



**Antonio Bernad**  
*Regenerative Cardiology Department*

“DE

LA REVOLUCIÓN  
NEOLÍTICA A  
LA REVOLUCIÓN  
TRANSGÉNICA”

Aula de Ciencia y  
Tecnología

U. de Granada

11 Junio, 2012



**Medicina Regenerativa:  
Realidades y Promesas**



MINISTERIO  
DE CIENCIA  
E INNOVACIÓN



**cnic**

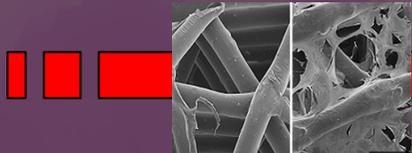
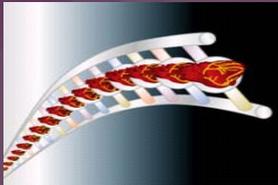
# Medicina Regenerativa

- Congénitas
- Infecciosas
- Tumores
- Funcionales
- Degener.
- Cardiov.
- Autoinm.
- Diabetes

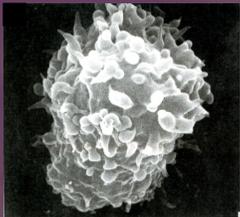


enfermo

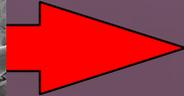
T G



bioMat

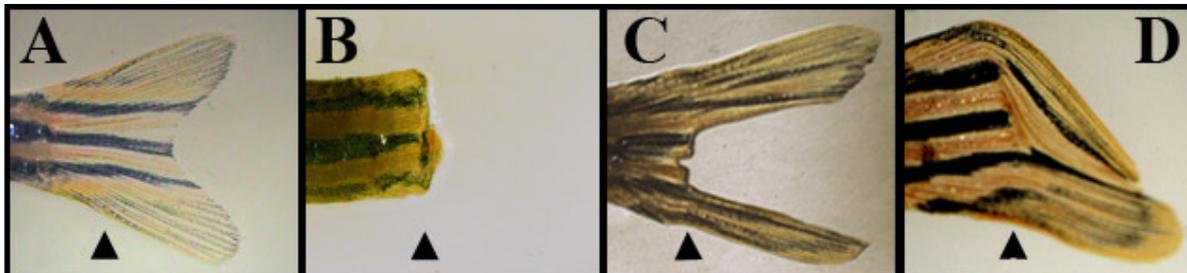
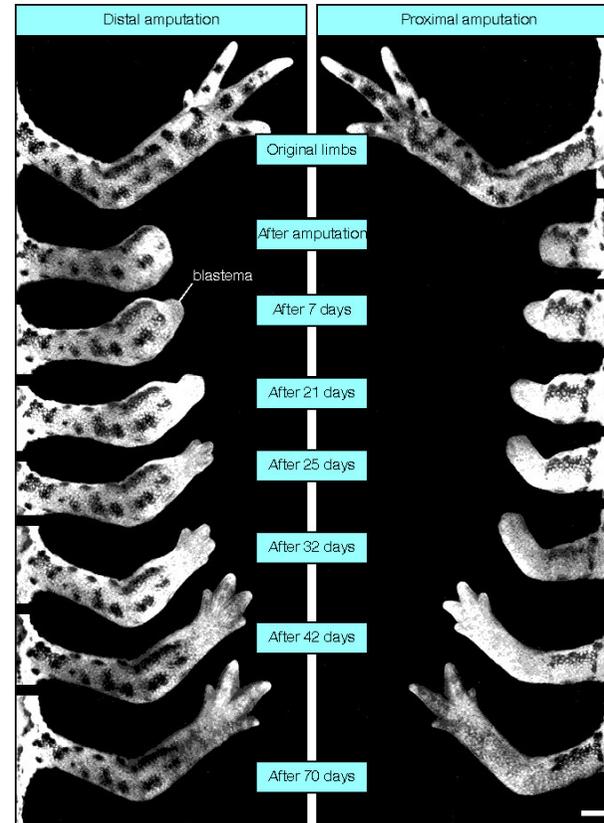


T C

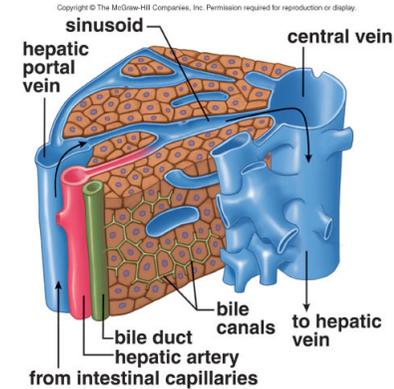
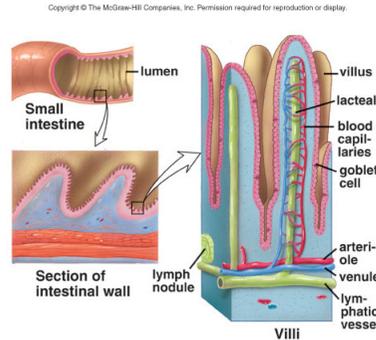
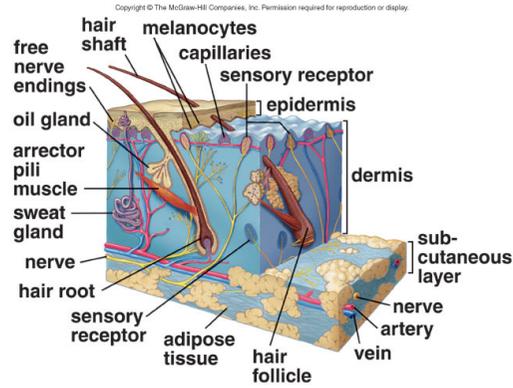


Mejora objetiva/  
Sano

# Regeneración. Vertebrados inferiores

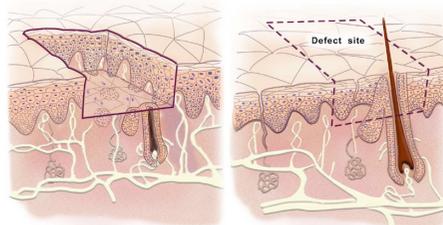


# Regeneración/Reparación. Mamíferos Adultos



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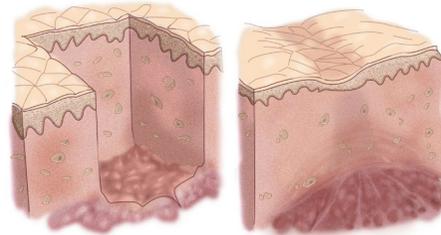
The epidermis is regenerative



Spontaneous regeneration of excised epidermis

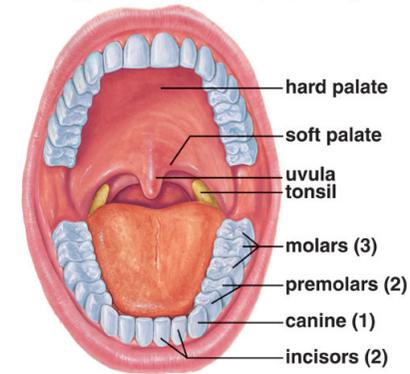
Figure by MIT OCW.

The dermis is nonregenerative

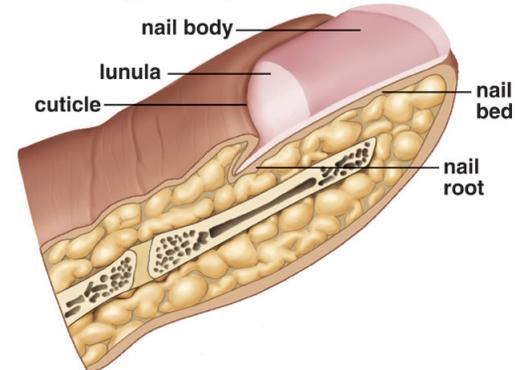


Spontaneous healing of skin excised to full thickness by contraction and scar formation. The dermis does not regenerate.

Figure by MIT OCW.



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# Regeneración/Reparación. Mamíferos Adultos

9830-9835 | PNAS | August 14, 2001 | vol. 98 | no. 17

## Heart regeneration in adult MRL mice

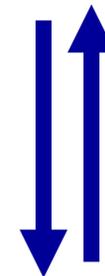
John M. Leferovich\*, Khamilia Bedelbaeva\*, Stefan Samulewicz\*, Xiang-Ming Zhang\*, Donna Zwas†, Edward B. Lankford†, and Ellen Heber-Katz\*§

\*The Wistar Institute, Philadelphia, PA 19104; and †Division of Cardiovascular Medicine, Thomas Jefferson University, Philadelphia, PA 19107

Communicated by Hilary Koprowski, Thomas Jefferson University, Philadelphia, PA, June 29, 2001 (received for review April 20, 2001)

The reaction of cardiac tissue to acute injury involves interacting cascades of cellular and molecular responses that encompass inflammation, hormonal signaling, extracellular matrix remodeling, and compensatory adaptation of myocytes. Myocardial regeneration is observed in amphibians, whereas scar formation characterizes cardiac ventricular wound healing in a variety of mammalian injury models. We have previously shown that the MRL mouse strain has an extraordinary capacity to heal surgical wounds, a complex trait that maps to at least seven genetic loci. Here, we extend these studies to cardiac wounds and demonstrate that a severe transmural, cryogenically induced infarction of the right ventricle heals extensively within 60 days, with the restoration of normal myocardium and function. Scarring is markedly reduced in MRL mice compared with C57BL/6 mice, consistent with both the reduced hydroxyproline levels seen after injury and an elevated cardiomyocyte mitotic index of 10–20% for the MRL compared with 1–3% for the C57BL/6. The myocardial response to injury observed in these mice resembles the regenerative process seen in amphibians.

Reparación:Regeneración



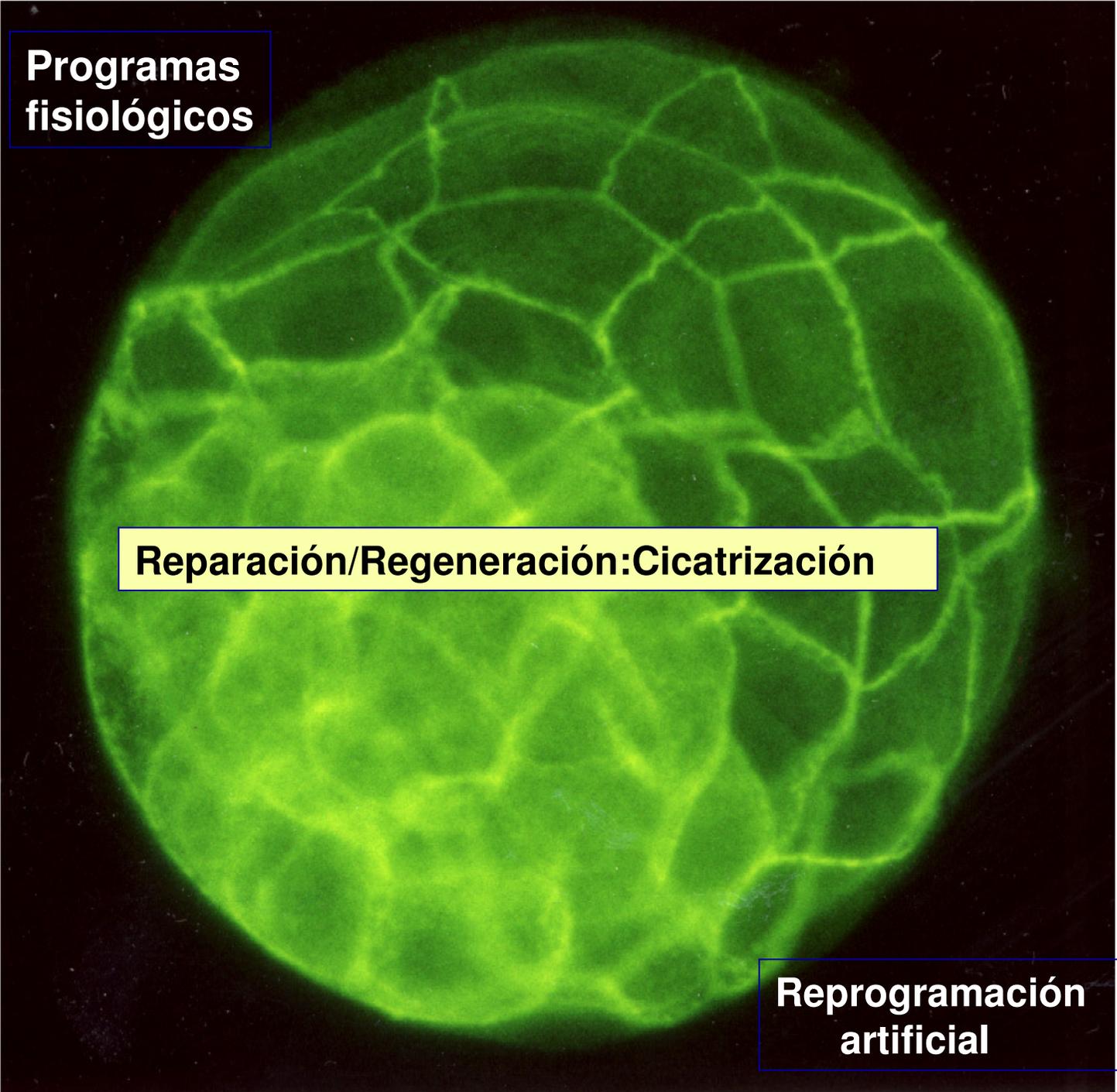
Reparación:Cicatrización

**Programas  
fisiológicos**

**Reparación/Regeneración:Cicatrización**

**Reprogramación  
artificial**

\*



# Reprogramación artificial

Manipulación Genética-----Terapia Génica

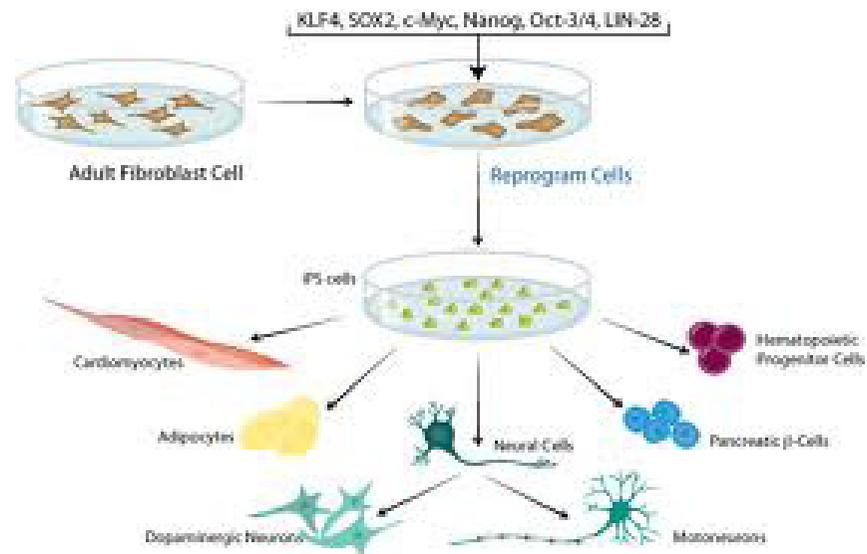
Obtención Células Madre-----Terapia Celular

