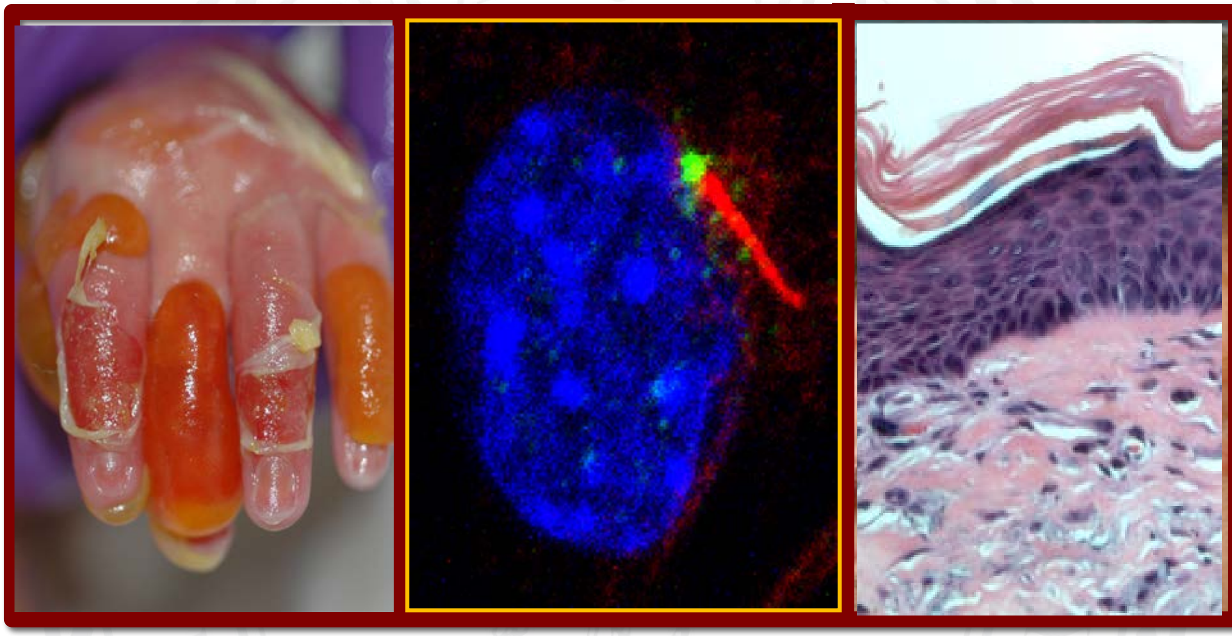
Cellular Therapies for Skin Disease



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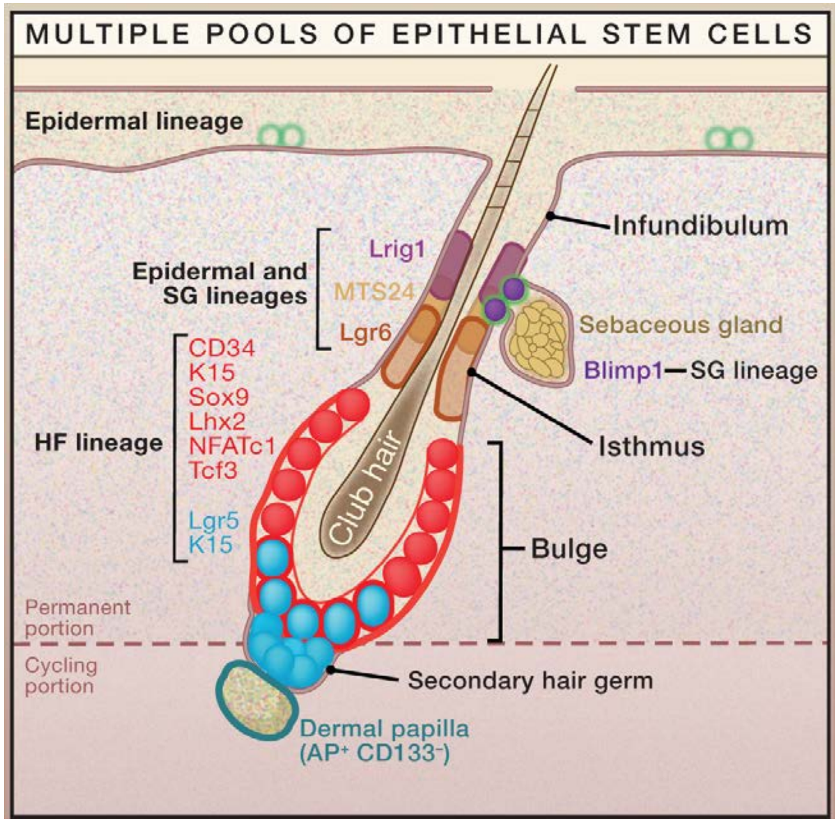
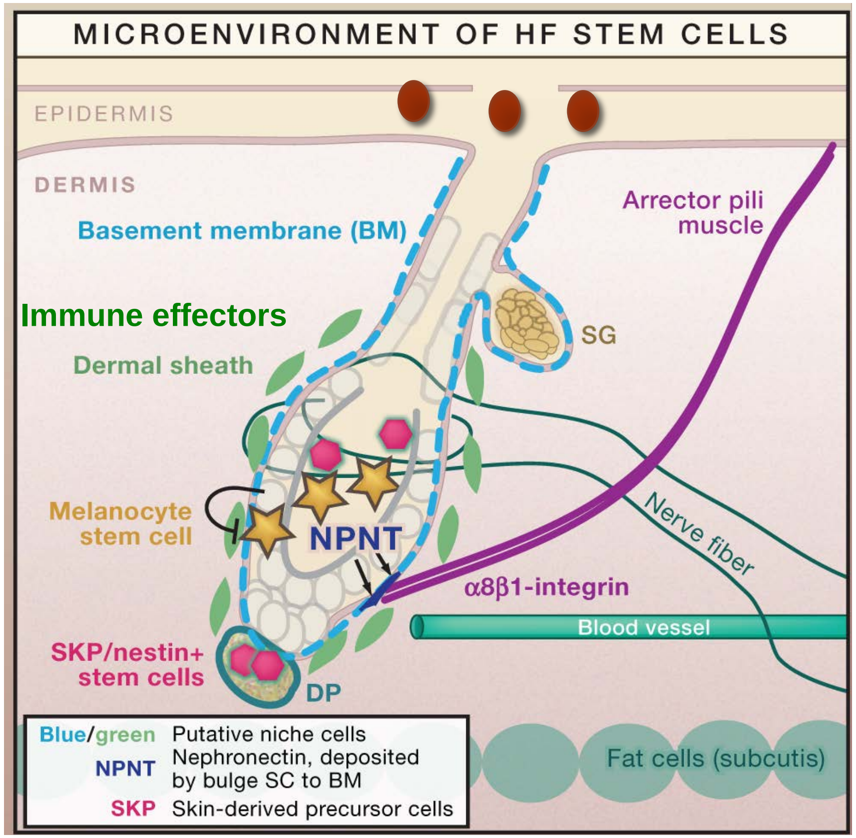
PROGRAM IN EPITHELIAL BIOLOGY

