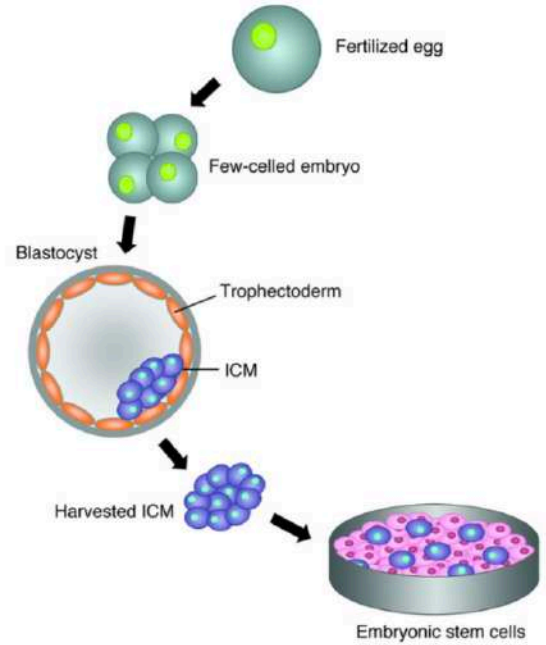
Stem cells and Regenerative Medicine

Educazione Civica – classi quinte LAS

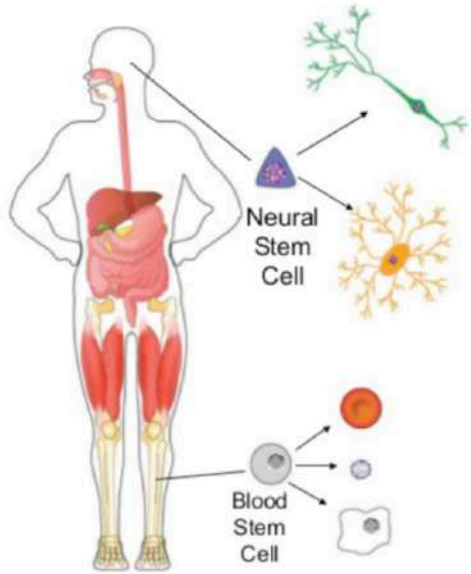
Regenerative medicine refers to innovative advanced therapies aimed at the permanent restoration/regeneration of diseased/destroyed tissues and organs.

STEM CELLS

EMBRYONIC



SOMATIC (adult)

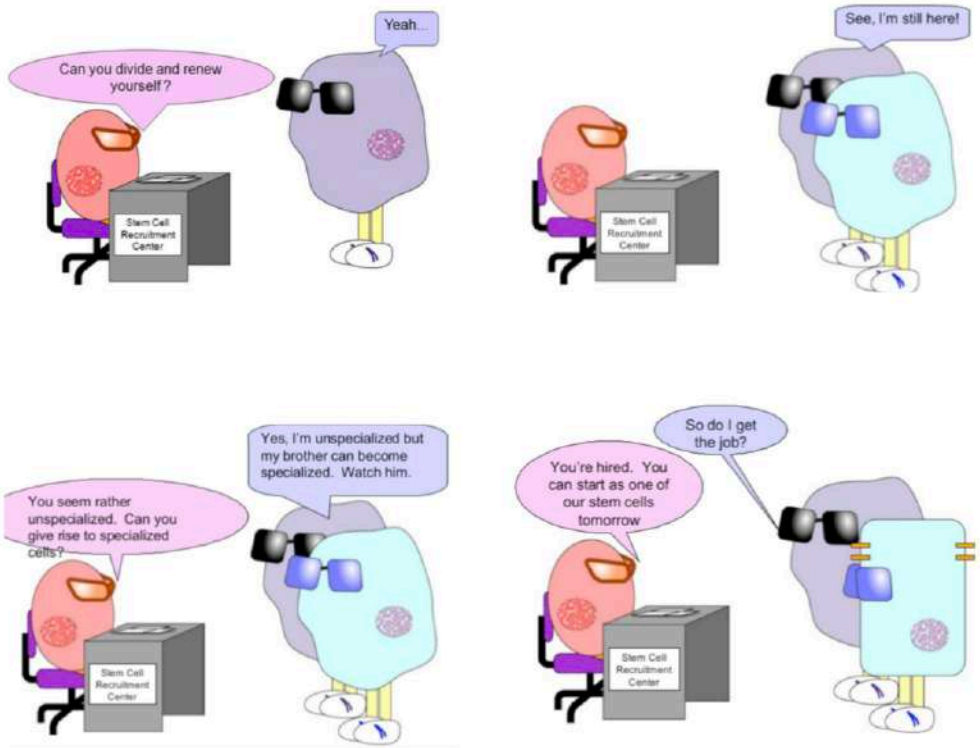


Adult stem cells contribute to homeostasis

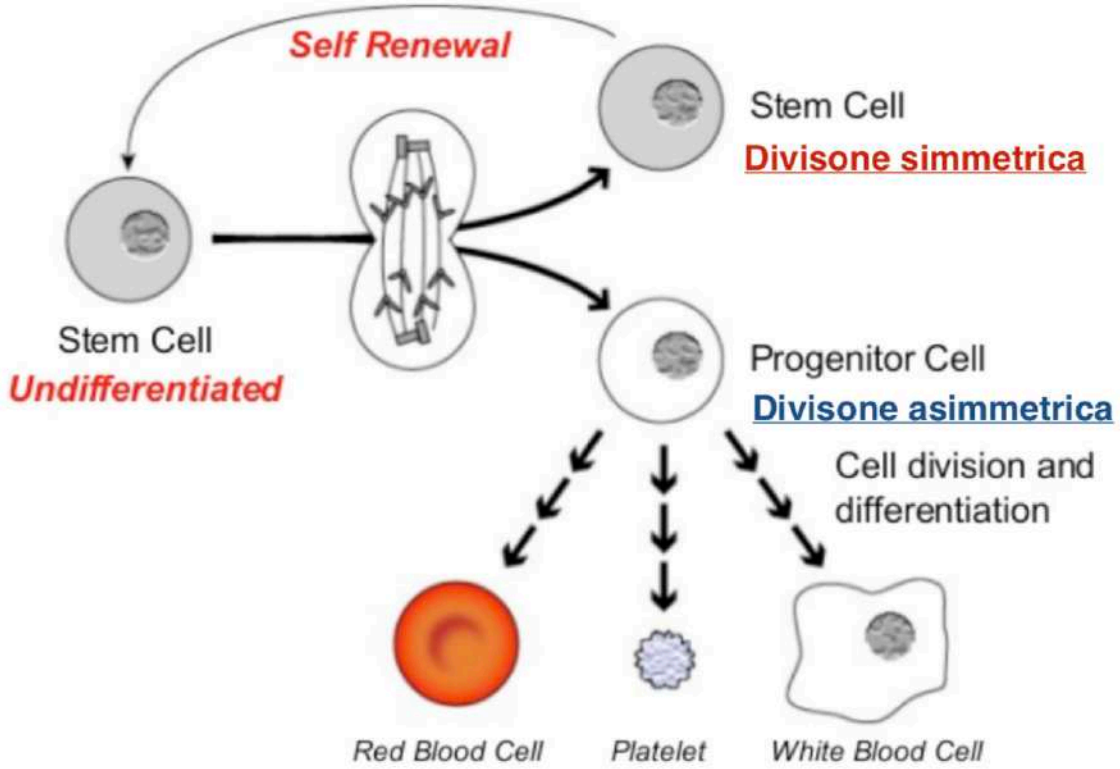
They **divide only when needed**

Their **progeny differentiate into cells that perform essential body functions.**

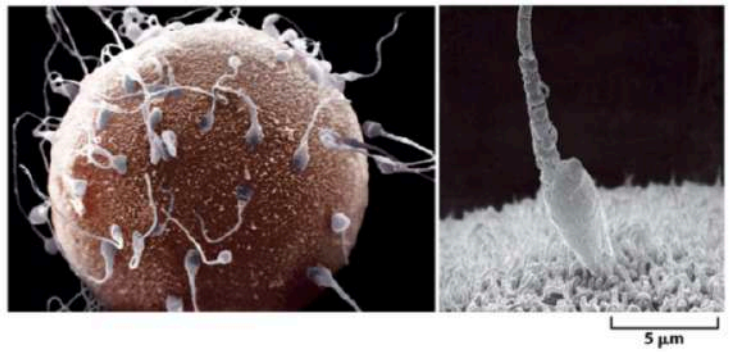
Stem Cells: What are they?

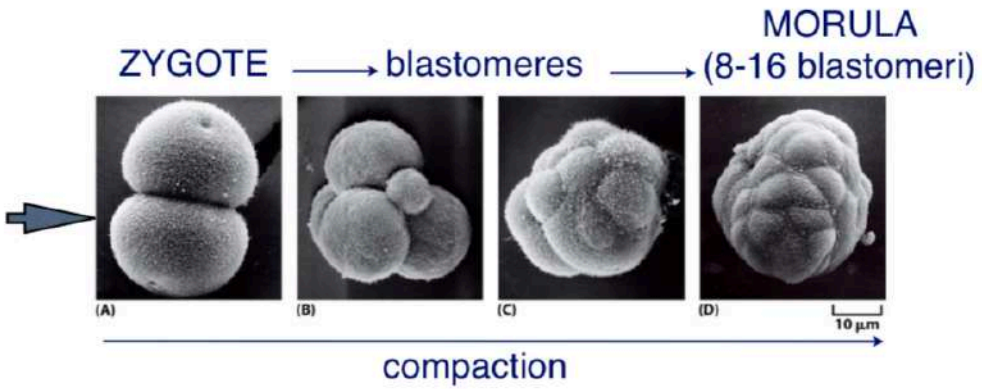


Stem Cells: Not like other cells

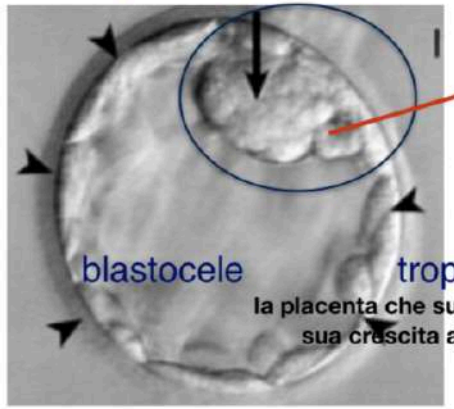


EMBRYONIC STEM CELLS





embryoblast (inner cell mass)



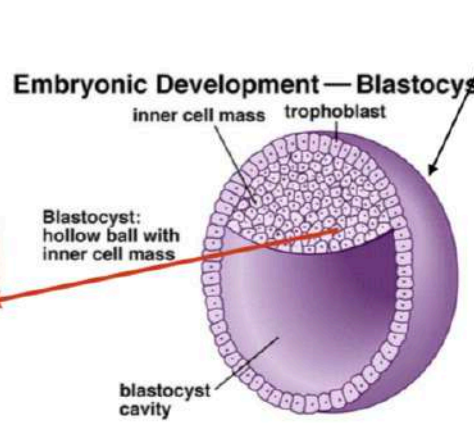
la placenta che supporta l'embrione nella sua crescita all'interno dell'utero

BLASTOCYST

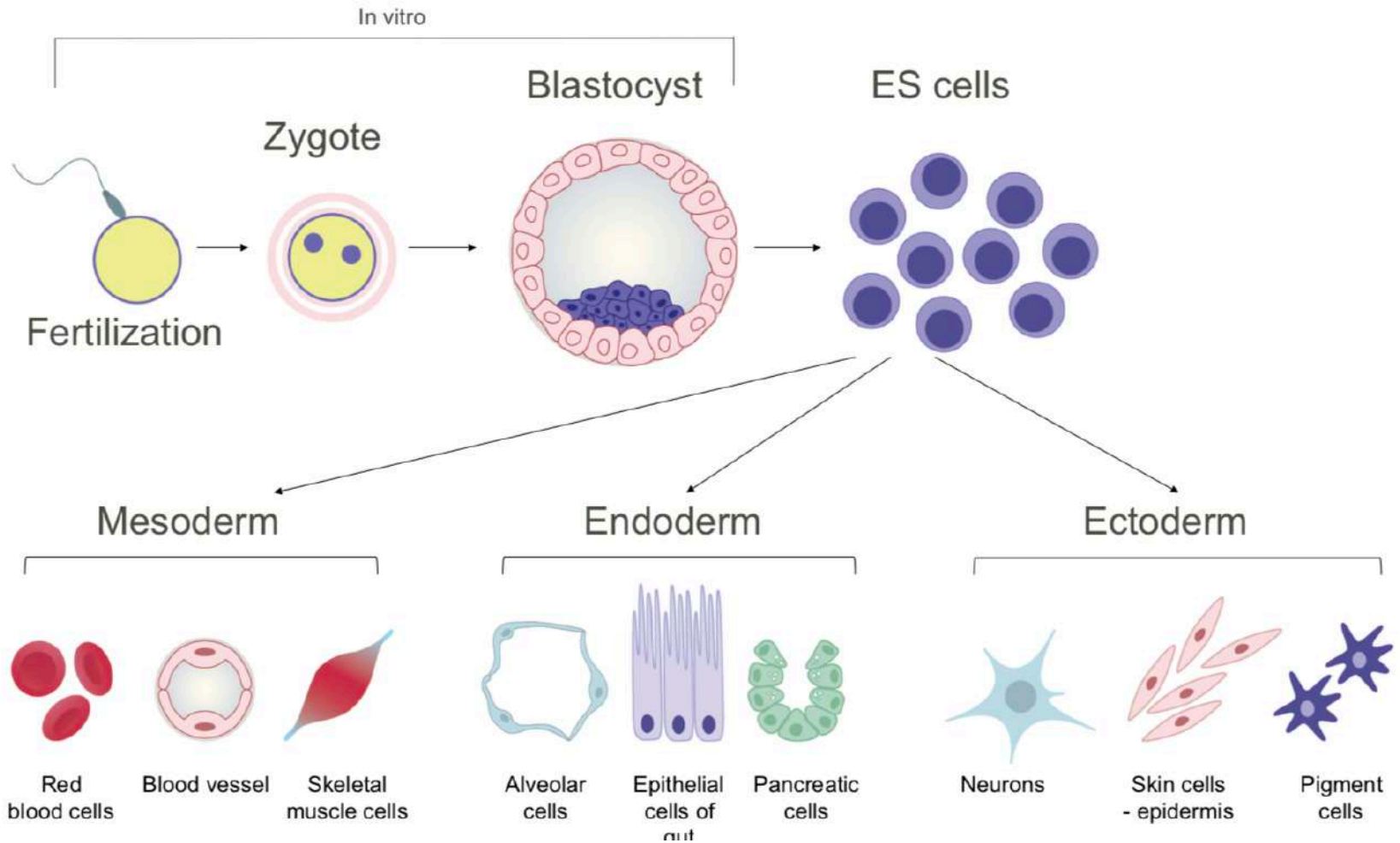
Dal 4° al 14 giorno di gestazione

EMBRYONIC STEM CELLS

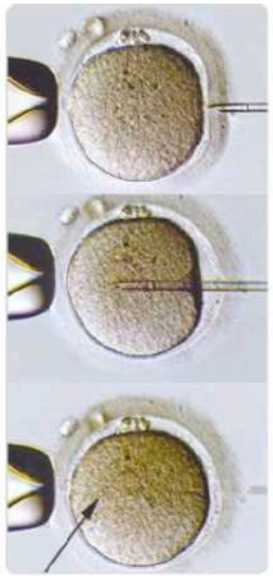
circa 100 cellule.