Células Madre Adultas

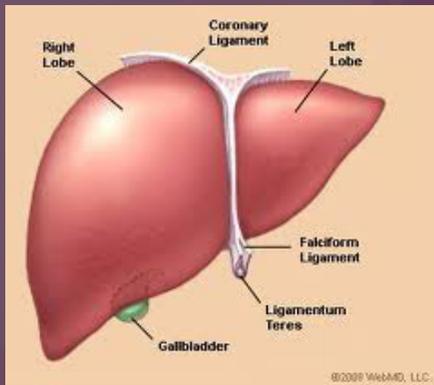
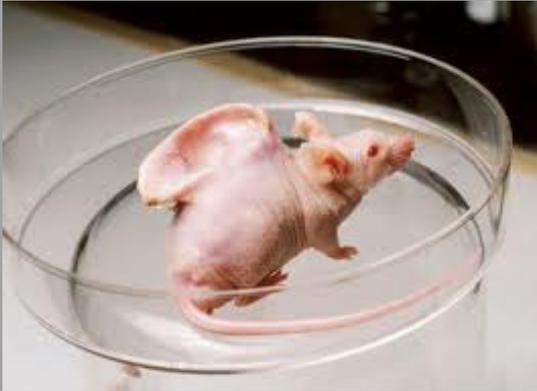
Líneas ES

Lineas iPS

Reprogramación Directa



# Organos artificiales (II): Biológicos



>>



>>>>

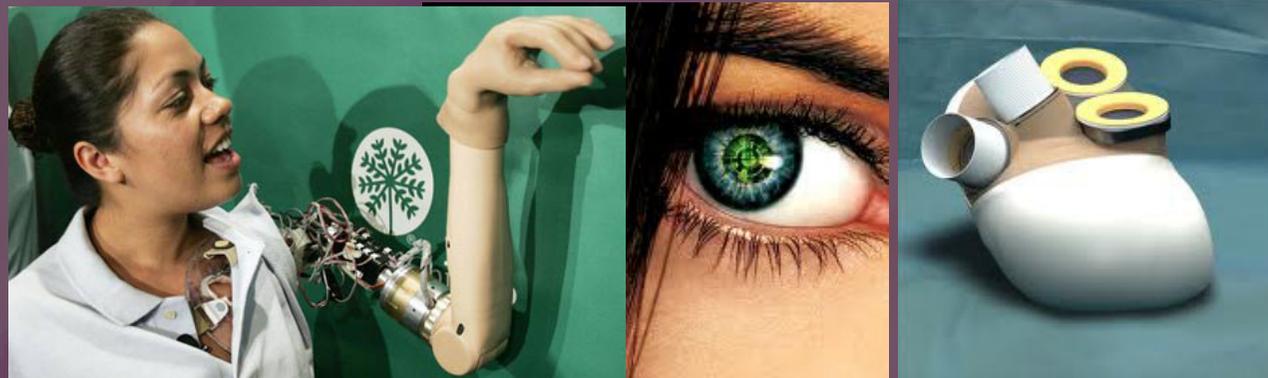


# Medicina Regenerativa

- ✓ Concepto muy amplio. Miles de combinaciones.
- ✓ Area muy multidisciplinar: biología, química, ingeniería y medicina



enfermo

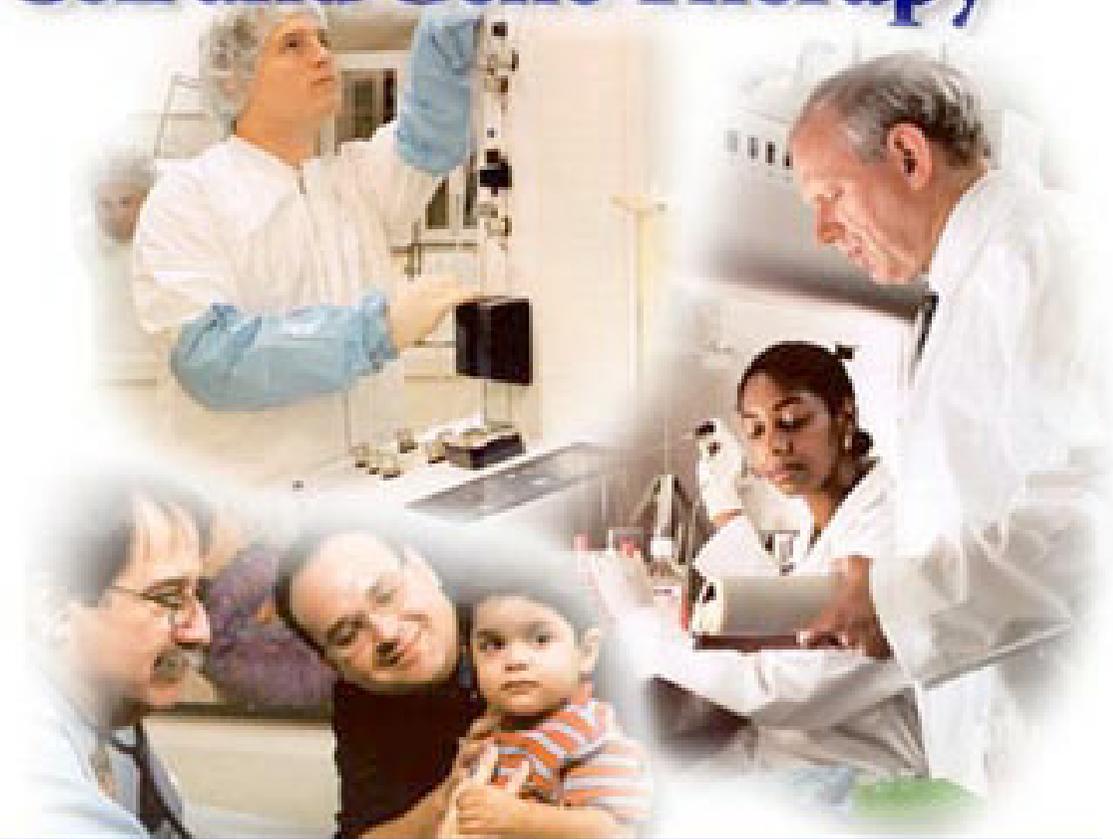


Organos artificiales (I)

Center for



# Cell and Gene Therapy



BAYLOR  
COLLEGE OF  
MEDICINE

*Methodist*

The Methodist Hospital



Texas  
Children's  
Hospital

# Terapia Génica Somática

**ADICIÓN**

**SUSTITUCIÓN**

**Alta eficiencia de transferencia**

**Regulación óptima**

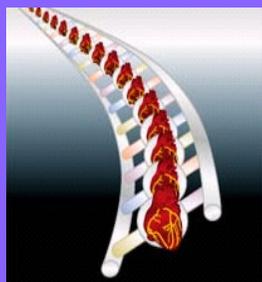
Regulación subóptima  
(dependiente del vector)

Baja eficiencia  
de recombinación  
homóloga

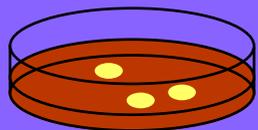
Células  
diana

Vectores

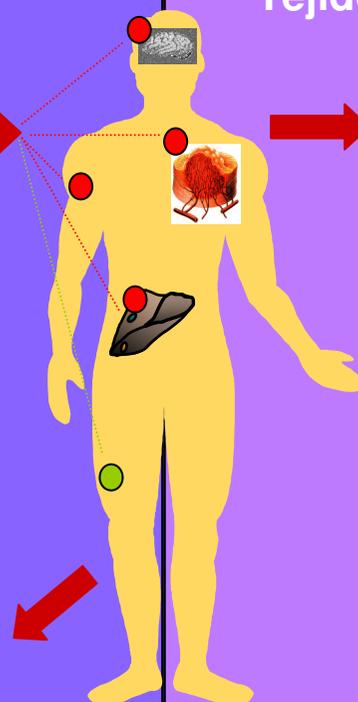
## In vivo



V. Viral  
V. No-viral  
DNA desnudo

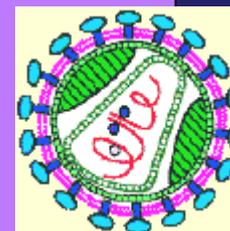
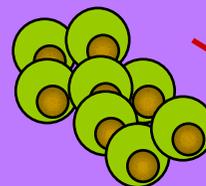


Seguimiento &  
Toxicidad



## Ex vivo

Obtención de  
Tejido Diana



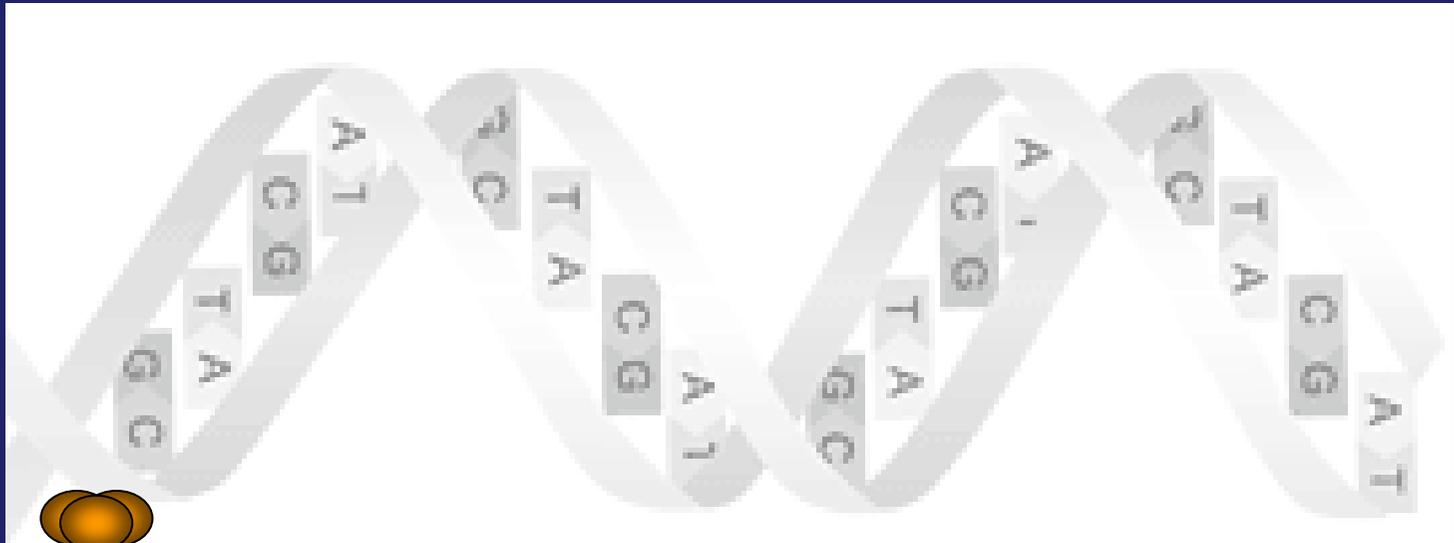
Selección



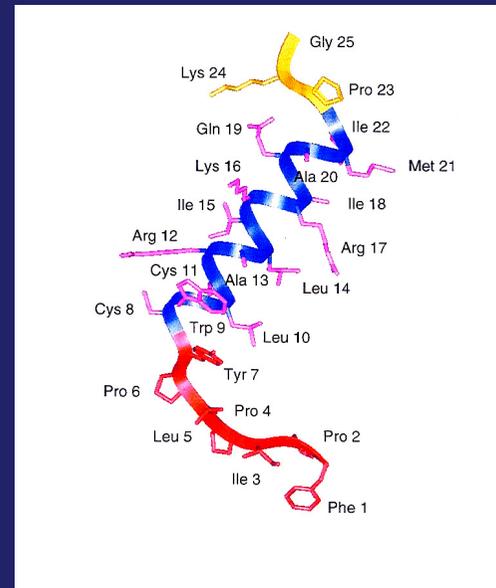
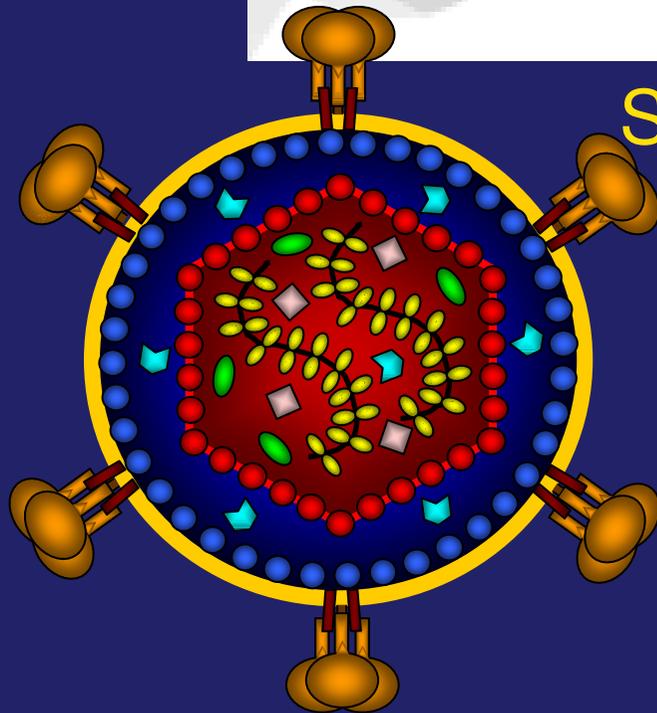
Amplificación