Many cell lineages compose the skin





From Woo and Oro Cell 2011

History of Autologous and Allogeneic Cell Therapy in Skin
Road Block: Scaling autologous tissue

Autologous or Allogeneic Partial Thickness Skin Graft

2500 BCE

Autologous Follicular Unit Transfer

1930s

Autologous Melanocyte Transfer

1970s

Over 200 monogenic skin disorders exist in humans, most with only palliative treatment available

Recessive Dystrophic Epidermolysis Bullosa
Collagen VII Deficiency

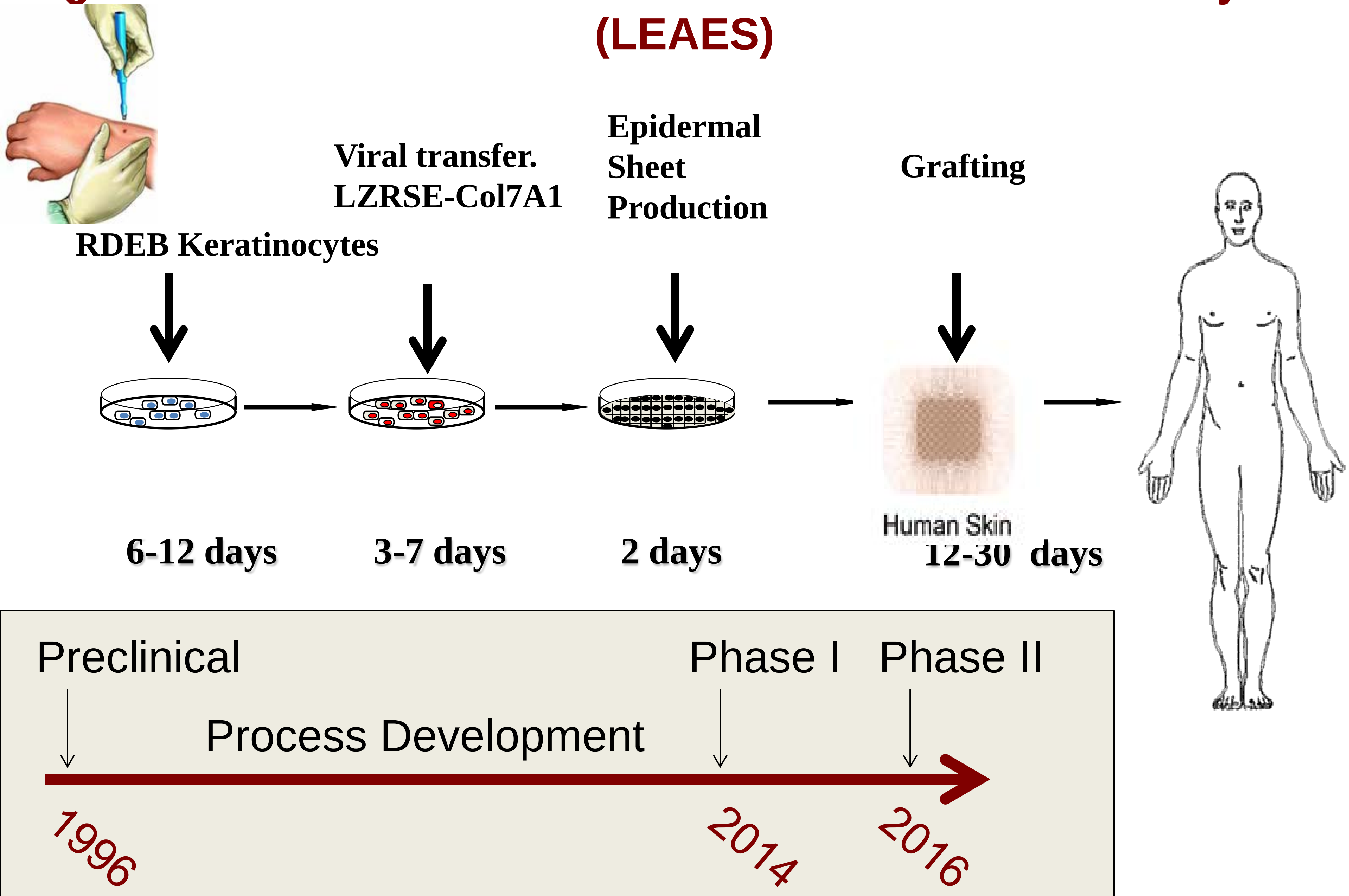
Therapeutic Approaches for EB

Inducers	Protein	Keratinocyte	Bone Marrow
sulforaphane	COL7A1 injections	Retroviral Transduction	Allogeneic BMT
		Genetic Correction	Allogeneic mesenchymal Stem Cells
		Spontaneous Revertant Cells	Genetic Correction

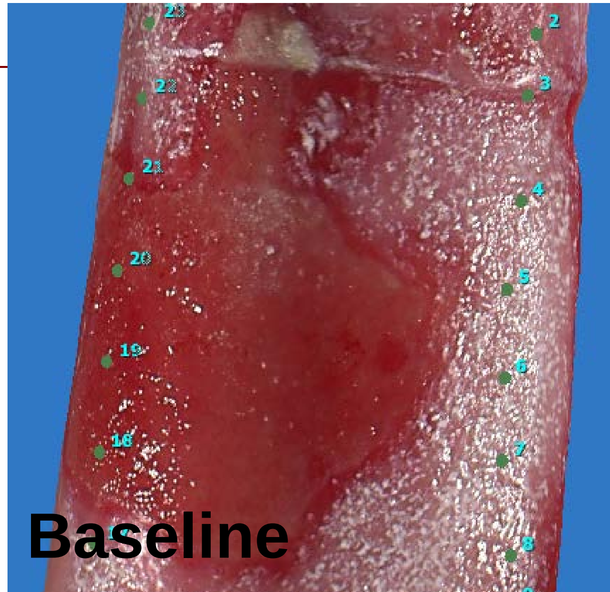
Development of Autologous Retroviral Corrected Keratinocyte Sheet Product (LEAES)

Keratinocyte

Proof of Concept: Genetic correction of RDEB by retroviral gene transfer of COL7A1 cDNA into somatic keratinocytes (LEAES)



Effective Wound Healing at 12 months



Subject	Site	Location	Description	Wound history	1 month[2]	3 months	6 months
1	A	R distal forearm	Erosion	>5 yrs			
	B	L forearm	Erosion	>5 yrs			
	C	R proximal forearm	Erosion	>5 yrs			
	D	R shoulder	Inflamed erosion	>5 yrs			
	E	L arm	New blister	1 wk			
	Z	R arm	Induced wound	New			
2	A	Central chest	Erosion	>5 yrs			
	B	L shoulder	Erosion and scar	>5 yrs			
	C	R forearm	Erosion and scar	3-5 yrs			
	D	R posterior shoulder	Inflamed erosion	>5 yrs			
	E	Lower back	Erosion	>5 yrs			
	Z	R upper chest	Induced wound	New			
3	A	R lateral hand	Erosion	3-5 yrs			
	B	R medial hand	Scar tissue	3-5 yrs			
	C	L ventral foot	Erosion and scar	3-5 yrs			
	D	L hand	Scar tissue	3-5 yrs			
	E	R foot	Erosion and scar	3-5 yrs			
	Z	L ventral foot	Induced wound	New			
4	A	L distal forearm	Inflamed erosion	>5 yrs			
	B	L medial forearm	Inflamed erosion	>5 yrs			
	C	L proximal forearm	Inflamed erosion	>5 yrs			
	D	R lateral forearm	Inflamed erosion	>5 yrs			
	E	R distal forearm	Inflamed erosion	>5 yrs			
	Z	R medial forearm	Induced wound	New			