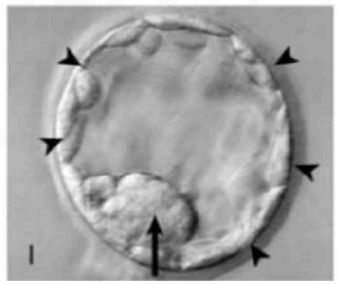
Embryonic stem cells are pluripotent and can make all mature cell types of the body



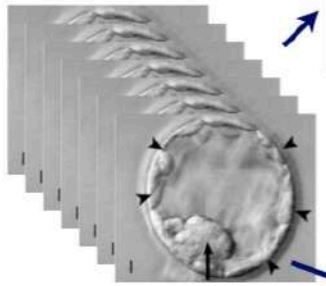
IVF
fecondazione assistita



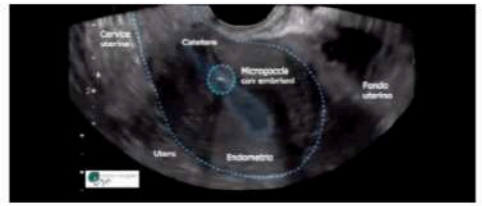
BLASTOCISTI



CELLULE STAMINALI EMBRIONALI



in utero



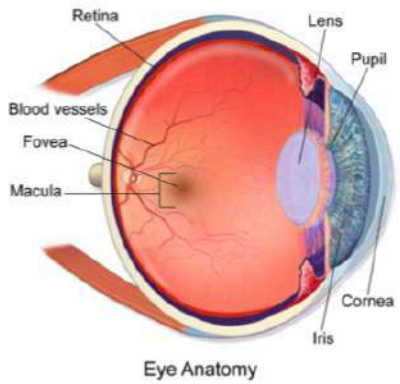
congelamento

blastocisti congelate in numero superiore all'uso, non saranno mai utilizzati per scopi di riproduzione

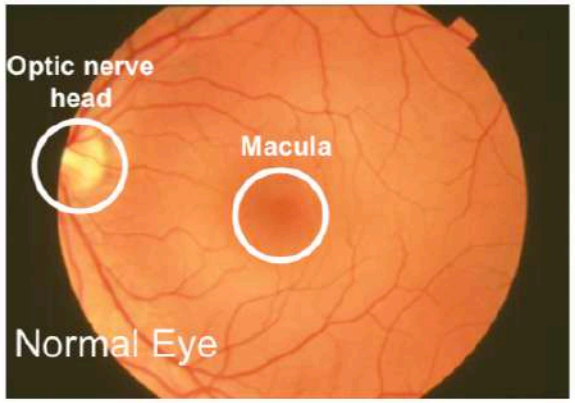
in Italia possiamo fare ricerca sulle staminali embrionali importandole dall'estero solo alcune linee.

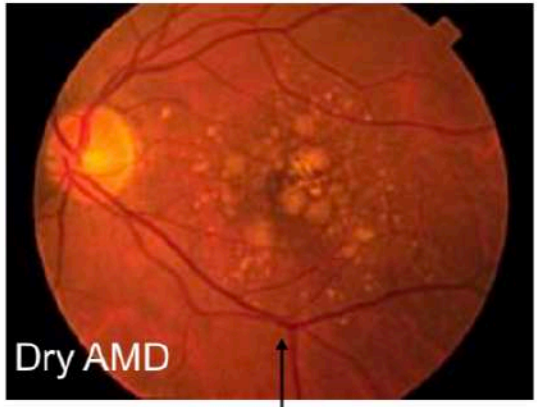
Age related macular degeneration (AMD)

In Italia 15.000 nuovi casi di AMD neovascolare/anno

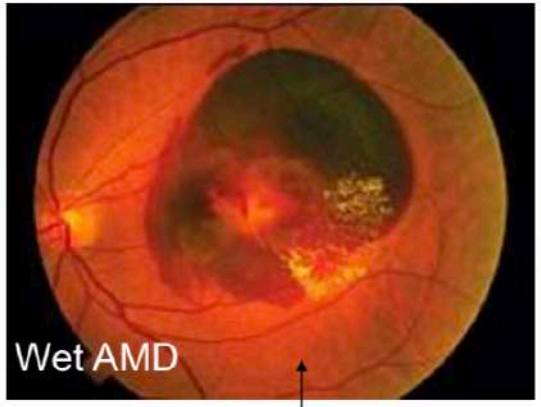


Eye Anatomy





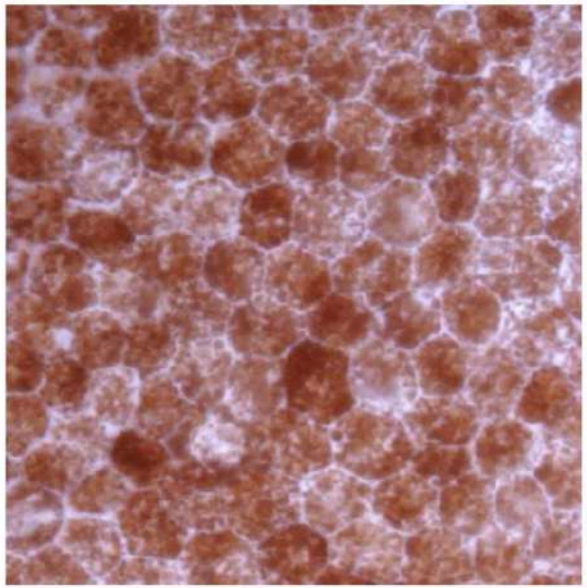
Deposito di materiale lipidico



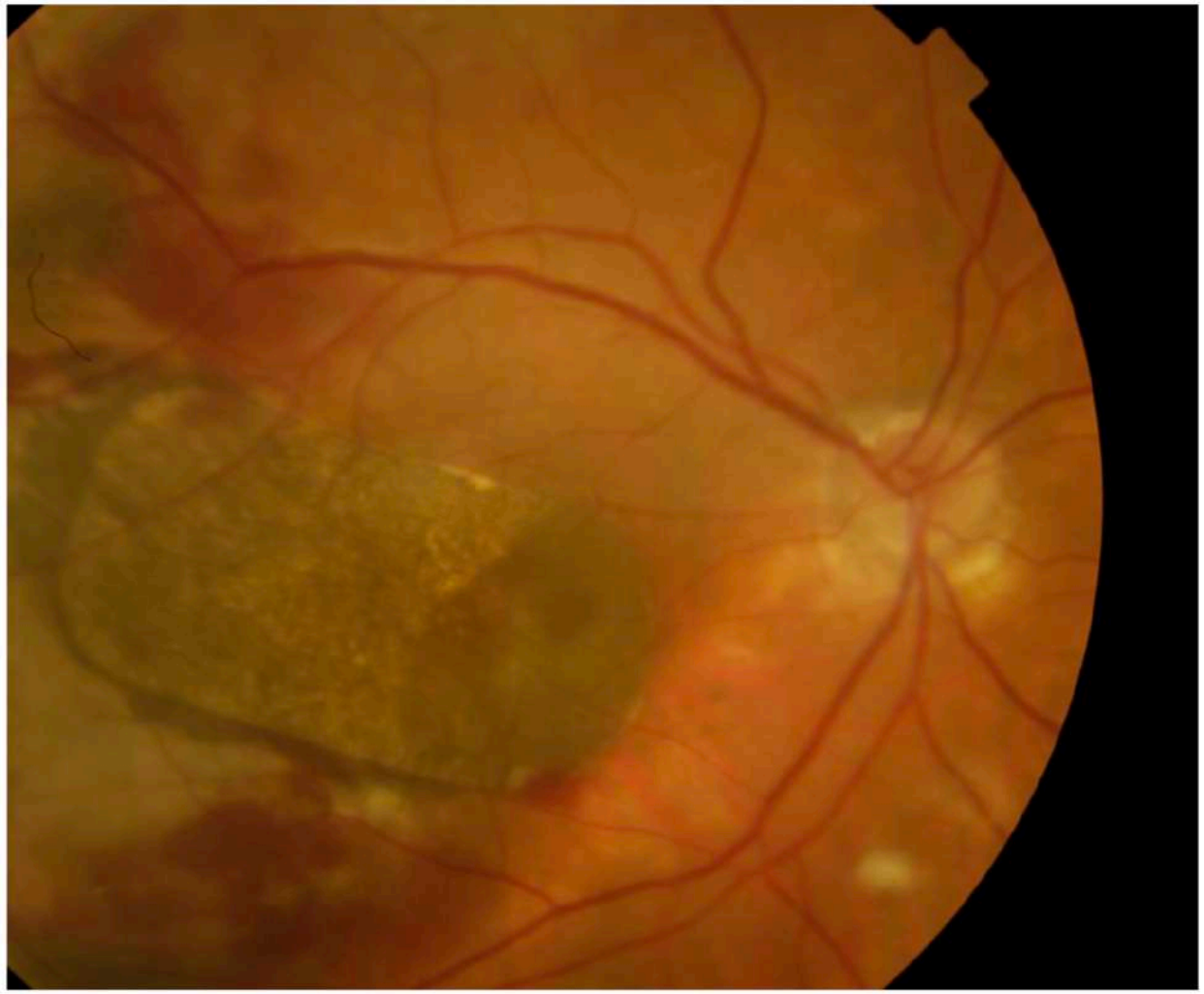
irregolarità dell'epitelio pigmentato retinico

Images from Peter Coffey, UCL

Stem Cell Treatments for macular degeneration



17 year old human

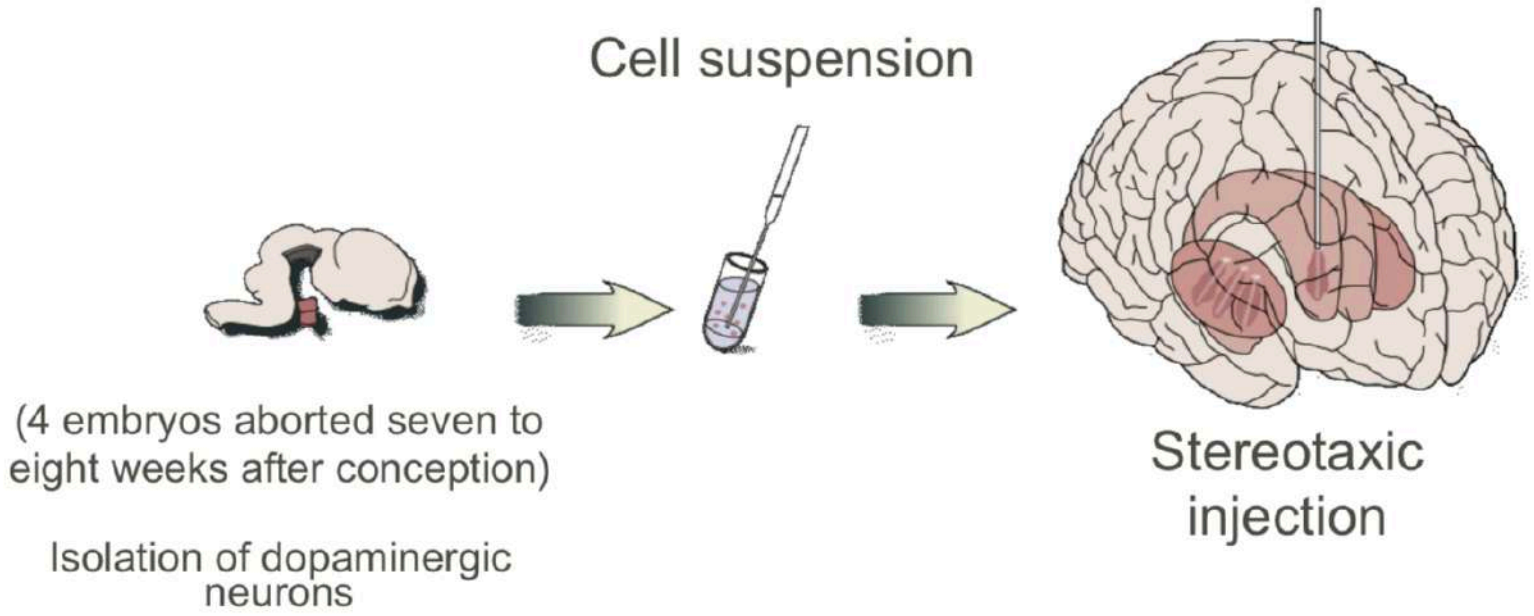


Images from Peter Coffey, UCL

Parkinson's Disease

- Tremor
- Muscle rigidity
- Difficulties initiating movements
- Posture and balance
- Non-motor symptoms

Transplantation of human fetal DA neuroblasts



significativo miglioramento nel paziente -> prova di principio

ci sono cellule «primitive» nel nostro organismo in grado di sostenere la rigenerazione di un tessuto

