ips cells – a new therapy for epidermolysis bullosa

marius wernig
stanford university

eb clinet meeting, debra
salzburg, austria september 24, 2017
Gene repair in iPS cells

- Reprogramming
- iPS cells
- Repaired iPS cells
- Transplantation
- Differentiated donor cells
- Adult cells

[Diagram showing the process of gene repair in iPS cells]
Basal keratinocytes attach to dermis via 12 protein linker complex

Adapted from Fitzpatricks, Marinkovich P, Chapter 62
Dystrophic Epidermolysis Bullosa
Retroviral gene transfer of COL7A1 cDNA into somatic keratinocytes

Viral transfer. LZRSE-Col7A1
Epidermal Sheet Production
Grafting

RDEB Keratinocytes

6-12 days 3-7 days 2 days 12-30 days

Phase I/IIA Trial IND #13708
(Phase III approved May 2017)
PI: Peter Marinkovich, MD
4 Patients 6 wounds / patient 18 mo follow-up
Safety Efficacy Pain
The Need for Improved Cell-Based Therapy

- Low keratinocyte stem cell number in patients
- Limited keratinocyte growth capacity
- Gene Transfer ineffective for dominant negative diseases
- Potential oncogene activation by randomly integrated retroviruses (squamous cell carcinoma!)
- Constitutive COL7A1 overexpression: Potential immunological side effects

*everything addressed by iPS cell approach!*
DEBCT v1 - iPS cell reprogramming:
excisable lentiviral cassette

Floxed 4F constructs:
EF1a-Oct4
2A
Klf4
IRES
Myc
2A
Sox2

(Sommer et al. 2009)

- Reprogramming requires presence of mouse feeders
- Expansion of iPS cells while maintaining normal KT requires mouse feeders
DEBCT v1 – gene targeting: AAV-DJ mediated

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<th>Method</th>
<th>Line</th>
<th>Picked clones</th>
<th>Targeted</th>
<th>Corrected allele</th>
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</tbody>
</table>
DEBCT v1 – differentiation

DEBCT efficacy: COLLAGEN VII production and stratification of corrected keratinocytes \textit{in vitro}

\textbf{COL7 Western blot}

\textbf{organotypic cultures}

Sebastiano, Zhen et al., \textit{Sci Transl. Med.} 2014
DEBCT efficacy:
Skin formation and Collagen VII production *in vivo*

3-week xenografts on NSG mice

SCC Predisposition 700X
Targeted Resequencing
BWA Pipeline Analysis

Notch1    H-ras
Notch2    K-ras
Notch3    RasA1
Notch4    CDKN2A
Jagged1   TP53
Jagged2   KLF4
CHUK
Proposal for DEBCT version 2

STEP M1
Biopsy and culture donor cells

Seed Bank Skin cells

STEP M2
Single step reprogramming and correction

MCB #1 Autologous Corrected iPSC lines

STEP M3
Produce Keratinocytes

STEP M4
Generate and transport epithelial sheets

ICB #1 - iPSC-derived keratinocytes
The lab
EB partnership:
Dennis Roop, UC Denver
Angela Christiano, Columbia University