Reinfusión de las  
células corregidas





## Sistemas de manipulación Génica



## Principales Familias de Vectores Virales:

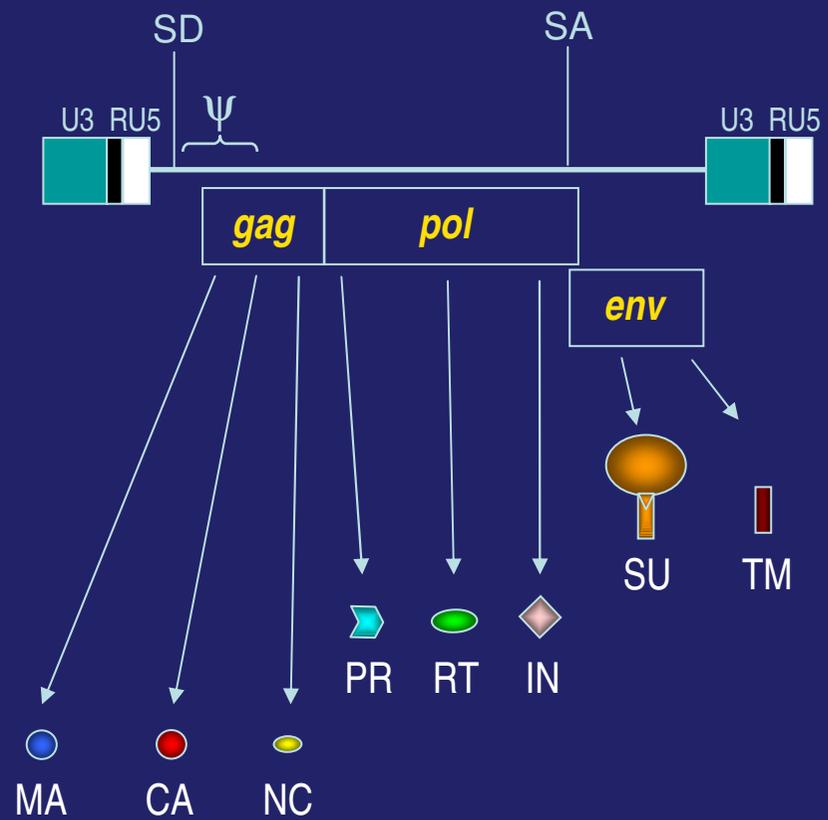
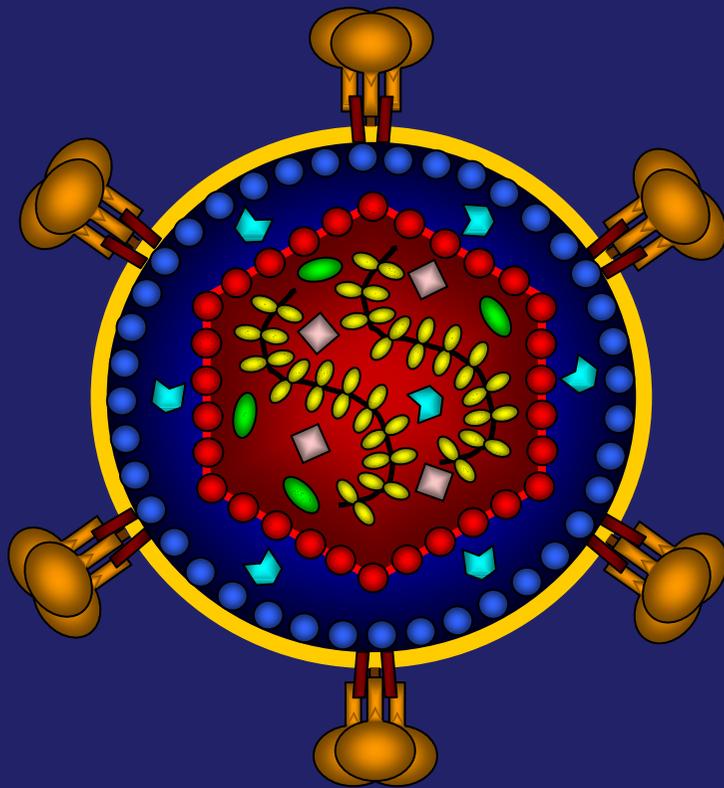
### Genoma RNA

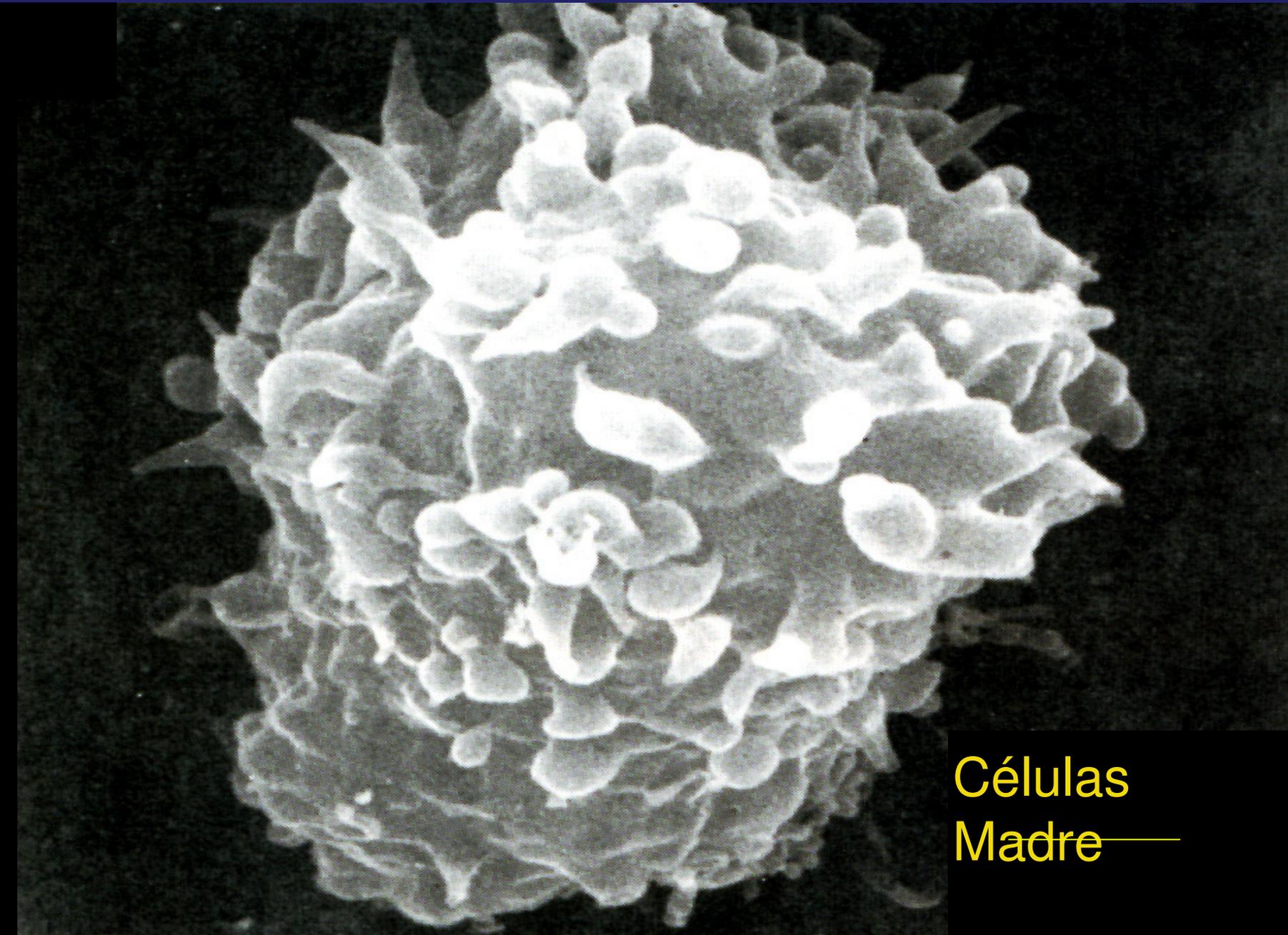
- Vectores Retrovirales (Mo-MLV)
- Vectores Lentivirales (VIH-1)

### Genoma DNA

- Vectores Adenovirales (Ad-5)
- Vectores Adeno-Asociados (AAV; Parvovirus MMV)
- Vectores derivados de Herpes (HSV)