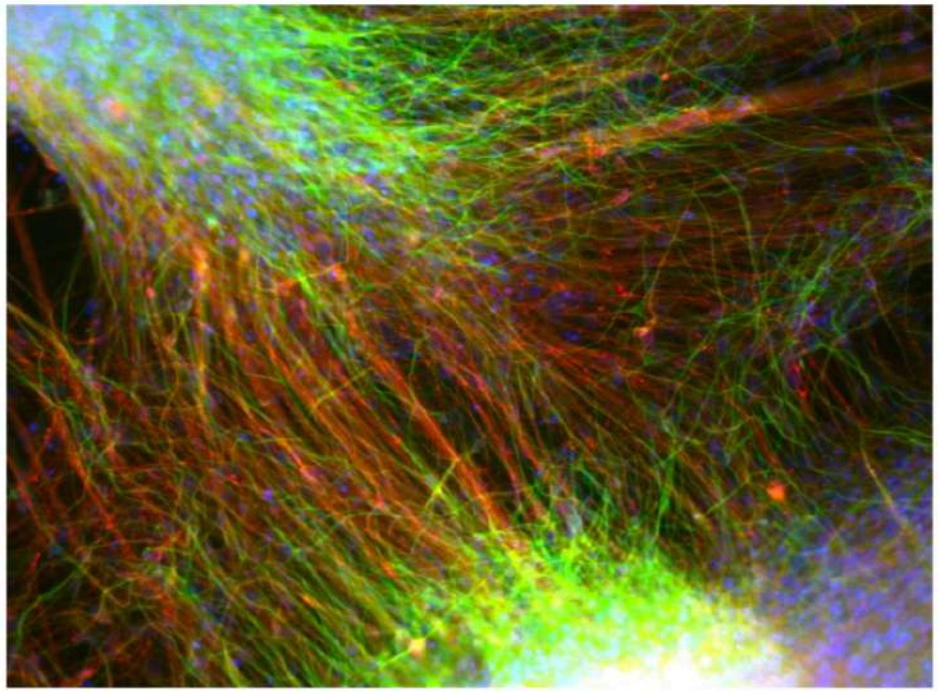
Difficulties using fetal cells

- Large variation in outcome
- Lack of material
- Ethical and societal considerations that vary with national regulations
- Logistical and practical issues

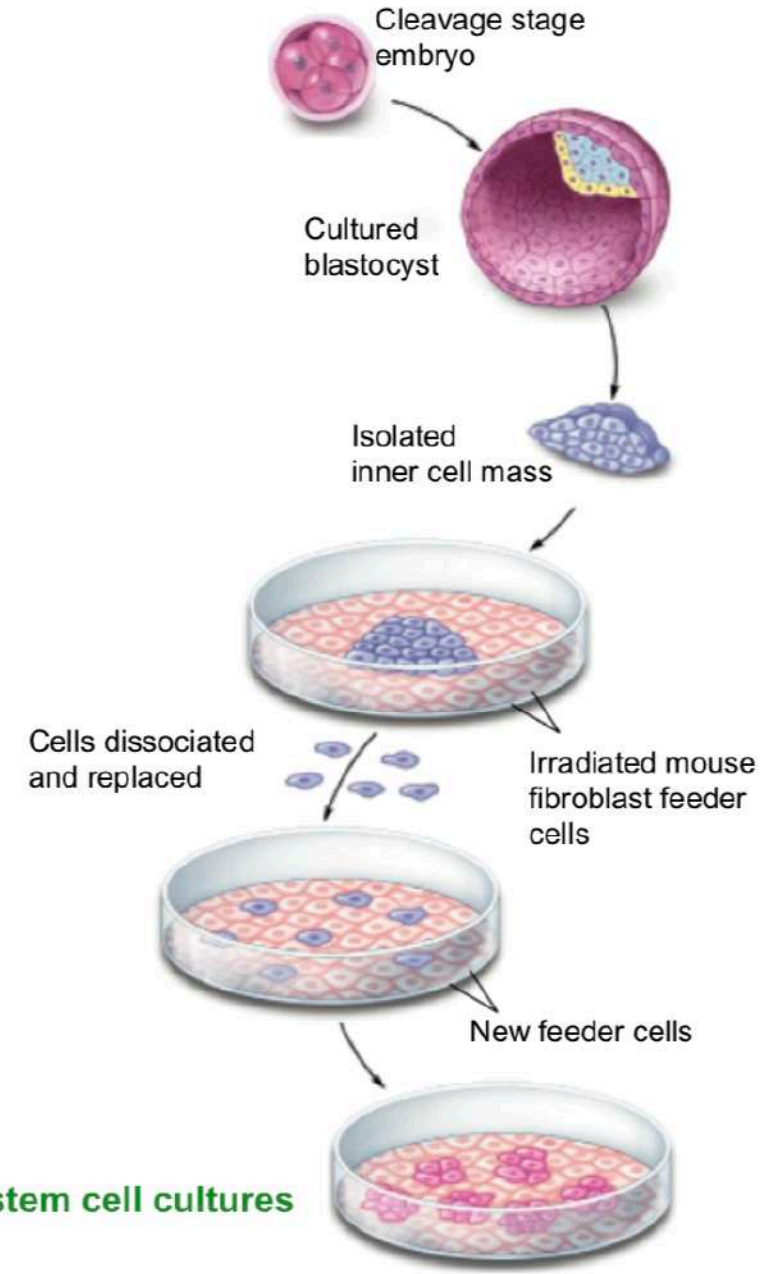
But….Stem cells!

Stem cells are an unlimited source from which transplantable cells can be derived

Dopaminerginc neurons derived from embryonic stem cells



(courtesy of Malin Parmar)

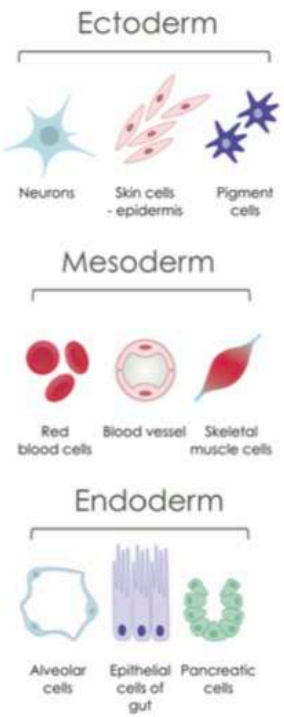


Established embryonic stem cell cultures

STEM CELLS

EMBRYONIC

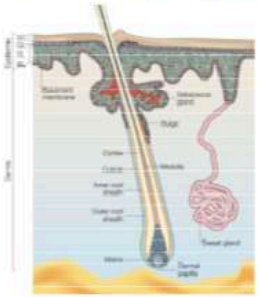
all tissues and organs



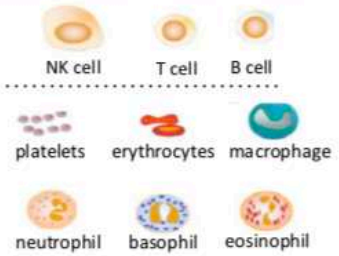
SOMATIC (adult)

is the cell in a tissue that is ultimately responsible for its generation and regeneration during the lifetime of the animal

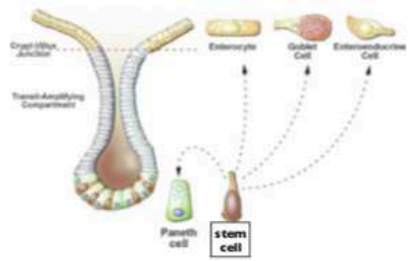
Skin



Blood



Intestine

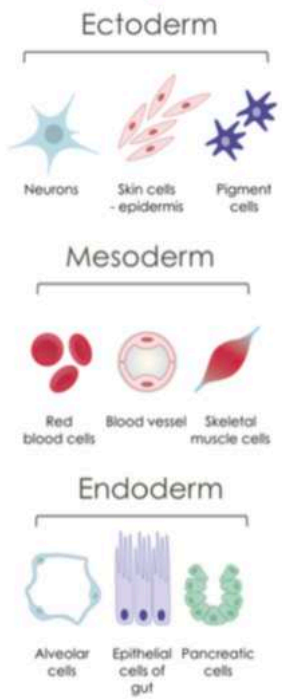


STEM CELLS

PLURIPOTENT

EMBRYONIC

all tissues and organs



?

Klf4 *Oct4* *Sox2* *c-Myc*

Induced pluripotent stem cells
iPS

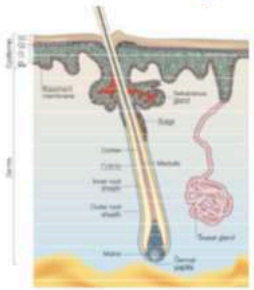
Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

Kazutoshi Takahashi,[1] Koji Tanabe,[1] Mari Ohnuki,[1] Megumi Narita,[1,2] Tomoko Ichisaka,[1,2] Kiichiro Tomoda,[3] and Shinya Yamanaka[1,2,3,4,*]

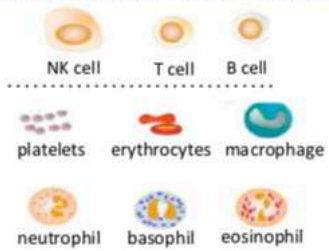
OLIGO/MULTIPOTENT

SOMATIC (adult)

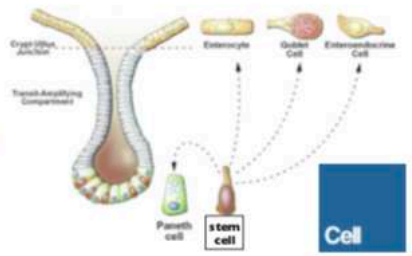
Skin

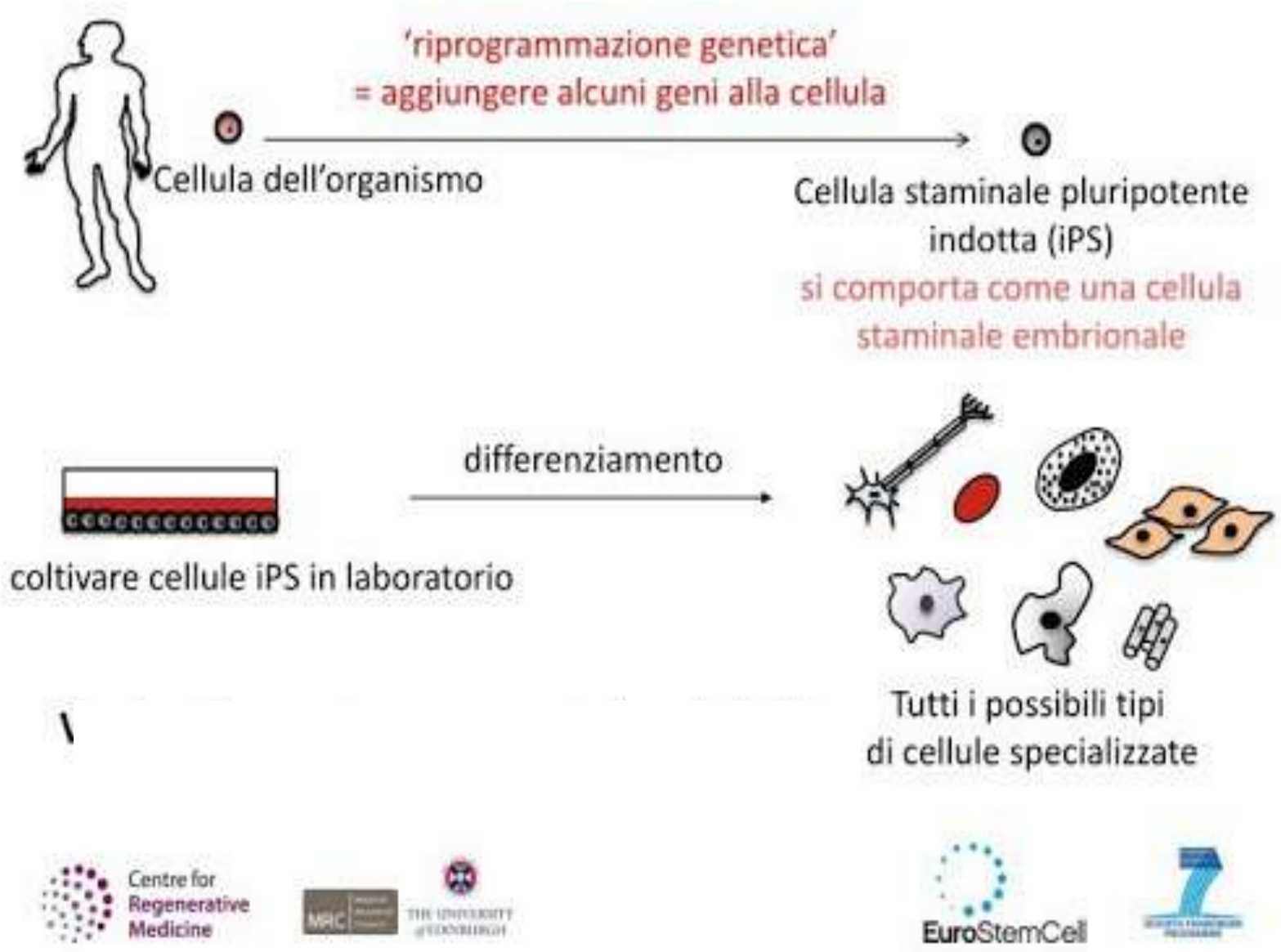


Blood



Intestine





Vantaggi:

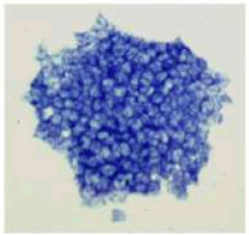
- Possibilità di trapianto autologo
- No problemi etici

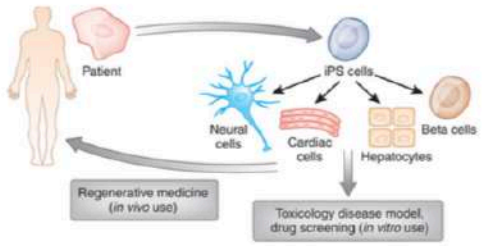
Limiti:

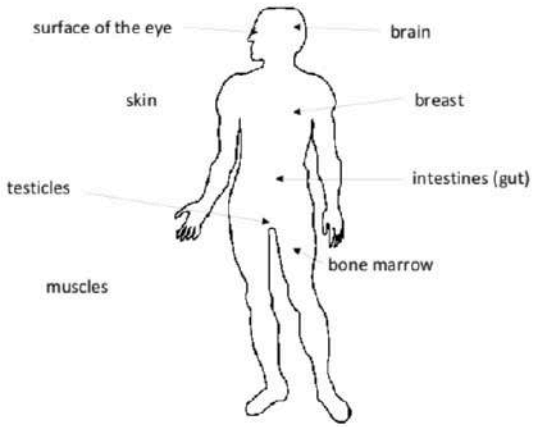
- Non complketa riprogrammazione
- epigenoma
- Bassa percentuale di cellule riprogrammate

TISSUE REGENERATION BY STEM CELLS

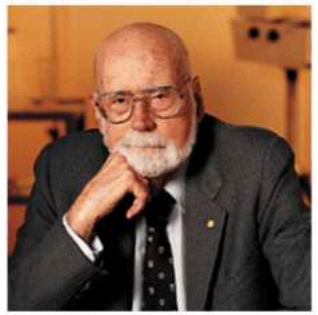
EMBRYONIC	iPS	ADULT
Phase I/II clinical trials	the first clinical trial approved in Japan	hematopoietic and epithelial stem cells in established clinical protocols, others in clinical trials







