

COURS CELULES SOUCHES (D. Aberdam) 2010

Ma 2/3: cours 1: Définitions et généralités sur les CS

Ve 5/3: cours 2: CS somatiques et plasticité

Ma 8/3: cours 3: CS hématopoétiques (Dr. Rouleau)

Ve 12/3: cours 4: CS embryonnaires

Ma 16/3: Partiel 1

Ve 19/3: cours 5: CS adultes et physiopathologie

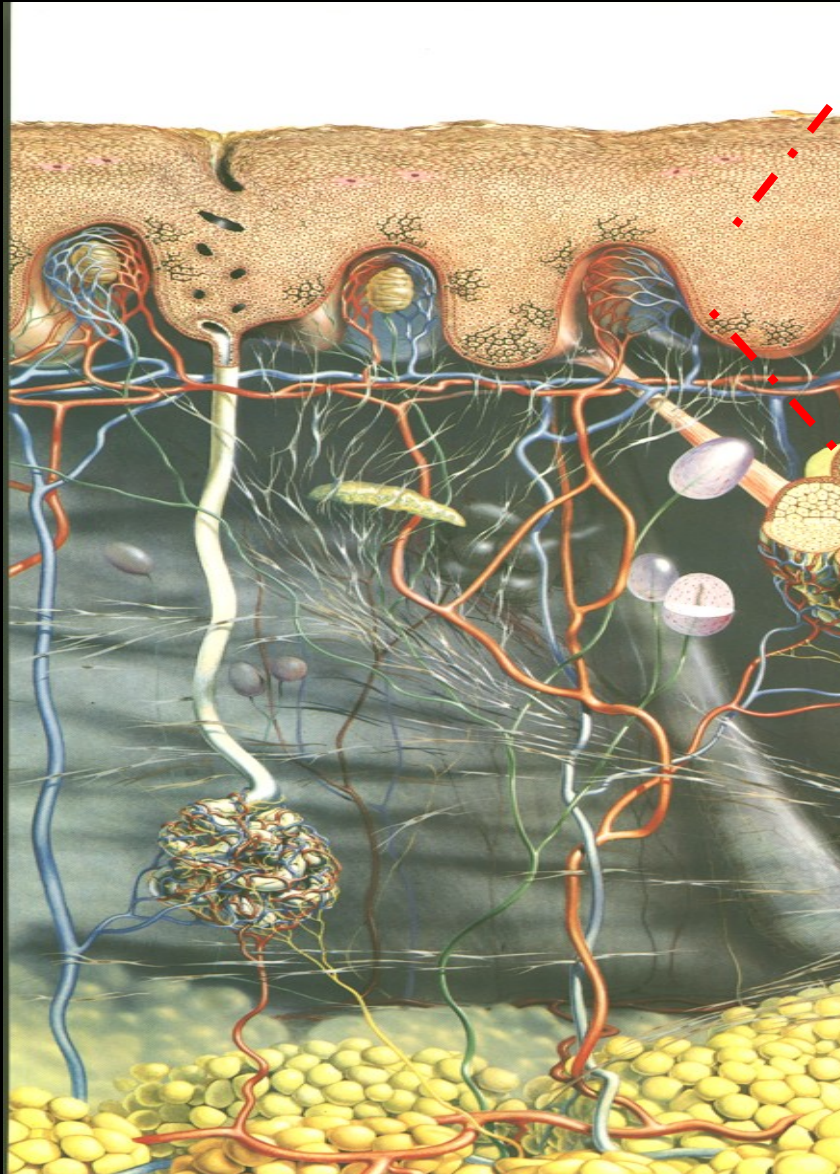
Ma 23/3: cours 6: CS reprogrammées (iPS) + labo

Ve 26/3: ~~Cours 7: CS tumorales~~ **Partiel 2**

~~Ma 30/3: Partiel 2~~

Examen: 28 mai

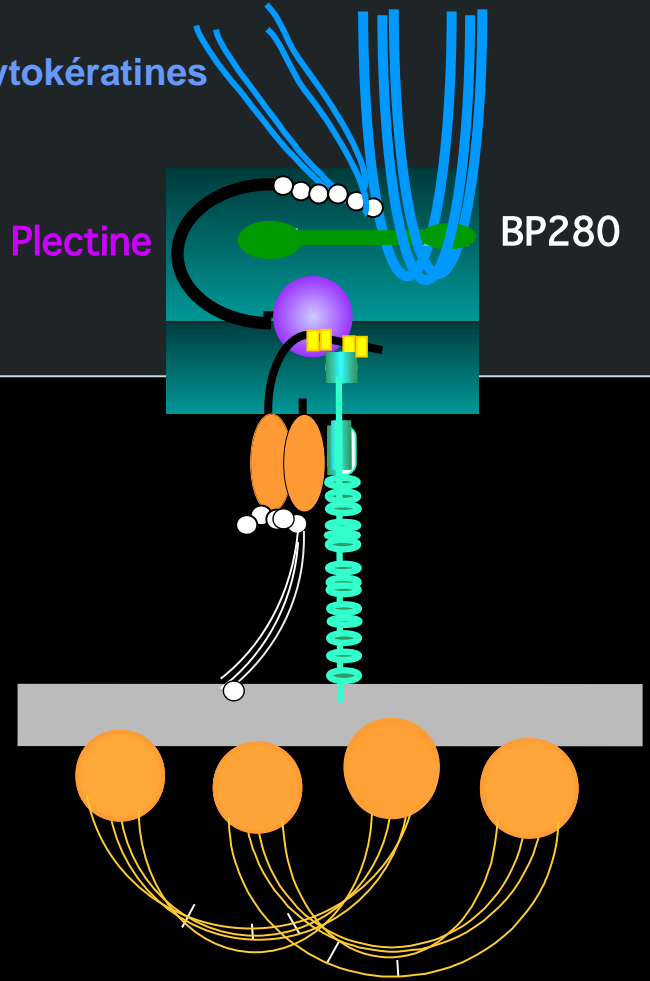
kératinocyte



Cytokératines

Plectine

BP280

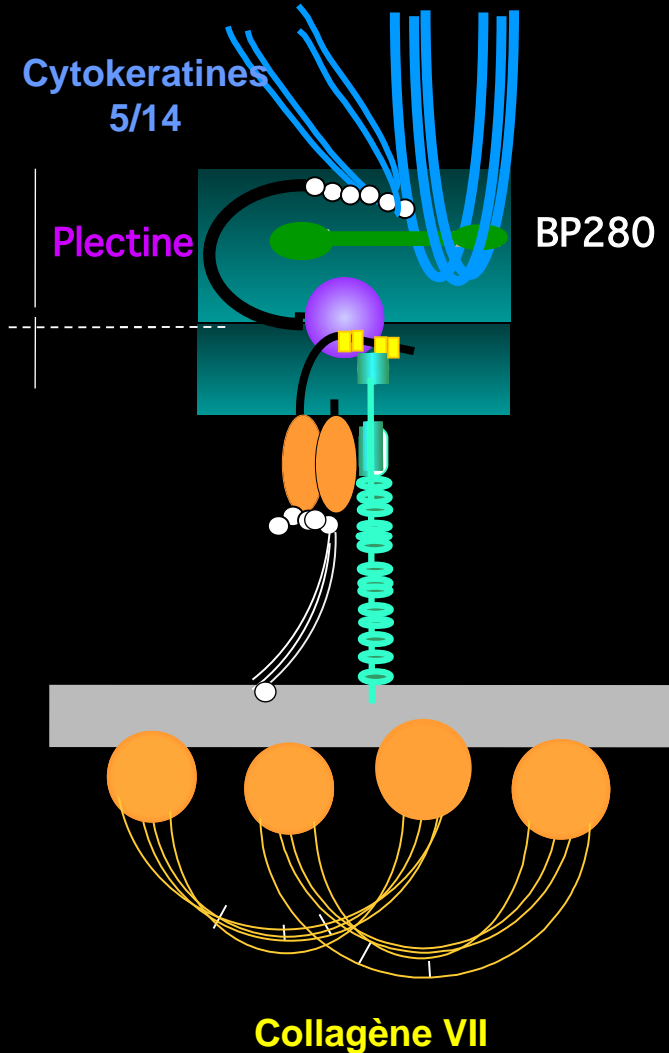


Collagène VII

EPIDERMOLYSIS BULLOSA

(EB)

Epidermolyse Bulleuse Simplex
Epidermolyse Bulleuse Jonctionnelle
Epidermolyse Bulleuse Dystrophique

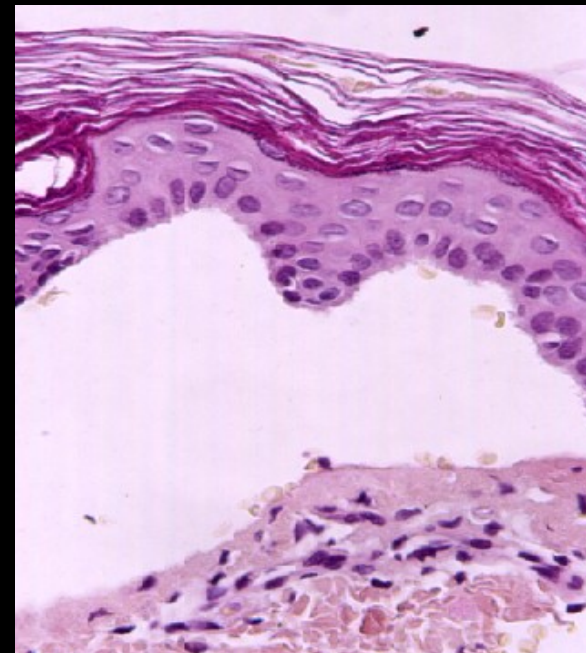


EBS
(R)

EBJ
(R)

EBD
(D et R)