# Estructura de las partículas retrovirales

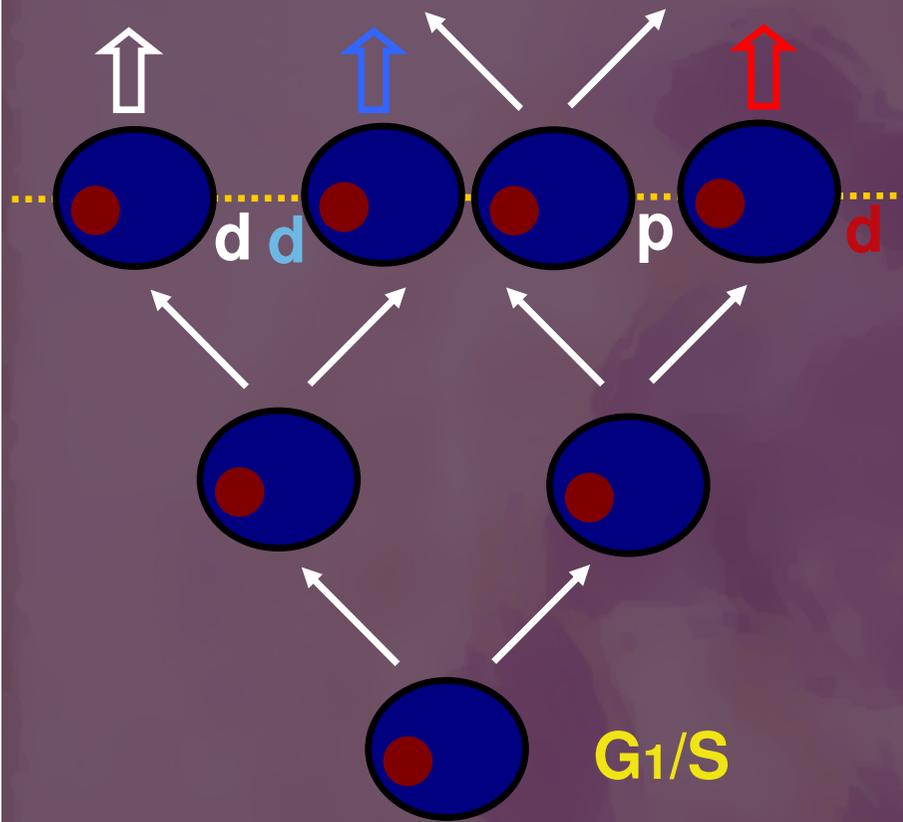




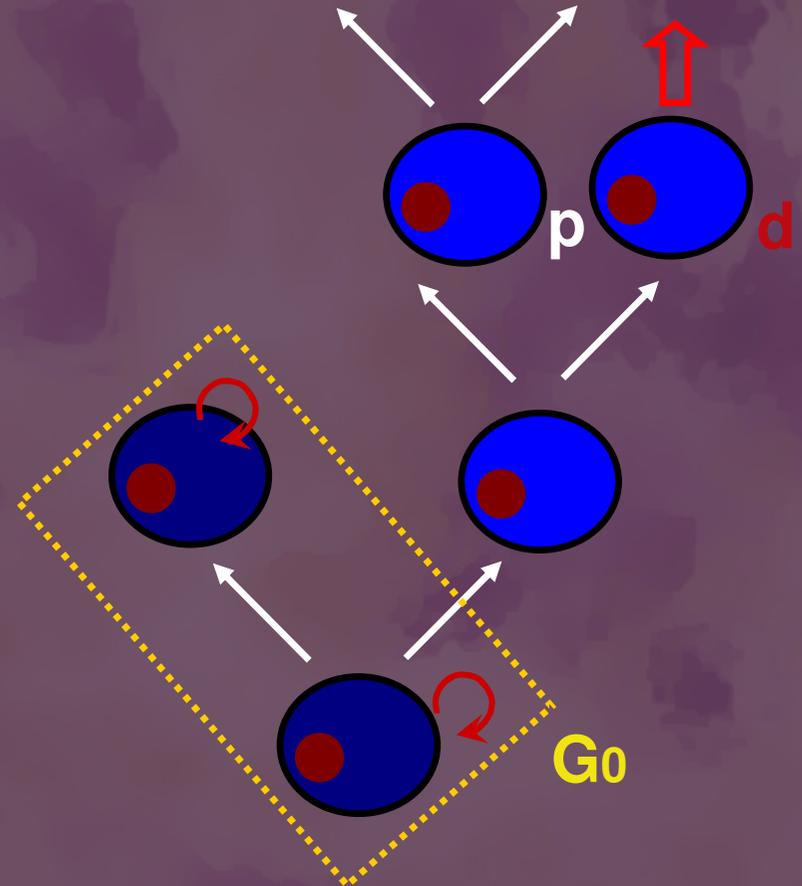
Células  
Madre

*Stem Cells*

# Célula Madre-- *Stem Cells*

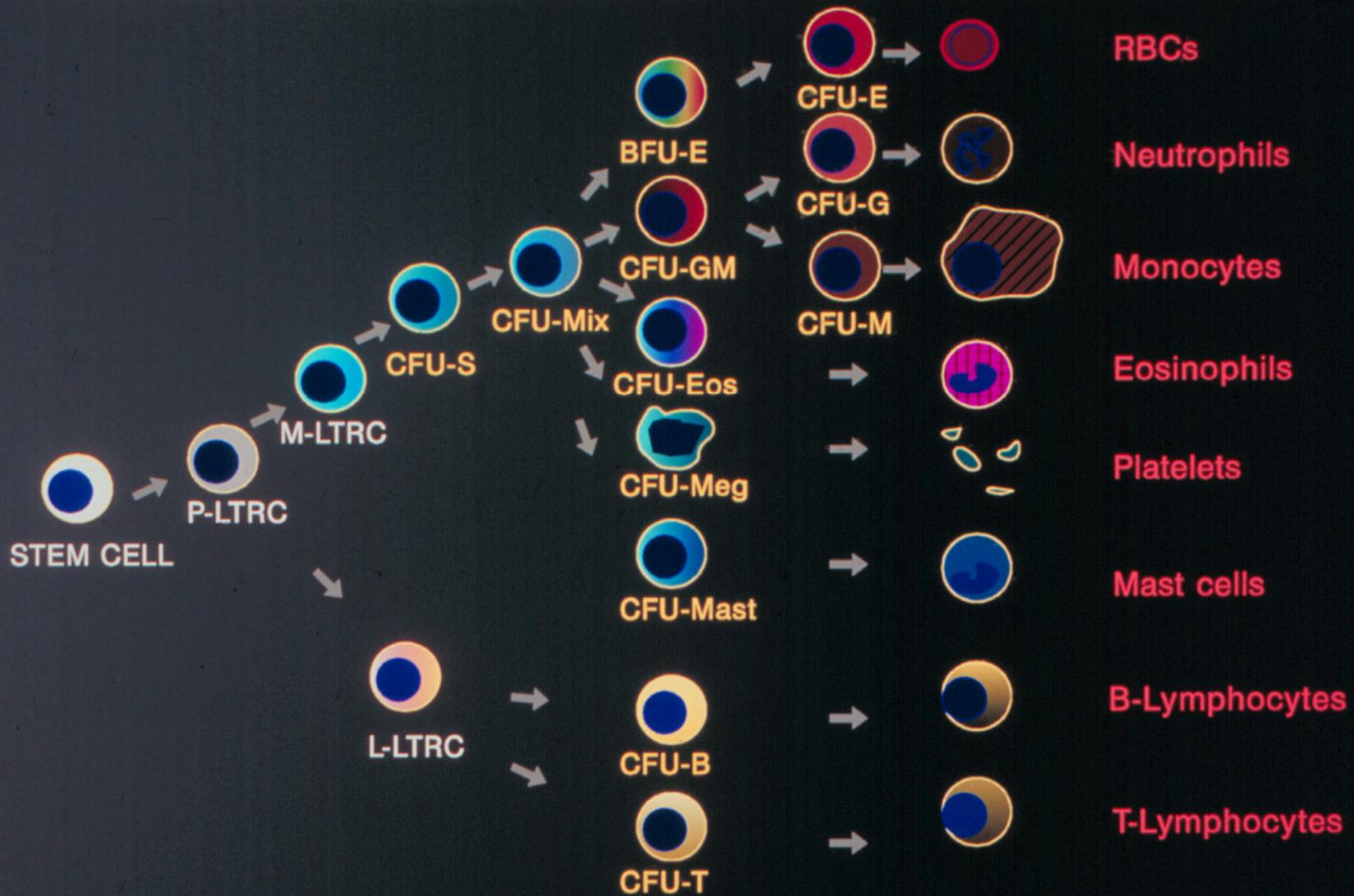


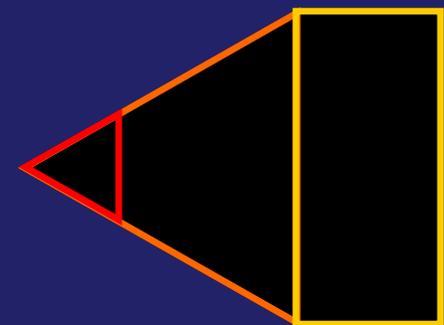
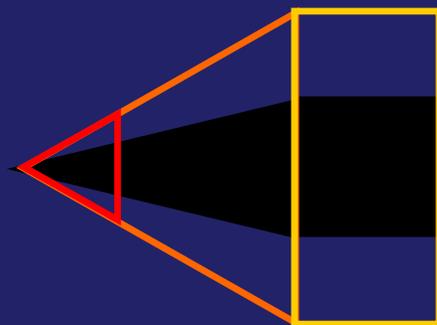
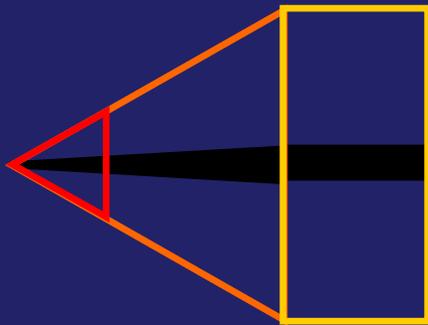
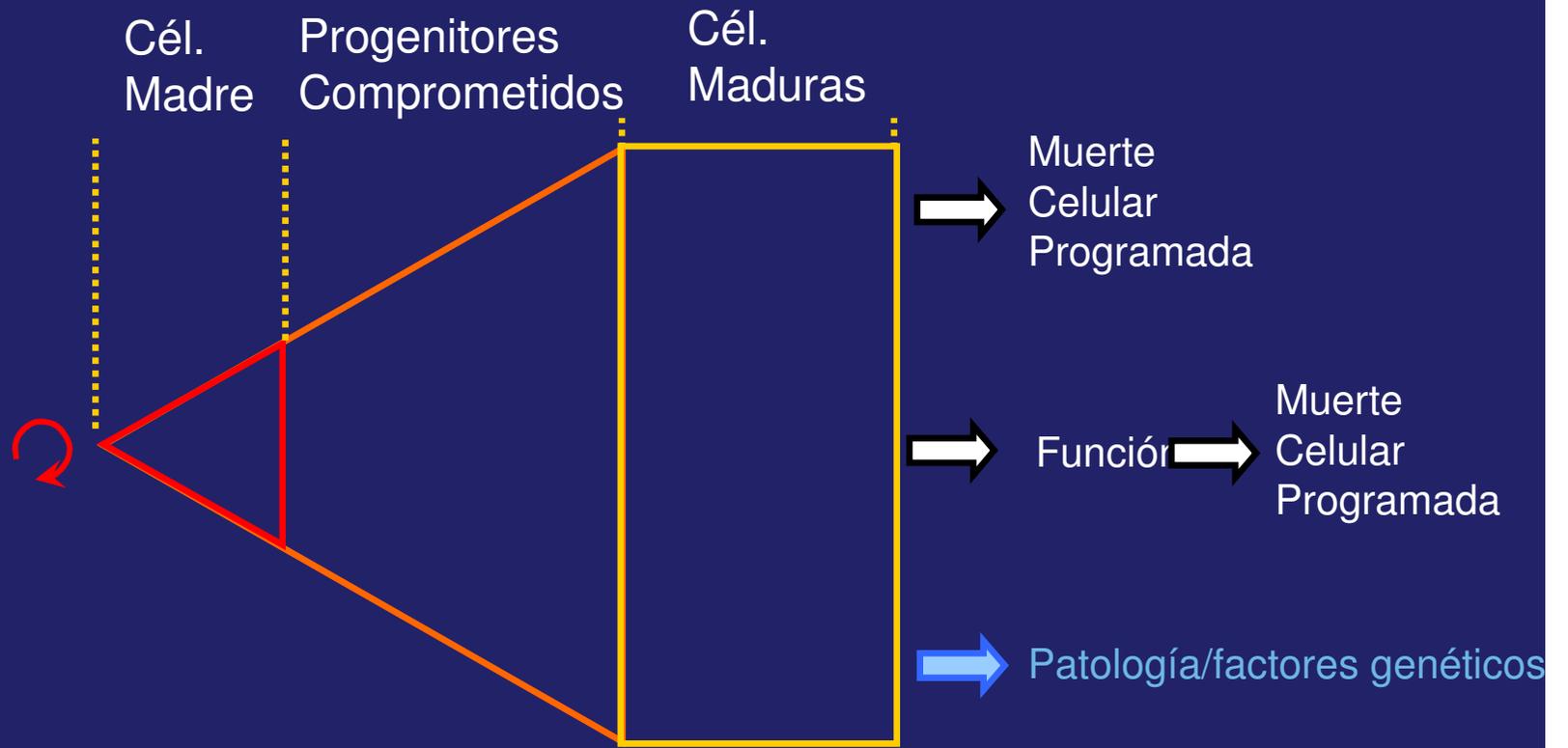
*División Simétrica*



*División Asimétrica*

# Organization of the mouse hematopoietic system





- **Gene Therapy of human severe combined immunodeficiency (SCID)-X1 disease**

*Cavazzana-Calvo M., Hacein-Bey S., de Saint Basile G., Groiss F., Yvon E., Nusbaum P., Selz F., Hue C., Certain S., Casanova J.L., Bousso P., Deist F.L., Fisher A.*

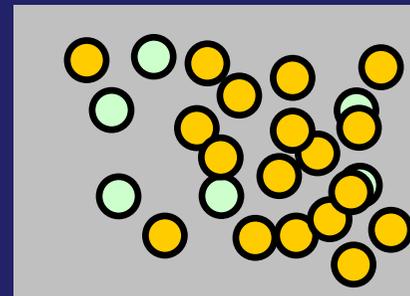
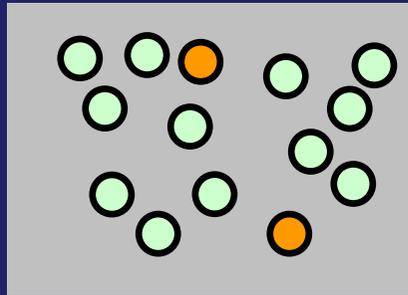
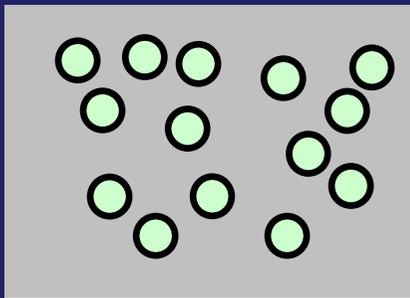
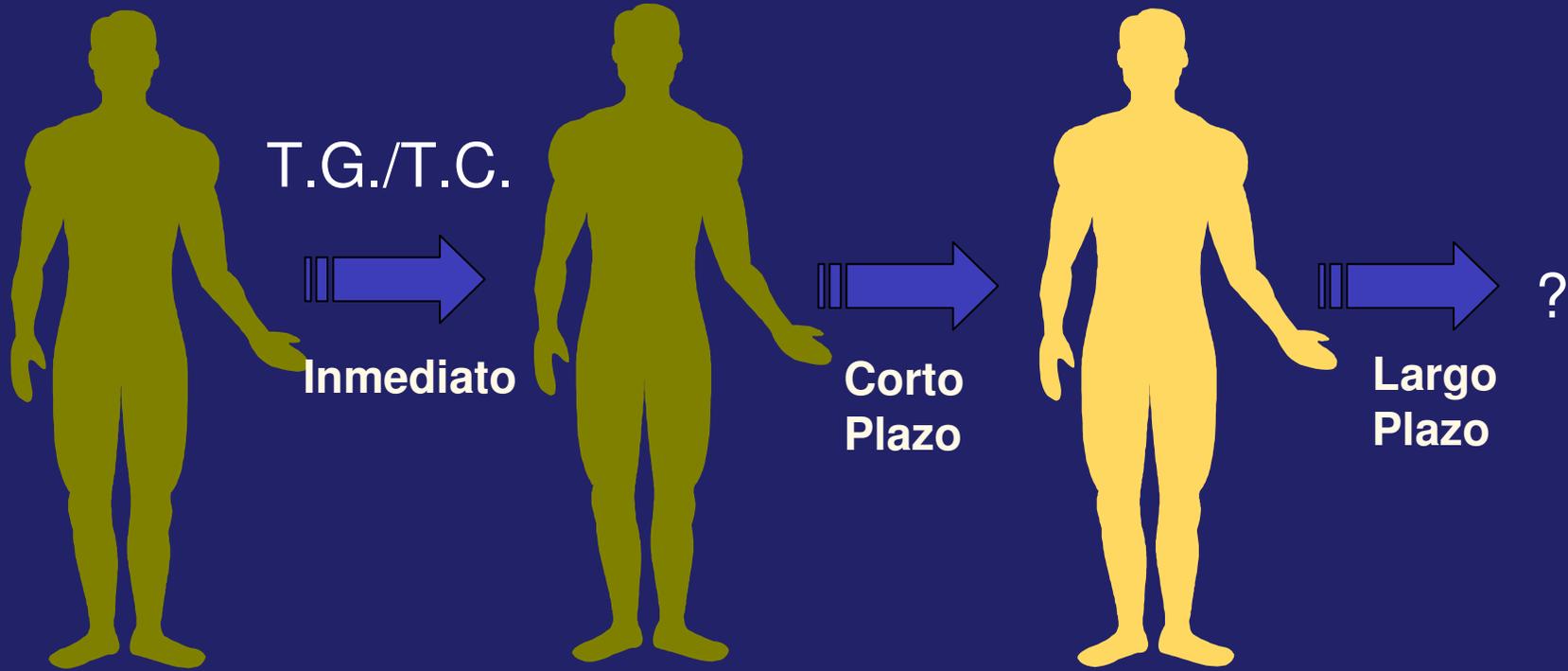
Science (2000) 288 (5466): 669-72

- **Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning**

*Aiuti A., Slavin S., Aker M., Ficara F., Deola S., Mortarello A., Morecki S., Andolfi G., Tabucchi A., Carlucci F., Marinello E., Cattaneo F., Vai S., Servida P., Miniero R., Roncarolo MG.*

Science (2002) 296 (5577):2410-3

# Selección positiva "In Vivo"



- **AAV2 gene therapy readministration in three adults with congenital blindness**

*Bennett J, Ashtari M, Wellman J et al.*

- **A phase I trial of adeno-associated virus serotype 1- $\gamma$ -sarcoglycan gene therapy for limb girdle muscular dystrophy type 2C**

*Herson S, Hentati F, Rigolet A et al.*

- **B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells**

*Kochenderfer JN, Dudley ME, Feldman SA et al.*

## ***Terapia génica como una nueva alternativa terapéutica***

**“La Medicina se encuentra a un paso de una nueva era, la Medicina Molecular. Como caso de otras revoluciones conceptuales y técnicas, nos ha tocado ser testigos de etapas de un cambio cualitativo en la forma en que el hombre comprende y confronta la enfermedad.**

**Como en anteriores revoluciones asociadas al desarrollo de la Ciencia (anatomía humana, fisiología, patología, patología médica, microbiología y patología química), la revolución de la medicina molecular está abriendo puertas a nuevas posibilidades terapéuticas que previamente eran fruto de sueños u objeto de la fantasía científica.”**

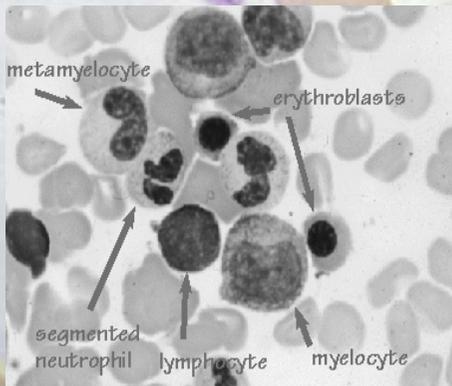
**Theodore Friedman**

*“The development of human gene therapy”; CSHL press (1999)*

**1972**

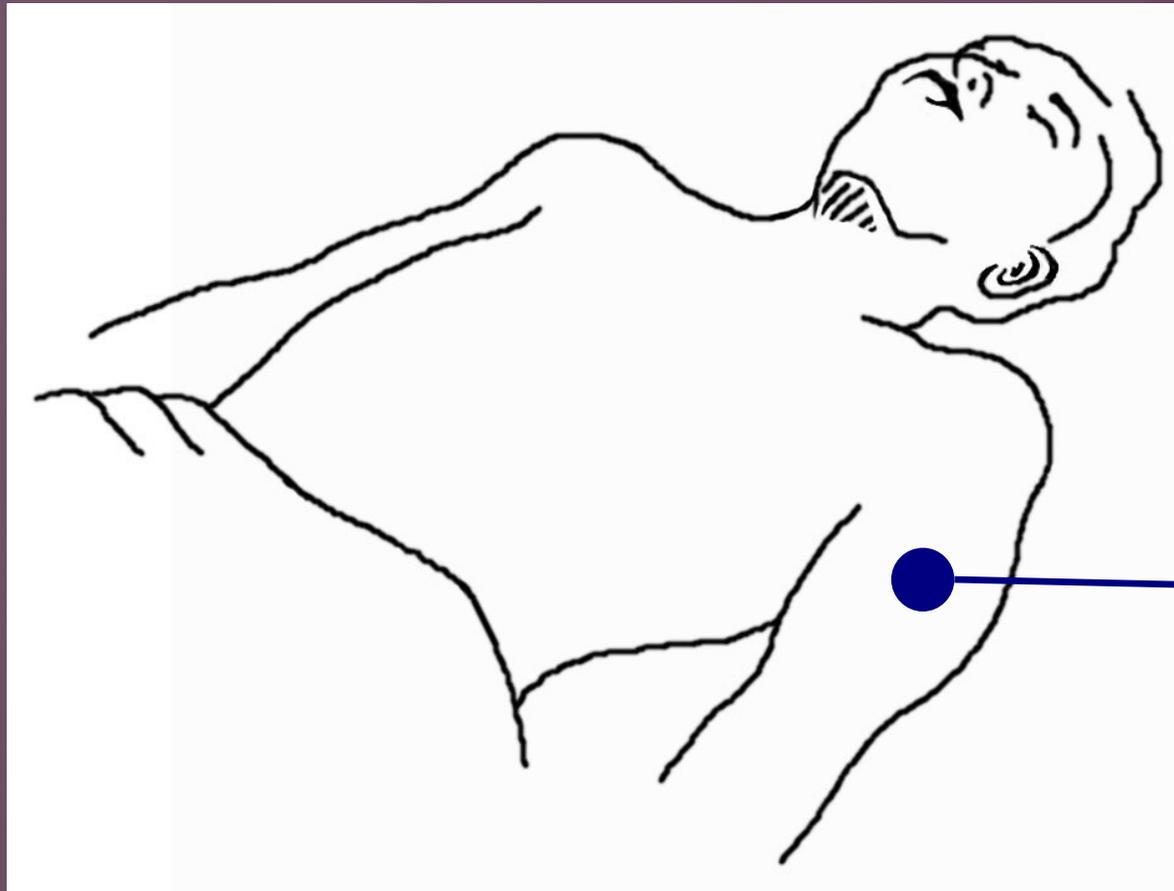
***Medicina Regenerativa:  
De la realidad clínica actual  
a un futuro prometedor***