HEMATOPOIETIC STEM CELLS: THE FIRST CELL THERAPY WITH STEM CELLS



Edward Donnall Thomas

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE ARCHIVE

Intravenous Infusion of Bone Marrow in Patients Receiving Radiation and Chemotherapy

1957

September 12, 1957 | Thomas , E. Donnall , M.D.† Lochte , Harry L. Jr. , M.D.‡ Lu , Wan Ching , Ph.D.§ Ferrebee , Joseph W. , M.D.¶

EPIDERMAL STEM CELL: THE FIRST CELL THERAPY WITH *CULTURED* STEM CELLS

Howard Green

The NEW ENGLAND JOURNAL of MEDICINE

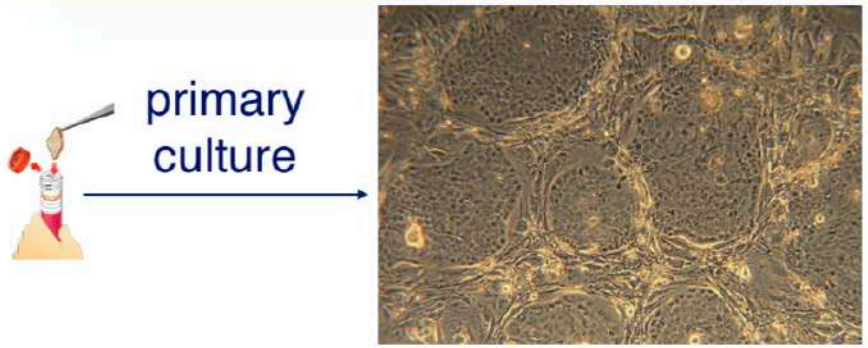
MEDICAL INTELLIGENCE

PERMANENT COVERAGE OF LARGE BURN WOUNDS WITH AUTOLOGOUS CULTURED HUMAN EPITHELIUM

1984

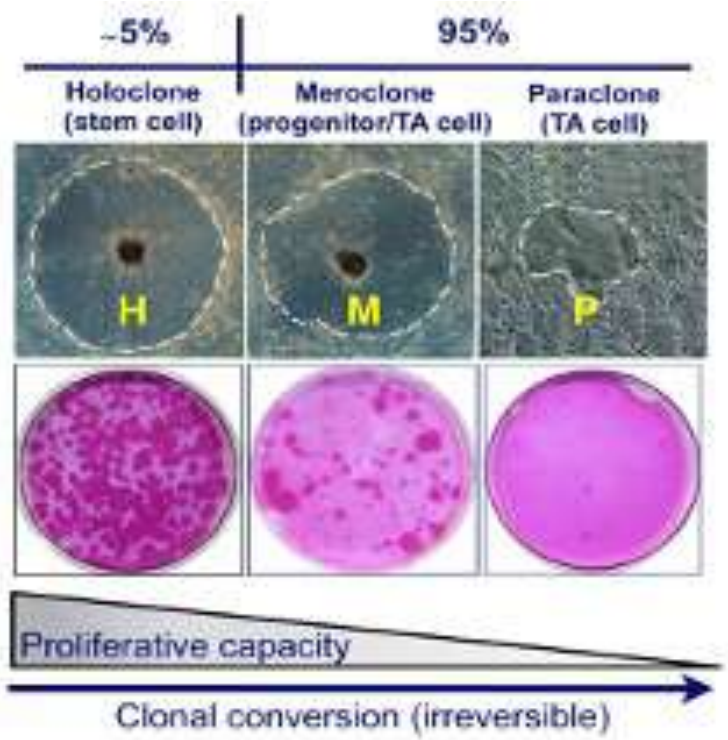
G. Gregory Gallico, III, M.D.,
Nicholas E. O'Connor, M.D.,
Carolyn C. Compton, M.D.,
Olaniyi Kehinde, B.A., and Howard Green, M.D.

STEM CELLS OF HUMAN STRATIFIED EPITHELIA



primary culture

isolation (and cultivation) of single clonogenic cells



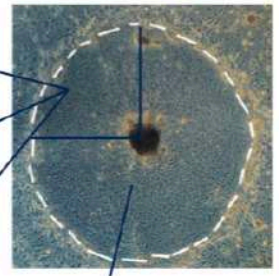
clonal analysis

stem cells ← holoclone

progenitors (TA cells) ← meroclone

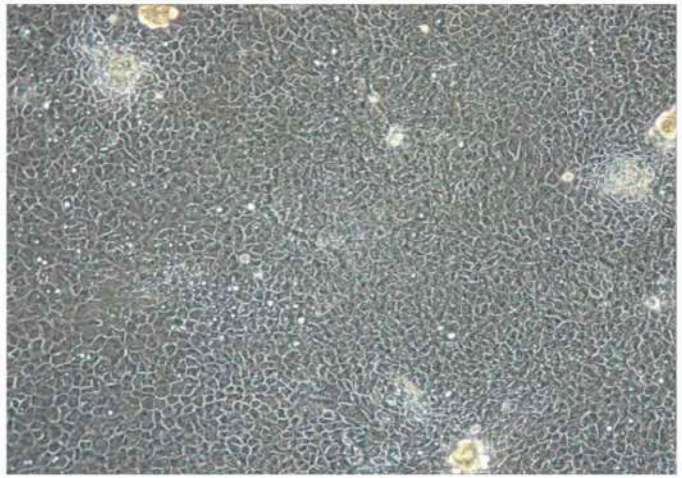
paraclone

isolation of single clones



clonal culture

primary culture



fibrin-cultured human epidermis

fibrin-cultured corneal epithelium

CELL THERAPY

epidermis, cornea
conjunctiva, urethra
oral mucosa

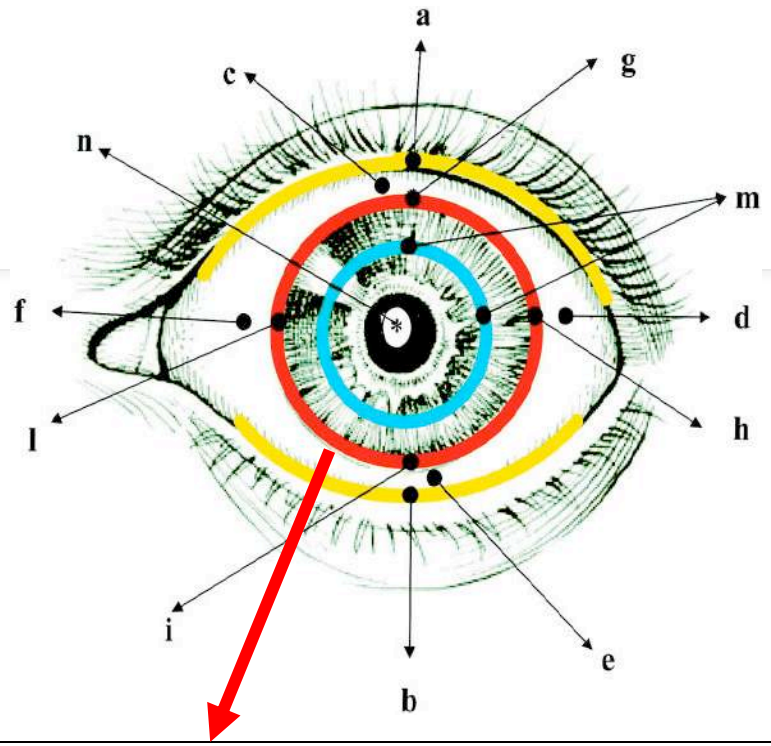
→ Skin and ocular burns

GENE THERAPY

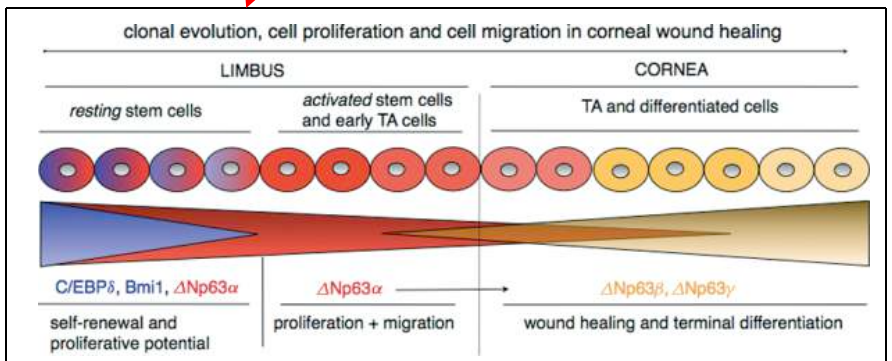
Epidermolysis Bullosa

O'Connor et al. *Lancet* 1981
Gallico et al. *N. Engl. J. Med.* 1984
Pellegrini et al. *Lancet* 1997
Pellegrini et al. *Transplantation* 1999
Ronfard et al. *Transplantation* 2000
Mavilio et al. *Nature Med.* 2006
Rama et al. *N. Engl. J. Med.* 2010
Hirsch et al. *Nature* 2017

Location of stem cells in the Human Ocular Surface



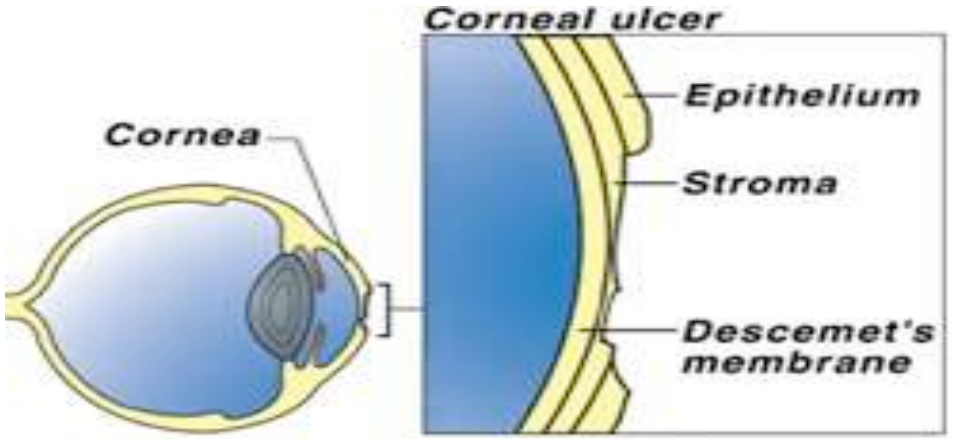
The ocular surface. This figure shows areas of the ocular surface from where biopsies were taken. Fornix, limbus, and paracentral cornea are colored in yellow, red, and blue, respectively. The central cornea is indicated by the asterisk. The bulbar region is indicated by the white color.



C/EBPδ regulates cell cycle and self-renewal of human limbal stem cells

Barbaro V, Testa A. Di Iorio E, Mavilio F, Pellegrini G and De Luca M.

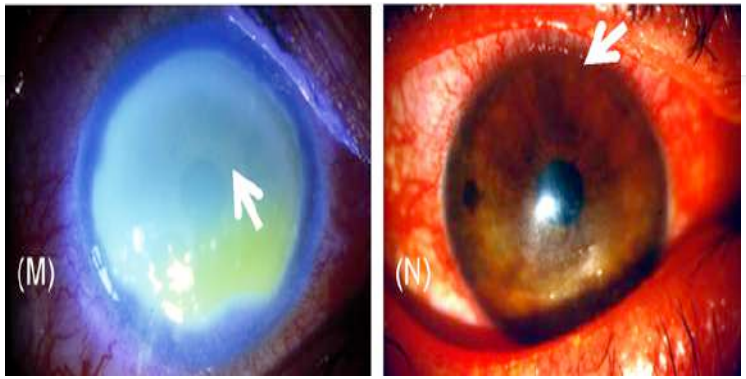
The Journal of Cell Biology, Vol. 177, No. 6, June 18, 2007 1037–1049
http://www.jcb.org/cgi/doi/10.1083/jcb.200703003 JCB 1037

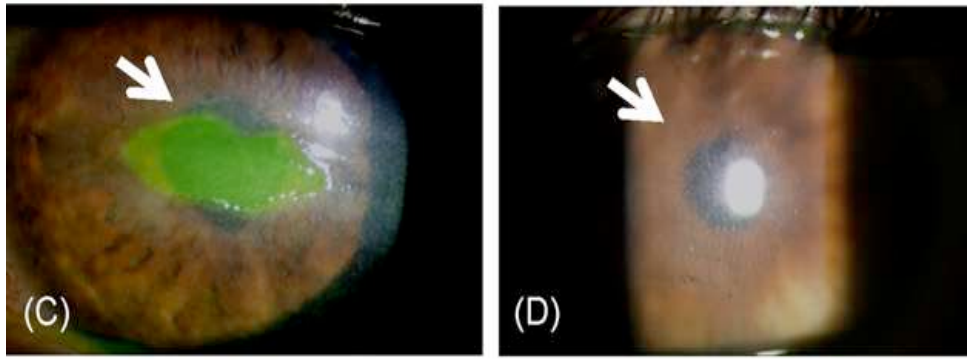


Corneal Neurotrophic ulcers

is caused by several ocular and systemic diseases and circumstances:

- fifth-nerve palsy
- viral infections
- chemical burns
- corneal surgery
- abuse of topical anesthetics
- neurotrophic keratisis
- diabetes mellitus
- multiple sclerosis





These ulcers results from loss of the sensory innervation of the cornea which leads to a decrease in the number of corneal stem cells, decreased metabolic and mitotic rates in the corneal epithelium.

LIMBAL STEM CELLS AND CORNEAL REGENERATION 1997-2015

THE LANCET

Long-term restoration of damaged corneal surfaces with autologous cultivated corneal epithelium

Graziella Pellegrini, Carlo E Traverso, Adriano Tito Franzi, Mario Zingirian, Ranieri Cancedda, Michele De Luca

Vol 349 • April 5, 1997

The NEW ENGLAND JOURNAL of MEDICINE

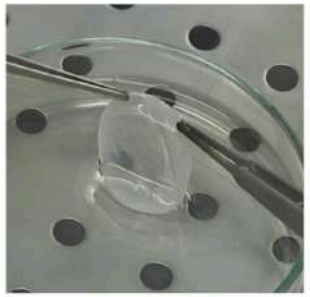
ORIGINAL ARTICLE

Limbal Stem-Cell Therapy and Long-Term Corneal Regeneration

Paolo Rama, M.D., Stanislav Matuska, M.D., Giorgio Paganoni, M.D., Alessandra Spinelli, M.D., Michele De Luca, M.D., and Graziella Pellegrini, Ph.D.

