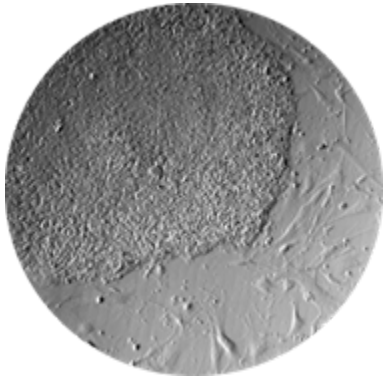
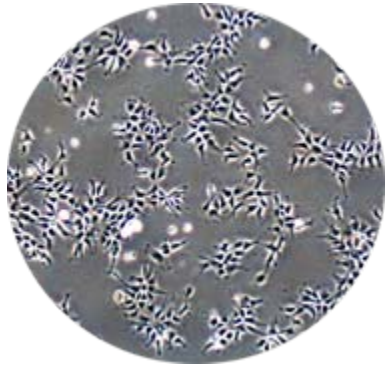
iPS for regenerative medicine

Hopes, dreams and nightmares…





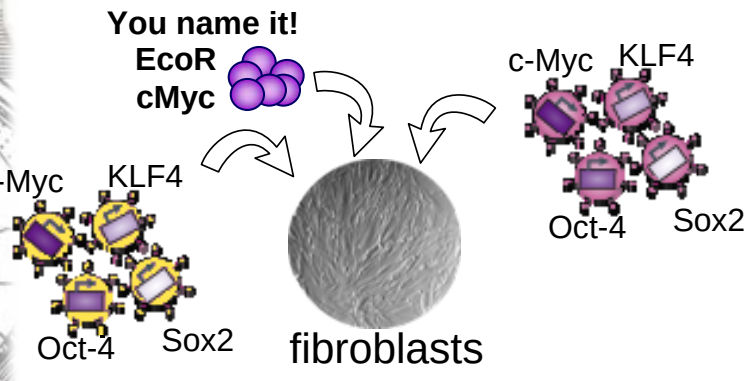
Marc Peschanski

I-STEM

Evry (Paris district) France

mpeschanski@istem.fr

Producing iPS for regenerative medicine



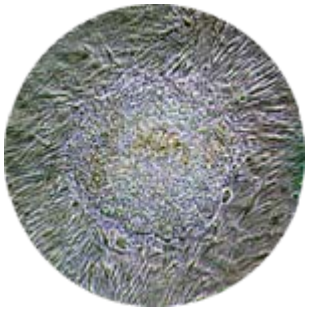
1d

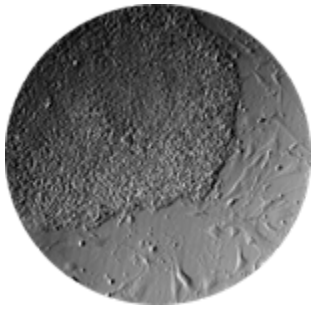
Plating on gelatin

1d

Culture in hES medium + **VPA**

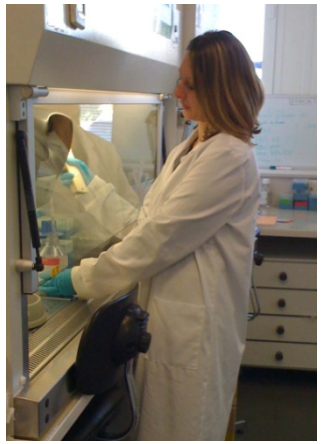
3-5w





iPS clones

Emergence of « ES-like » clones → From controls or patients fibroblasts

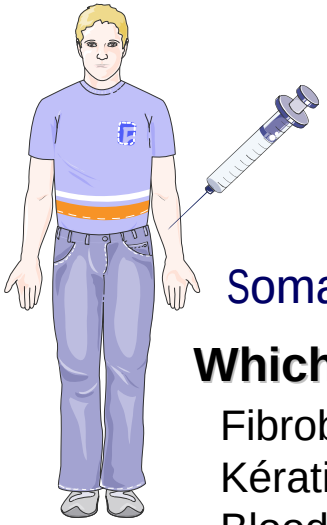


iPS, what do we expect from them in regenerative medicine?

- Like ES cells
  - Unlimited resource at the undifferentiated stage
    - *Industrialization of the production process*
    - *GMP compliance and comprehensive control*
  - Fully versatile capacity at differentiation
    - *Access to « any » cell type at « any » stage of differentiation*
    - *Cell populations at near homogeneity or enriched cultures*
  - Open ability at genetic engineering
    - *To provide them with additional phenotypes of interest*
    - *To improve protocols of production/differentiation*
    - *To address safety issues*

- Unlike ES cells,
  - A way to create pluripotent stem cell lines expressing any desired genotype

iPS production : methods and potential developments



Somatic cells

Which cells?