**Trasplante de médula ósea  
Reparación Cartílago  
Piel. Quemados**



***Sistema Linfohematopoyético***

# Células Madre en el Organismo Adulto

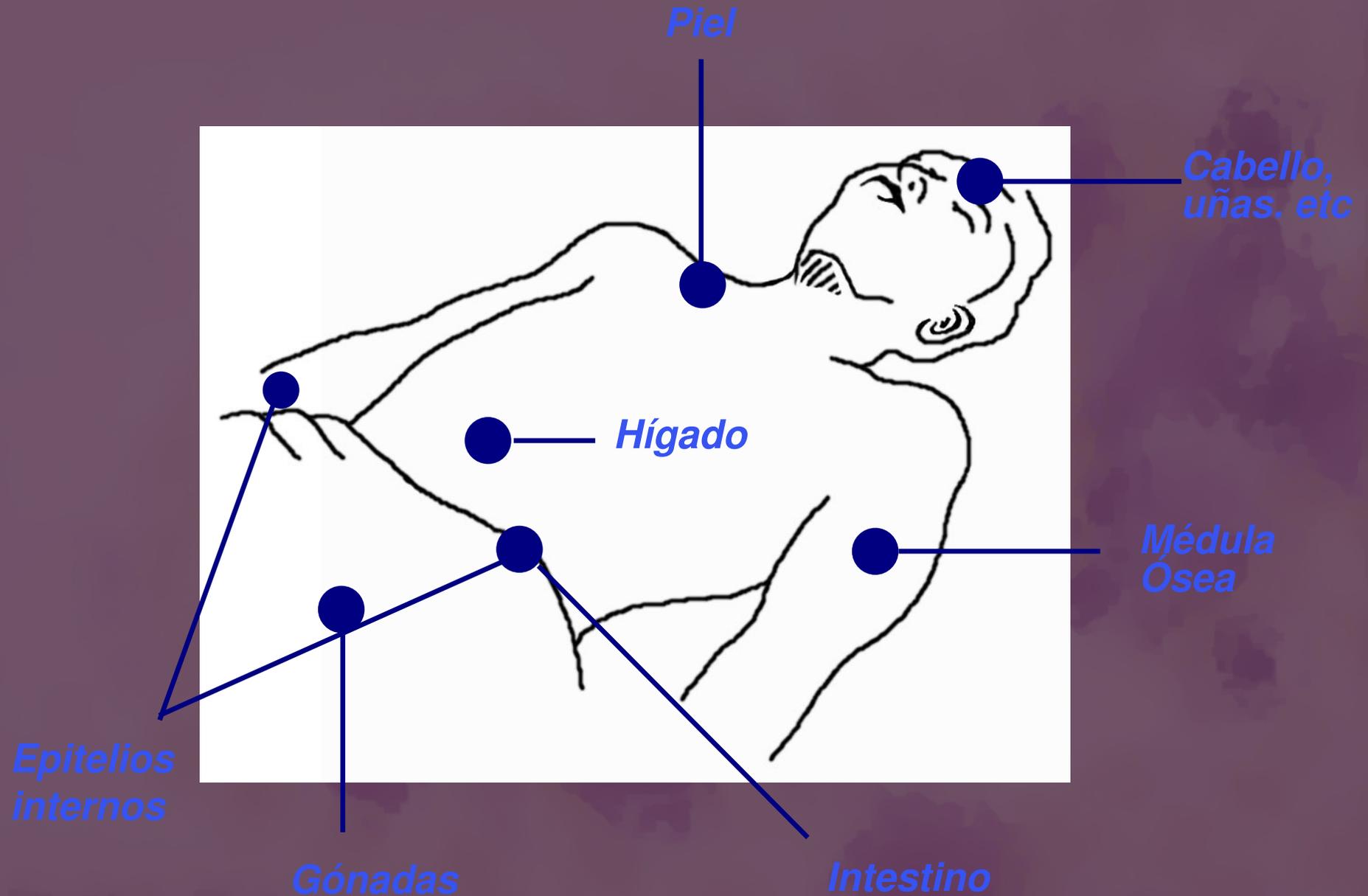


*Médula Ósea*

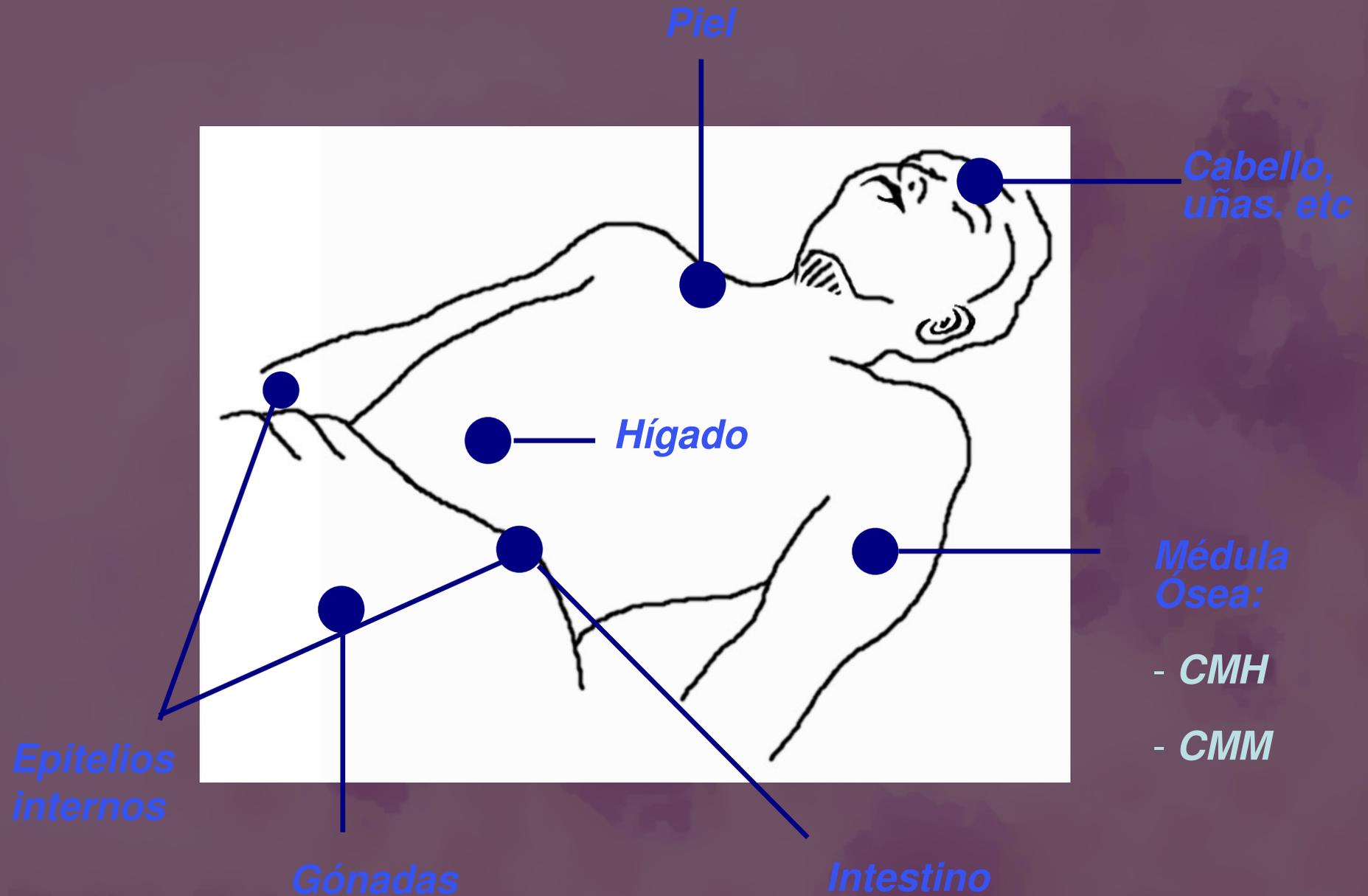
*Dr. E.D. Thomas  
(1958)*

*Ferrebee, J.W., Lochte, H.L., Jr., Jaretzki, A., 3<sup>rd</sup>, Sahler, O.D., Thomas, E.D.  
Successful marrow homograft in the dog after radiation.  
Surgery 1958; 43: 516-520.*