Ziprashvili et al. JAMA in press

The Need for Improved Cell-Based Therapy

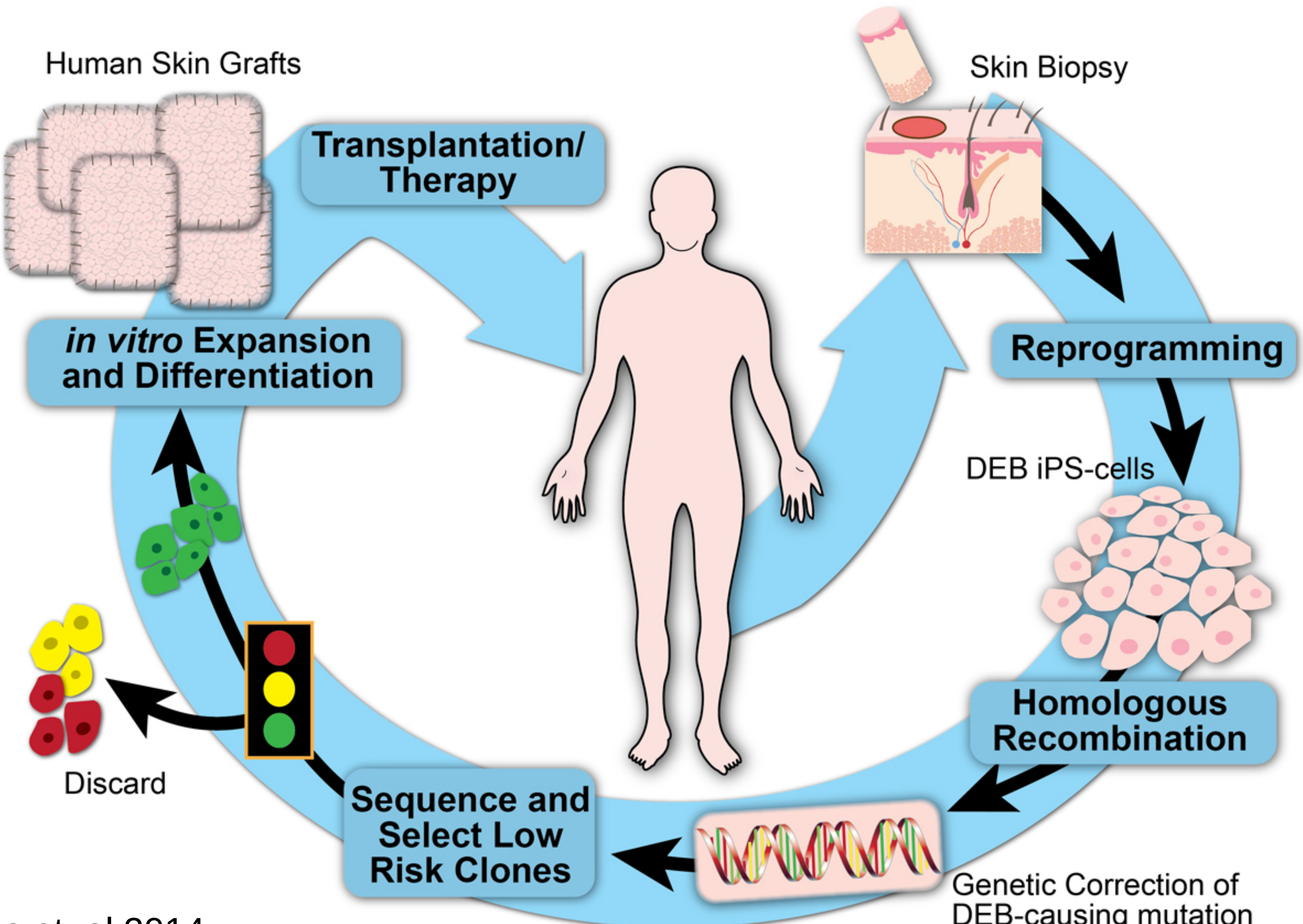
Low keratinocyte stem cell number in patients and inability to scale autologous production to clinical levels

Gene Transfer ineffective for dominant negative diseases