Fibroblasts
Kératinocytes
Blood cells

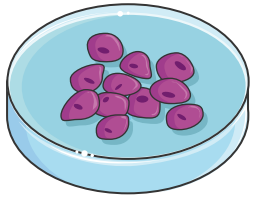
Which RF? Which way of delivery?

Oct-4/Sox2	Viruses (lenti/rétro)
KLF4/c-Myc	Episomes
LIN28/Nanog	Polycistronic vector
hTERT/SV40	Proteins

Reprogramming

Optimization of the reprogramming process?

Small molecules
Culture conditions



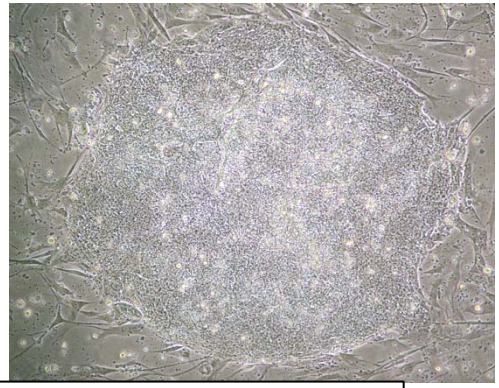
Induced pluripotent stem cells (iPS)

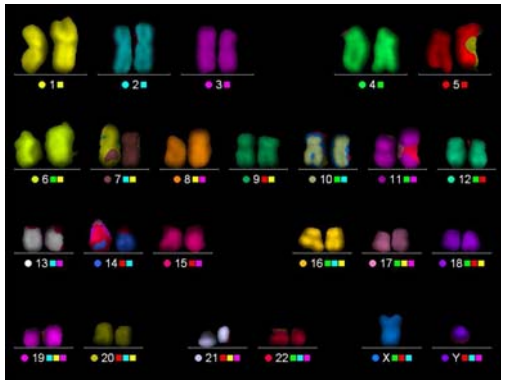
Selection?
Quality control?

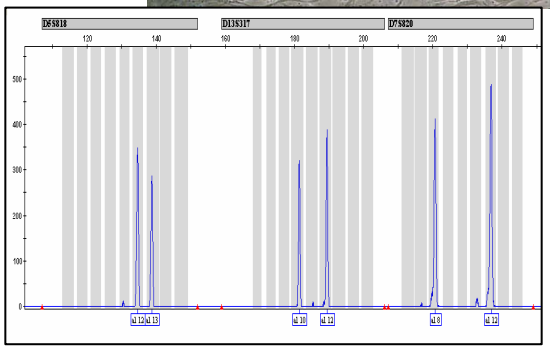
Morphology
Live markers

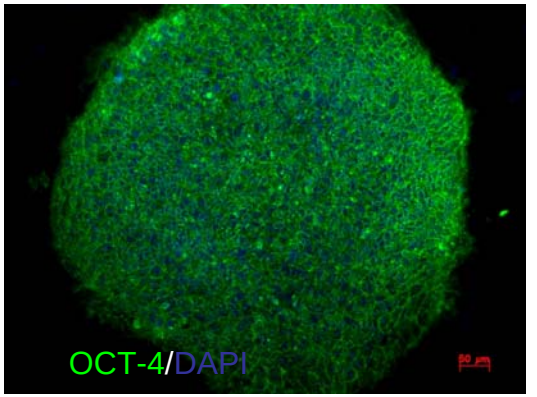
Quality control of iPS clones: QC similar to ES

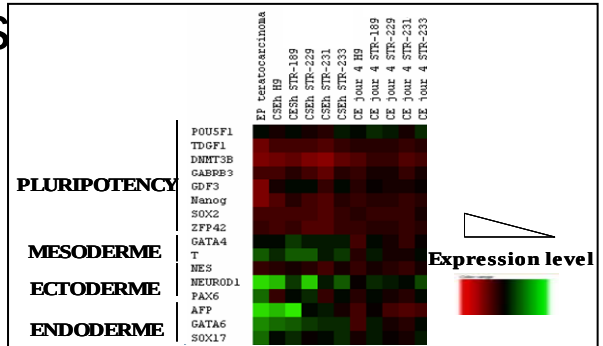
- Morphology
- Karyotype
- Genotype
- Pluripotency markers
- Differentiation capacities
  - Embryoid Bodies/3 germ layers
  - Directed differentiation