Peau EB



Epiderme

Derme

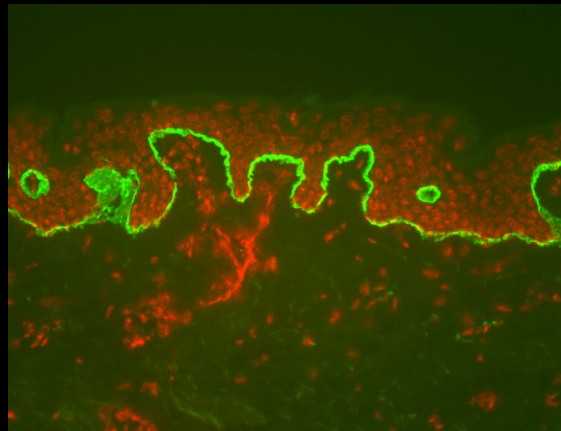
Anticorps monoclonal GB3

Immunisation de souris par des antigènes

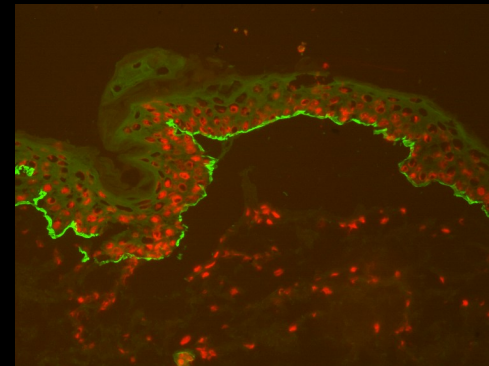
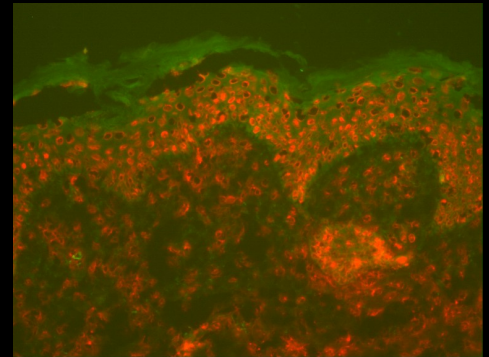
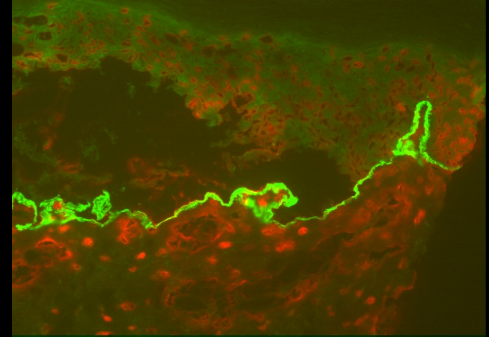
Collecte du sérum (lymphocytes B)

Fusion des lymphocytes B avec une lignée lymphoma: hybridome

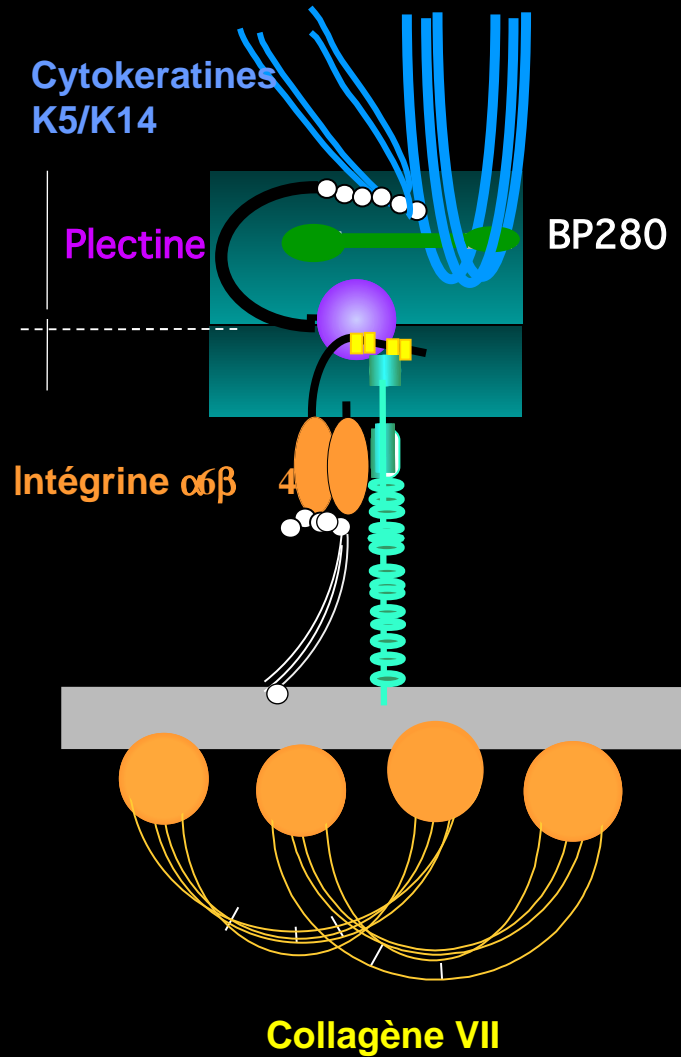
Sélection clonale d'un hybridome



Anticorps monoclonal GB3



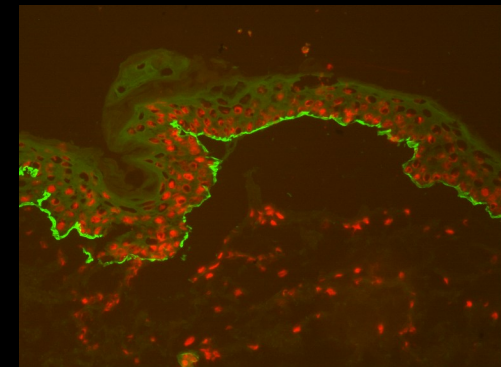
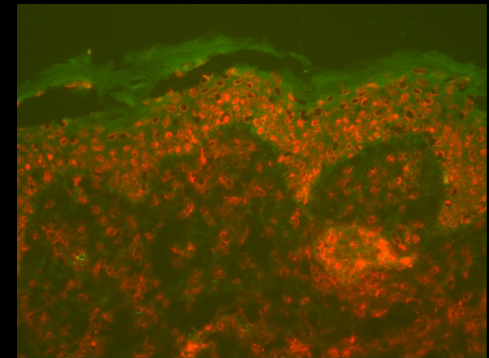
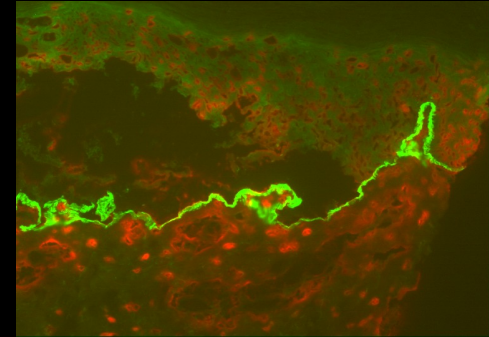
Anticorps monoclonal GB3



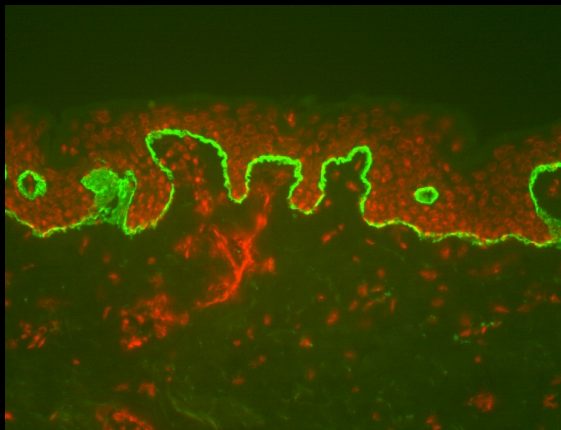
EBS

EBJ

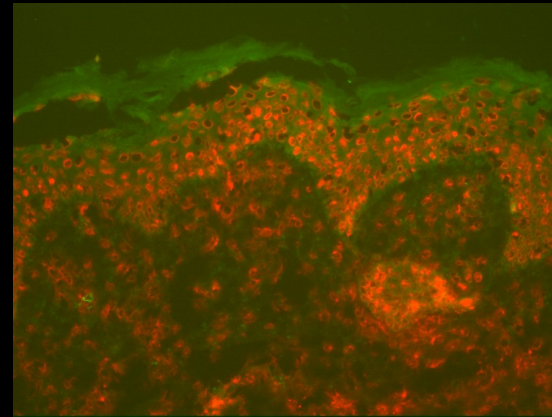
EBD



•Epidermolyse bulleuse jonctionnelle(EBJ)



contrôle



EBJ



Cellules du patient

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graph TD; A[Cellules du patient] --> B[ADN génomique]; A --> C[ARNm]; A --> D[Protéines]; B --- B1[Southern blot]; B --- B2[PCR]; B --- B3[Banque génomique]; B --- B4[ChIP]; C --- C1[Northern blot]; C --- C2[RT-PCR]; C --- C3[Banque d'ADNc]; C --- C4[Banque d'expression]; D --- D1[Western blot]; D --- D2[IP]; D --- D3[Anticorps];
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ADN génomique