2010;363:147-55.

University of Modena and Reggio Emilia H. San Raffaele, Milan



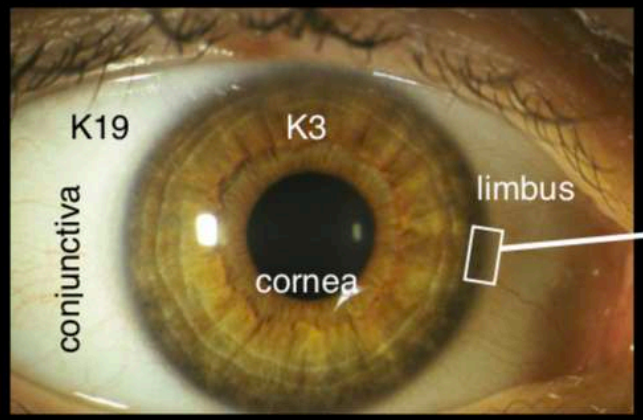
fibrin-cultured corneal epithelium

Pellegrini et al., *The Lancet*, 1997
Rama et al., *Transplantation*, 2001
Pellegrini et al., *N.Engl.J.Med.*, 2004
Rama et al., *N.Engl.J.Med.*, 2010
Pellegrini et al., *Regen.Med.* 2013

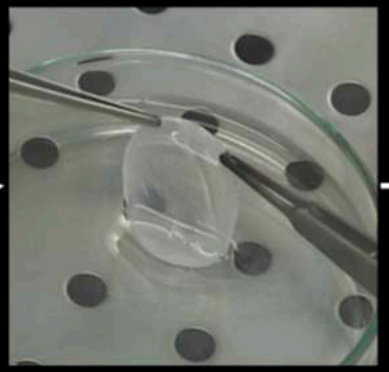
Pellegrini et al., *J.Cell Biol.*, 1999
Pellegrini et al., *PNAS*, 2001
Di Iorio et al., *PNAS*, 2005
Barbaro et al., *J.Cell Biol.*, 2007
Pellegrini et al., *Stem Cells* 2013

Corneal regeneration by cultures of limbal stem cells

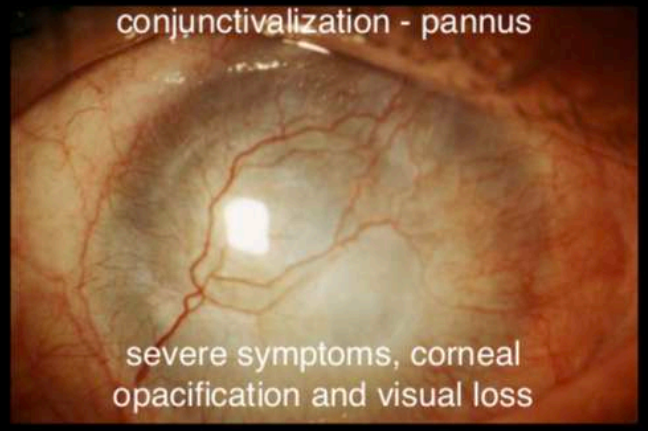
THE HUMAN CORNEAL EPITHELIUM



fibrin-cultured
corneal epithelium



limbal stem cells deficiency
conjunctivalization - pannus



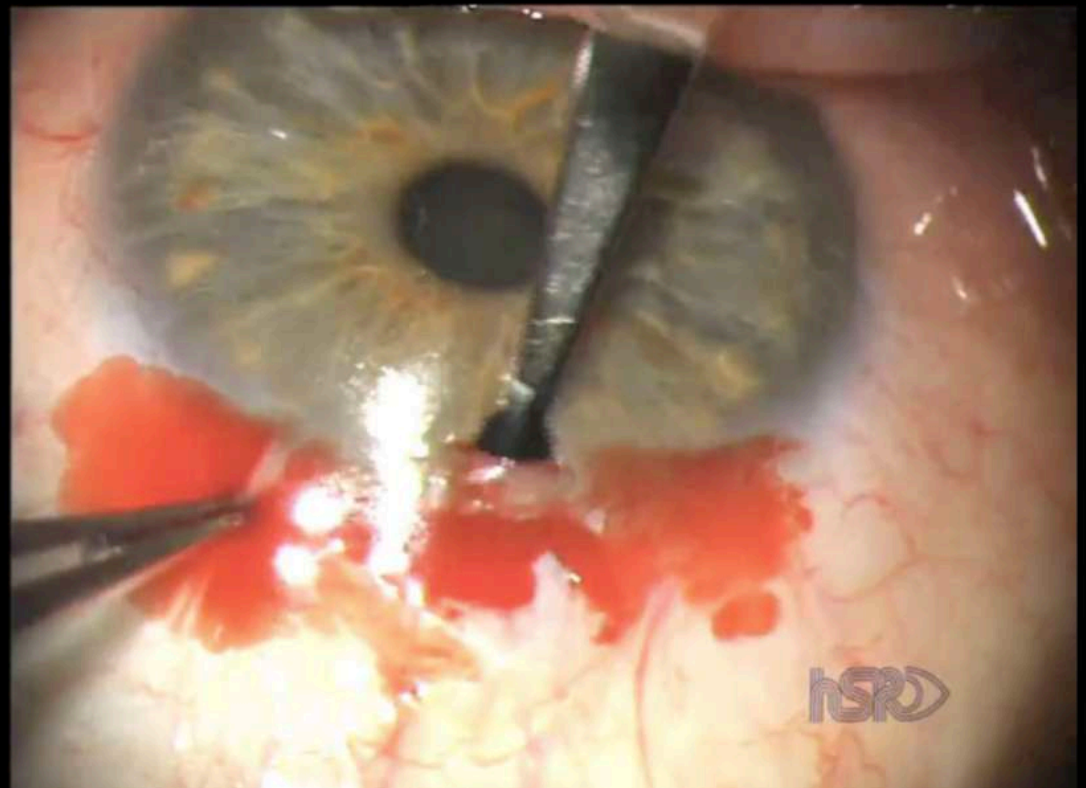
2 different population of stem cells
(cornea and conjunctiva)

The conjunctival stem cell is bipotent (goblet)

Corneal holoclones are found only in the limbus
(central cornea only meroclones and paraclones)

Δp63α regulates the proliferative/regenerative
potential of human limbal stem cells

C/EBPδ (and Bmi1) regulate their
self-renewal and mitotic quiescence

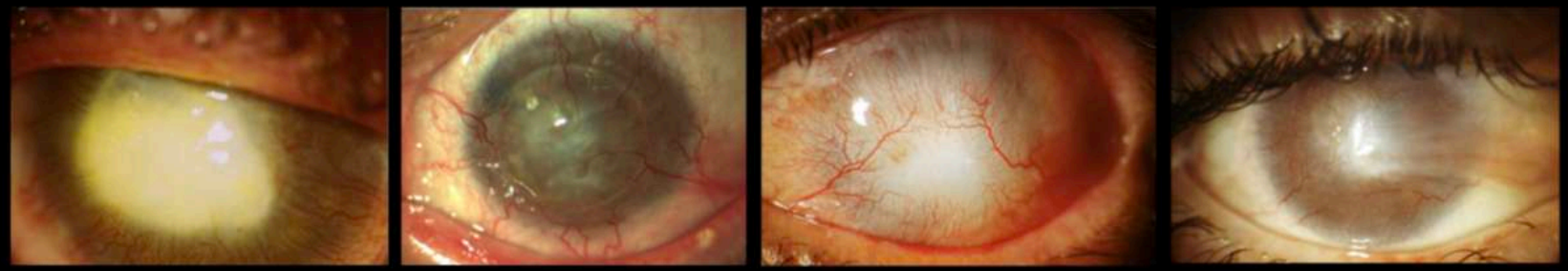


Corneal regeneration by cultures of limbal stem cells

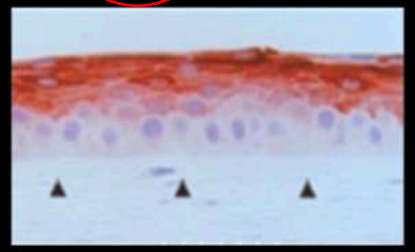
Rama et al., *N.Engl.J.Med.* 2010
Pellegrini et al., *Regen.Med.* 2013

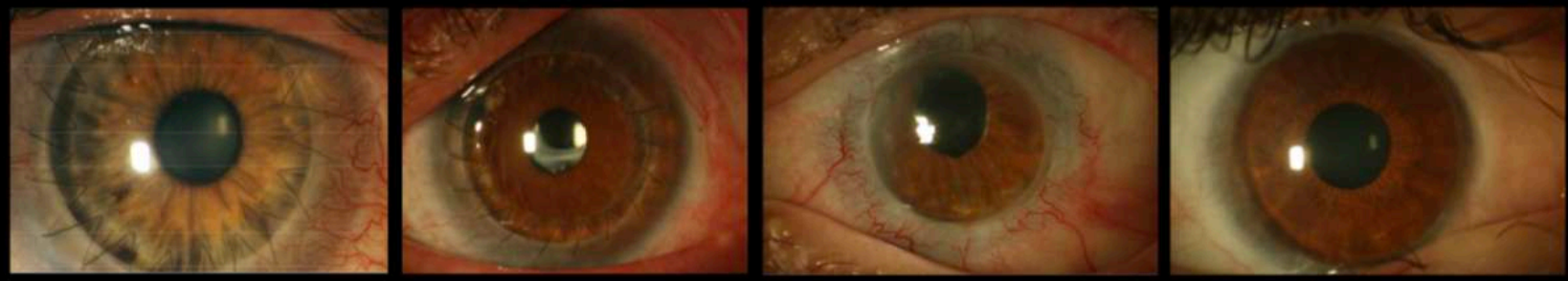
BEFORE: severe symptoms and loss of vision

$K3^{-}/K19^{+}$



$K3^{+}/K19^{-}$





2 years | 2 years | 2 years | 4 years

AFTER: complete restoration

FULL RECOVERY OF VISUAL ACUITY

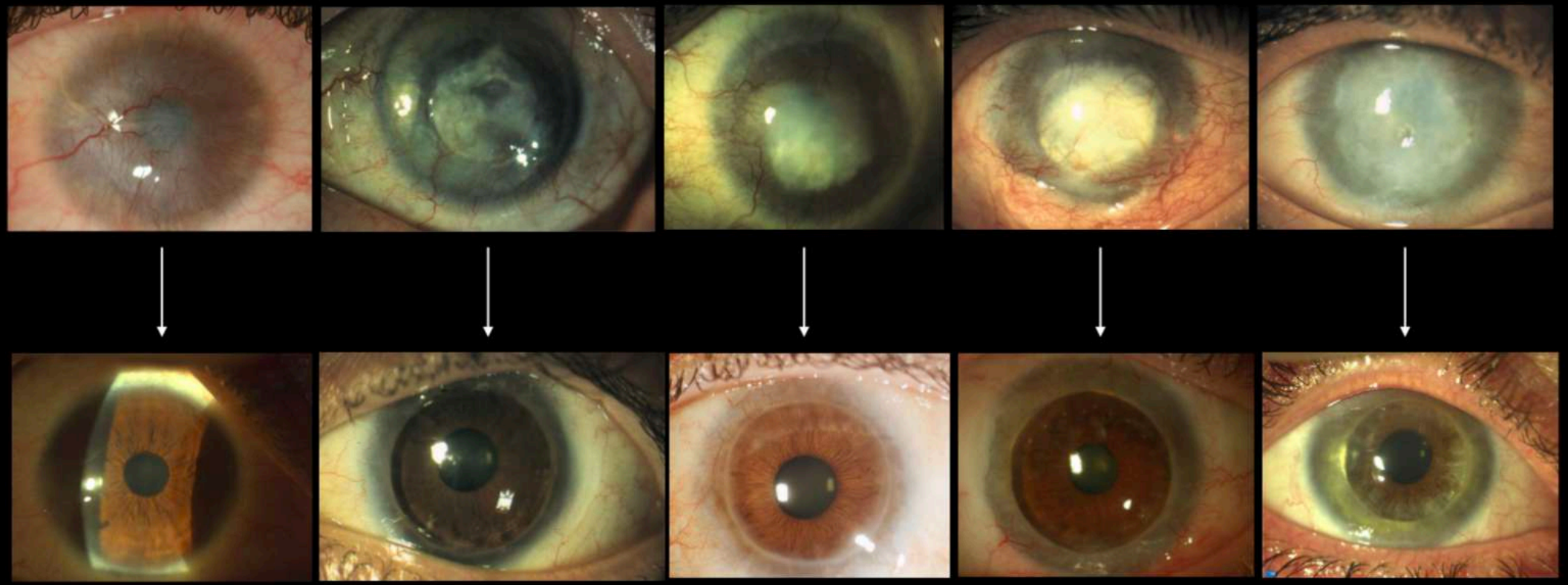
K3 : marker d
cellule cornea

K19: marker d
cellule
congiuntivali

Corneal regeneration by cultures of limbal stem cells

Rama et al., *N.Engl.J.Med.* 2010
Pellegrini et al., *Regen.Med.* 2013

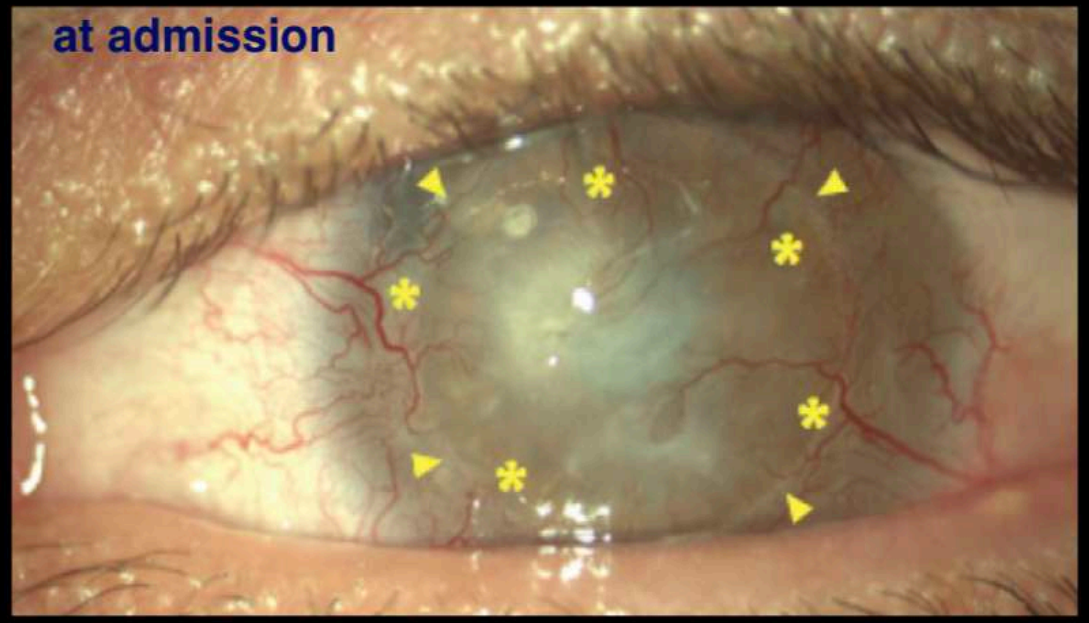
BEFORE: severe symptoms and loss of vision