Safety concerns with permanently integrated retroviruses and premalignant donor skin

Therapeutic Reprogramming through corrected iPS therapy

Long-term collaboration with Marius Wernig



Sebastiano et. al 2014

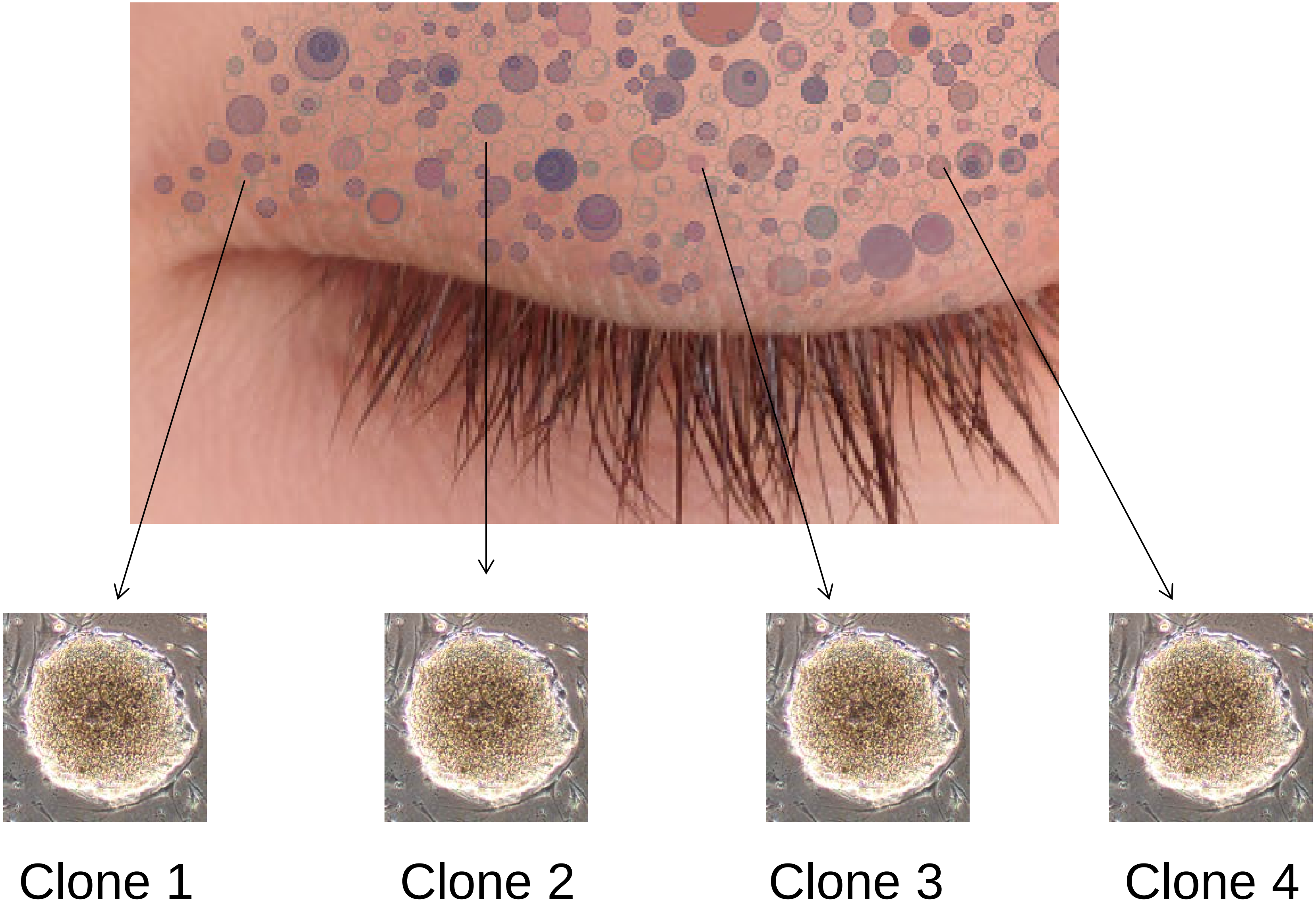
Issue I:
Improved cell bank safety by clone selection