Southern blot

PCR

Banque génomique

ChIP

ARNm

Northern blot

RT-PCR

Banque d'ADNc

Banque d'expression

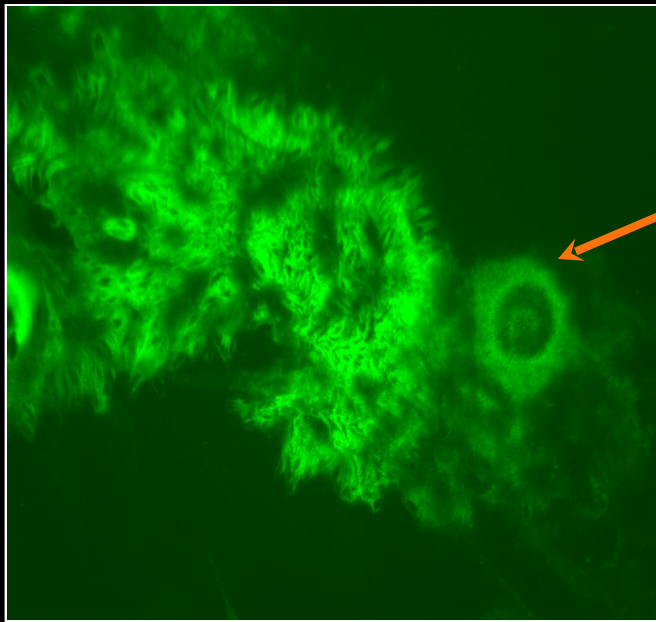
Protéines

Western blot

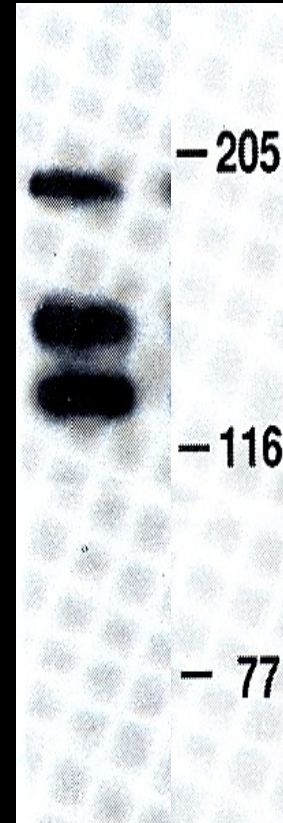
IP

Anticorps

Molécule trimérique, sécrétée dans la MEC



IF

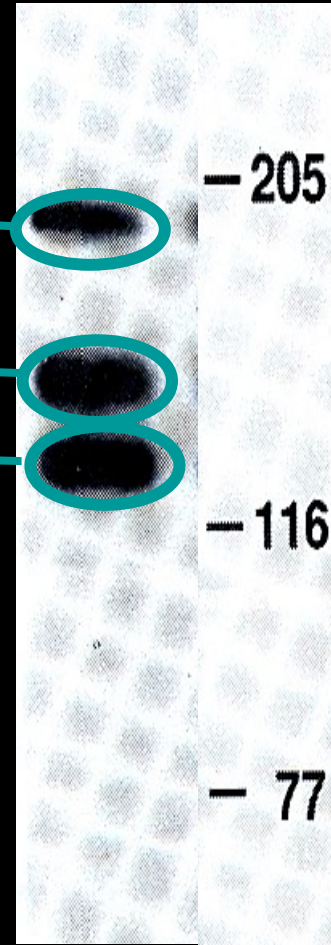


IP

Colonne d'affinité GB3

Criblage de
banque
d'expression

Anticorps
polyclonaux

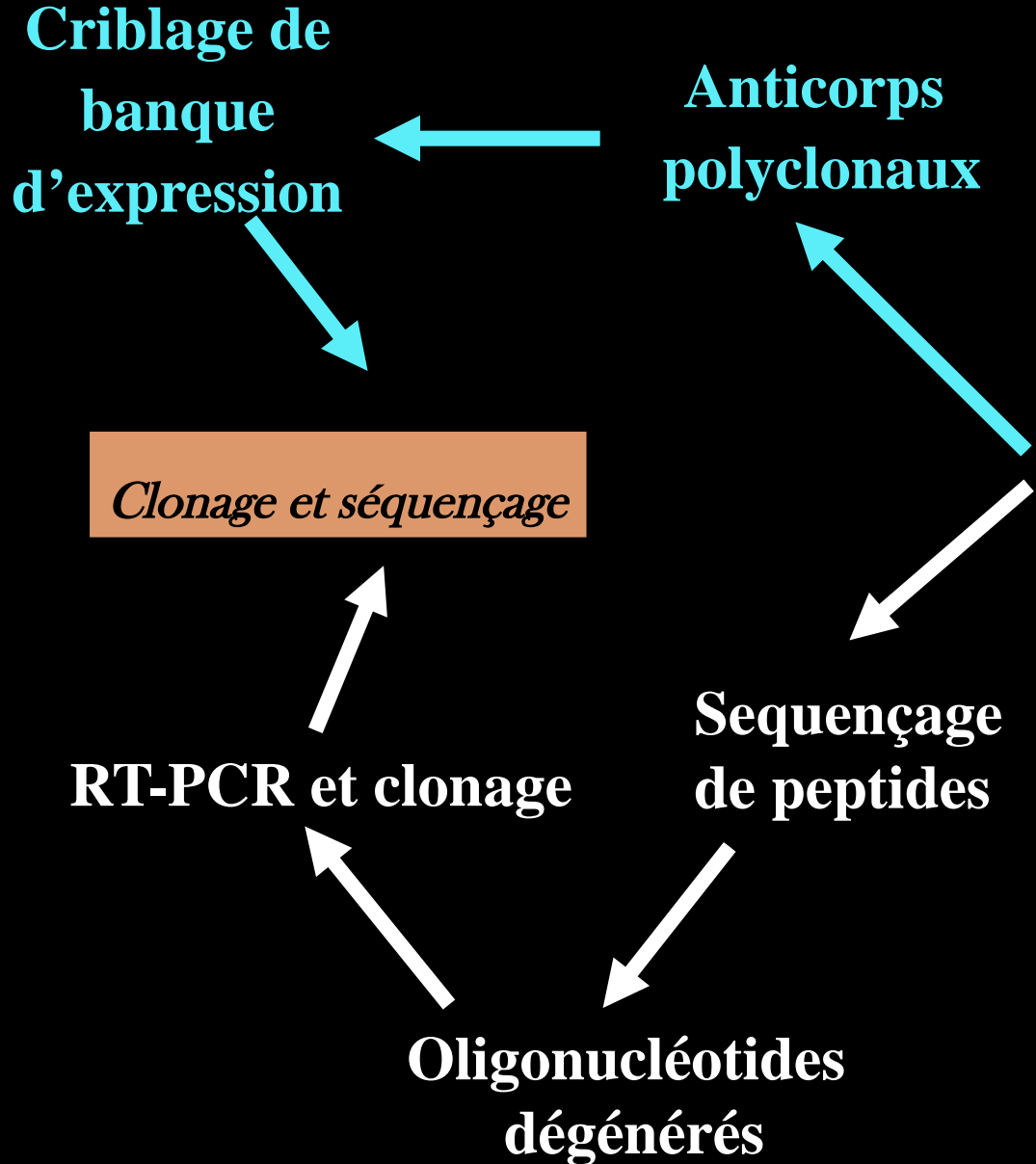


Clonage et séquençage

RT-PCR et clonage

Séquençage
de peptides

Oligonucléotides
dégénérés



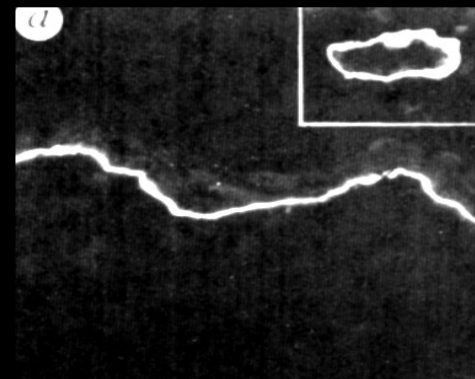
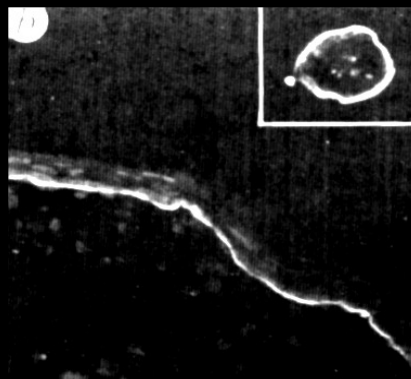
Immunofluorescence sur biopsies cutanées

$\alpha\beta$

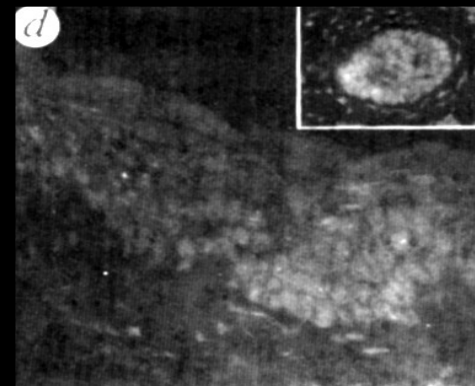
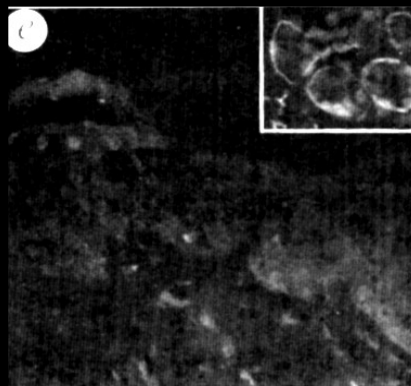
β 3