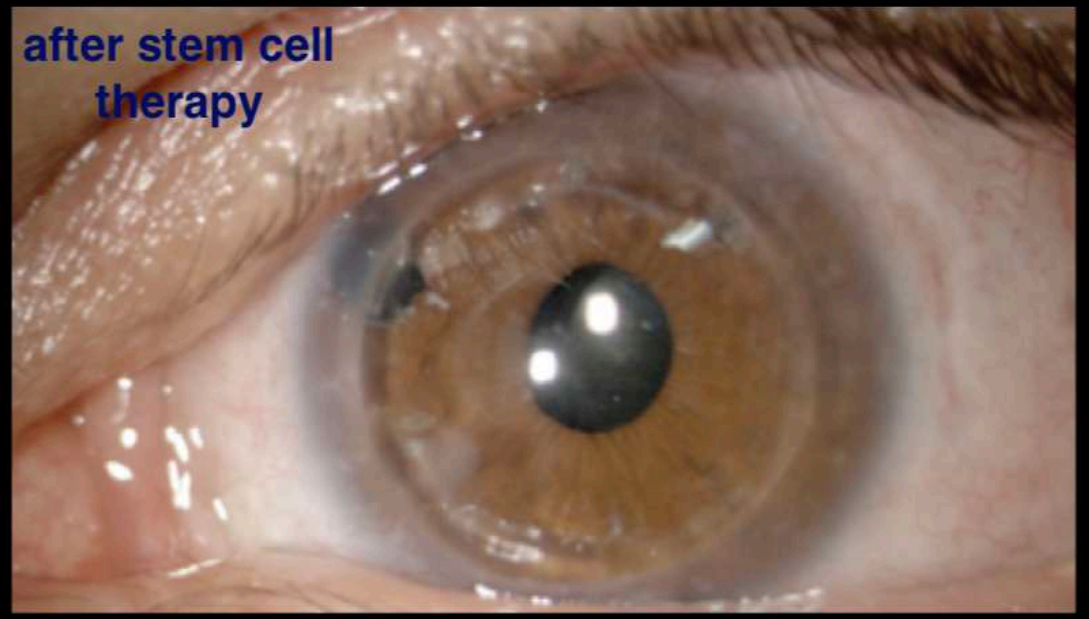


4 years | 5 years | 6 years | 7 years | 10 years

AFTER: complete restoration

FULL RECOVERY OF VISUAL ACUITY





strictly dependent on a precise number of p63^{high} holoclone stem cells

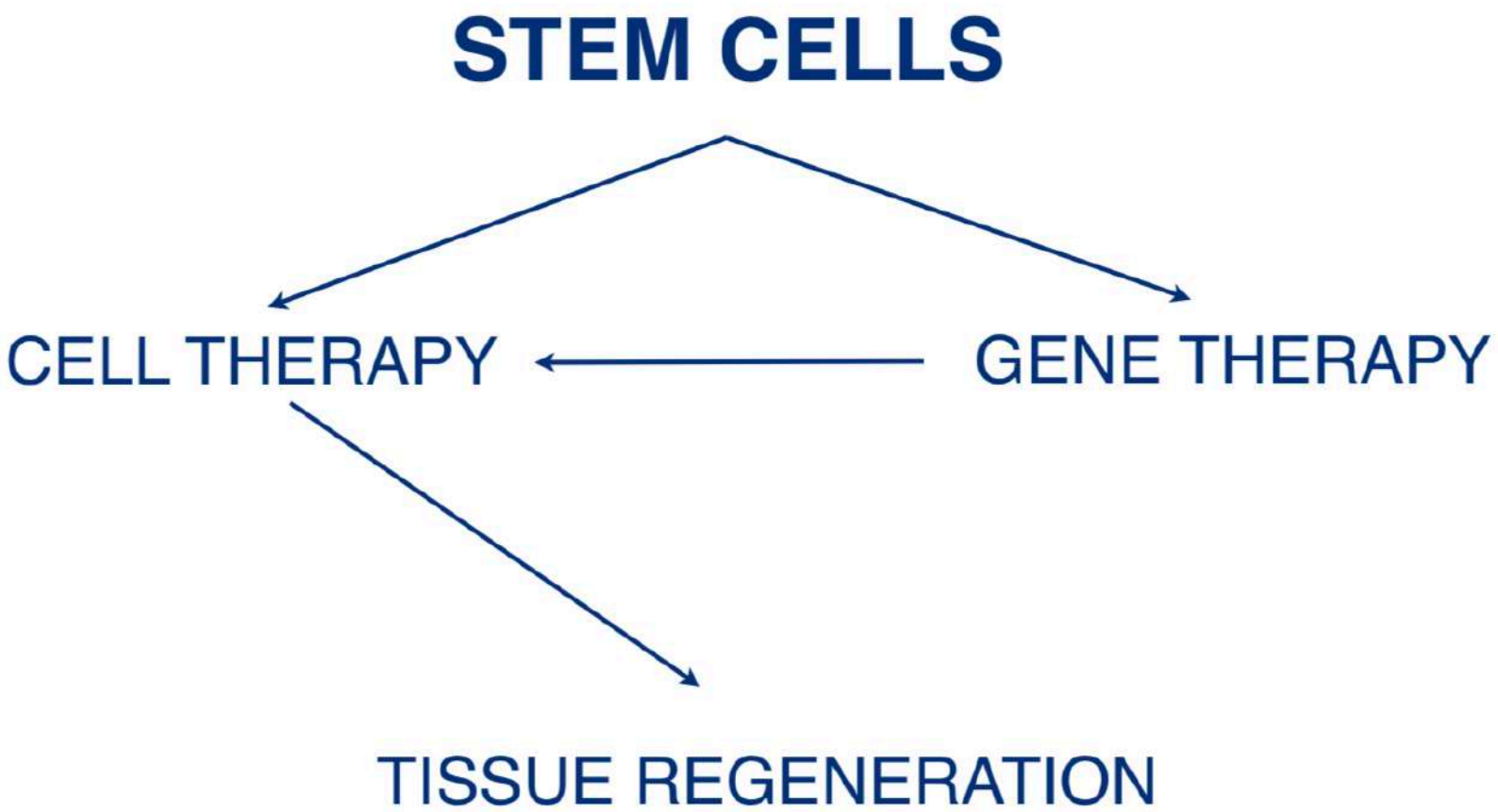
permanent corneal restoration and recovery of visual acuity (up to 20 years F.U.)

2008: EMA, OMPD

February 2015:
EMA, Registration: HOLOCLAR®

Pellegrini et al., *The Lancet* 1997
Rama et al., *Transplantation* 2001
Rama et al., *N.Engl.J.Med.* 2010
Pellegrini et al., *Regen.Med.* 2013

THE ROAD TO EX VIVO GENE THERAPY



GENE THERAPY WITH HEMATOPOIETIC STEM CELLS

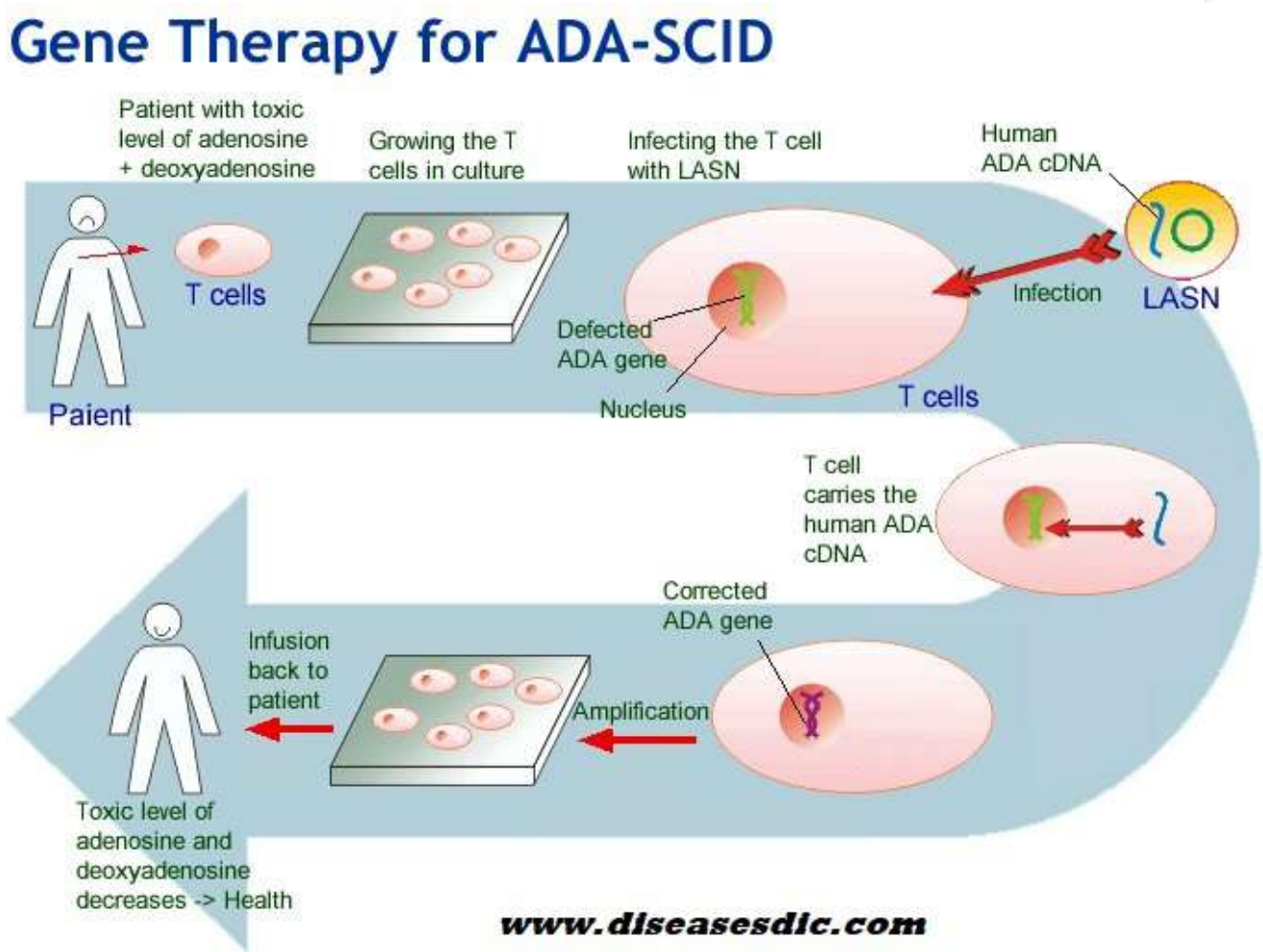
The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JANUARY 29, 2009 VOL. 360 NO. 5

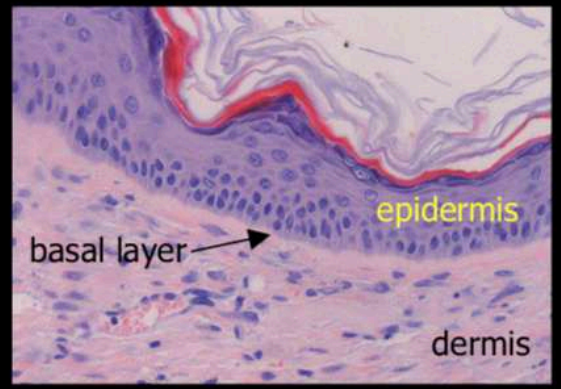
Gene Therapy for Immunodeficiency Due to Adenosine Deaminase Deficiency

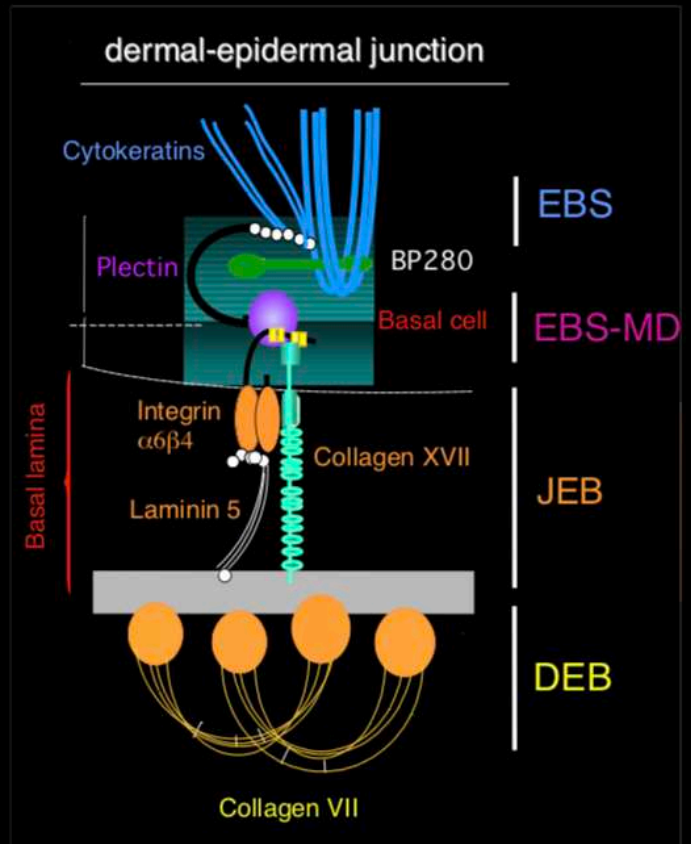
Alessandro Aiuti, M.D., Ph.D., Federica Cattaneo, M.D., Stefania Galimberti, Ph.D., Ulrike Benninghoff, M.D., Barbara Cassani, Ph.D., Luciano Callegaro, R.N., Samantha Scaramuzza, Ph.D., Grazia Andolfi, Massimiliano Mirolo, B.Sc., Immacolata Brigida, B.Sc., Antonella Tabucchi, Ph.D., Filippo Carlucci, Ph.D., Martha Eibl, M.D., Memet Aker, M.D., Shimon Slavin, M.D., Hamoud Al-Mousa, M.D., Abdulaziz Al Ghonaium, M.D., Alina Ferster, M.D., Andrea Duppenthaler, M.D., Luigi Notarangelo, M.D., Uwe Wintergerst, M.D., Rebecca H. Buckley, M.D., Marco Bregni, M.D., Sarah Marktel, M.D., Maria Grazia Valsecchi, Ph.D., Paolo Rossi, M.D., Fabio Ciceri, M.D., Roberto Miniero, M.D., Claudio Bordignon, M.D., and Maria-Grazia Roncarolo, M.D.