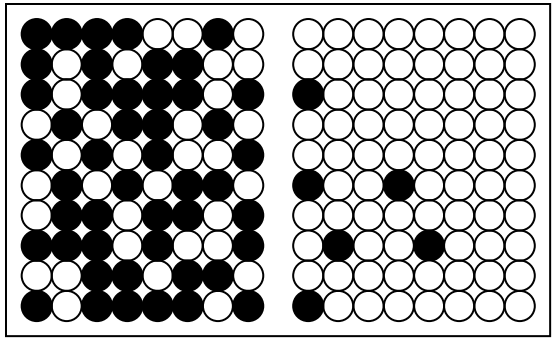




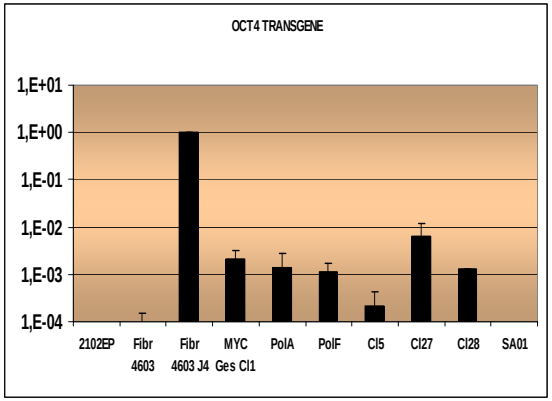


Controlling Quality of iPS lines for regenerative medicine: seeking which specific controls?