$\gamma 2$

Contrôle



Patient



Liaison génétique entre le gène LAMC2 et l'EBJ-H

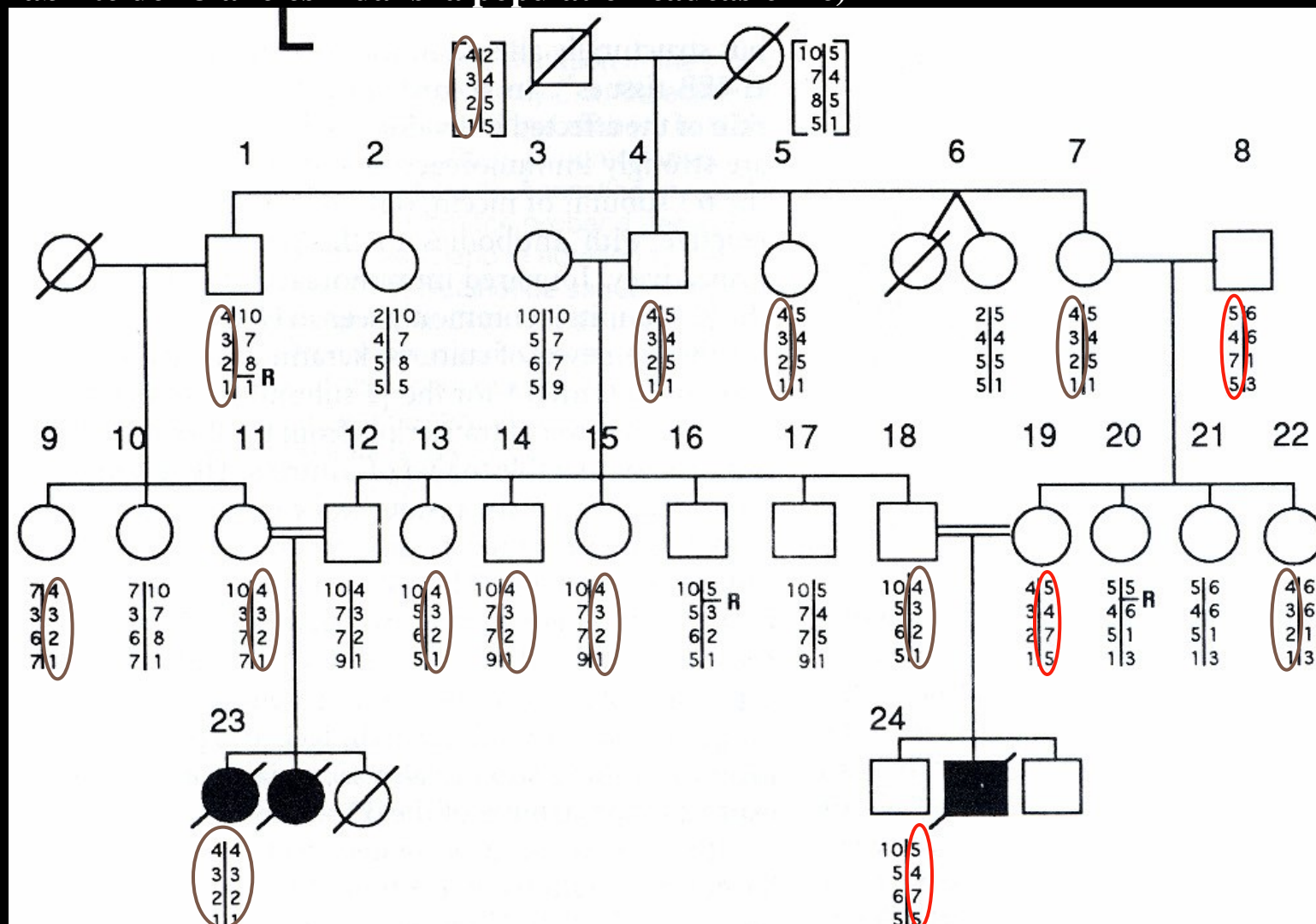
Diff cultés: trois gènes. caractère récessif et léthal 3 familles consanguines

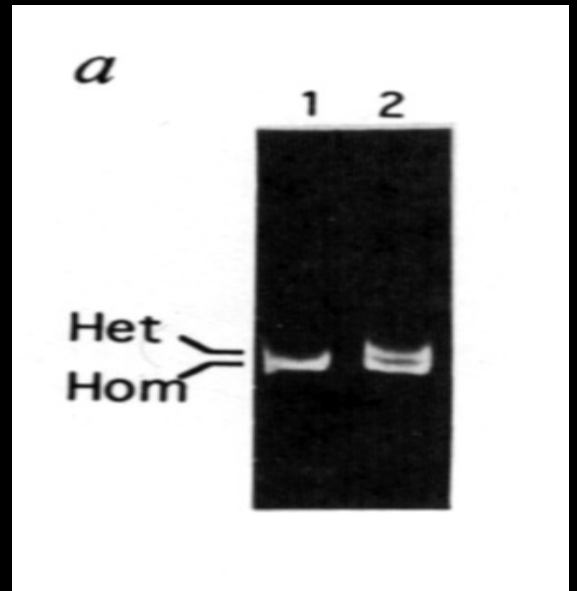
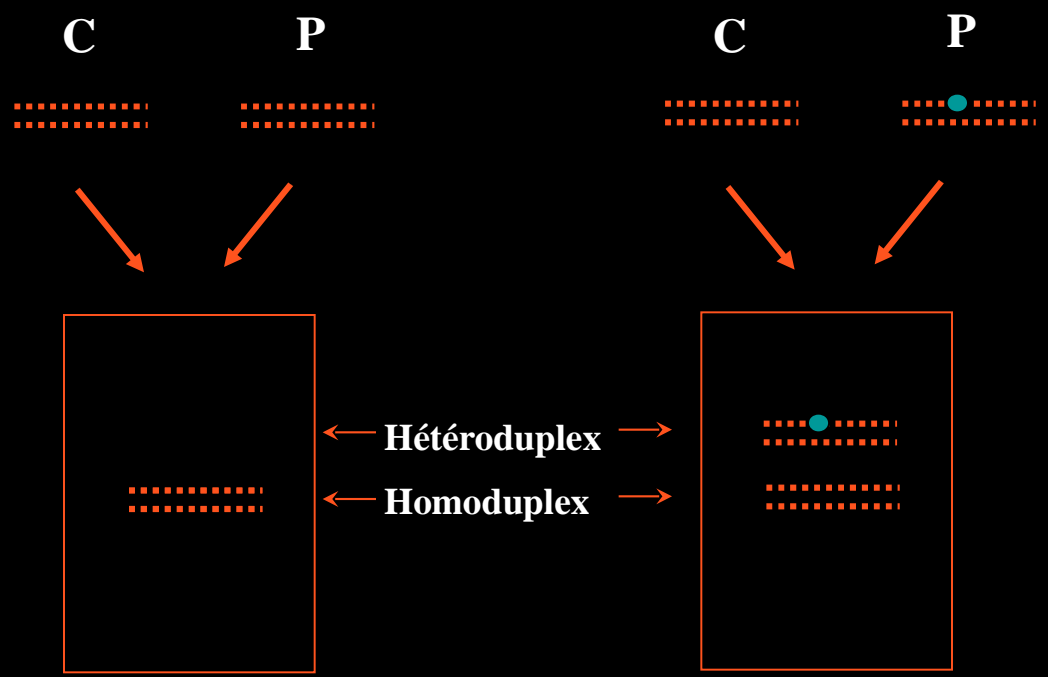
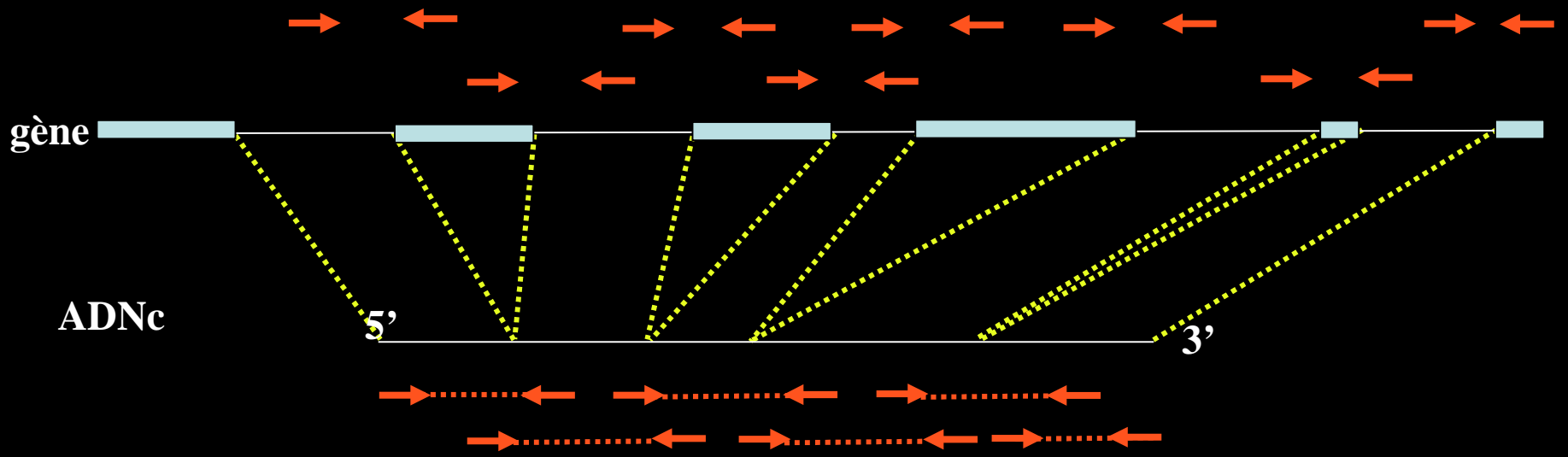
Recherche de microsatellites CA/GT proches (ou dans le locus LAMC2)