55% of RDEB patients who survive to early adulthood will die of squamous cell carcinoma.

Can we select out SCC-predisposed corrected iPS clones to form a “clean iPS cell bank”?

Lee et al., Nature Genetics 46, 106–1062 (2014)
Atwood et al., 2015, Cancer Cell 27, 342–353

Each iPS clone comes from one cell in skin



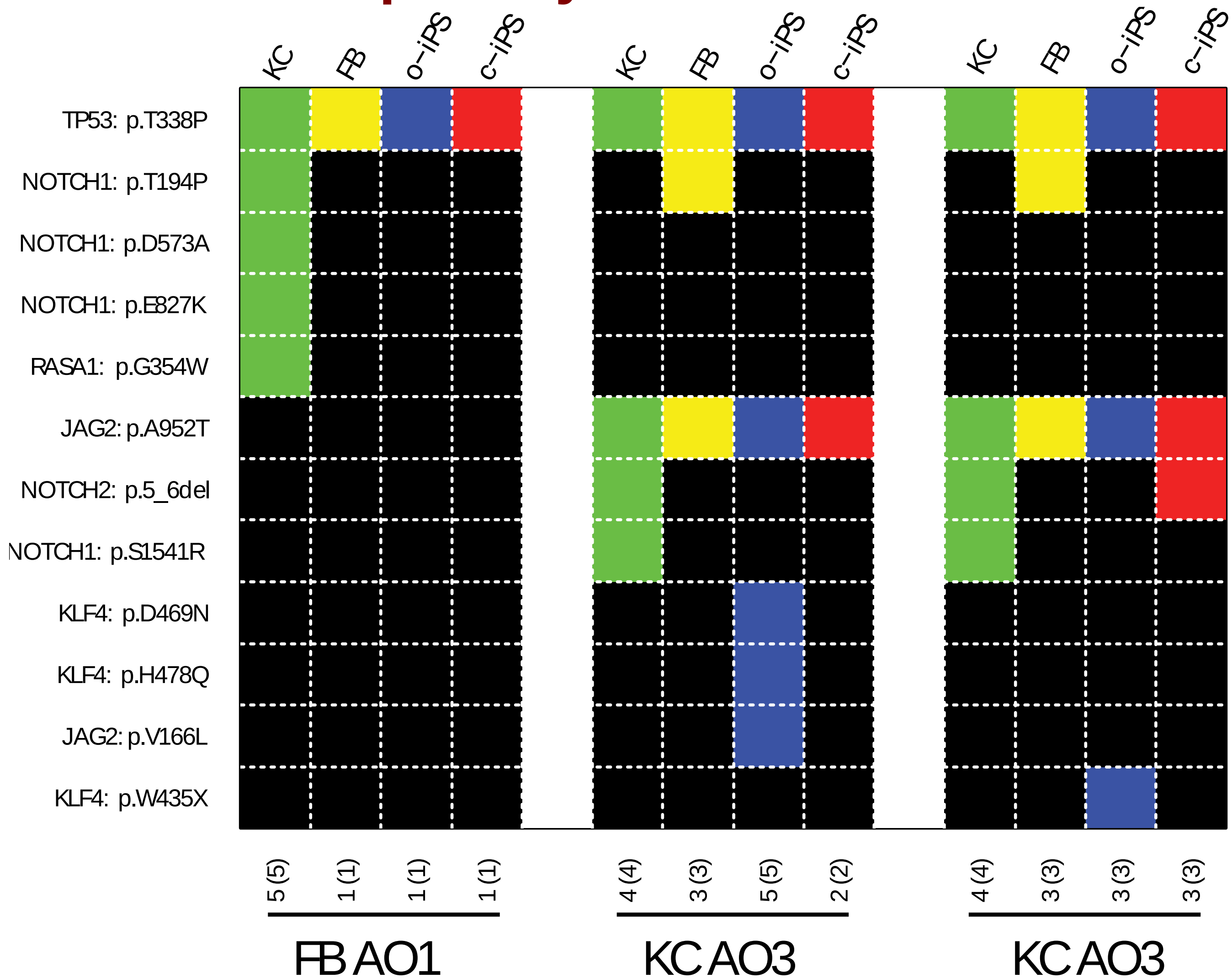
http://www.the-scientist.com/?articles.view/articleNo/43041/title/Cancer-Driving-Mutations-Common-in-Normal-Skin-Cells/

Selecting out IPS with both pre-existing and acquired cancer-susceptibility mutations

SCC Predisposition 700X Targeted Resequencing

BWA Pipeline Analysis

Notch1	H-ras
Notch2	K-ras
Notch3	RasA1
Notch4	CDKN2A
Jagged1	TP53
Jagged2	KLF4
CHUK	



Issue 2:
Which genes in the genome should we be editing?

