Update on the European GENEGRAPH project:
*Ex-vivo* Gene Therapy for Recessive Dystrophic Epidermolysis Bullosa

Alain Hovnanian

Department of Genetics
Inserm UMR 1163 *Imagine Institute*
Necker hospital, Paris, France

alain.hovnanian@inserm.fr
Tel: +33 1 42 75 42 89
Dermal-epidermal junction: hemidesmosomes and focal contacts
Recessive Dystrophic Epidermolysis Bullosa

Rare disease (1 in 450 000 living births)
Caused by COL7A1 defects
Genotype/phenotype correlations
Wide spectrum of clinical severity
Orphan disease
No standard satisfactory treatment
Extensive heterogeneity of COL7A1 mutations in RDEB

- 118 exons
- 8.9 kb cDNA
- > 350 mutations registered
Type VII collagen: main features

- **COL7A1** gene, 32 kb, chromosomal region 3p21
- 118 exons
- 8.9 kb cDNA, repeated sequences
- 290 kDa protein
- Locally secreted
- Produced by keratinocytes and fibroblasts
- Homotrimers form anchoring fibrils
- Long half-life (> 60 days)
Typ VII collagen and anchoring fibril formation

Varki et al. 2007
Type VII collagen staining and anchoring fibrils are reduced or absent in generalized RDEB
Epidermal stem cells and holoclones

Y. Barrandon and H. Green PNAS 1985

Alonso and Fuchs, PNAS 2003
Correction of junctional epidermolysis bullosa by transplantation of genetically modified epidermal stem cells

Fulvio Mavilio¹, Graziella Pellegrini¹,², Stefano Ferrari², Francesca Di Nunzio¹, Enzo Di Iorio², Alessandra Recchia¹, Giulietta Maruggi¹, Giuliana Ferrari³, Elena Provasi⁴, Chiara Bonini⁴, Sergio Capurro⁵, Andrea Conti⁶, Cristina Magnoni⁶, Alberto Giannetti⁶ & Michele De Luca¹,²

Received 8 June; accepted 11 October; published online 19 November 2006; doi:10.1038/nm1504
Preclinical study supporting the rationale

SIN Retroviral Vectors Expressing COL7A1 Under Human Promoters for Ex Vivo Gene Therapy of Recessive Dystrophic Epidermolysis Bullosa

Matthias Titeux¹,², Valérie Pendaries¹,², Maria A Zanta-Boussif³, Audrey Décha¹,², Nathalie Pironon¹,², Laure Tonasso², José E Mejia¹,², Agnes Brice³, Olivier Danos⁴ and Alain Hovnanian¹,⁵,⁷

¹Inserm, US63, Toulouse, France; ²University Paul-Sabatier, Toulouse, France; ³Génétion, Evry, France; ⁴Inserm U781, Necker Hospital for Sick Children, Paris, France; ⁵CHU Necker for Sick Children, Department of Dermatology, Paris, France; ⁶CHU Necker for Sick Children, Department of Genetics, Paris, France; ⁷University René Descartes, Paris, France

Molecular Therapy, 2010
Retroviral vectors design

**Classical retrovirus**

- **pMSCV**
  - 5' LTR
  - U3 R US
  - ppt
  - MfeI
  - EcoRI
  - COL7A1 cDNA

- **pMSCV-COL7A1**

**SIN retrovirus**

- **pBullet** (MFG derived)
  - 5' LTR
  - U3 R US
  - ppt
  - pol
  - gag
  - SA

- **pCM**
  - Deletion of non-essential sequences

- **pCMS**
  - Deletion of U3 enhancer

- **pCMS-pCOL7A1-COL7A1**
  - 5' LTR
  - U3 R US
  - ppt
  - MluI
  - EcoRV
  - COL7A1 cDNA
  - 0.6 kb
  - 8.9 kb

- **pCMS-pEF1α-COL7A1**
  - 5' LTR
  - U3 R US
  - ppt
  - pEF1α
  - COL7A1 cDNA
  - 0.2 kb
  - 8.9 kb

**Genethon**
Genetic correction of RDEB keratinocytes and fibroblasts using SIN COL7A1 retroviral vectors

Titeux et al. Mol Ther 2010
Fibrin-based human skin equivalent

Adapted from LLames et al. *Transplantation* 2004
Skin equivalent grafted on nude mouse

D0

D20

D60

D90
Functional correction 5 months post-grafting

H/E | Type VII collagen | TEM
--- | --- | ---

NHK/NHF

Krdeb/Frdeb

Kcol7/Fcol7

Kef1α/Fef1α

Titeux et al. *Mol Ther* 2010
Orphan drug designation
by the European Medicines Agency (E.M.A.)
March 2009

Medicinal product:

“Skin equivalent graft genetically corrected with a
COL7A1-encoding SIN retroviral vector”
GENEGRAFT European Project: Gene therapy for RDEB

- Phase I/II *ex vivo* gene therapy clinical trial for RDEB using skin equivalent grafts genetically corrected with a $COL7A1$-encoding SIN retroviral vector

www.genegraft.eu
Project concept and objective

- The project relies on the concept that transplantation of autologous skin equivalents made of genetically corrected epidermal stem cells and dermal fibroblasts using a safe SIN retroviral vector has a therapeutic potential.