2009



Inherited EPIDERMOLYSIS BULLOSA: “the butterfly children”





Devastating skin BLISTERING

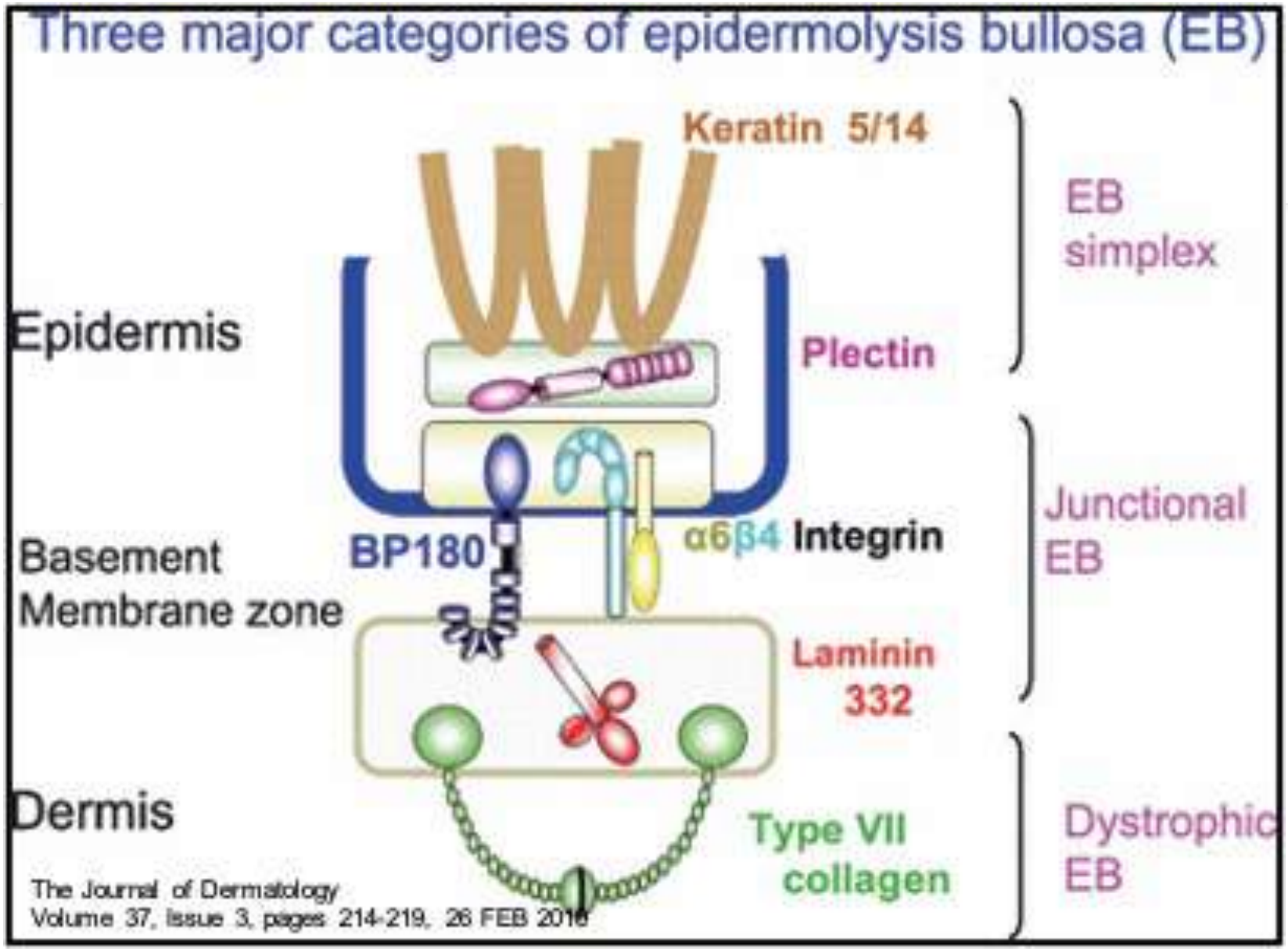
Very POOR QUALITY OF LIFE

SHORT LIFE EXPECTANCY
Squamous Cell Carcinoma

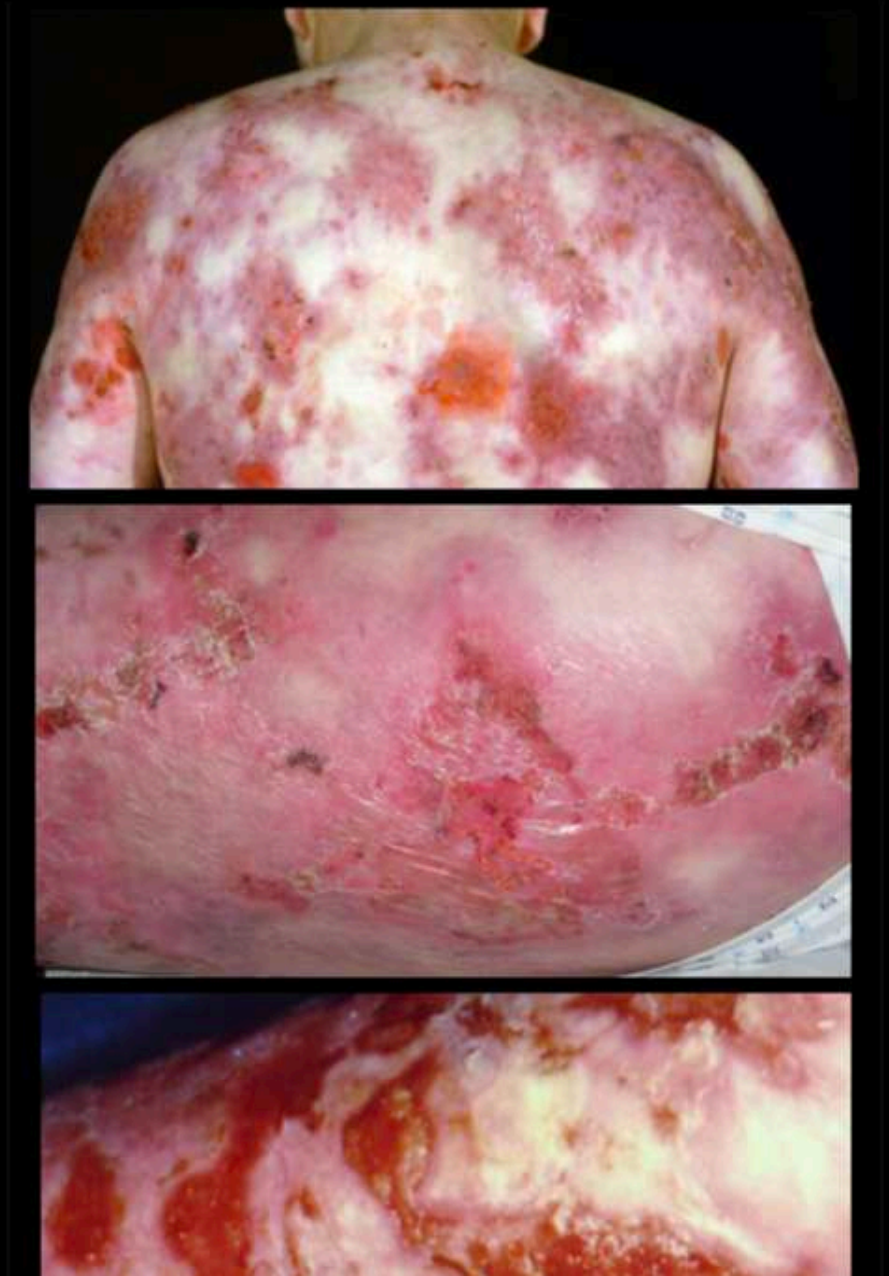
SIMPLEX:
keratins 5/14

JUNCTIONAL:
laminin 5, α6β4, collagen XVII

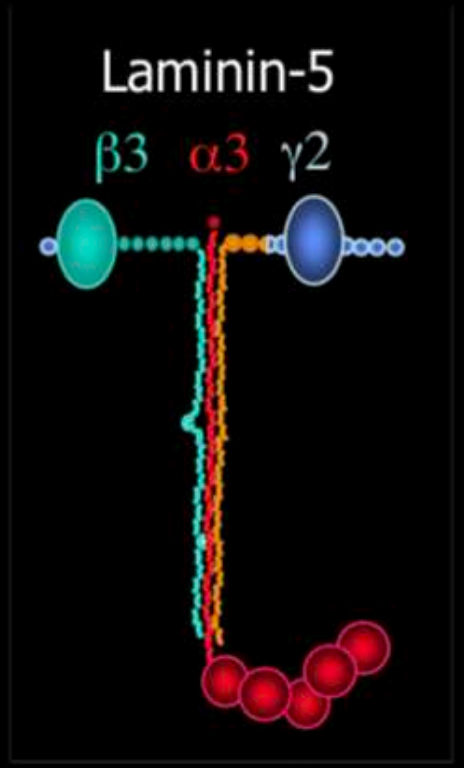
DYSTROPHIC:
Collagen VII


Three major categories of epidermolysis bullosa (EB)
Keratin 5/14
Epidermis
Plectin
EB simplex
Junctional EB
BP180
α6β4 Integrin
Basement Membrane zone
Laminin 332
Dermis
Type VII collagen
Dystrophic EB
The Journal of Dermatology
Volume 37, Issue 3, pages 214-219, 26 FEB 2010

CLAUDIO: 37 years old man



Phase I/II clinical trial:
ex vivo combined
cell and gene therapy

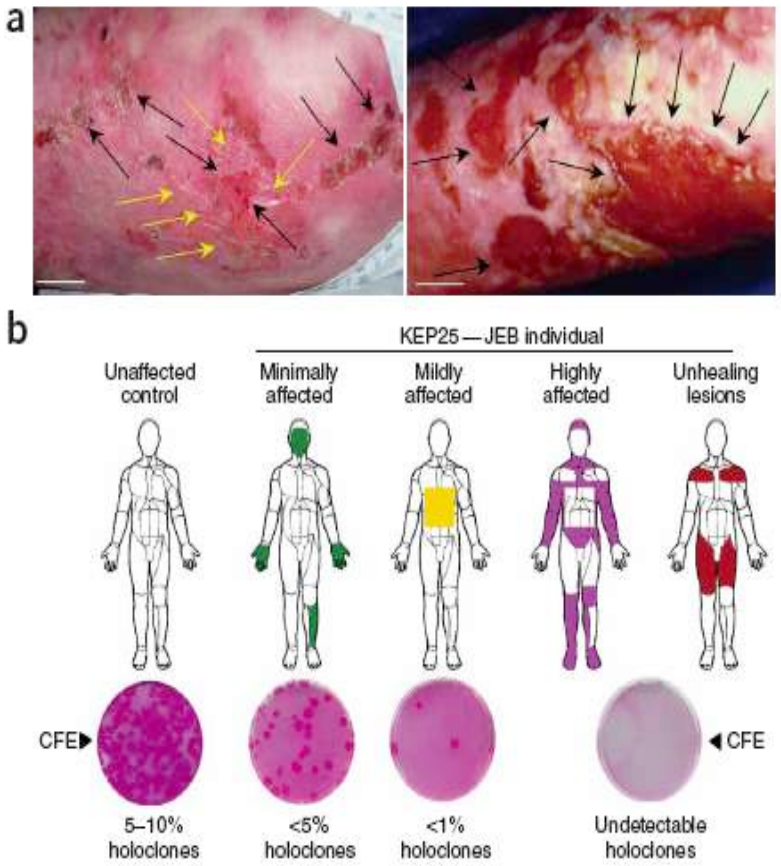


Laminin 5 (β3 chain)

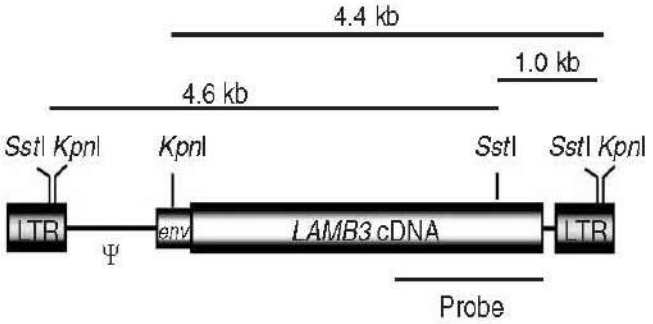
Allele: splice site mutation (E210
Allele: frameshift (PTC)