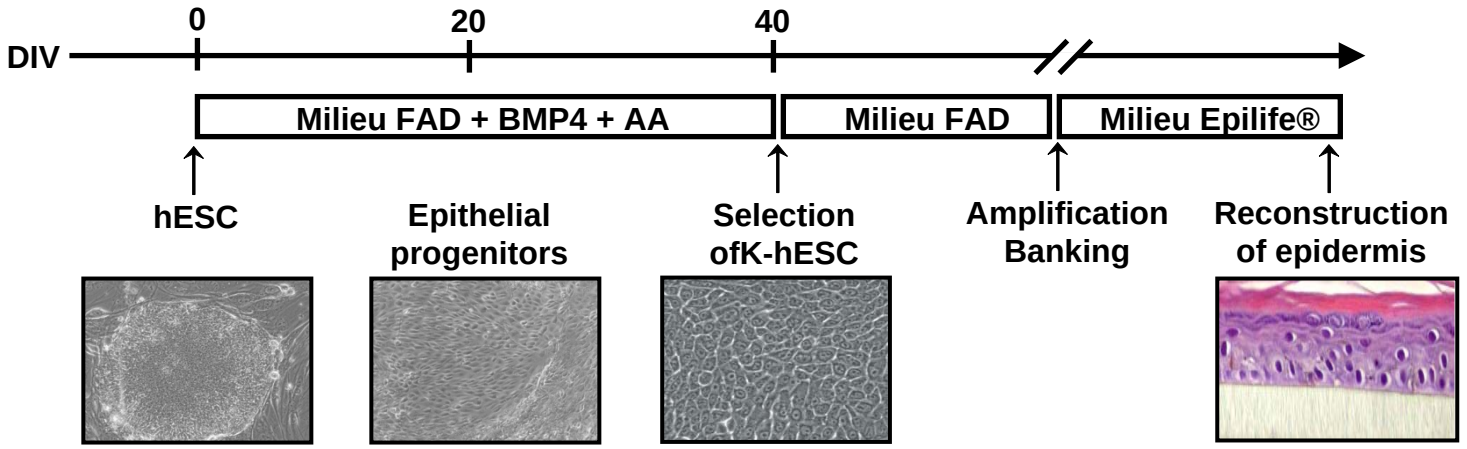
- Methylation status

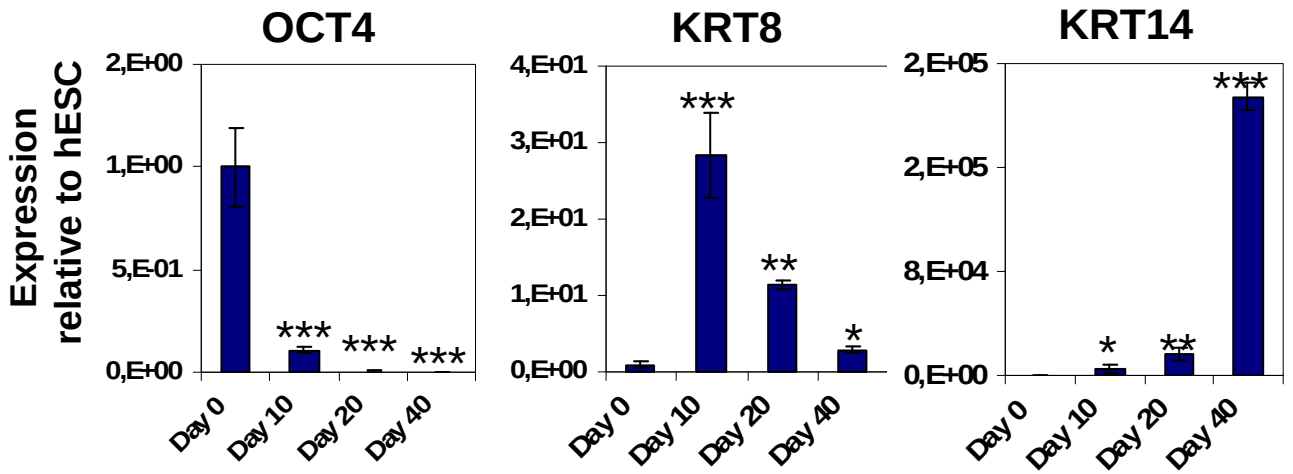


- Transgenes silencing



Pluripotent stem cells differentiation: How we do it with ES cells

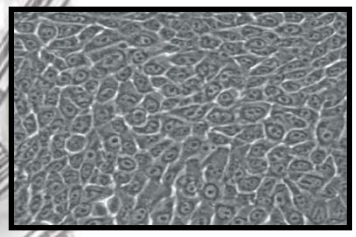




(Guenou et al., The Lancet, Nov 2009)

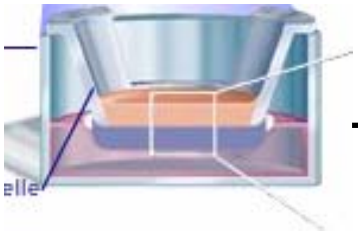
ES cells differentiation: how we check for potential in regenerative medicine

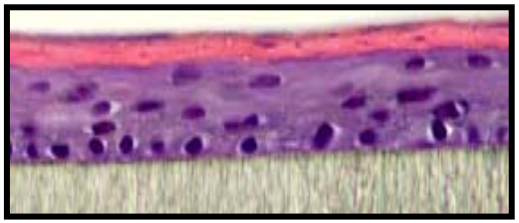
In vitro reconstruction of epidermis



Keratinocytes derived from hESC

In vitro

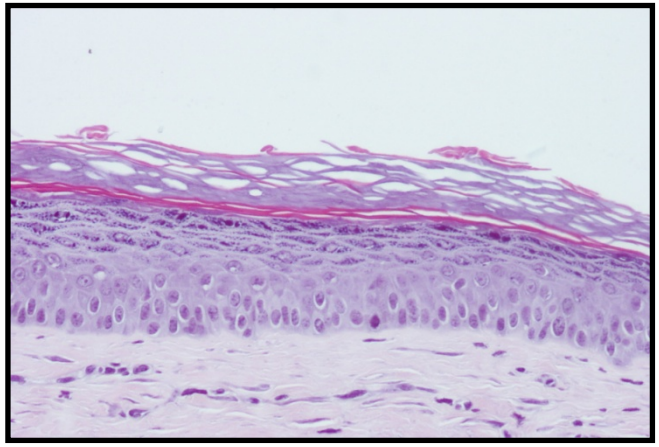




In vivo reconstruction of epidermis

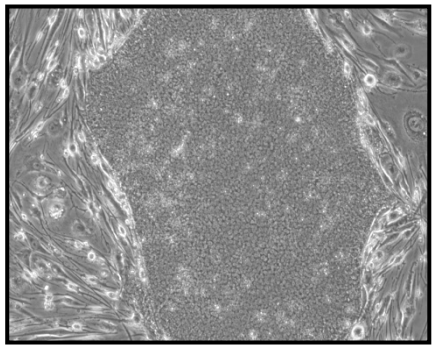
In vivo

Grafting in immunodeficient mice



iPS for regenerative medicine: is there a limit to their pluripotency?