3 marqueurs AFM + un microsatellite lié à LAMC2

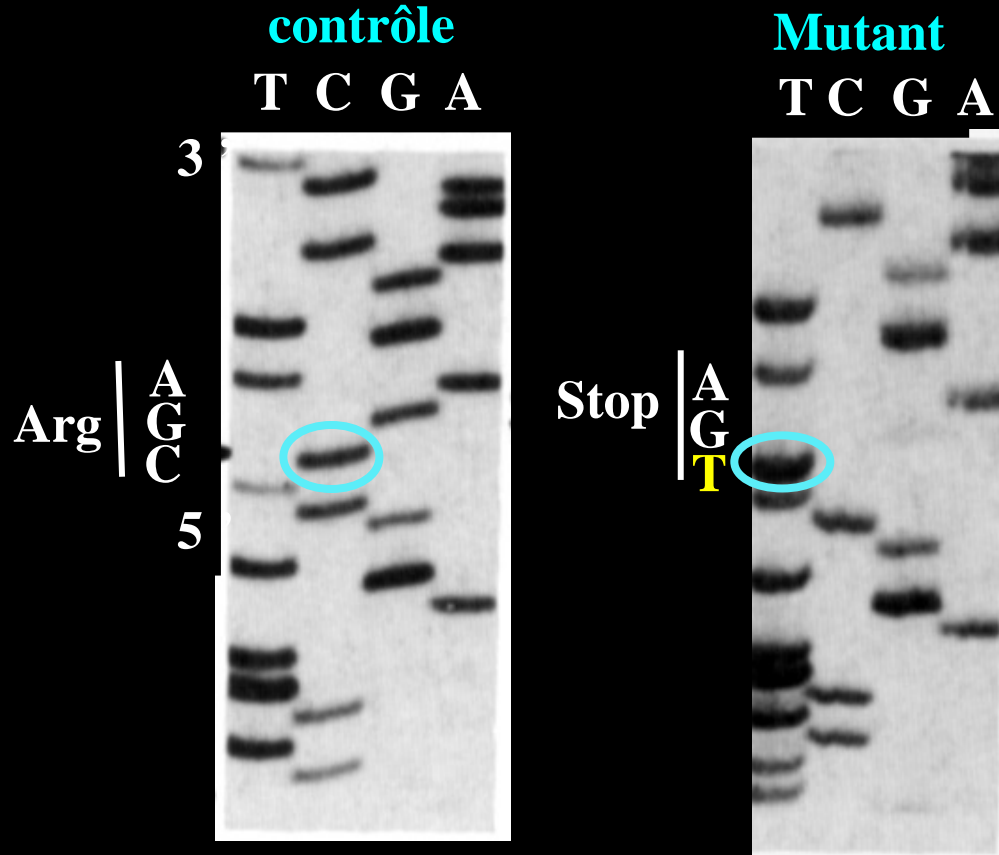
(variabilité de 13 allèles # dans la population caucasienne)

— CACA...CACA —
 → ←

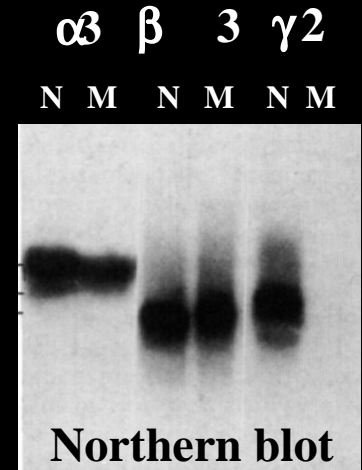
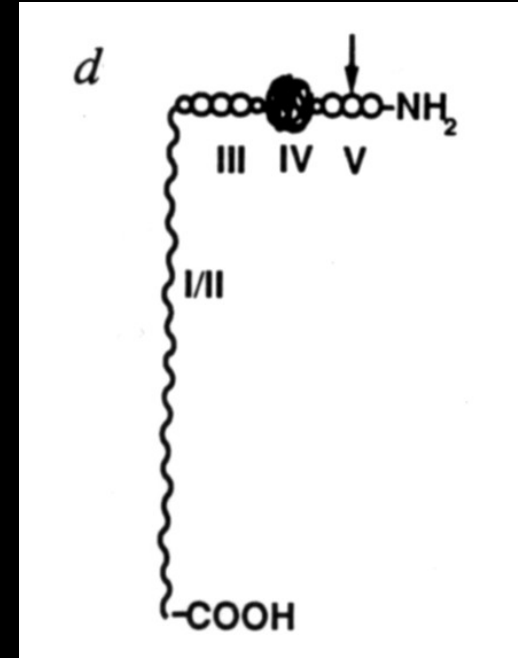




Famille consanguine. Patient homozygote



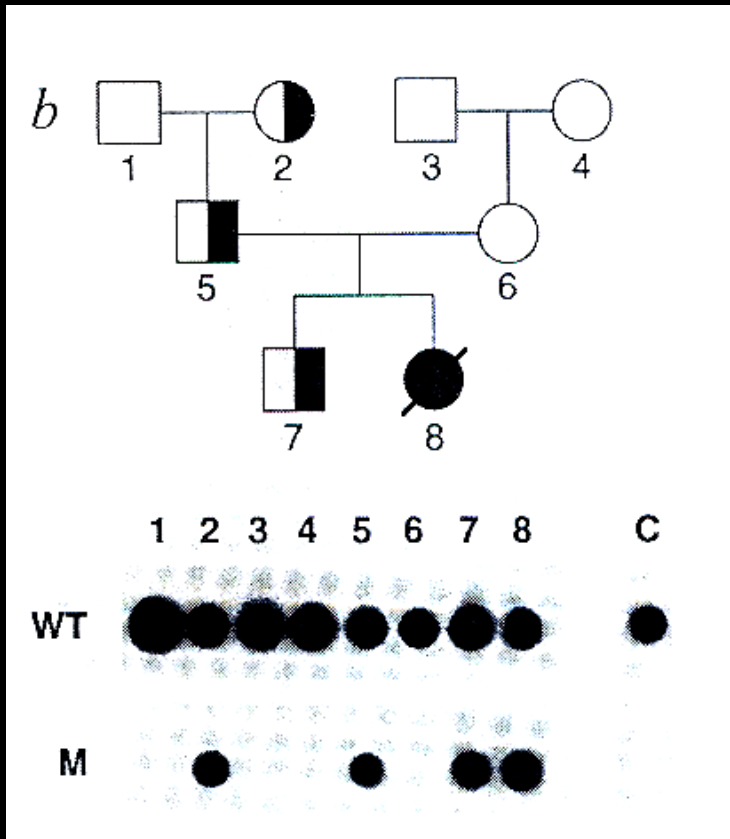
Exon 3



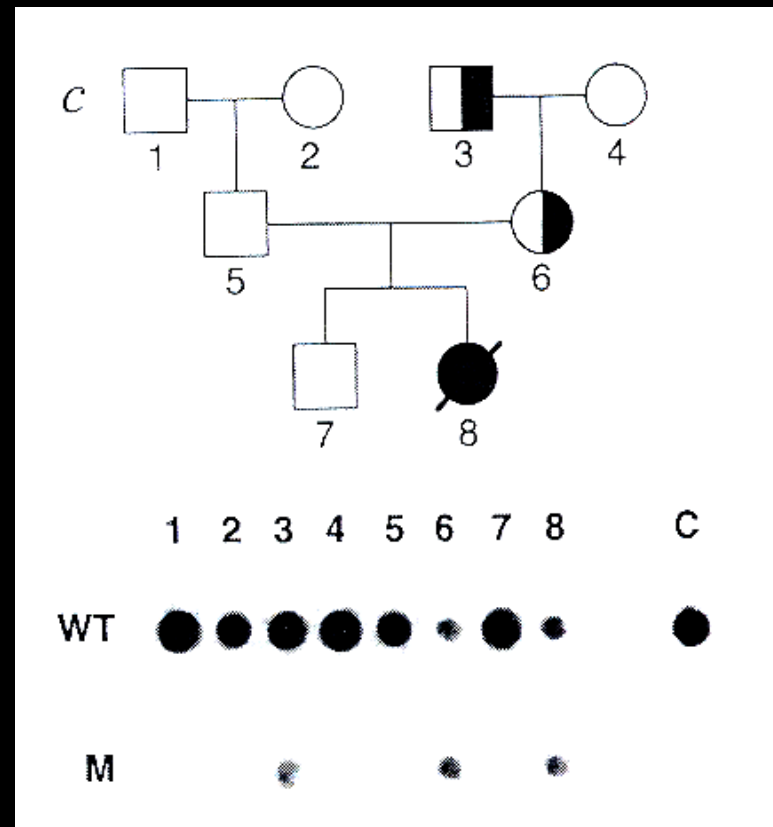
Allele-Specific Oligonucleotide (ASO)

