Advancing Next-Generation Gene and Cell Therapies for Epidermolysis Bullosa

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Rare Disease Day 2018
Epidermolysis Bullosa

- Group of inherited connective tissue disorders
- Total occurrence is ~20 per 1 million births
- Subset of rare severe forms of EB
  - Recessive dystrophic (RDEB) – type VII collagen
  - Junctional (JEB) – multiple genes (laminin)
- Frequently fatal early in life
- Treatment options largely palliative
- Predisposition to squamous cell carcinoma
Hematopoietic stem cell transplant

Bone Marrow Transplantation for Recessive Dystrophic Epidermolysis Bullosa

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John A. McGrath, M.D., Maria Hordinsky, M.D., Douglas R. Keene, B.S.,
David T. Woodley, M.D., Mei Chen, Ph.D., Megan J. Riddle, B.A.,
Mark J. Osborn, Ph.D., Troy Lund, M.D., Ph.D., Michelle Dolan, M.D.,
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Gene and cellular engineering for RDEB

From Marrow to Matrix: Novel Gene and Cell Therapies for Epidermolysis Bullosa

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Gene therapy for RDEB

Current gene therapy trials utilize randomly integrating viral vectors

- Risk of insertional mutagenesis
- Non-physiological expression levels
- Vector silencing

Precision correction is highly advantageous, but historically impractical
Precision genome editing for RDEB

Targeted nucleases dramatically enhance precision genome modification

TAL-effector Nuclease (TALEN)

RDEB  RDEB unmodified  RDEB TALEN edited

Collagen 7/Cytokeratin 5/DAPI

TALEN-based Gene Correction for Epidermolysis Bullosa

Mark J Osborn1, Colby C Starker1, Amber N McBroy1, Beau R Webber1, Megan J Riddle1, Lily Xia1, Anthony P Defeo1, Richard Gabriel1, Manfred Schmidt1, Christoph von Kalle2, Daniel F Carlson1, Morgan L Maeder1, J Keith Joung3,5, John E Wagner1, Daniel F Voytas3,5, Bruce R Blazer1 and Jakub Tolar1
Precision genome editing for RDEB

Targeted nucleases dramatically enhance precision genome modification

CRISPR/Cas9-based genetic correction for recessive dystrophic epidermolysis bullosa

Beau R Webber, Mark J Osborn, Amber N McElroy, Kirk Twaroski, Cara-in Lonetree, Anthony P DeFeo, Lily Xia, Cindy Eide, Christopher J Lees, Ron T McElmurry, Megan J Riddle, Chong Jai Kim, Dharmeshkumar D Patel, Bruce R Blazar, and Jakub Tolar.
iPSC-based cellular engineering

Gene corrected iPSC can generate multiple therapeutic cell types *in vitro*

- Corrected iPSC
- Mesenchymal Stem Cells
- Keratinocytes
- Hematopoietic Progenitors
- Blood Cells
Enabling efficacy studies through genome engineering

CRISPR/Cas9 can be used to generate animal models of disease for pre-clinical studies

![Diagram showing CRISPR/Cas9 application in animal models](image)

**Percent survival**

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P=0.01
The next generation of genome editing

Even with nucleases targeted gene correction is inefficient (<10%)

New technologies have dramatically increased efficiency

- Recombinant adenoassociated virus (rAAV)
- Cas9 base editor
Cas9 base editor

Mediates targeted enzymatic conversion of single DNA bases (C>T and A>G)

Requires no DNA donor molecule

Does not generate double strand break

Human T Cells

Human Hematopoietic Stem Cells

BE4 Alone
C₅: 0%
C₆: 0%

BE4+gRNA
C₄: 39%
C₆: 46%

ACT₄AC₆GCTGGATAGCCTCC

gRNA Protospeacer
What’s next?

Optimize and employ base editor technology

Preclinical studies

Clinical trials
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Lily Xia
Megan Riddle
Elise Durgin

**Osborn Lab**
Dr. Mark Osborn

**MGL**
Kyle O’Connor

THANK YOU!