Approccio terapeutico

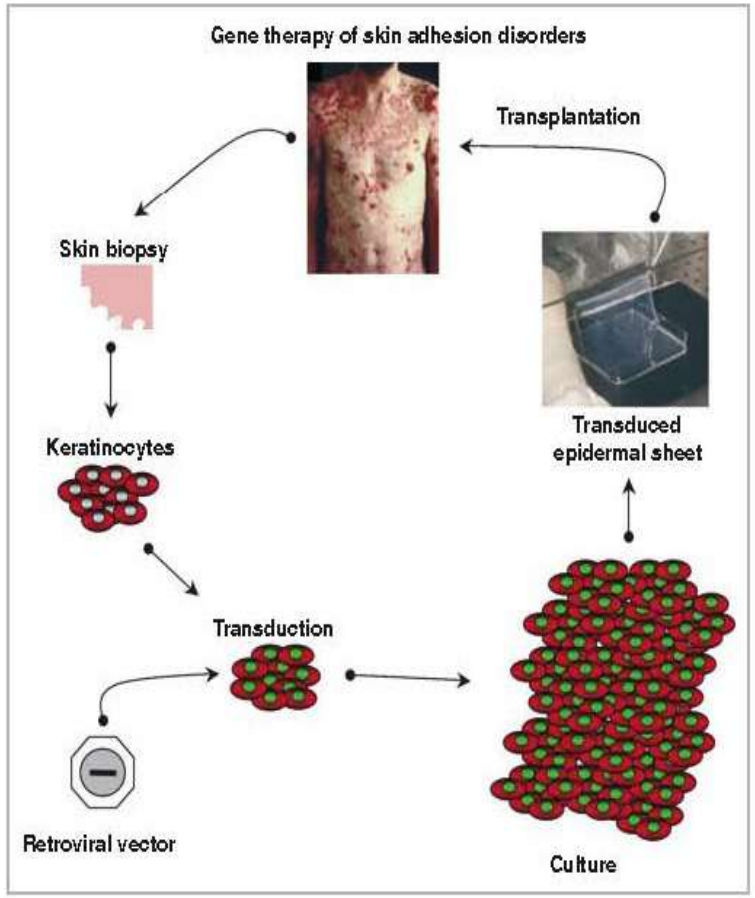
Biopsia dal paziente



Vettore retrovirale



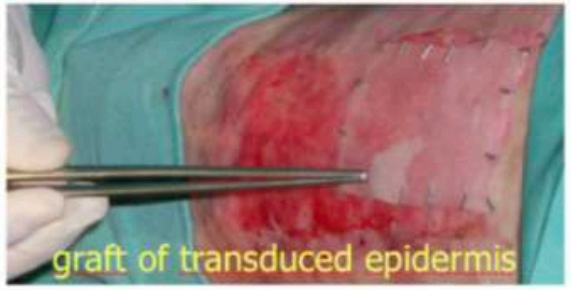
Schema del trattamento

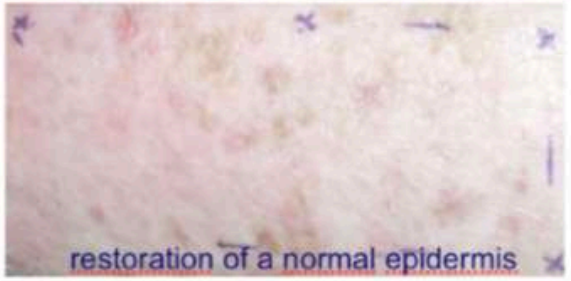


Trapianto

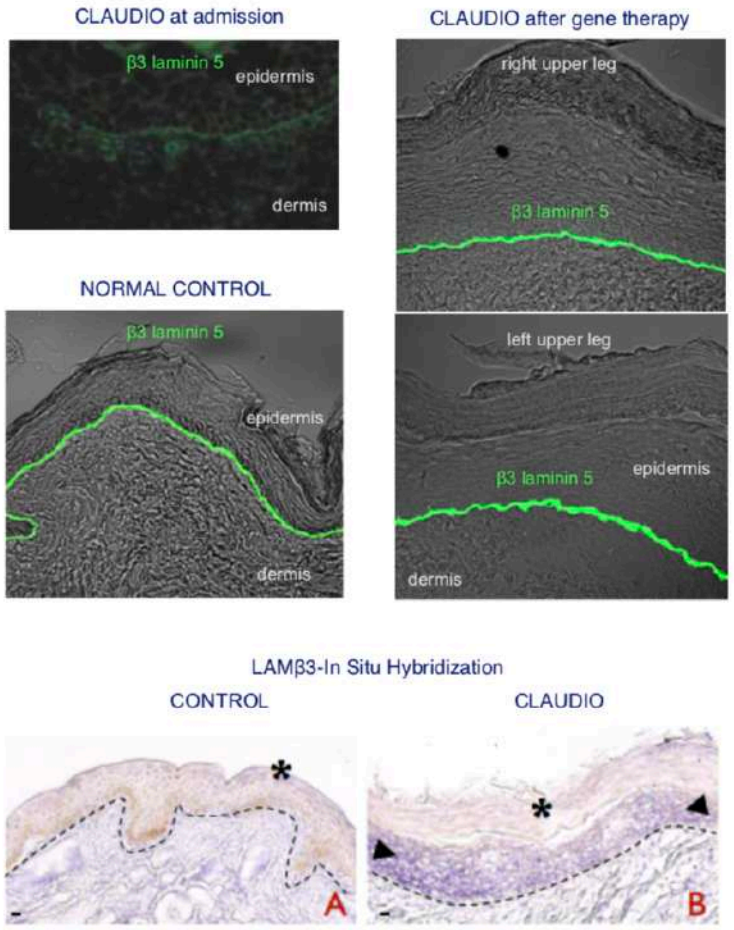
right upper leg

left upper leg





Follow-up a 12 anni dal trapianto



Mavilio et al., *Nature Med.* 2006
De Rosa et al., *Stem Cell Rep.* 2014

La pelle trapiantata si è rigenerata correttamente, questo significa che in vitro sono state corrette le cellule staminali e le nuove cellule portano il gene corretto della Laminina 5 che viene espressa.

GENE THERAPY OF JUNCTIONAL EPIDERMOLYSIS BULLOSA

nature medicine

2006

Correction of junctional epidermolysis bullosa by transplantation of genetically modified epidermal stem cells

Fulvio Mavilio[1], Graziella Pellegrini[1,2], Stefano Ferrari[2], Francesca Di Nunzio[1], Enzo Di Iorio[2], Alessandra Recchia[1], Giulietta Maruggi[1], Giuliana Ferrari[3], Elena Provasi[4], Chiara Bonini[4], Sergio Capurro[5], Andrea Conti[6], Cristina Magnoni[6], Alberto Giannetti[6] & Michele De Luca[1,2]

2014

Stem Cell Reports
Report
ISSCR
OPEN ACCESS

Long-Term Stability and Safety of Transgenic Cultured Epidermal Stem Cells in Gene Therapy of Junctional Epidermolysis Bullosa

Laura De Rosa,[1,5] Sonia Carulli,[1,5] Fabienne Cocchiarella,[1] Daniela Quaglino,[2] Elena Enzo,[1] Eleonora Franchini,[1] Alberto Giannetti,[1] Giorgio De Santis,[4] Alessandra Recchia,[1] Graziella Pellegrini,[1] and Michele De Luca[1,*]

Laminin 5-β3 JEB

Closure of a Large Chronic Wound through Transplantation of Gene-Corrected Epidermal Stem Cells

Journal of Investigative Dermatology (2017) 137, 778–781; doi:10.1016/j.jid.2016.10.038

Johann W. Bauer[1,9,*], Josef Koller[1,9], Eva M. Murauer[2,9], Laura De Rosa[3], Elena Enzo[3], Sonia Carulli[4], Sergio Bondanza[4], Alessandra Recchia[3], Wolfgang Muss[5], Anja Diem[6], Elisabeth Mayr[2], Pamina Schlager[2], Iris K. Gratz[2,7,8], Graziella Pellegrini[3,10] and Michele De Luca[3,10]

2017

Collagen VII

JAMA | Preliminary Communication

Safety and Wound Outcomes Following Genetically Corrected Autologous Epidermal Grafts in Patients With Recessive Dystrophic Epidermolysis Bullosa

2016

Zurab Siprashvili, PhD; Ngon T. Nguyen, BS; Emily S. Gorell, MS; Kylie Loutit, BS; Phuong Khuu, MD; Louise K. Furukawa, MD; H. Peter Lorenz, MD; Thomas H. Leung, MD, PhD; Douglas R. Keene, BS; Kerri E. Rieger, MD, PhD; Paul Khavari, MD, PhD; Alfred T. Lane, MD, MA; Jean Y. Tang, MD, PhD; M. Peter Marinkovich, MD

ARTICLE

doi:10.1038/nature24487

Regeneration of the entire human epidermis using transgenic stem cells

Tobias Hirsch[1]*, Tobias Rothoeft[2]*, Norbert Teig[2]*, Johann W. Bauer[3]*, Graziella Pellegrini[4,5]*, Laura De Rosa[5]*, Davide Scaglione[6], Julia Reichelt[3], Alfred Klausegger[3], Daniela Kneisz[3], Oriana Romano[7], Alessia Secone Seconetti[5], Roberta Contin[5], Elena Enzo[5], Irena Jurman[8], Sonia Carulli[9], Frank Jacobsen[1], Thomas Luecke[10], Marcus Lehnhardt[1], Meike Fischer[2], Maximilian Kueckelhaus[1], Daniela Quaglino[7], Michele Morgante[8], Silvio Bicciato[7], Sergio Bondanza[9] & Michele De Luca[5]

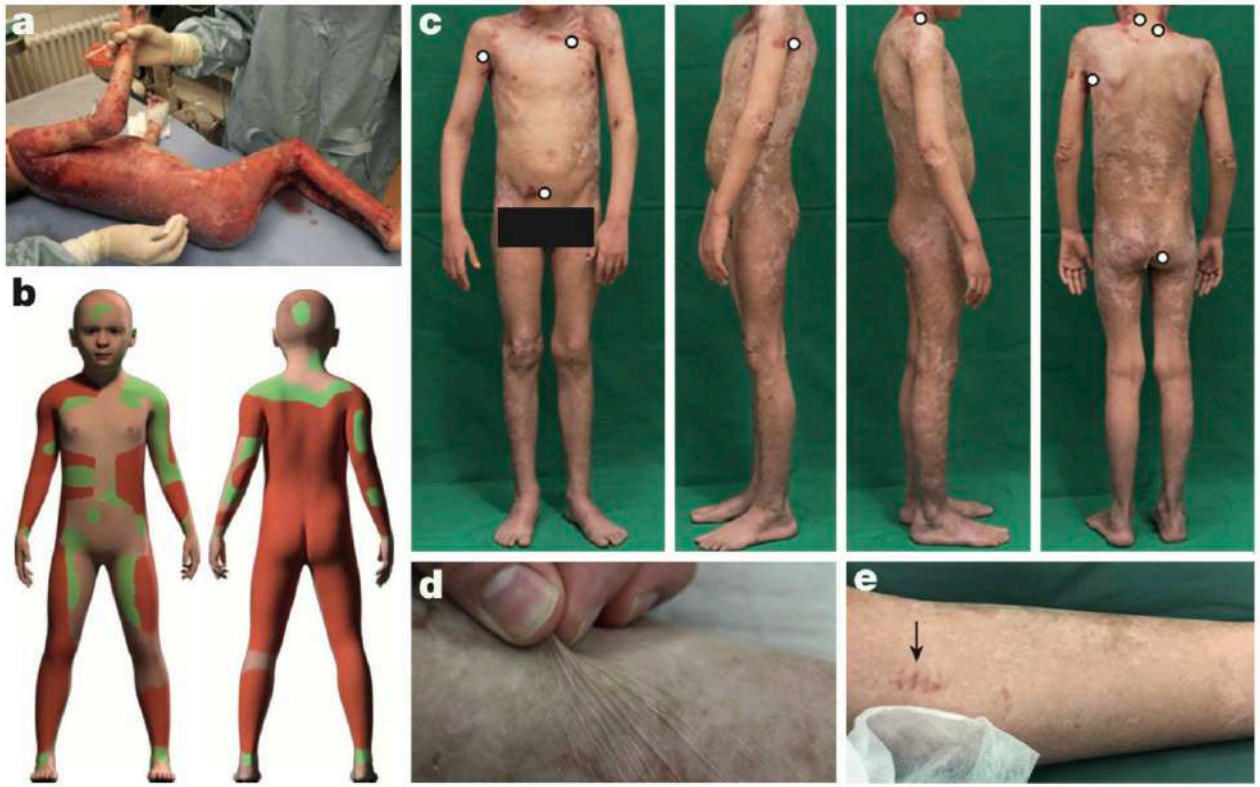


Figure 1 | Regeneration of the transgenic epidermis. a, Clinical picture of the patient showing massive epidermal loss. **b**, Schematic representation of the clinical picture. The denuded skin is indicated in red; blistering areas are indicated in green. Flesh-coloured areas indicate currently non-blistering skin. Transgenic grafts were applied on both red and green areas. **c**, Restoration of patient's entire epidermis, with the exception of very few areas on the right thigh, buttocks, upper shoulders/neck and left axilla (white circles, altogether $\leq 2\%$ of TBSA). **d**, Normal skin functionality and elasticity. **e**, Absence of blister formation at sites where post-graft biopsies were taken (arrow).

ARTICLE

doi:10.1038/nature24487

Regeneration of the entire human epidermis using transgenic stem cells

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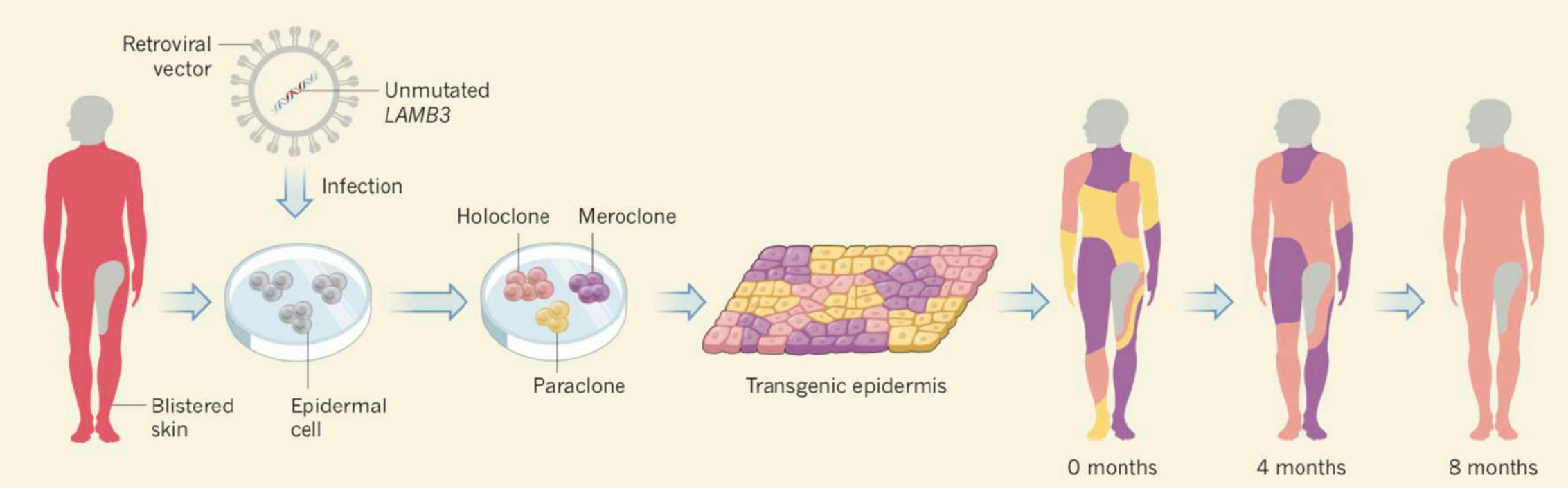


Tu puoi fare la differenza!