Exemple d'une famille non consanguine

Mutation portée par la mère



Mutation portée par le père



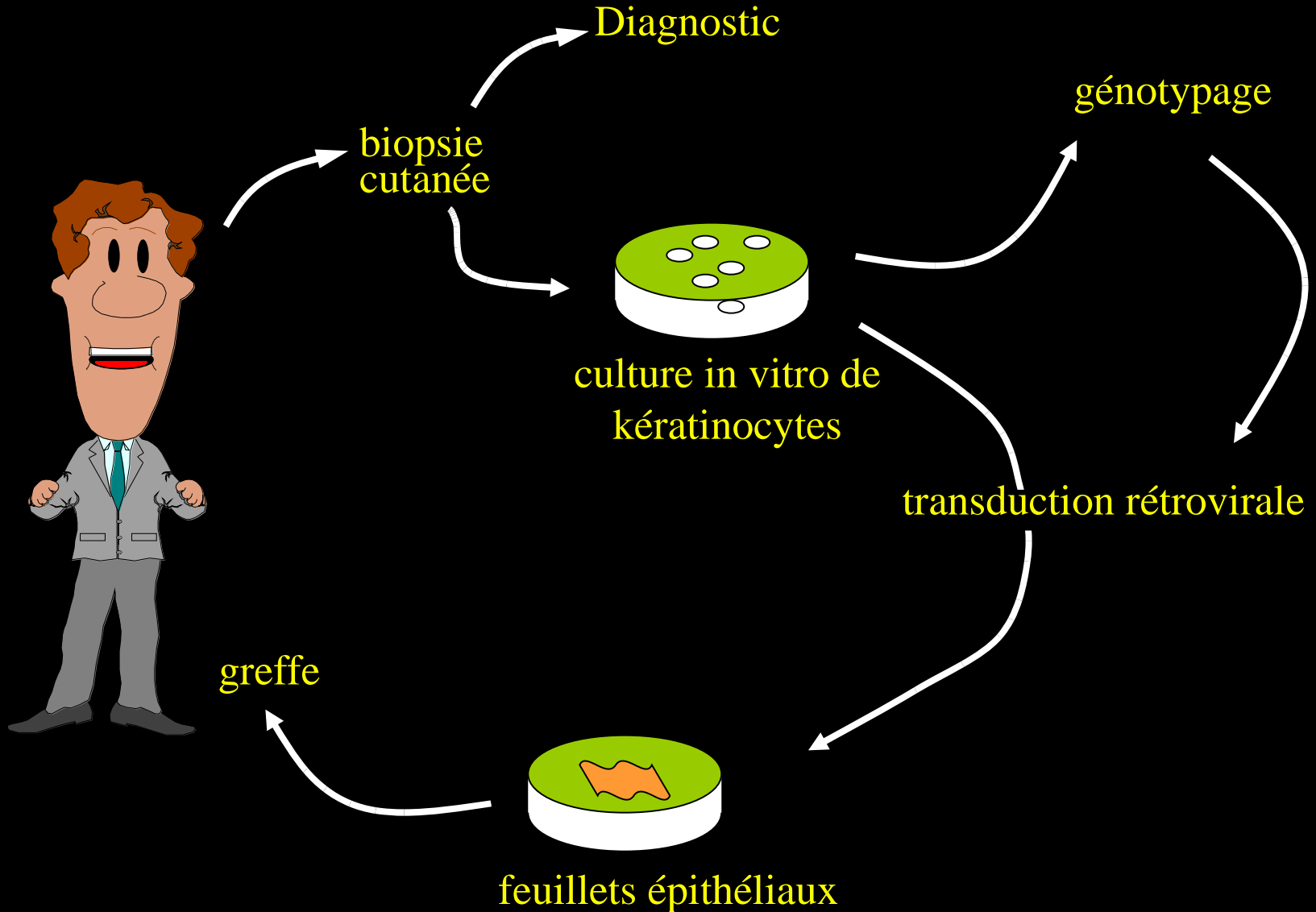
Formes non léthales d'EBJ



- **Expression réduite de laminine-5 mutée**
- **Etat traumatique, sans soins efficaces**

THERAPIE GENIQUE CUTANEE

Ex vivo



Thérapie génique des EB

Pré-diagnostic/génotype:

Diagnostic (immunohistologie, moléculaire)

Recherche de mutation

Préparation de gène:

Clonage in vitro

Choix du vecteur (lentivirus, intégrases,...)

Transduction phénotypique

Modèles:

Modèles animaux

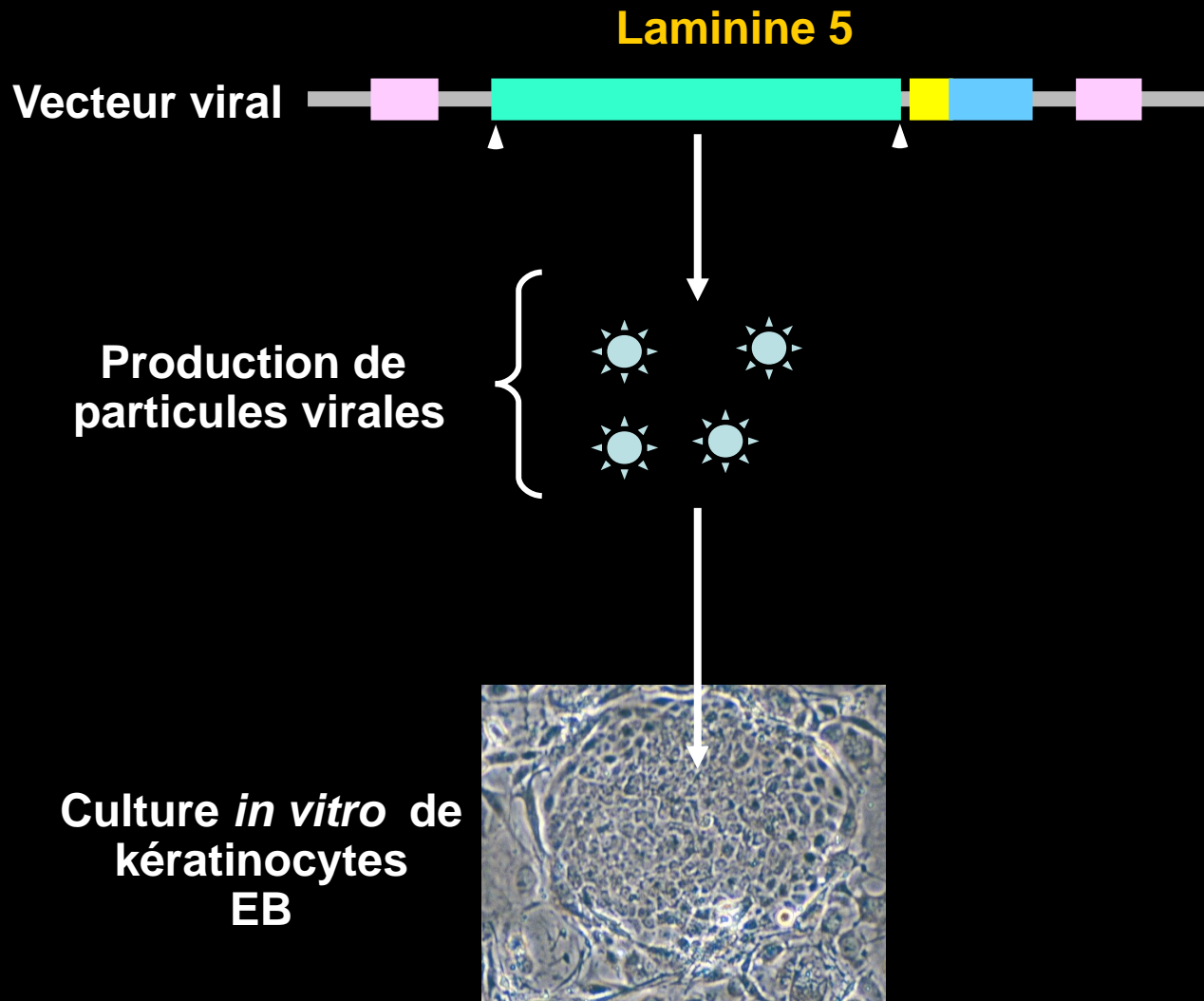
Transplantation de greffe

Évaluation de la réponse immunitaire

Expression du transgène in vivo sur du long terme

Évaluation des risques de mutation insertionnelle du transgène

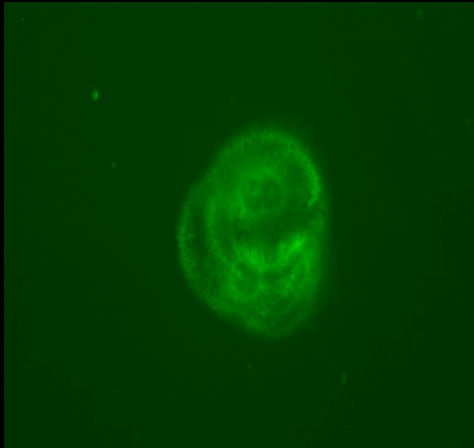
PRODUCTION D 'UN VIRUS « CURATIF »



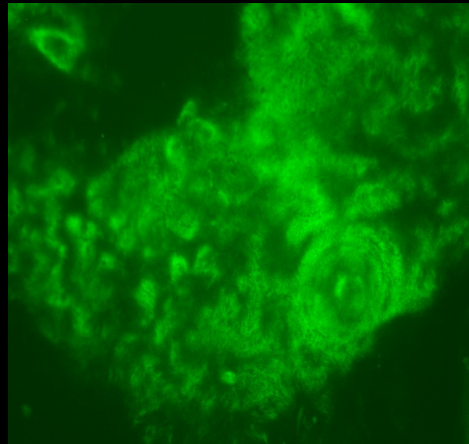
Réversion phénotypique de kératinocytes EBJ