Powerful new gene-editing technologies, such as CRISPR-Cas9, hold great promise for advancing science and treating disease, but they also raise concerns and present complex challenges, particularly because of their potential to be used to make genetic changes that could be passed on to future generations, thereby modifying the human germline.

International Summit on Human Gene Editing

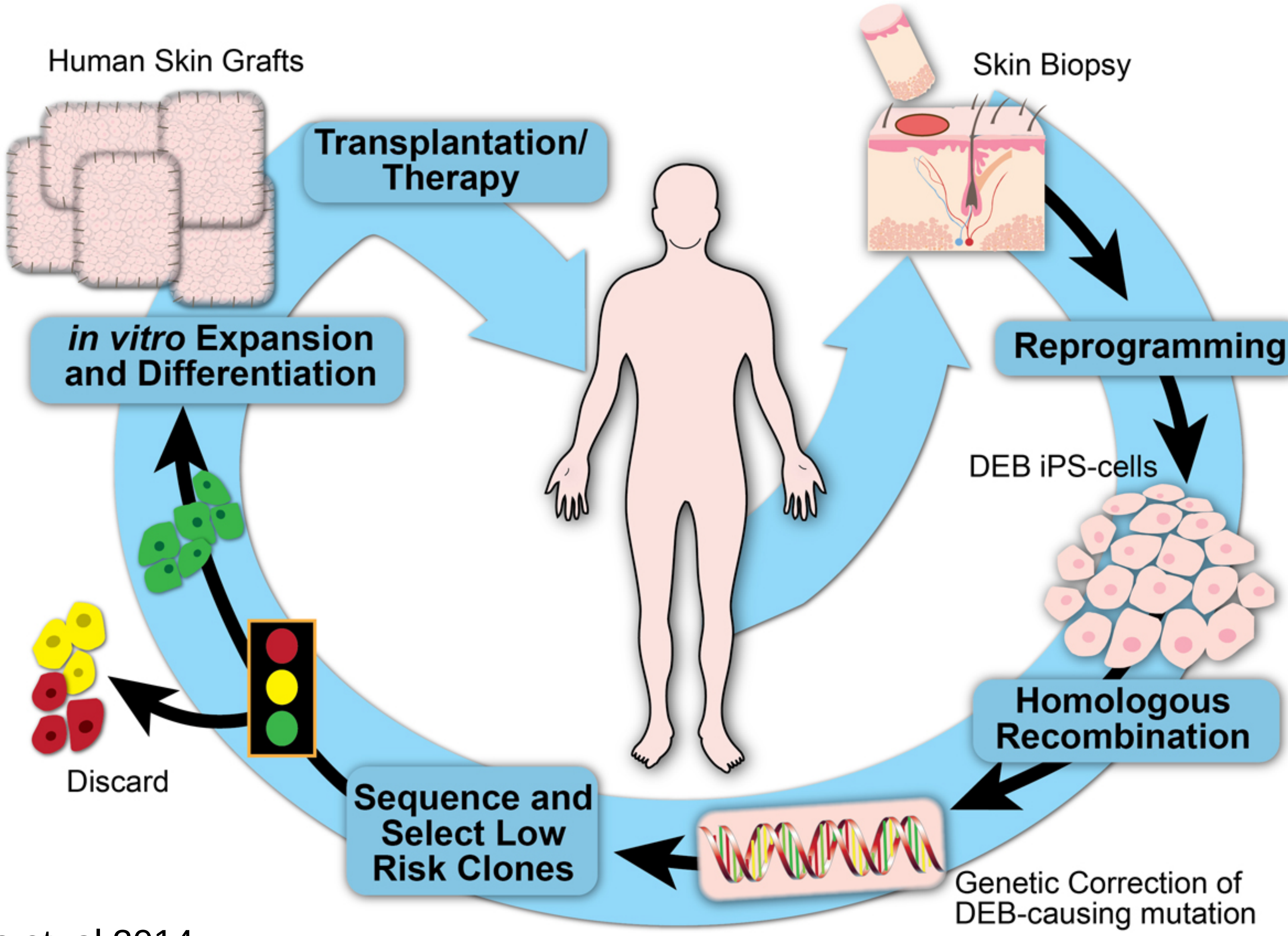
Dec 1-3, 2015

US National Academy of Sciences
Chinese Academy of Scienes
Royal Society

http://www.nationalacademies.org/gene-editing/index.htm

Therapeutic Reprogramming through corrected iPS therapy

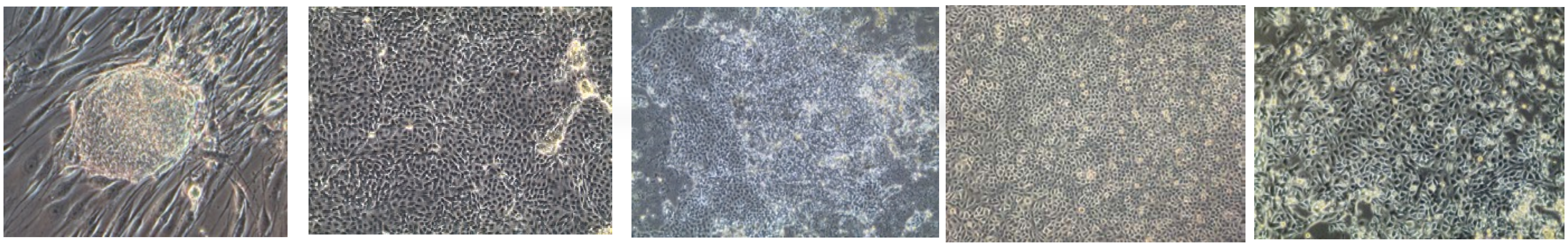
Long-term collaboration with Marius Wernig and Al Lane