- GENEGRAFT aims to evaluate the feasibility and safety of autologous genetically corrected skin equivalents for the treatment RDEB. It will also evaluate whether COL7A1 gene transfer can restore DEJ adherence.
GENEGRAFT - FP7 European project 2011-2016
Phase I/II clinical trial for *ex vivo* RDEB gene therapy

1. **Clinical trial authorization (ANSM)**
2. **Biopsy**
3. **SIN vector Pilot batch**
4. **Genetic correction K+F**
5. **Transfert to GMP protocols**
6. **Safety studies**
7. **GMP viral batch**
8. **Corrected Skin equivalent**
9. **Patient recruitment and selection**
10. **Engraftment**

**GENEGRAFT - FP7 European project 2011-2016**
Phase I/II clinical trial for *ex vivo* RDEB gene therapy

**GENEGRAFT - FP7 European project 2011-2016**
Phase I/II clinical trial for *ex vivo* RDEB gene therapy

**GENEGRAFT - FP7 European project 2011-2016**
Phase I/II clinical trial for *ex vivo* RDEB gene therapy

**GENEGRAFT - FP7 European project 2011-2016**
Phase I/II clinical trial for *ex vivo* RDEB gene therapy

**GENEGRAFT - FP7 European project 2011-2016**
Phase I/II clinical trial for *ex vivo* RDEB gene therapy

**GENEGRAFT - FP7 European project 2011-2016**
Phase I/II clinical trial for *ex vivo* RDEB gene therapy
WP1
Patient selection
Patient pre-selection criteria

1. Moderate to severe form of RDEB

2. Low levels of detectable type VII collagen NC-1 domain (skin biopsy and/or WB)

3. Both COL7A1 mutations are known

4. Patient has multiple suitable skin sites for grafting (blistered and non-blasistered areas)

5. Good general condition

6. Above 7 years of age and compatible with the anesthetic, surgical and post-surgical procedures

7. Strong personal motivation and/or parental support
Patient pre-selection and selection process

Recruitment

300 RDEB patients

Pre-selection

Pre-selection criteria

- Moderate RDEB
- One \( \text{COL7A1} \) mutation is not a PTC
- Type VII collagen is detectable on skin biopsy
- Skin areas without blister on the arms
- Good general condition

Selection

Selection criteria

- Clinical criteria
- Immune tolerance to type VII collagen (ELISA, ELISPOT and \textit{in silico} prediction (HLA genotyping))
- Proliferative capacity of keratinocytes and fibroblasts

Validation and scoring of pre-selected patients (optimal clinical, biological, molecular and immunological features)

10 pre-selected RDEB patients

3 to 6 selected RDEB patients
GENEGRAFT - EB Gen study

Study of immune tolerance and capacity for wound healing of patients with recessive dystrophic epidermolysis bullosa

**Primary outcome:**
Determination of the proliferative capacity of keratinocytes and fibroblasts in characterized RDEB patients

**Status:** Currently recruiting

**Principal investigator:** Prof. Alain Hovnanian

**Contact:** alain.hovnanian@inserm.fr
tel: +33 1 42 75 42 89 or +33 6 08 98 67 11
WP2
Viral vector optimization and production
Viral vector development

**Vector**
- **Optimize** viral SIN-vector
- **Decision** based on:
  - transient production of col7AI vectors
  - Physical titer (BeFA)
- **Combine**
  - best SIN-vector
  - optimized orf
- **Integrate into**
  - exchange construct

**Gene**
- **Optimize** col7AI orf
- **Decision** based on:
  - Frequency of mutants in viral population
  - Identified hot-spot(s)

**Cell**
- **Basic cell line**:
  - HEH293 VacAMPHO
  - Expression of MMLV gag/pol and ampmo envelope
- **Integration**:
  - Tagging vector (prETAG.fcN)
  - **Select tagged packaging cell**:
    - Tested for high titer
    - Tag confirmed
  - **Targeting**
    - exchange construct pbibETAR.fc-CMS(+).col7AI
    - **Select targeted producer cell**
      - Tested for high titer
      - tag/target/clean-up confirmed
- **Establish**
  - primary/secondary seed bank (PSB, SSB)
  - master cell bank (MCB)