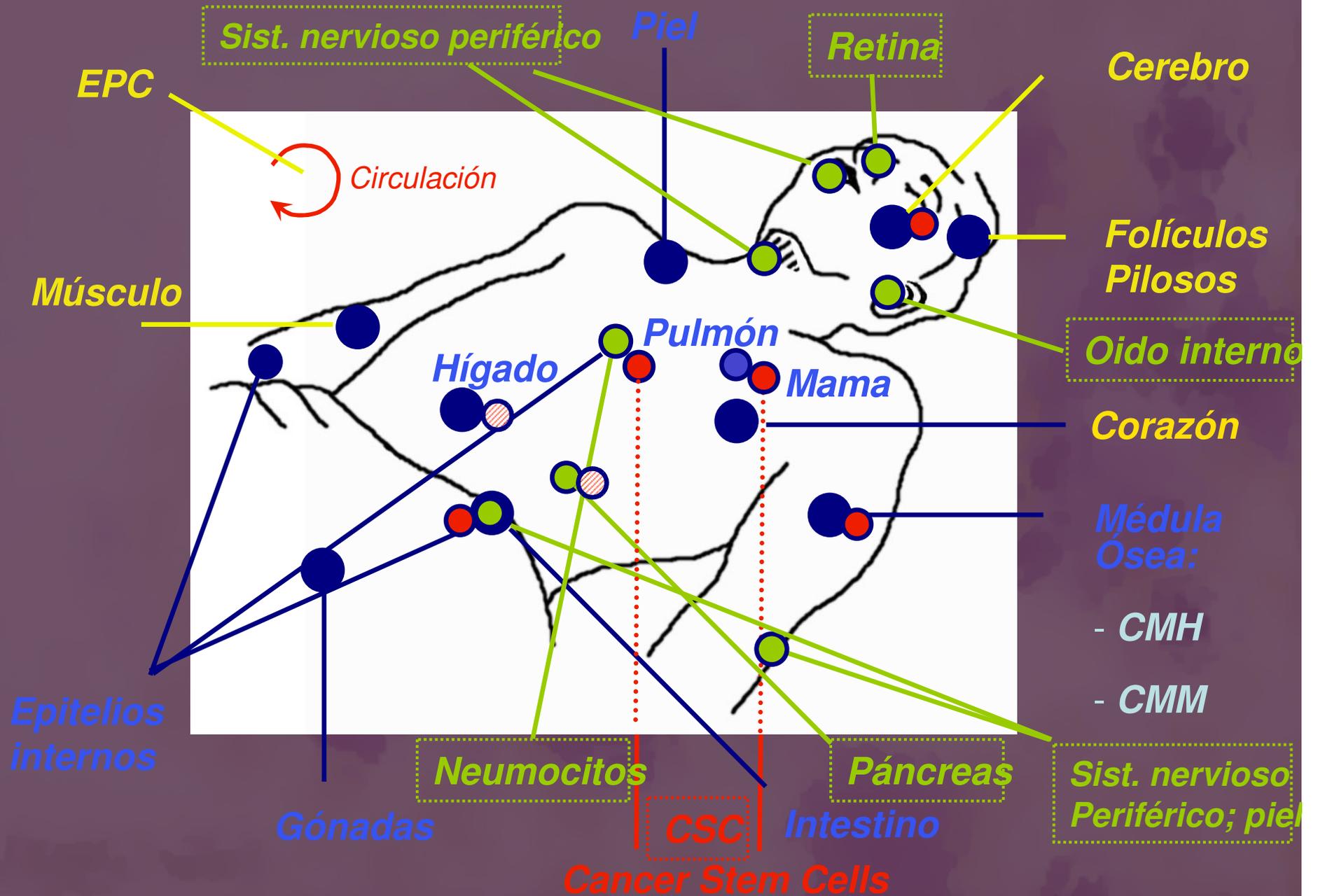
# Células Madre en el Organismo Adulto



# Células Madre en el Organismo Adulto

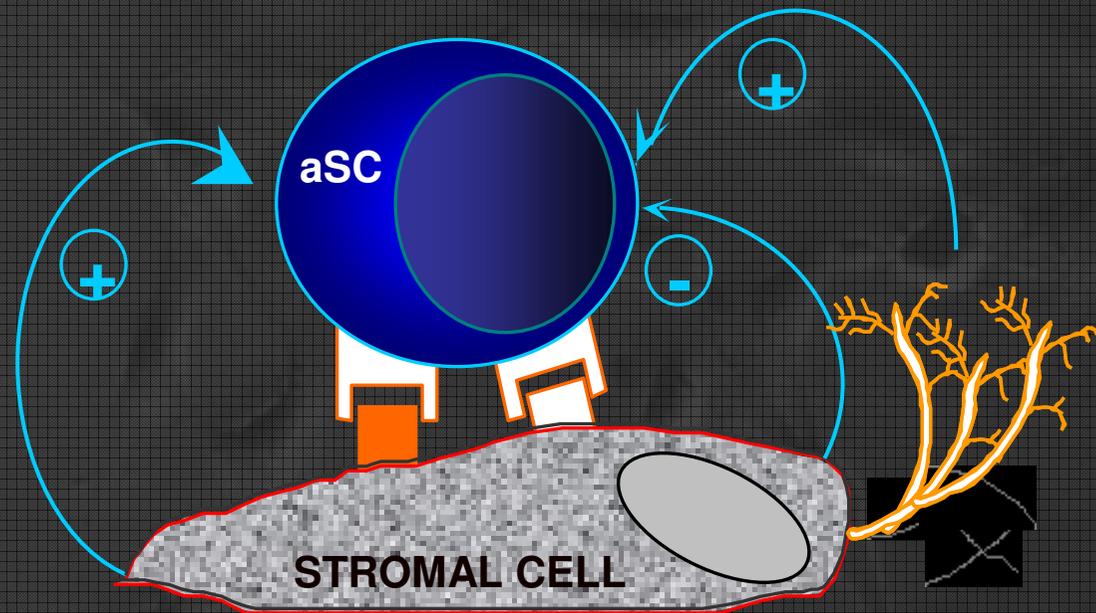


# Células Madre en el Organismo Adulto (IV)



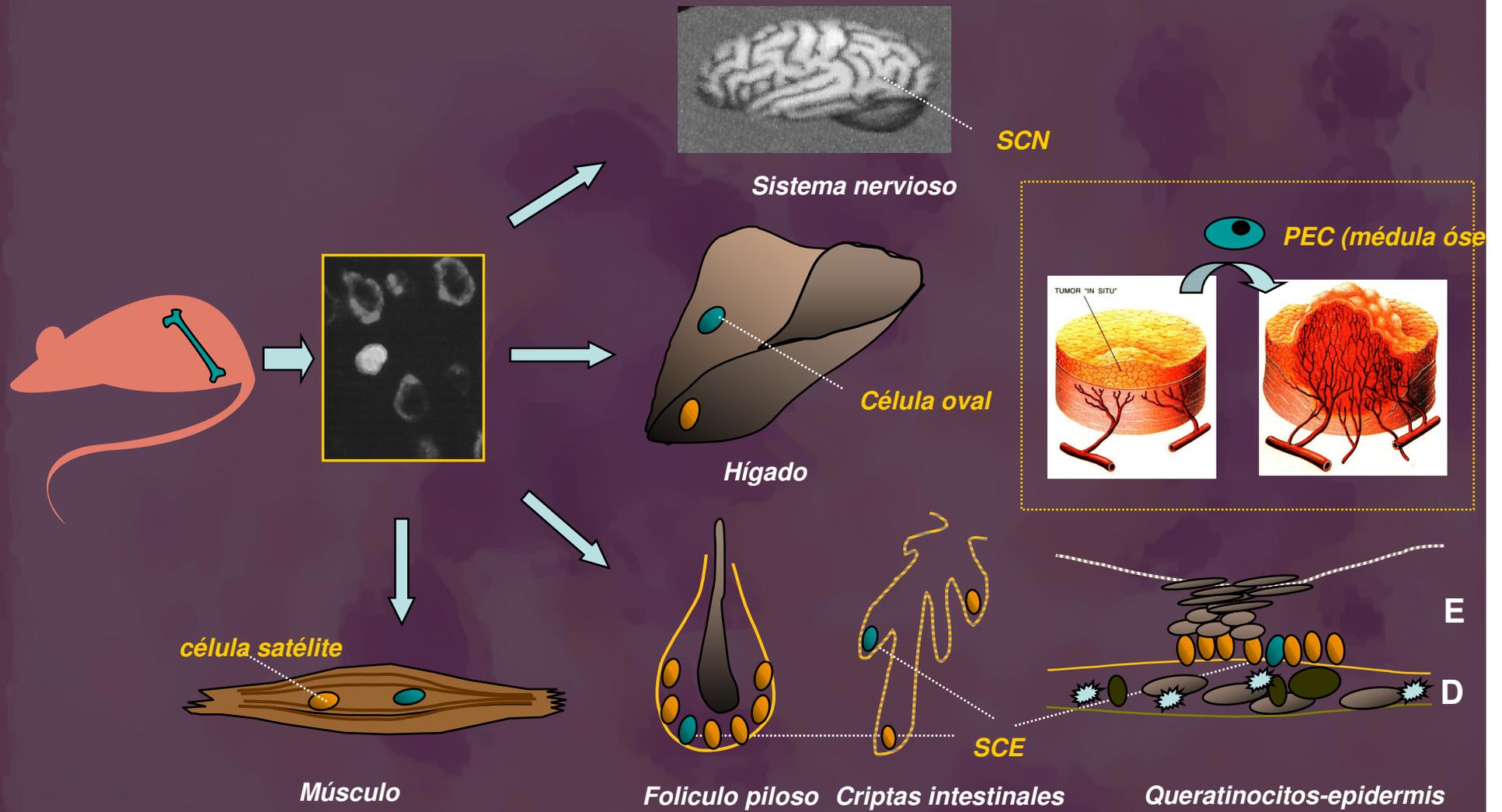
“el nicho”

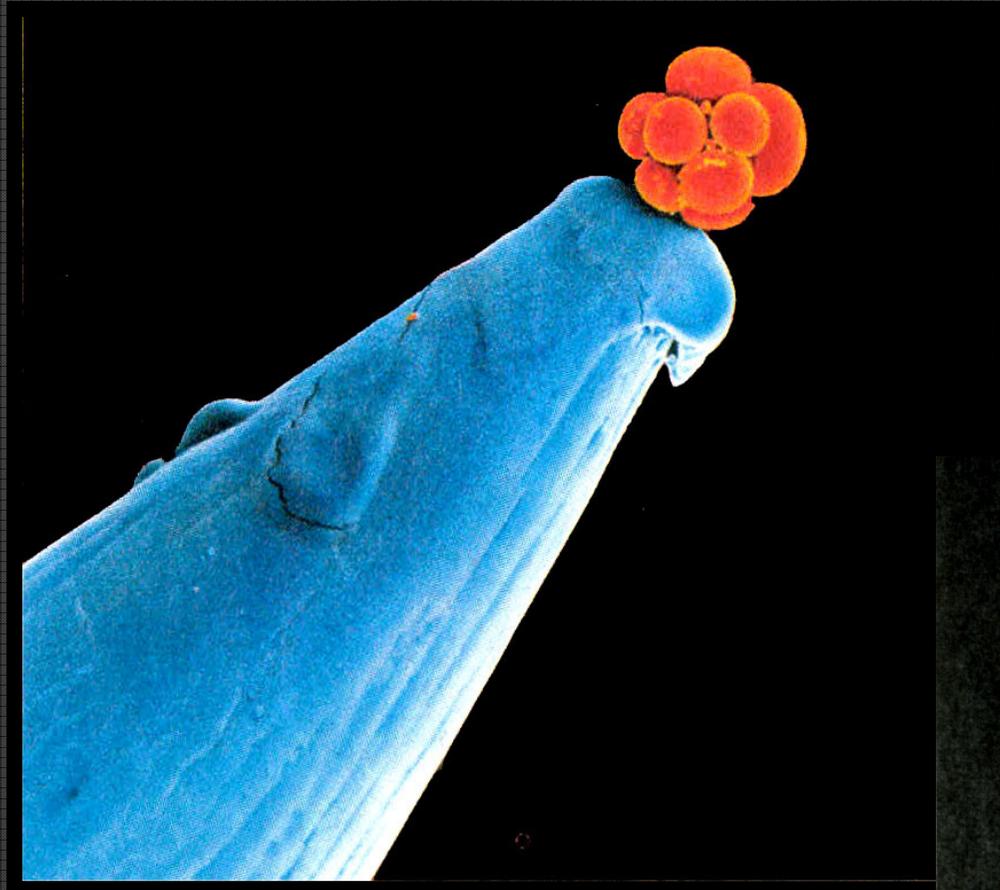
## Adult Stem Cells



Pittenger et al. Science 1999

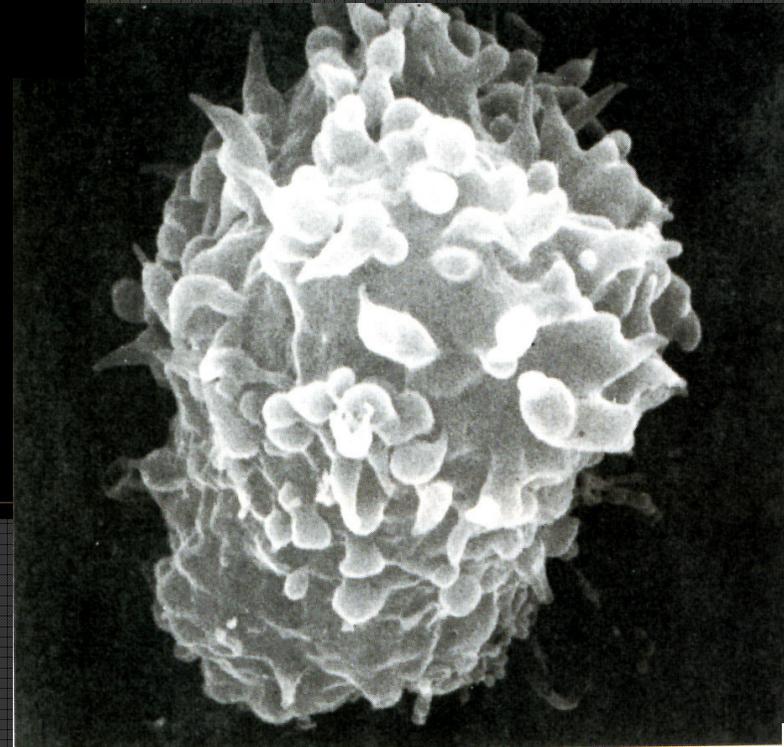
# "Plasticidad" de las células madre hematopoyéticas





embrionarias

adultas



**Antigua controversia-- probablemente obsoleta**

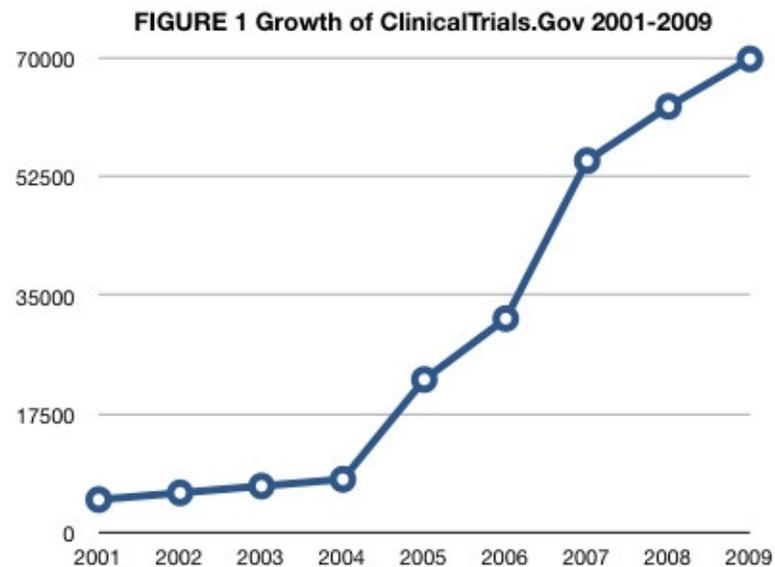
## *LOS ENSAYOS CLÍNICOS*





**EUROPEAN SOCIETY OF GENE & CELL THERAPY**  
A SOCIETY DEDICATED TO THE DEVELOPMENT OF GENE THERAPY, CELL THERAPY & GENETIC VACCINES

<http://www.esgct.eu/>



### ***Stem Cell Clinical Trials: An Index***

<http://thestemcellblog.com/2007/07/16/stem-cell-trials-a-harpers-index/>

### ***The BioTech weblog***

<http://www.biotech-weblog.com/50226711/first-clinical-trial-using-stem-cell-therapy-for-spinal-cord-injuries.php>

### ***Terapia Génica***

<http://www.wiley.co.uk/genmed/clinical/>

<http://www.genetherapynet.com/clinicaltrials.html>

## Who is who !!



<http://clinicaltrials.gov/>

*“ClinicalTrials.gov is a registry of federally and privately supported clinical trials conducted in the United States and around the world. ClinicalTrials.gov gives you information about a trial's purpose, who may participate, locations, and phone numbers for more details. This information should be used in conjunction with advice from health care professionals.*

*Read more... “*

**Actualmente contiene 72.268 ensayos en 165 naciones**

De todos, cuantos implican el uso de células madre?

1. Ensayos totales con “células madre” = **2.319** (aprox. 3%)
2. Ensayos totales con “terapia génica” = 334
3. E. cardiovascular + “células madre” = 118
4. Diabetes + “células madre” = 21
5. Parkinson + “células madre” = 0
6. “Células madre” + China = 17
7. “Células madre” + Alemania = 128
8. “Células madre de cordón umbilical” = 18
9. “Células madre” + “transferencia nuclear” = 0
10. “Células madre embrionarias” = 1

Actualizado a 21 de Marzo, 2009

**Study of Autologous Fat Enhanced w/ Regenerative Cells Transplanted to Reconstruct Breast Deformities After Lumpectomy**

IP: Alexander M Milstein, MD

Sponsor: **Cytori Therapeutics**

Adipose-derived stem cells (ADRCs); direct transplantation in patient breast

**Phase IV (70); no placebo**

Locations:

Belgium	Jules Bordet Institute of Cancer. Brussels	[Rika Deraemaecker]
Italy	Università degli Studi di Firenze	[Claudio Calabrese]
<b>Spain</b>	<b>Hospital General Universitario Gregorio Marañón. Madrid</b> <b>Instituto Valenciano de Oncología</b>	[Rosa Pérez] [Carlos Vázquez-Albaladejo]
UK. Scotland	Glasgow Royal Infirmary. Glasgow	[Eva Weiler-Mithoff]

## LOS ENSAYOS CLÍNICOS

***PERO.....***

***Cuidado donde te metes ó metes a tus familiares!!!!***



# Donor-Derived Brain Tumor Following Neural Stem Cell Transplantation in an Ataxia Telangiectasia Patient

Ninette Amariglio<sup>1,2</sup>, Abraham Hirshberg<sup>3</sup>, Bernd W. Scheithauer<sup>4</sup>, Yoram Cohen<sup>1</sup>, Ron Loewenthal<sup>5</sup>, Luba Trakhtenbrot<sup>2</sup>, Nurit Paz<sup>1</sup>, Maya Koren-Michowitz<sup>2</sup>, Dalia Waldman<sup>6</sup>, Leonor Leider-Trejo<sup>7</sup>, Amos Toren<sup>6</sup>, Shlomi Constantini<sup>8</sup>, Gideon Rechavi<sup>1,6\*</sup>

**1** Cancer Research Center, Sheba Medical Center and Sackler School of Medicine, Tel Aviv University, Tel-Aviv, Israel, **2** Institute of Hematology, Sheba Medical Center, Tel Hashomer, Israel, **3** Department of Oral Pathology, School of Dental Medicine, Tel Aviv University, Tel-Aviv, Israel, **4** Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, United States of America, **5** Tissue Typing Laboratory, Sheba Medical Center and Sackler School of Medicine, Tel Aviv University, Tel-Aviv, Israel, **6** Department of Pediatric Hemato-Oncology, Sheba Medical Center and Sackler School of Medicine, Tel Aviv University, Tel-Aviv, Israel, **7** Institute of Pathology, Tel-Aviv Medical Center, Tel-Aviv, Israel, **8** Pediatric Neurosurgery, Dana Children's Hospital, Tel-Aviv Medical Center, and Sackler School of Medicine, Tel Aviv University, Tel-Aviv, Israel

## Methods and Findings

A boy with ataxia telangiectasia (AT) was treated with intracerebellar and intrathecal injection of human fetal neural stem cells. Four years after the first treatment he was diagnosed with a multifocal brain tumor. The biopsied tumor was diagnosed as a glioneuronal neoplasm. We compared the tumor cells and the patient's peripheral blood cells by fluorescent in situ hybridization using X and Y chromosome probes, by PCR for the amelogenin gene X- and Y-specific alleles, by MassArray for the ATM patient specific mutation and for several SNPs, by PCR for polymorphic microsatellites, and by human leukocyte antigen (HLA) typing. Molecular and cytogenetic studies showed that the tumor was of nonhost origin suggesting it was derived from the transplanted neural stem cells. Microsatellite and HLA analysis demonstrated that the tumor is derived from at least two donors.

## *Turismo celular / Tratamientos milagro ??*

### **Embryonic Tissues Center (EmCell)**

Centro de tejidos embrionarios. Kiev, Rusia.