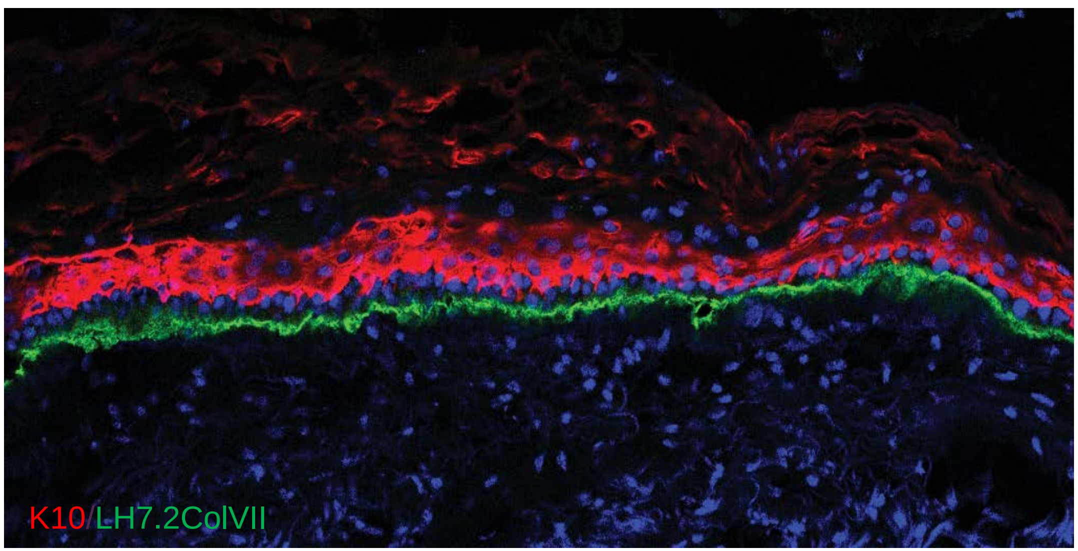


Sebastiano et. al 2014

Skin Tissue Bioengineering

ES/iPS cells → Aggrewell → **Keratinocyte**





Sebastiano, Zhen et al. Science Trans Med 2014

Issue III.
Safety and Efficacy of iPS-derived Tissue

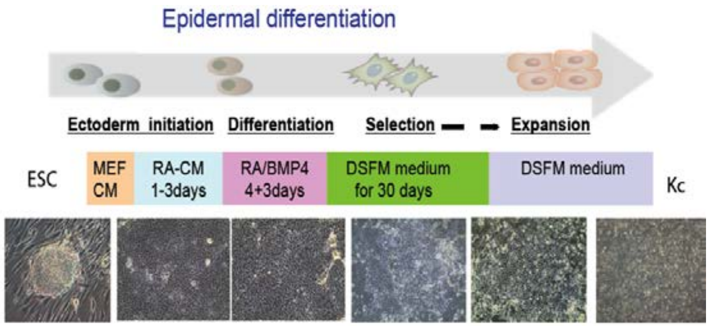
Cell Line	Production Runs	Emergence (wks)	Stratification Quality
KC IPS1	8	6	+++
KC IPS3	8	7	+
FB IPS2	12	8	+/-
FB IPS1	2	6	+++

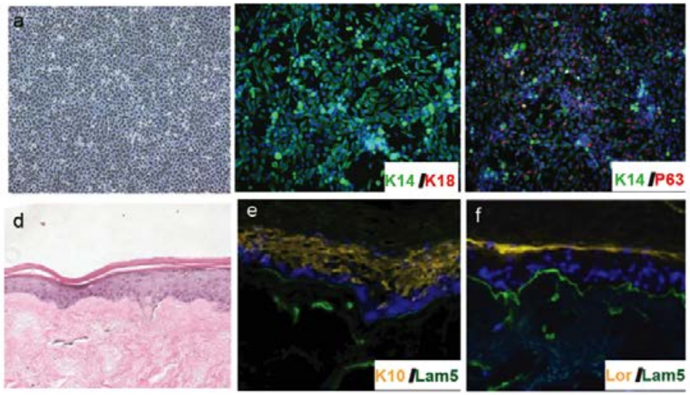
GOAL: TO PRECISELY DEFINE EPIDERMAL DIFFERENTIATION


Google
self-driving car
HYBRID


Map
Satellite
Terrain
Menlo Oaks
VA Healthcare System of Menlo Park
East Palo Alto
Palo Alto Airport of Santa Clara County
The Willows
Willow Rd
82
Menlo Park
Linfield Oaks
University Ave
101
Crescent Park
Middlefield Rd
Duveneck/ St. Francis
Allied Arts
Alma St
University South
Palo Alto
Triple El
Greer Park