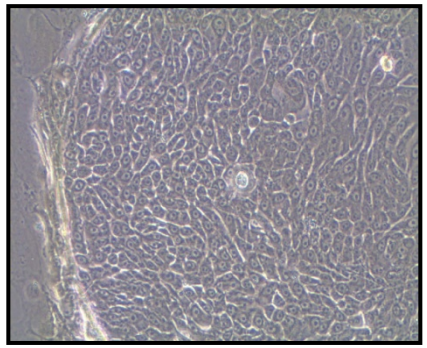
iPS-IMR90 / MRC5



BMP4 + AA

40 Jours

K-iPSC



Lentiviral Reprogrammation

OCT4 NANOG SOX2 LIN28

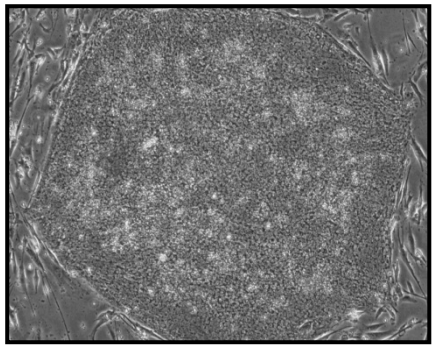
Fibroblasts

iPS

OCT4 KLF4 SOX2 c-MYC

Rétrovirale Reprogrammation

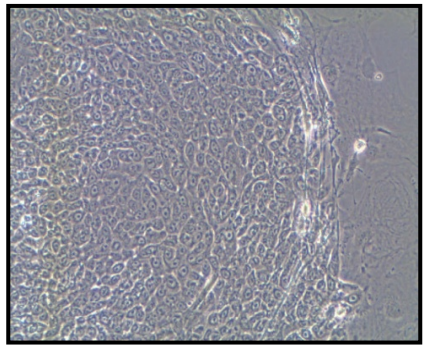
iPS-4603



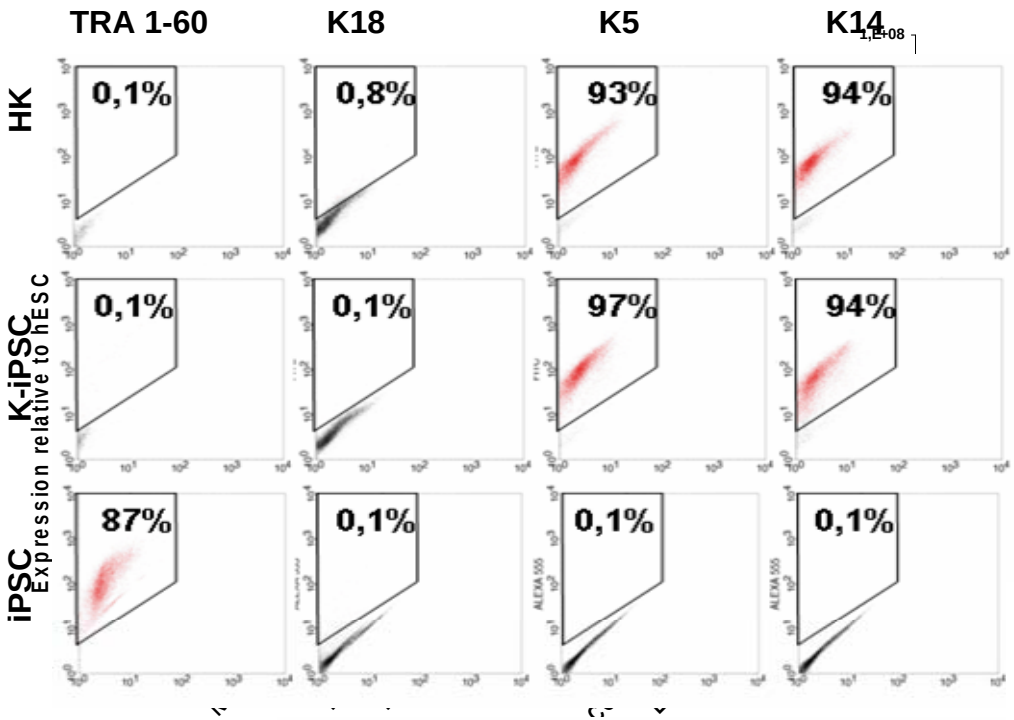
BMP4 + AA

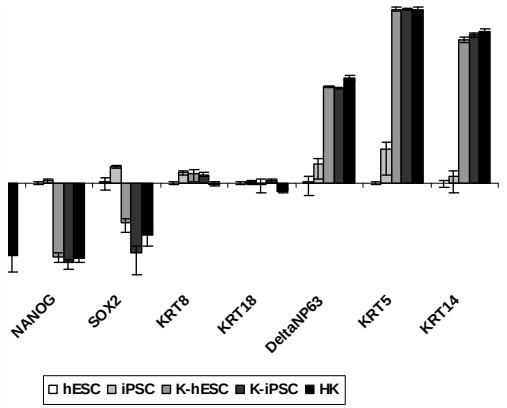
40 Jours

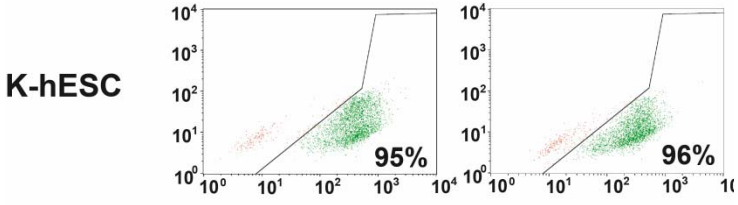
K-iPSC

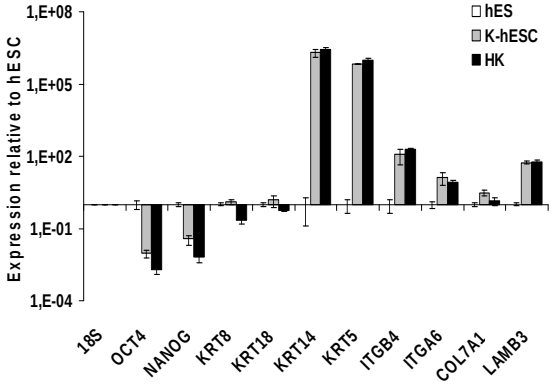


iPS derived keratinocytes: identical to ES-derived cells?