**GMP-produced**
- Therapeutic SIN-vector
Viral vector development

Optimisation of the MLV-based SIN vector

- **Backbone construct:**
  Psi(+) significantly improves the viral titres
- **Promoter:**
  pCMS-Psi+/EFS.COL7A1-SIN.1
  better than pCMS-Psi+/COL7A1-COL7A1-SIN.1

Establishment of a packaging cell line

- Human packaging cell line (HEK293)
- Amphotropic pseudotyped
- Using the tagging/targeted system through Recombinase Mediated Cassette Exchange (Flp-recombinase system)
- Producing high viral titers (2 to 5 $\times 10^6$ IP/ml)

Ghani *et al.* 2007, Löw *et al.* *Gene Ther* 2009
Testing pre-GMP viral vector batches

A new generation cassette-exchange based HEK293 stable producer cell line allows for the production of a high titre of COL7A1 SIN vectors

Primary RDEB keratinocytes

Primary RDEB fibroblasts
WP3
Preparation of the graft under GMP conditions
Transfer and adaptation of the knowhow and protocols from research to GMP standards (UNIMORE and CIEMAT)

**Identification of the best GMP-certified reagents:**
- Human fibrinogen
- Human Thrombin
- Retronectin
- 3T3-J2 feeder layer

**Elaboration of standard operating procedures (SOPs):**
- Keratinocyte and fibroblast culture
- Keratinocyte and fibroblast transduction
- Preparation of skin equivalents
Skin transplantation procedure

- 2 to 6 grafts of 7 x 5 cm
- unblistered and blistered areas
- surgical excision
- middle dermis
- under light general anesthesia (no intubation)
- pre- and post-operative care and follow-up
WP4
Safety considerations
Safety considerations

A. Insertional mutagenesis

B. Immune tolerance

C. Transgene integrity
A - Insertional mutagenesis

1. Subcutaneous tumorogenicity test
2. Monitoring of grafted mice
3. Integration site analysis by LAM-PCR and NGS to study the integration pattern over time
B - Immune tolerance

1. Detection of circulating antibodies (ELISA and IIF)
2. *Ex vivo* T-cell activation assays (ELISPot)
3. HLA genotyping for antigenic prediction
C - Transgene integrity

- Genomic level
  Southern blot
  PCR and sequencing

- Protein level :
  western-blot analysis
Characterization of integrated \textit{COL7A1} provirus by Southern-blot analysis

Titeux \textit{et al.} \textit{Mol Ther} 2010
Assessment of type VII collagen protein integrity by western-blot analysis

Titeux et al. Mol Ther 2010
WP5
Reglementary and Ethical issues
EBGen study
Study of immune tolerance and capacity for wound healing of patients with recessive dystrophic epidermolysis bullosa

INSERM sponsorship
COSSEC agreement
ANSM agreement
CPP Necker agreement
NHS REC agreement
GENEGRAFT : major steps achieved

- Pre-selection of 20 RDEB patients
- **Human HEK293 packaging cell line** producing high viral titers of improved SIN COL7A1 clinical-grade gamma retroviral vector
- Two major new partners for the preparation of genetically corrected skin equivalents under GMP:
  - **Michele De Luca**, University of Modena
  - **Marcela del Rio and Fernando Larcher**, CIEMAT, Madrid
- Agreements by INSERM (sponsorship), COSSEC, ANSM, REC, CPP for EBGen
Next steps include:

- Completion of pre-inclusion to 30 patients
- Testing 10 pre-selected patients for keratinocyte proliferative capacity to select **3 to 6 patients with optimal criteria**
- Production of the **master cell bank**, production and validation of GMP-grade viral batch
- **Skin equivalent production** under GMP conditions
- **Banking** of « Clinical grade » keratinocytes and fibroblasts from selected RDEB patients
- Pre-submission to the ANSM
Generation and release of the investigational medicinal product (IMP)

5 mm skin biopsy

Isolation of keratinocytes and fibroblasts

Culture of keratinocytes and fibroblasts

Transduction of keratinocytes and fibroblasts

Skin equivalent

Harvest for QA/QC

Transduction of keratinocytes and fibroblasts

Skin equivalent

Harvest skin equivalent

Meet specification

Sterility, copy number, C7 expression, RCR

Transport of skin equivalents to the hospital for grafting
Conclusion and perspectives

- GENEGRRAFT will assess the feasibility, safety and the potential of genetically corrected skin equivalents to restore DEJ.

- Other gene therapy trials using COL7A1 gene addition are encouraging (Khavari’s laboratory, Stanford, USA).

- Other approaches being developed in parallel (cell and protein therapy) by other groups and by our laboratory.

- Combination of gene, cell and protein therapy to address the wide spectrum of COL7A1 mutations and disease variability for personalized medicine.
GENEGRAFT annual meeting, Modena, July 15th 2014