[www.emcell.com](http://www.emcell.com)

### **Biomark International**

La FDA la cerró (web activa en España)

<http://www.biomark-intl.com>

### **Centro Médico Sheba**

Dep. de la Universidad de Tel Aviv. Israel

### **Centro Xcell**

Colonia (Alemania)

<http://www.xcell-center.es/>

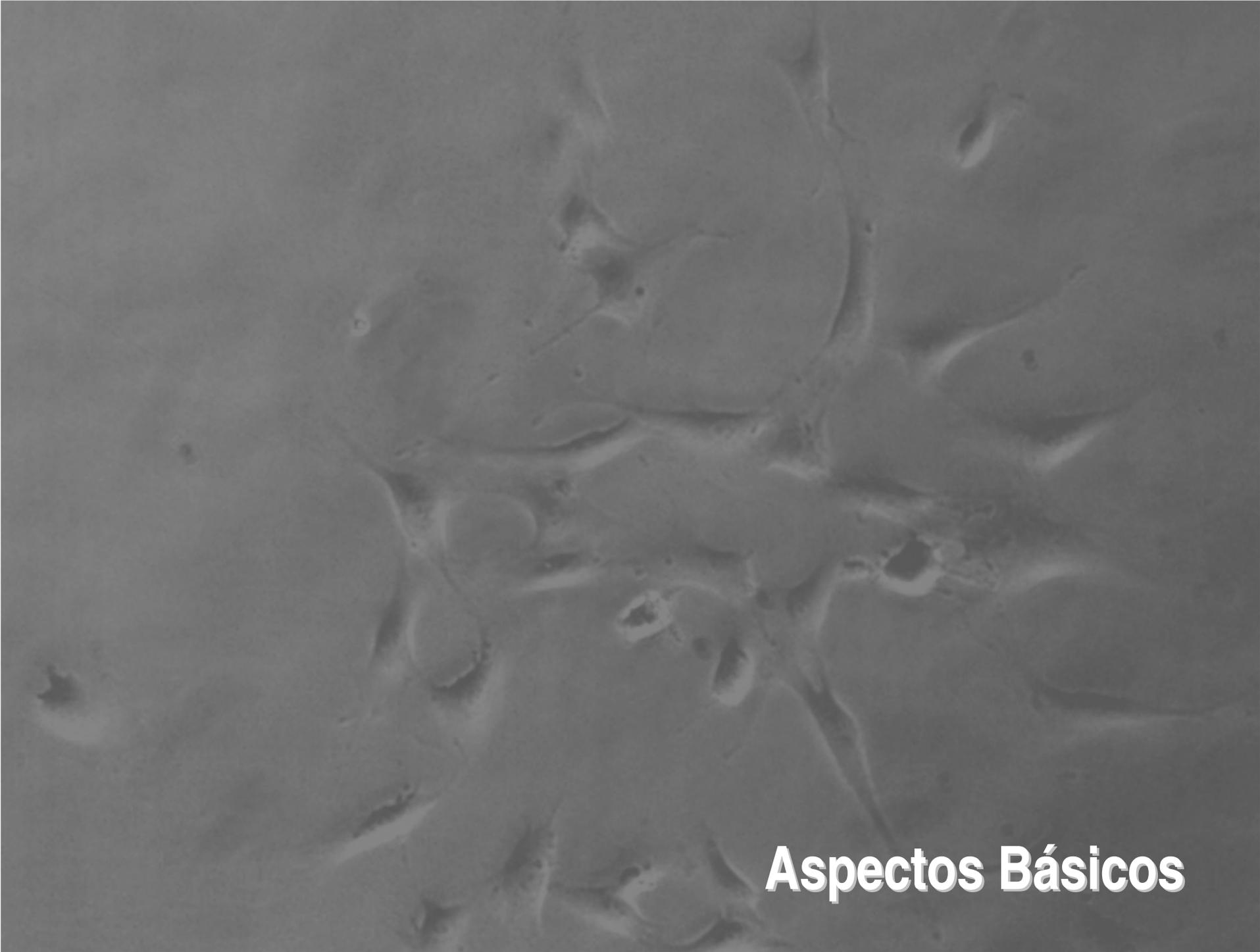
## *Sospechas (sobre 50 Instituciones)*

- La **clínica Medra** en las Bahamas  
<http://www.medra.com/>
- **EmbryoTech**  
[www.embryotech.com](http://www.embryotech.com)
- **The Kharkov Clinic “Dr.Alex”**  
[www.doctor-alex-ua/e/clinica.html](http://www.doctor-alex-ua/e/clinica.html)
- **Stem Cell Therapy International (SCTI)**  
[www.scticorp.com](http://www.scticorp.com)
- **StemCure**  
[www.stemcure.com](http://www.stemcure.com)
- **Donetsk’s VitaCell**  
[www.vitacell.com.ua](http://www.vitacell.com.ua)

## *Corolario*

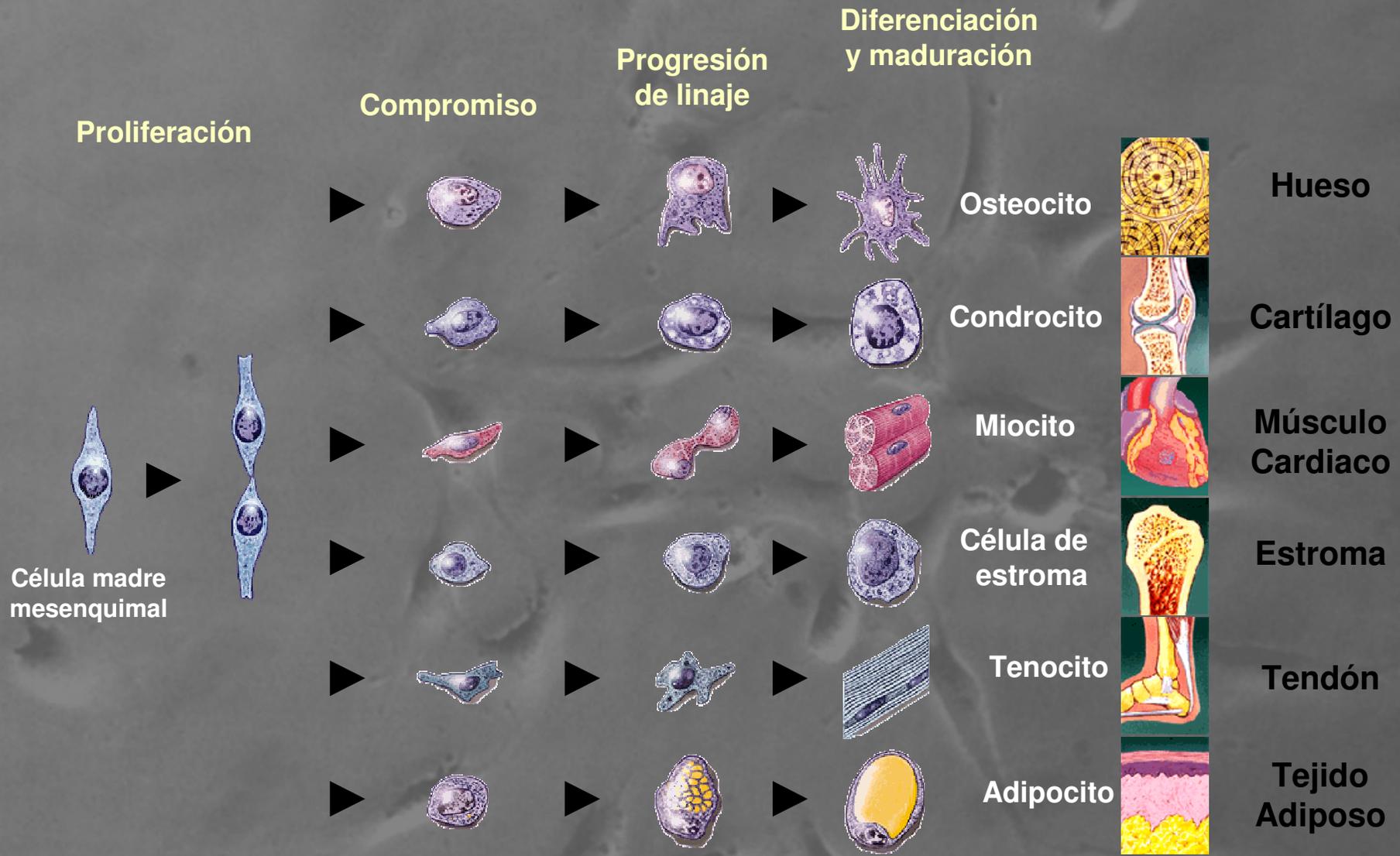
**En este momento es completamente necesario ponerse en manos de Instituciones avaladas por su prestigio y trayectoria y evitar los “tratamientos milagro” y las [instituciones] y/o [clínicas] nacidas de la nada.**

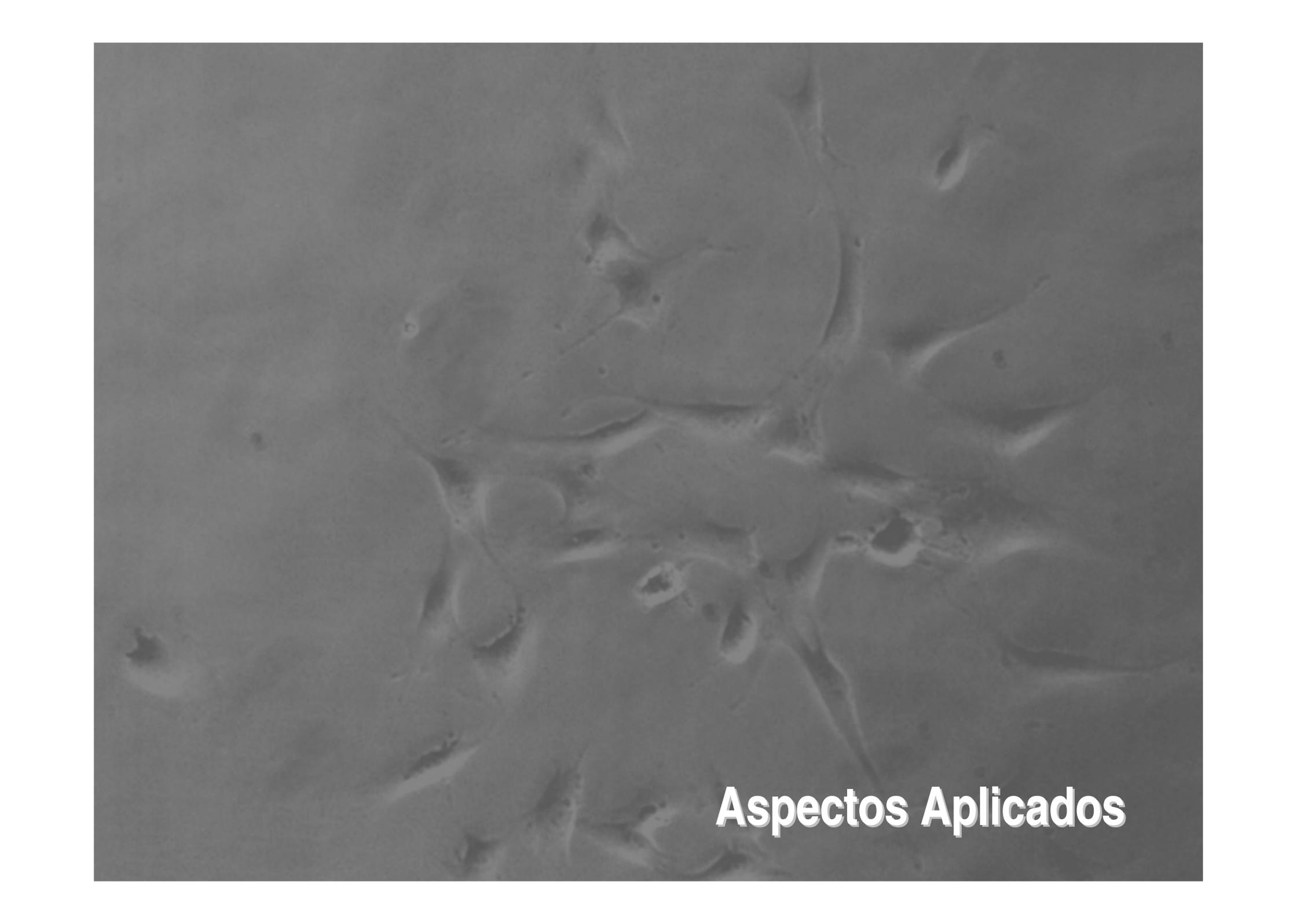
**En caso de duda acudir a los centros de información y control de las Administraciones autonómicas o nacionales.**



**Aspectos Básicos**

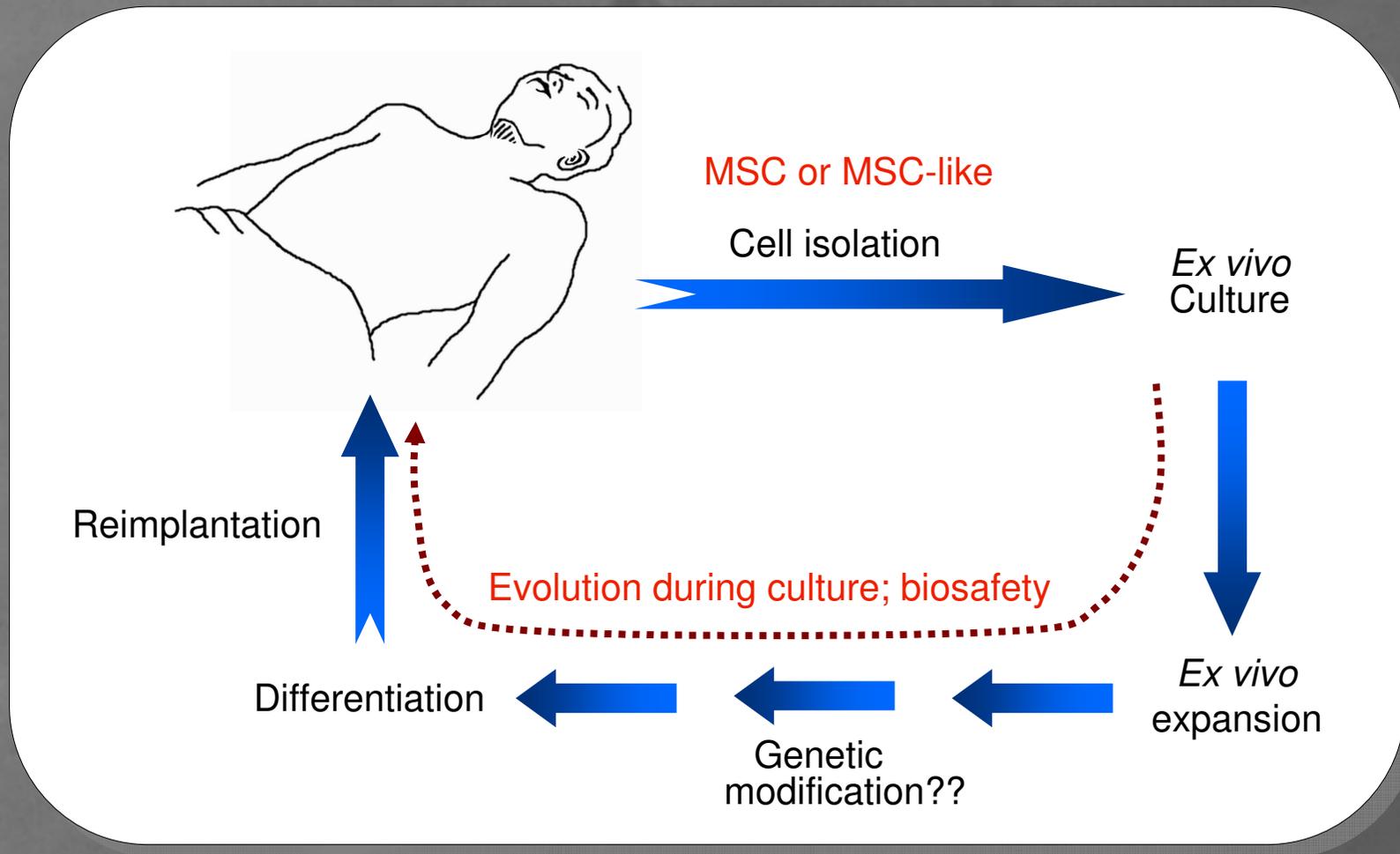
# Células madre mesenquimales



A grayscale micrograph showing a complex network of biological tissue, likely a histological section. The image displays various cellular structures, including what appears to be a large, rounded cell in the upper center and several elongated, spindle-shaped cells throughout the field. The overall texture is granular and fibrous. The text "Aspectos Aplicados" is overlaid in the bottom right corner in a white, bold, sans-serif font.