Chaîne $\beta 3$ - mutations R42X / E210K

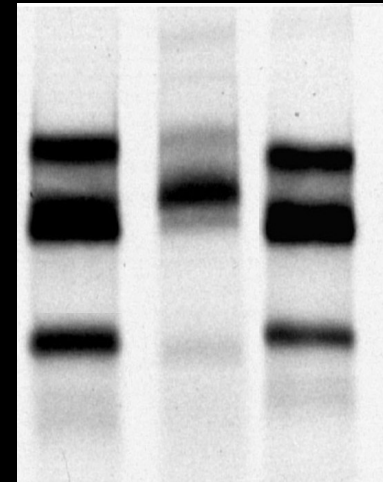
EBJ

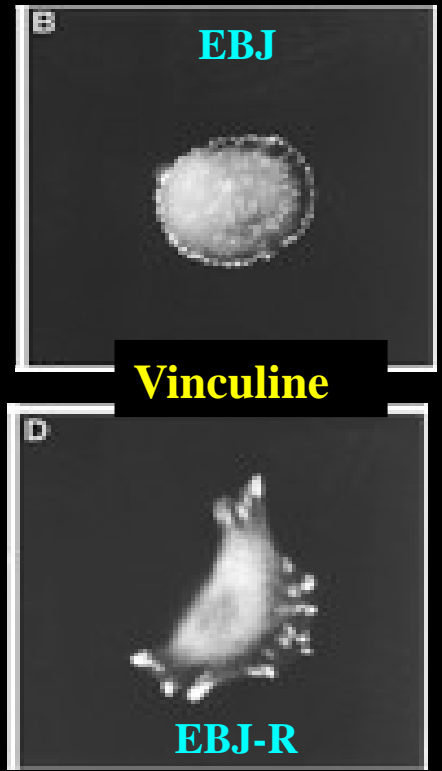
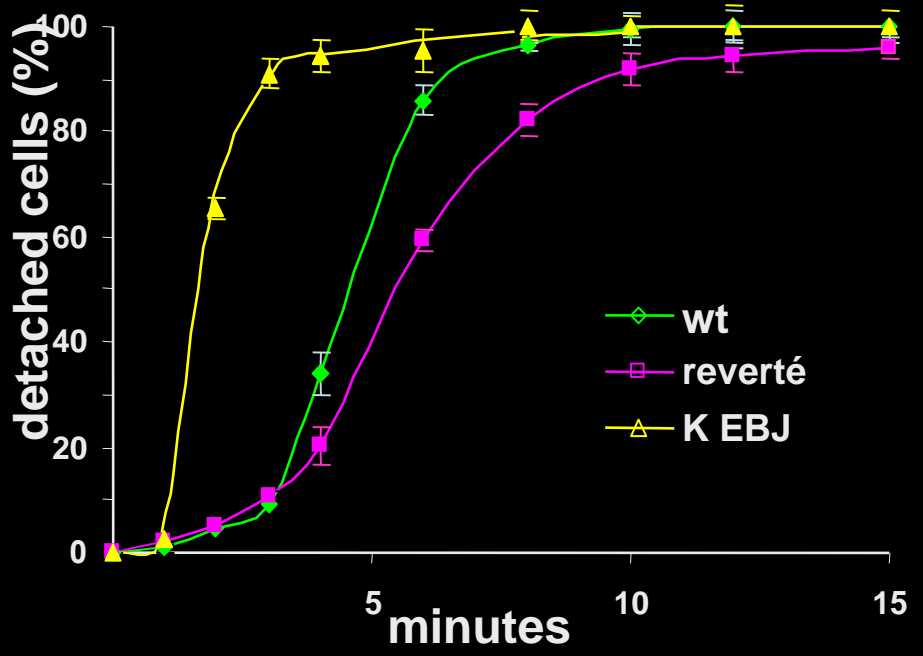
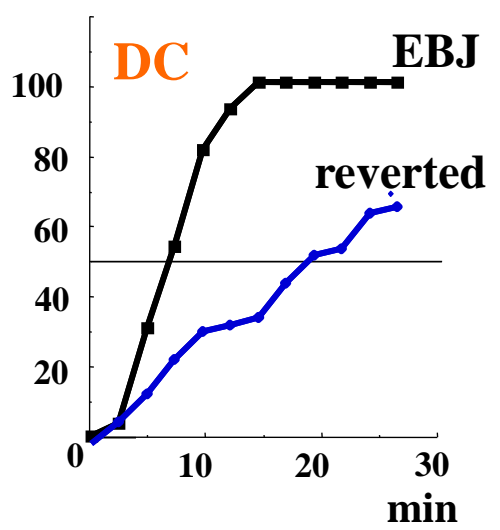
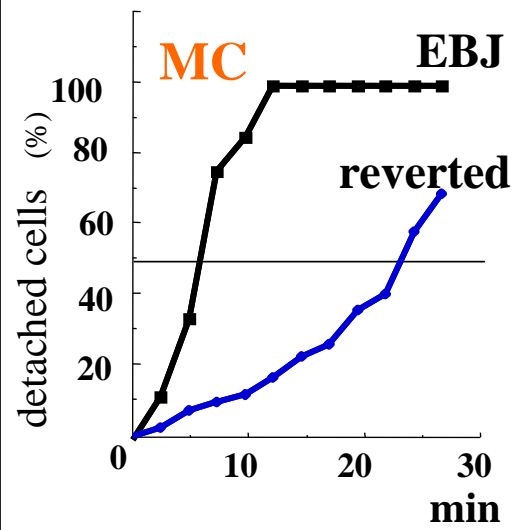


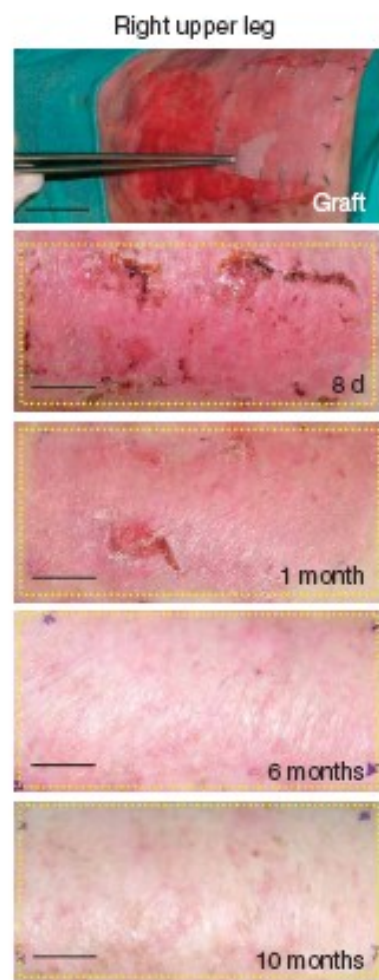
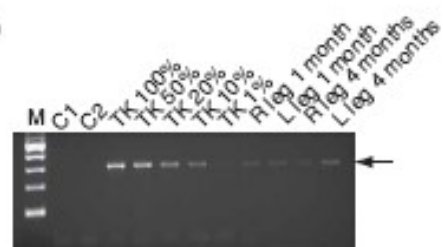
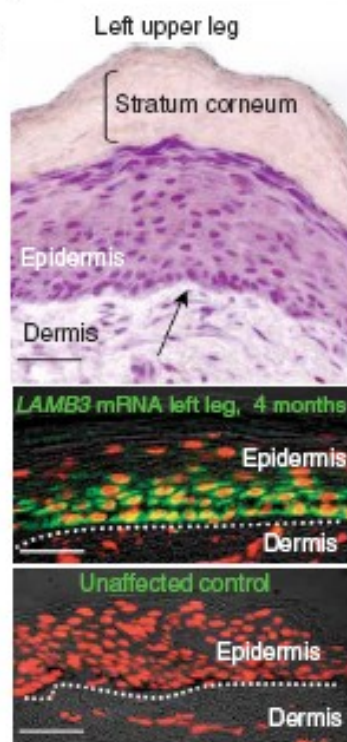
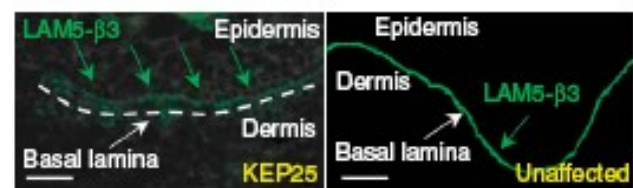
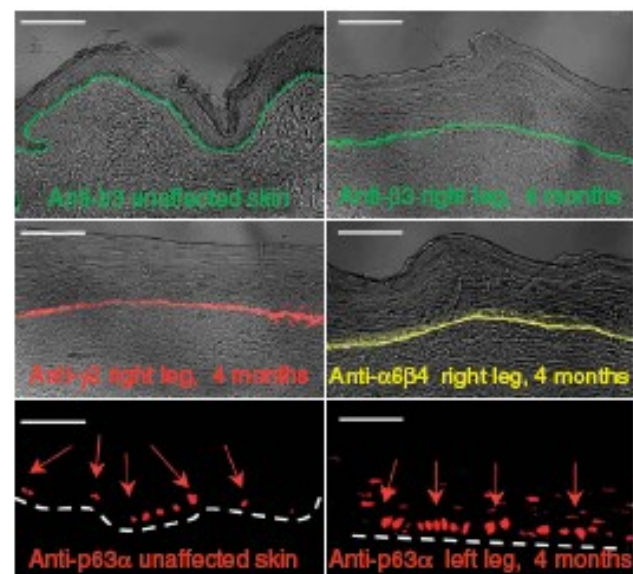
EBJ-R



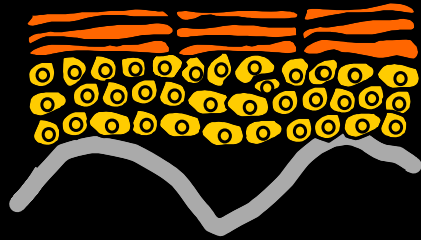
WT EBJ EBJ-R



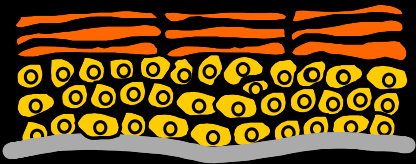
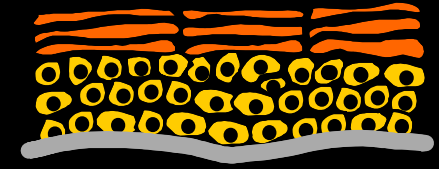


a**b****c****d****e**

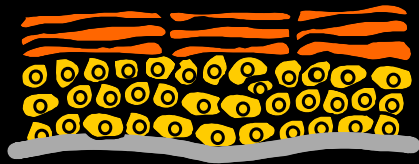
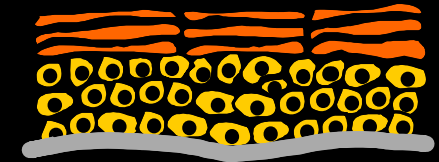
Applications potentielles de la thérapie génique cutanée