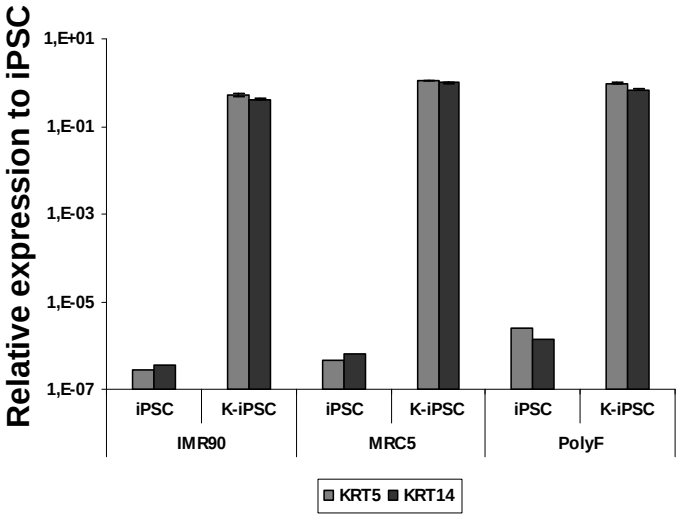


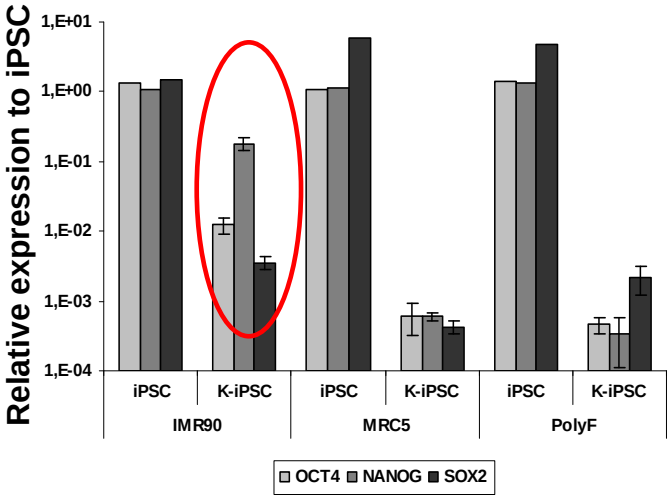




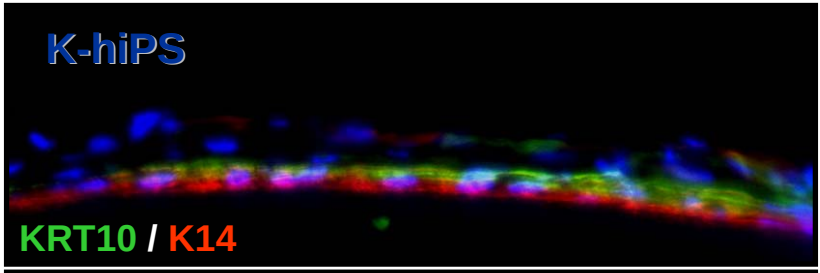


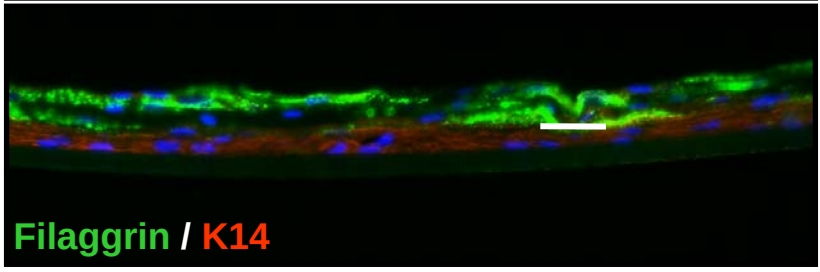
iPS for regenerative medicine: are all pluripotent stem cell lines alike?