**Aspectos Aplicados**

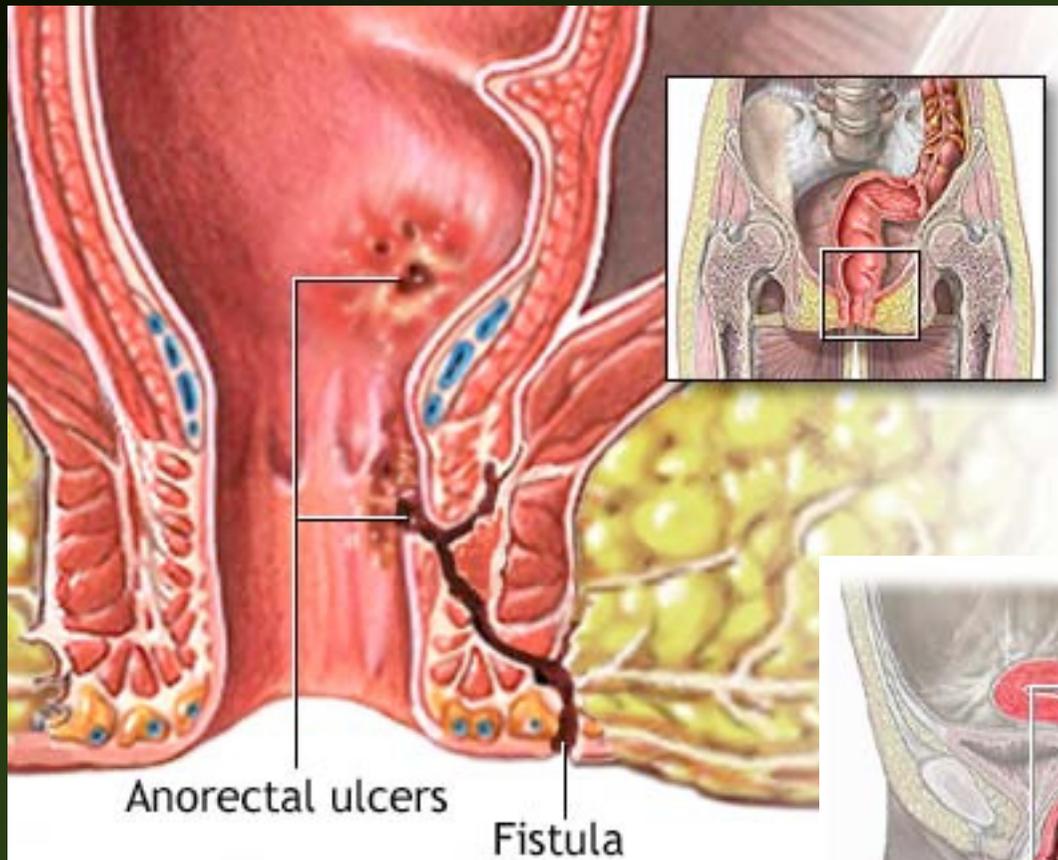
# Cell Therapy



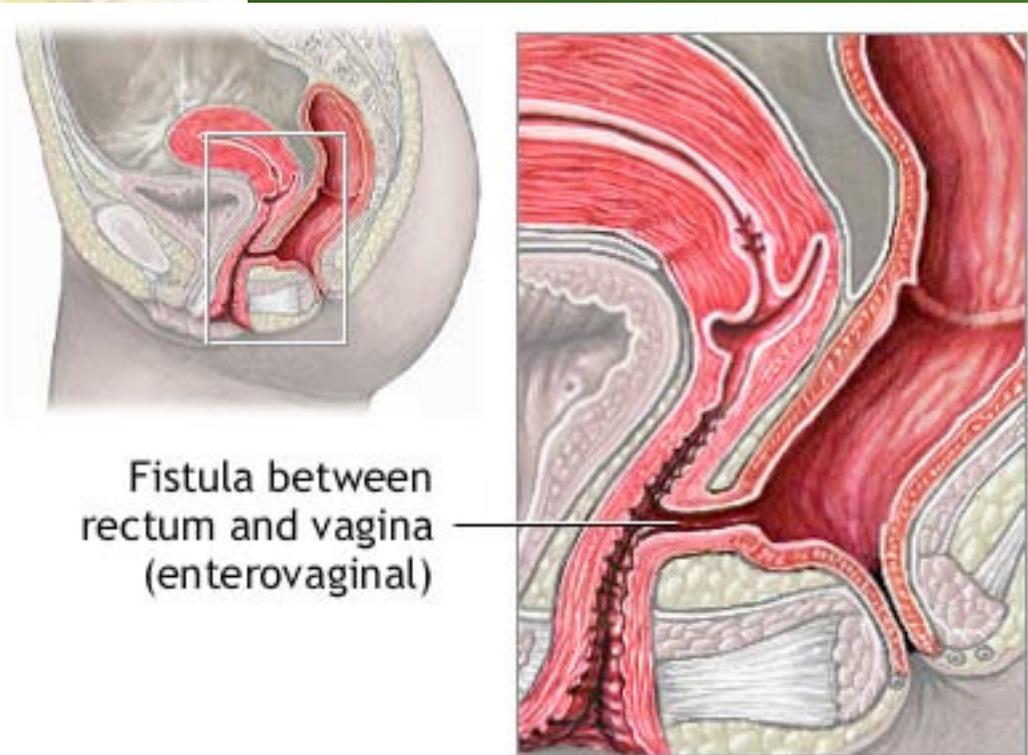
*Del laboratorio a la Clínica  
(2003-2009)*

*Células madre mesenquimales para  
mejorar el proceso de cicatrización  
espontáneo?*

## ¿Tratamiento de la fístula?



Una fístula es una conexión anormal entre un órgano, vaso o sección intestinal y otro órgano, vaso, sección intestinal o piel.





*Living Medicines*

## **Expanded Adipose-Derived Stem Cells (Cx401) for the Treatment of Complex Perianal Fistula; a Randomized, Controlled Clinical Trial**

D. Garcia-Olmo<sup>1</sup>, D. Herreros<sup>1</sup>, I. Pascual<sup>1</sup>, J. A. Pascual<sup>2</sup>, E. Del-Valle<sup>3</sup>, J. Zorrilla<sup>3</sup>, P. De-La-Quintana<sup>1</sup>, M. Garcia-Arranz<sup>1</sup>, M. A. Gonzalez<sup>4</sup>, J. Alemany<sup>4</sup>, G. Fernandez<sup>4</sup>, I. Portero<sup>4</sup>, M. Pascual<sup>4</sup>

*<sup>1</sup> Dept. of Surgery and Cell Therapy, La Paz University Hospital, Universidad Autónoma de Madrid (UAM); <sup>2</sup> Dept. of Surgery, Doce de Octubre University Hospital; <sup>3</sup> Dept. of Surgery, Gregorio Marañón University Hospital; <sup>4</sup> Cellerix S.L., Madrid, Spain*



# ¿Quiénes han participado?



- H. LA PAZ realiza y dirige el desarrollo clínico desde el inicio



- Genetrix actúa como PROMOTOR y desarrolla la producción con calidad clínica (GMP)

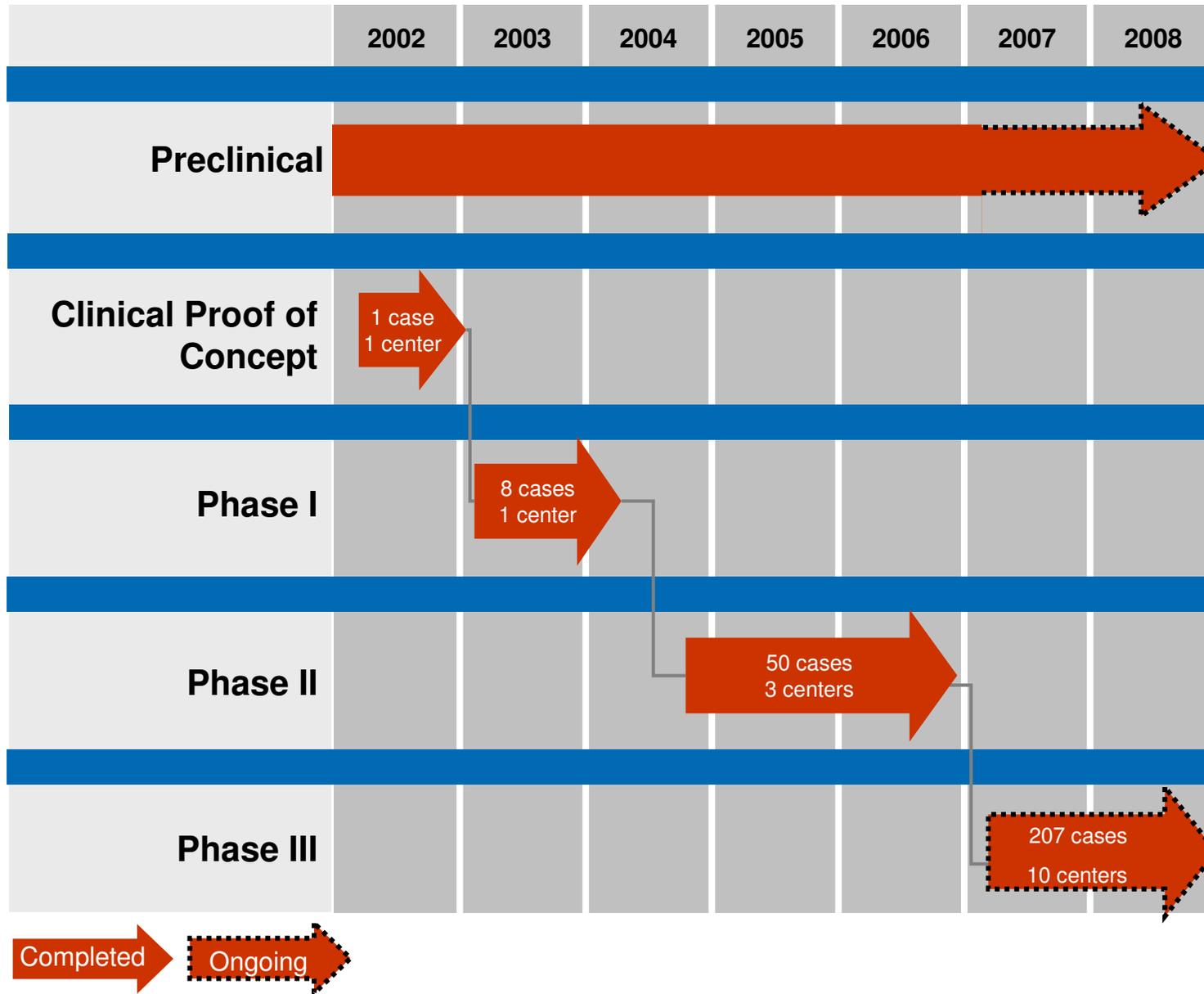
# Tratamiento propuesto

## Tratamiento no quirúrgico de fistulas complejas perianales usando células madre de tejido adiposo expandidas *ex vivo* (Cx401)

- **Células madre adultas** (no embrionarias)
- Tratamiento autólogo: No hay riesgo de rechazo
- Células madre **de tejido adiposo**
  - **Altos rendimientos** (entre 100 y 1000 veces superiores a la médula ósea)
  - **Accesible y expansible:** liposucción simple
- No hay manipulaciones mayores, ni uso de factores de crecimiento— **mayor seguridad**
- **No hay riesgo de incontinencia anal.**

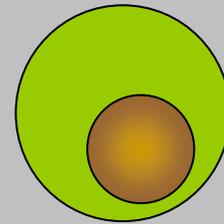


# Cx401 Clinical Development

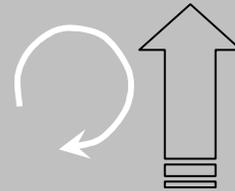


# Conclusiones

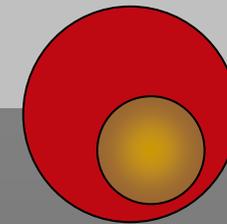
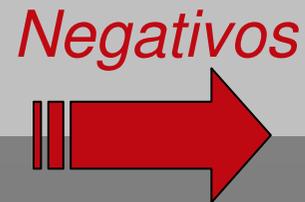
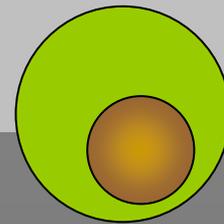
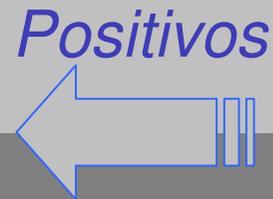
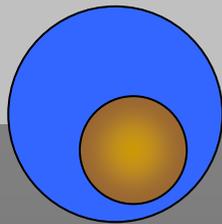
- **Cx401 es un **tratamiento efectivo** en fístula compleja, tanto en pacientes afectados de Crohn como en no-Crohn**
  - La probabilidad de obtener cicatrización completa es cuatro veces superior en pacientes tratados con Cx401 en comparación con los que se trataron con gel de fibrina.
- **El tratamiento con Cx401 parece **mejorar la Calidad de vida** del paciente**
  - Hay una diferencia estadísticamente significativa en términos de condición física general en pacientes tratados con Cx401.
- **Cx401 es un **tratamiento efectivo a largo plazo** en fístula compleja, tanto en pacientes afectados de Crohn como en no-Crohn**
  - Tras un año de seguimiento de todos los pacientes, la tasa de recurrencia se ha establecido en un 17.6% en el grupo tratado con con Cx401.
- **El tratamiento con Cx401 **es seguro****
  - No se han observado efectos secundarios severos en ninguno de los pacientes incluidos en las Fases I y II de los ensayos clínicos.



*Homeostasis  
de los tejidos & órganos*



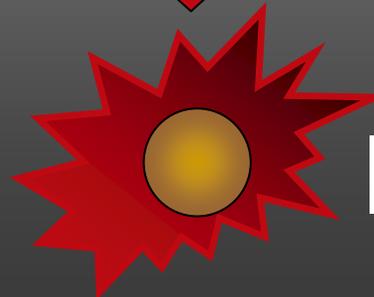
**Fisiología Normal**



**Nuevas  
Posibilidades  
Terapéuticas**

*Células Madre*

**Nuevas  
Implicaciones  
Fisio-Patológicas**



**Cáncer Stem Cells**

**Regulation of gene expression &  
genetic stability in adult stem cell  
(2011)**



***Cardiovascular Development and Repair Department***

Antonio Bernad  
Jefe del Dpto. Cardiología Regenerativa



*Laboratorio de expresión y estabilidad  
genética de células madre adultas*