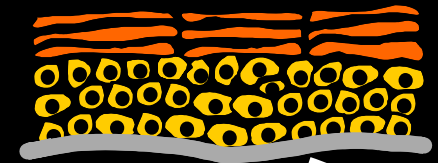
correction de
défauts géniques



catabolisme de toxines
circulantes



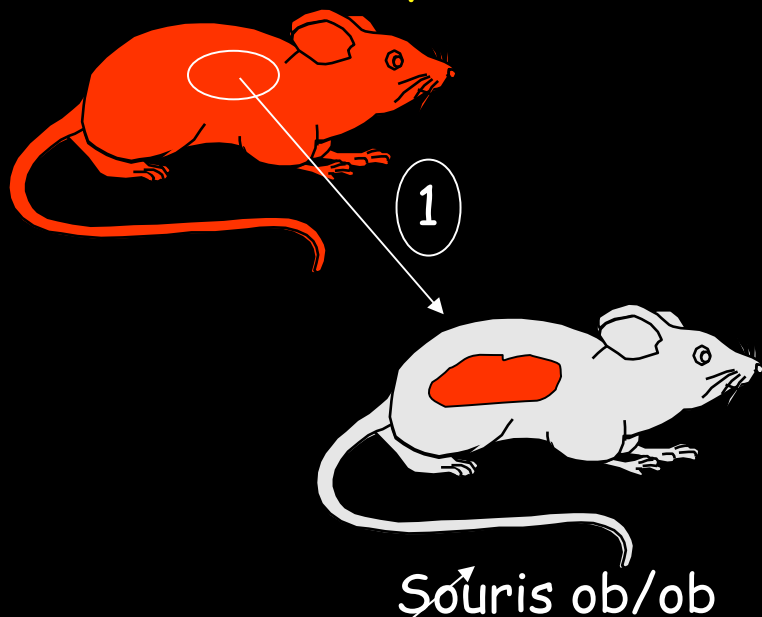
sécrétion de
médicaments diffusibles



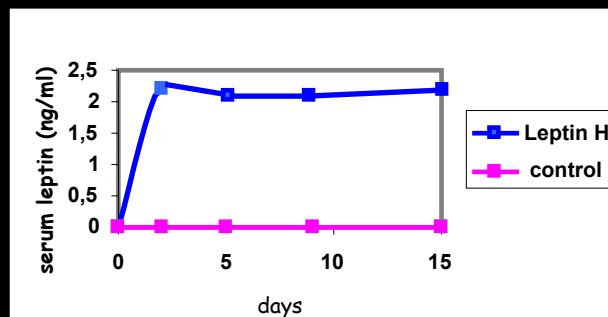
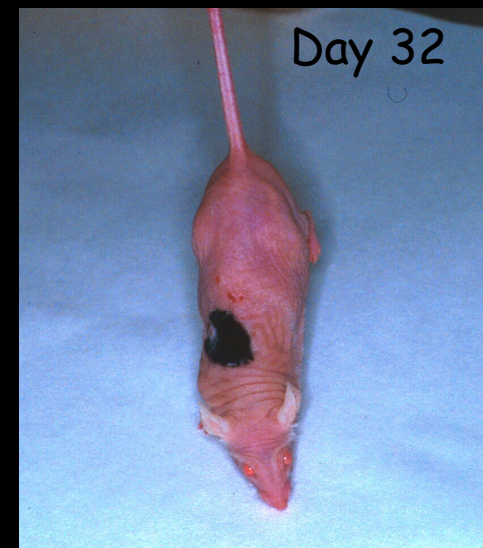
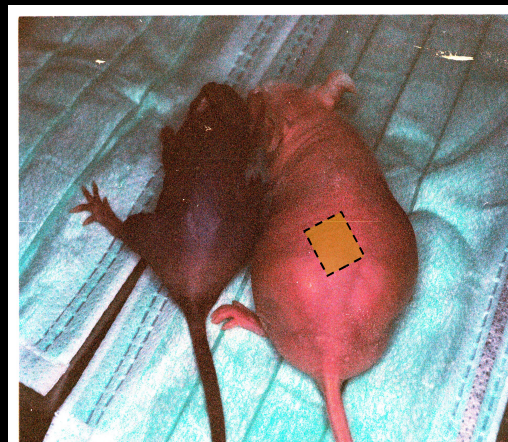
Correction du phénotype d'obésité par transplantation d'une peau transgénique

Larcher et al.(2001) . FASEB J 15: 1529-38

Promoteur K5-leptin-LacZ

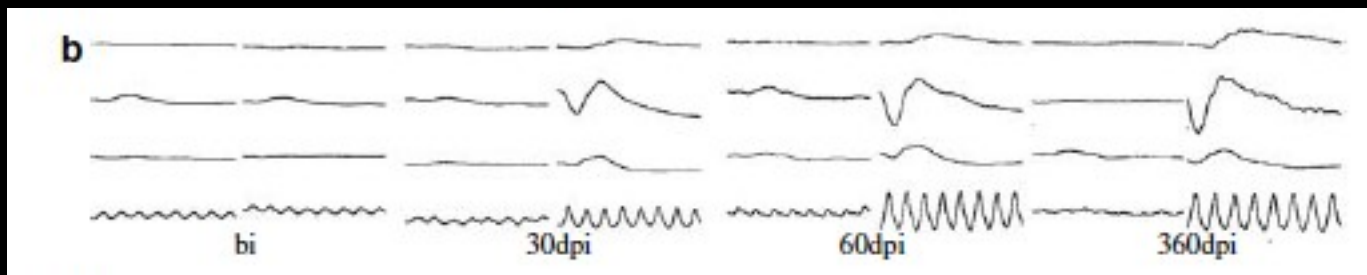
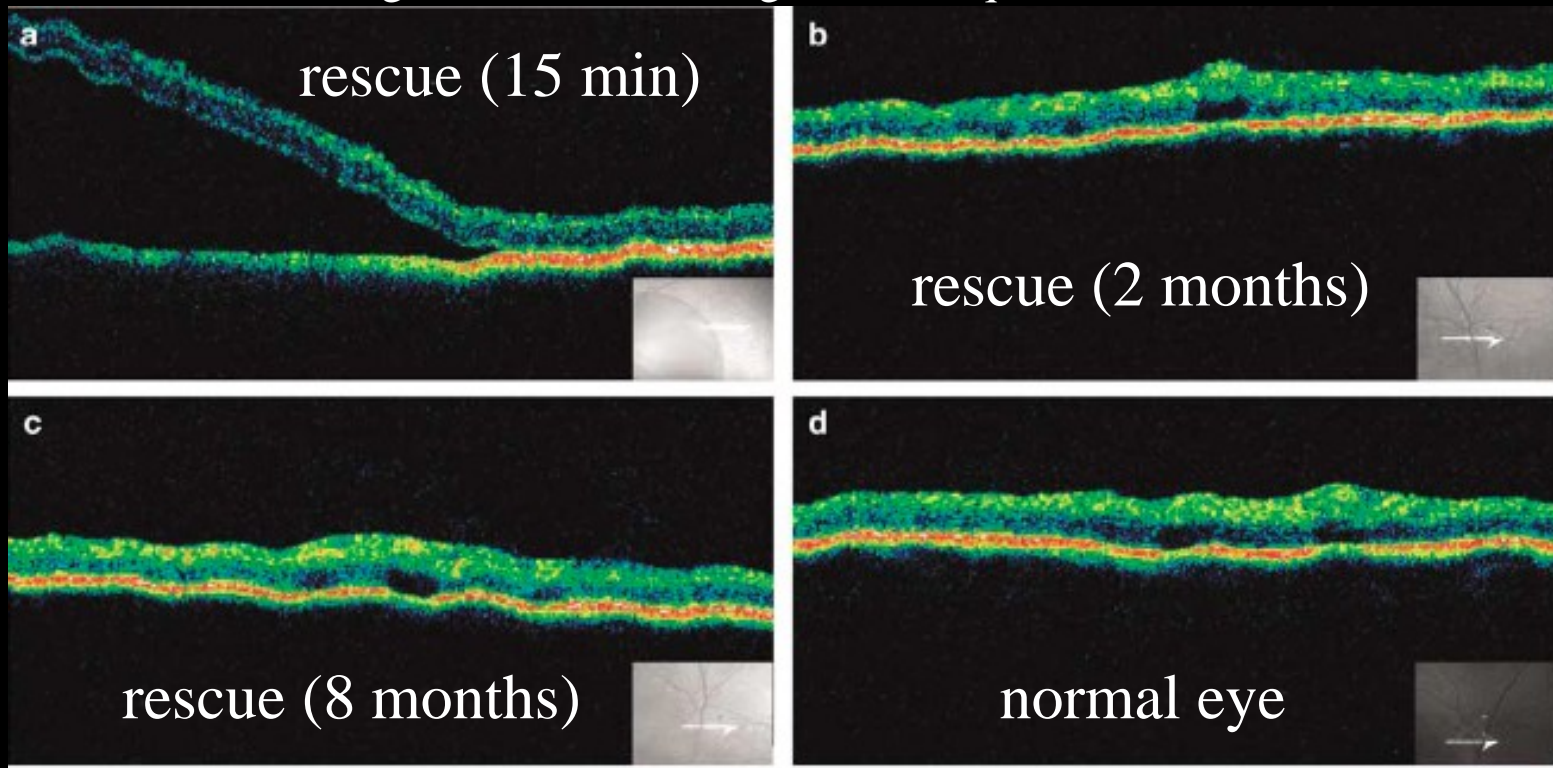


Grefe d'épiderme humaine exprimant la leptine



Restoration of vision in RPE65-deficient Briard dogs using an AAV serotype 4 vector that specifically targets the retinal pigmented epithelium

Retinite pigmentaire (cécité chez l'enfant). Touche les photorécepteurs (RPE) et l'enzyme RPE65. Permet la conversion du signal lumineux en signal électrique.





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

Fulvio Mavilio¹, Graziella Pellegrini^{1,2}, 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 Luca^{1,2}

nm/naturemedicine