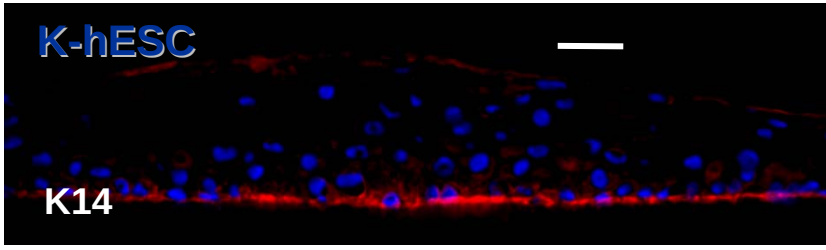


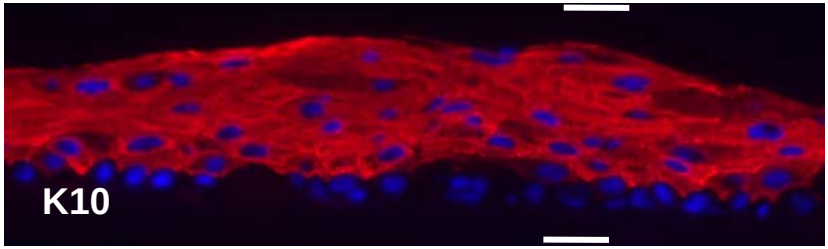


Organotypic culture







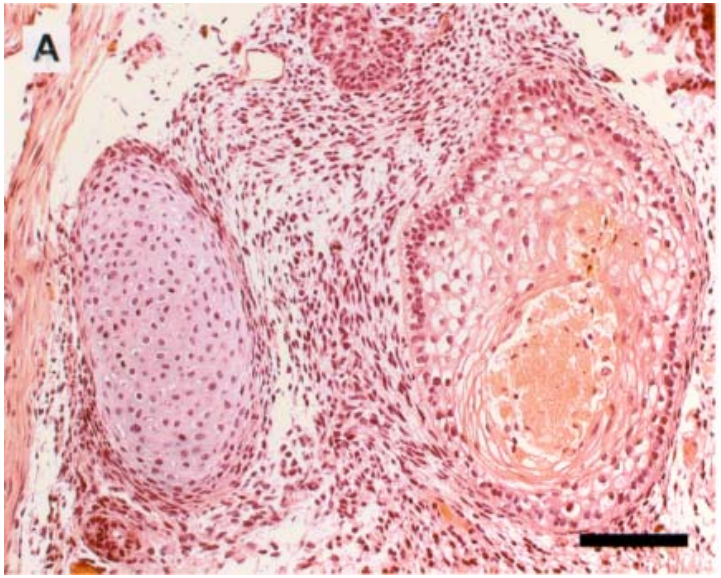


Engineering iPS cell lines

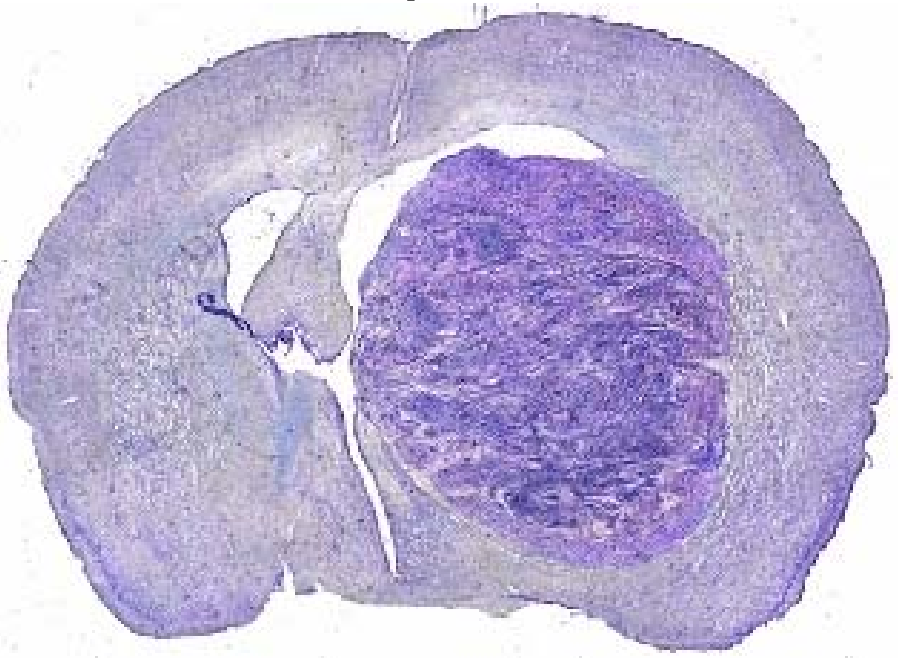
- **For improving protocols**
  - *One major logistic issue: « good » and « bad » clones?*
  - *Looking for new reprogrammation/differentiation protocols*
- **For improving the end-product**
  - *Introducing selection markers*
  - *Coaxing cells to specific cell phenotypes*
- **For addressing safety**
  - *Pre-graft action*
  - *Post-graft « off-switch »*

Real and fantasmatic safety issues with pluripotent stem cells tumorigenicity

Teratomas: actually not that worrysome (and let's be careful about terms!)



