immune phage library

**In silico predicted guides**

- **24 gRNA**

CD34+ editing

- **9 gRNAs**

Cell function

- **3 gRNA**

Lead guides selected based on in-vitro de-risking of the corresponding CD117 variant.

- **188 Abs**

- **72 clones**

Unique VH

- **66 clones produced**

Lead antibodies: Based on binding to WT and edit pair
mAb-7 binds minimally to CD117 variant (created through base editing) and blocks SCF

- mAb-7 binds CD117 with high affinity (pM)
- mAb-7 binds minimally to CD117 variant as purified protein with rapid dissociation
- mAb-7 blocks SCF binding to CD117

<table>
<thead>
<tr>
<th></th>
<th>M07e cells expressing wt CD117</th>
<th>M07e cells expressing CD117 ESCAPE variant</th>
<th>SCF blocking</th>
<th>KD(M) Human CD117</th>
<th>KD(M) Cyno CD117</th>
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<tbody>
<tr>
<td>mAb7</td>
<td>Yes</td>
<td>L/N</td>
<td>Yes</td>
<td>&lt;1.0E-12</td>
<td>&lt;1.0E-12</td>
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CD34+ cells edited with ABE8 + CD117-targeting sgRNA escape recognition by antibodies that bind to wild type CD117.
Exposure of mAb-7 to wild-type CD34+ cells mimics complete SCF withdrawal

CD34+ cells edited with CD117 sgRNA maintain viability upon exposure to mAb-7
BEAM-101: Recreating hereditary persistence of fetal hemoglobin (HPFH) with base editing

Naturally-occurring base changes cause Hereditary Persistence of Fetal Hemoglobin (HPFH), which protects patients from SCD/B-Thal.

- Base editors can reproduce these changes, leading to high, consistent levels of fetal hemoglobin.
- Higher fetal hemoglobin likely to correlate with further reductions in disease symptoms.

ESCAPE-1: Base editing enables efficient multiplex editing of both ESCAPE CD117 edit and HbF induction

- gRNA for therapeutic edit such as for BEAM-101 or BEAM-102 (Makassar)
- gRNA for surface antigen epitope change that does not impact HSC biology but protects from mAb
- Single base editor capable of efficient multiplex edit of both therapeutic edit and antigen modification
Highly efficient multiplex editing of CD117 and HBG1/2 can be achieved in CD34+ HSPCs

- Multiplexing of HBG1/2a and CD117 sgRNAs with ABE leads to efficient base editing of both targets (>85%)
- Single clonal analysis reveals that all cells have HBG1/2 editing in the multiplex editing condition.
- CD117 multiplex editing outcomes are: >97% bi-allelic, 1.5% mono-allelic, and 1.5% unedited

~60% gamma globin induction detected in IVED cells differentiated from multiplex edited CD34+ cells
- multi-plex editing (CD117 site + HBG1/2) leads to similar levels of gamma G in IVED cells as base editing of HBG1/2 alone.
SCF blocking by mAb-7 significantly inhibits erythroid colony formation

BFU-E colonies (n=24) were sequenced in each group

1. Prepare cells (1:1 mixture of CD117 base edited and unedited CD34+ cells) and adjust concentration of mAb-7
2. Add cells to methylcellulose
3. Plate and incubate
4. Count colonies

Without mAb-7, there is an even distribution of edited to non-edited cells. Increasing [conc.] of mAb-7 yielded higher proportion of CD117 edited colonies.
CD117 requires SCF binding and autophosphorylation to enable downstream signaling and HSC cell proliferation.

Feng et al, Diabetologia, 2015
Base edited CD117 cells can bind to ligand SCF and induce phosphorylation *in vitro*

- Cells edited with CD117 sgRNA retain the ability to bind SCF.
- Upon SCF binding, edited CD117 undergoes phosphorylation similar to WT protein.
- mAb-7 blocks SCF binding as well as CD117 phosphorylation in unedited cells.
- mAb-7 fails to block SCF binding to edited protein.
- Edited protein undergoes phosphorylation even in the presence of mAb-7.
Fc Engineered versions of anti CD117 mAb-7 do not produce mast cell degranulation \textit{in vitro}

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\end{axis}
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ESCAPE-2: Makassar Editing for SCD + CD117 edit paired with mAb-7 for conditioning
BEAM-102: Direct correction of the sickle causing mutation

- Base editing recreates naturally-occurring human variant Hb-G Makassar which has alanine (E6A) instead of sickle-causing valine (E6V)
- Hb-G Makassar is a normal β-globin variant and does not cause sickle disease, e.g., blood smear shows negative for sickle cells

Identification of guide that can be multiplexed with Makassar installation that evades mAb-7 binding

- High levels of base editing at the HBB gene locus for sickle to Makassar gene correction can be achieved with an ABE
- A second CD117 variant identified that escapes mAb-7 binding and can be accessed through ABE editing
Summary

- Genotoxic conditioning continues to be a major barrier to the adoption of HSC transplant.
- New conditioning agents show promise but cannot discriminate between disease and transplanted cells, and therefore are designed with short half-life or given at low doses well before transplant.
- To solve this, we developed a concept we call Engineered Stem Cell Antibody Paired Evasion (ESCAPE), where a base-edited antigen: antibody pair enables edited cells to be resistant to mAb binding. Contrastingly, this conditioning mAb, binds to and interferes with CD117:
  - mAb-7 binds wild-type CD117 with high affinity but minimally to base edited CD117 variant protein.
  - Edited CD117 behaves normally in vitro vs wild-type in proliferation, differentiation, viability, and phosphorylation assays.
  - Fc engineered mAb-7 does not induce mast-cell degranulation in vitro.
- (ESCAPE-1) Multiplexing CD117 sgRNA with therapeutic sgRNAs (e.g. HBG1/2) with a single ABE8 editor achieved >85% CD117 base editing in CD34+ cells also containing therapeutic edit.
  - Multiplex base edited CD34+ cells evade mAb-mediated effects and SCF-ligand blocking, allowing for escape in vitro.
- (ESCAPE-2) mAb-7:CD117 edit pair, that can be installed by an ABE, is also compatible with efficient base editing to convert the causative SCD mutation into a variant encoding the naturally-occurring, non-polymerizing hemoglobin Makassar.
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AND many more!!