Multi-dimensional genomic analysis of differentiation





IPS- START

DAY 7

DAY 14

DAY 21

DAY 43

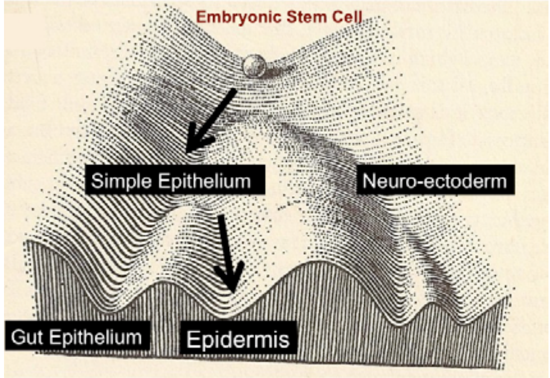
KC- TARGET

RNA SEQ

ATAC-SEQ

ChIP-SEQ

Capture C



Network Model

Perturbation Studies

Issue IV.
Consortia can increase speed of clinical development

EB iPS Consortium
(Founded May 2016)

Stanford	Columbia	UC Denver
Excisable LV Reprogramming	4 Factor virus	mRNA-miRNA
rAAV correction	Genetic Revertant	TALEN
DIFF Protocol 1	**DIFF Protocol 2**	**DIFF Protocol 3**

↓

Coordinated Process Development
Best Practices
Shared Resources

Stanford Children's Health | Lucile Packard Children's Hospital Stanford

Center for Definitive and Curative Medicine

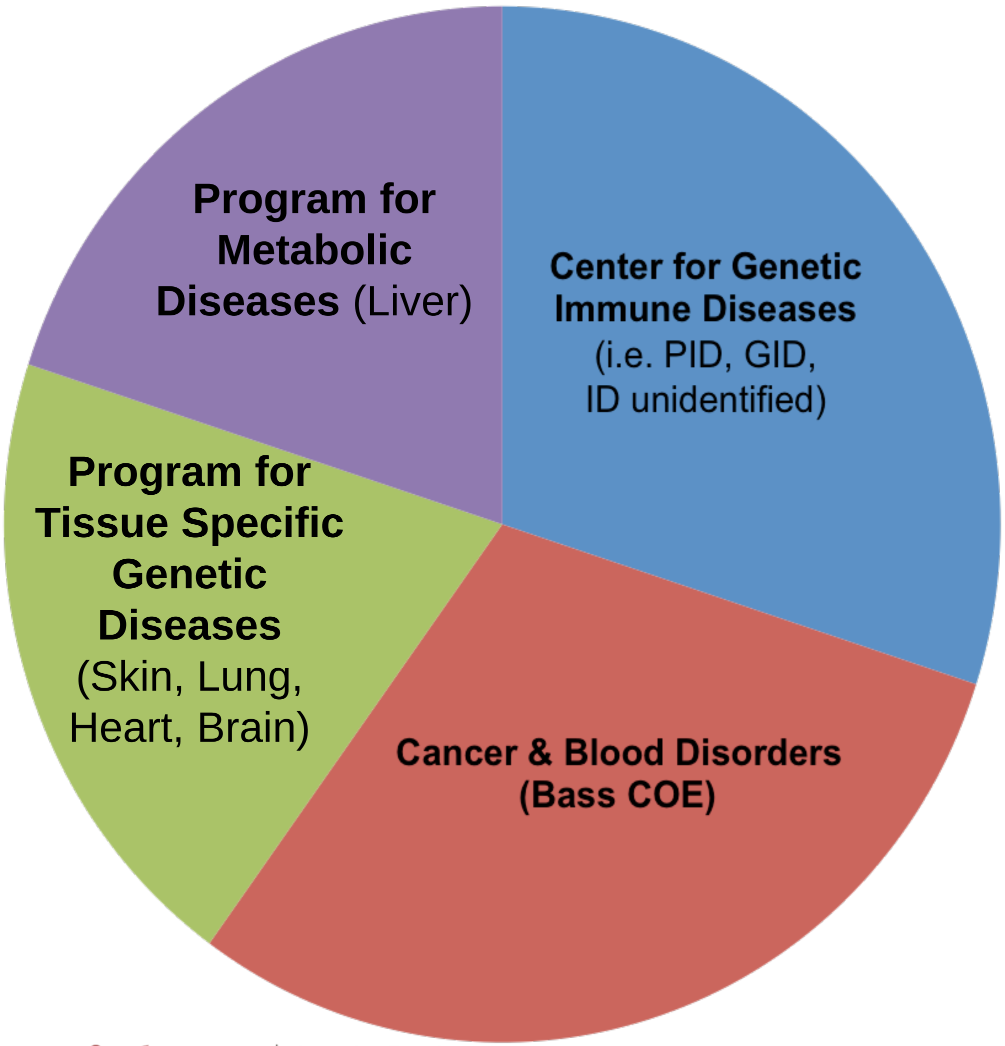
Director: Maria-Grazia Roncarolo

Associate Directors:
Matt Porteus
Anthony Oro
David Digiusto

Stanford | MEDICINE

Stanford Center for Definitive and Curative Medicine

Disease Areas



GMP Facility
Cell Manufacturing

David Digiusto, Director

Stanford Children's Health | Lucile Packard Children's Hospital Stanford

Stanford MEDICINE Stanford Children's Health | Lucile Packard Children's Hospital Stanford

Summary

Skin has a rich history of cell therapy development with scaling autologous tissues a major roadblock

For genetic diseases like Epidermolysis bullosa, autologous corrected keratinocyte sheets have powerful, long-lasting disease modifying activity

IPS-derived, genome-edited, autologous keratinocyte sheets brings the promise of scalability

Consortia and centers of cell therapy process development are needed to accelerate treatments