Post-graft control of normal progenitors proliferation: the actual problem



What iPS can do for regenerative medicine that ES cells can't: the international haplobank prospective

- **Objectives**

  Providing clinicians with clinically compatible iPS cell lines and, when relevant, cell therapy products

  Contributing to an international network of clinically compatible iPS cell banks for meeting cell therapy needs of the entire world population

- **Focus**

  Collecting and banking samples from haplotypically homozygous donors for HLA-A, -B and –DR (bone marrow registries contain 0.5-0.6%)

  Developing and implementing GMP technology for derivation of iPS cell lines, banking and differentiation into specific therapeutically relevant cell phenotypes

Hemi-similarity: a biological basis to make a cell bank foreseeable

Haplotypically homozygous donors in the French registry: altogether >200 haplotypes (broad types)

Haplotypes differ from one population to another in proportions, i.e. initiative for a world cell bank consortium is timely

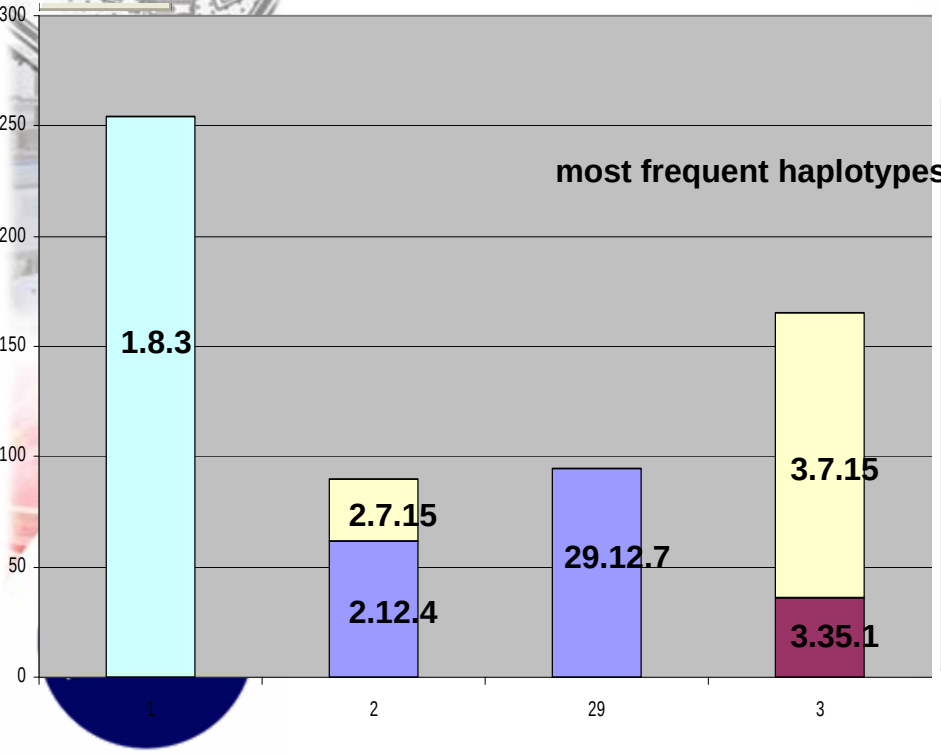


Tableau 5 : Fréquences géniques de différents allèles DRB1 dans des populations caucasiennes, africaines et asiatiques. (Colombani, 1993)Abréviations: FRA: France, DAN: Danemark, GER: Allemagne, ITA: Italie, ROU: Roumanie, SPA: Espagne, US: Etats-Unis, CAN: Canada, SEN: Sénégal, JAP: Japon.

Allèles	Populations caucasiennes								Autres	
	FRA	DAN	GER	ITA	ROU	SPA	US	CAN	SEN	JAP
DRB1*0101	9,3	13,0	6,7	6,5	7,6	6,6	7,3	5,6	0,6	4,9
DRB1*0301	10,9	10,2	9,4	10,5	11,4	6,7	9,5	12,3	10,2	0,4
DRB1*0401	5,6	17,6	8,1	2,3	4,2	5,6	6,7	9,5	-	1,8
DRB1*0701	14,0	14,8	12,3	12,5	8,3	18,9	14,4	9,4	7,8	0,6
DRB1*1101	9,2	0,9	9,2	12,4	7,3	1,0	4,4	2,6	9,3	2,0
DRB1*1301	6,0	8,3	4,5	4,8	4,4	4,5	5,1	4,7	4,7	0,7
DRB1*1501	8,0	17,6	7,8	5,6	6,2	9,4	10,3	10,4	-	6,8
Total	63,0	82,0	58,0	55,0	49,0	53,0	58,0	55,0	33,0	17,0
DRB1*1304	-	-	-	0,2	-	-	-	-	25,3	-
DRB1*0405	1,6	-	0,6	-	0,7	2,1	0,7	-	0,6	12,3


istem