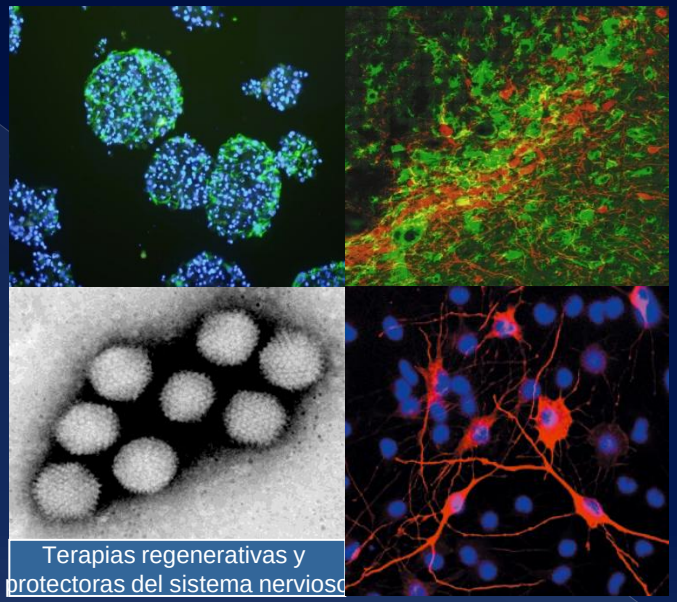
Células madre



Fernando J. Pitossi
Fundación Instituto Leloir
CONICET

Composición elemental del cuerpo humano

70% H_2O

Elemento	% de peso seco	% de peso total
C	61.7	18.5
N	11.0	3.3
O	9.3	2.8
H	5.7	1.7
Ca	5.0	1.5
P	3.3	1.0
K	1.3	0.4
S	1.0	0.3
Cl	0.7	0.2
Na	0.7	0.2
Mg	0.3	0.1

B, F, Si, V, Cr, Mn, Fe, Co, Cu, Zn, Se, Mo, Sn, I

En 70 kg de peso:

49 kg de H_2O
13 Kg de carbono
2,3 Kg de nitrógeno
Etc.

Organización vital

sistemas + sistemas: organismo

↑

órgano + órgano: sistemas

↑

tejido + tejido: órgano

↑

célula + célula: tejido

↑

Organelas + membrana: célula

↑

Conjunto de Biomoléculas + membrana: organelas

↑

Biomoléculas: proteínas, ADN, ARN, lípidos, azúcares

↑

Moléculas inorgánicas

Capacidad de cambio celular

El cuerpo está compuesto por 10 $^{13\text{-}14}$ células (10 -100.000.000.000.000)

Relativizado al espacio que ocupa una persona (0.25m2)>

2.500.000.000.000 m2=

> 65.000.000 de Plazas de Mayo

>10.000 x Buenos Aires

Organismo con alta capacidad de cambio

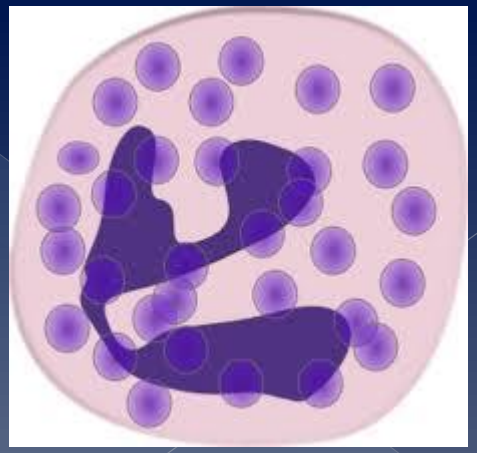
Organismo con alta capacidad de cambio

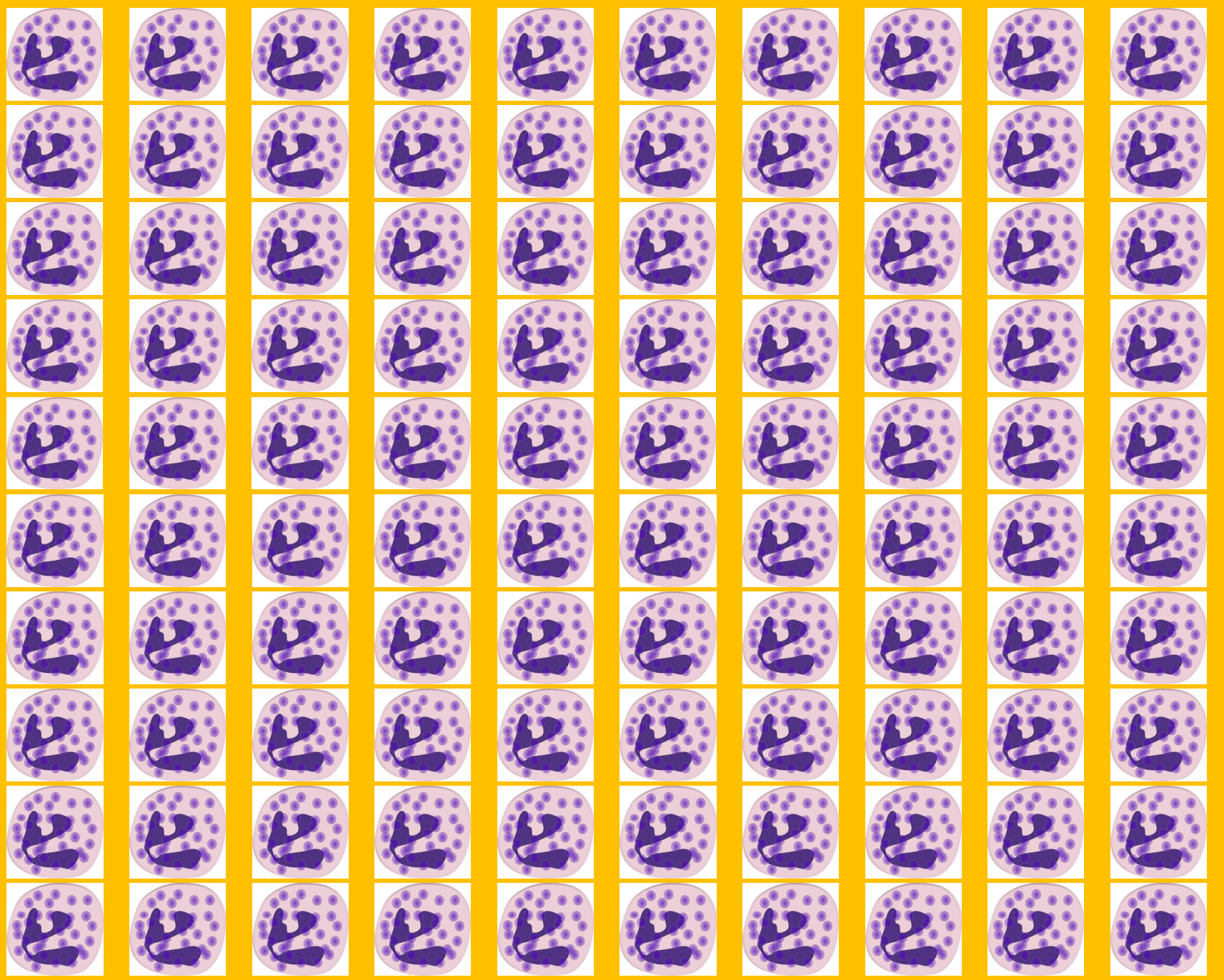
Neutrófilos

Célula mayoritaria de los glóbulos blancos del sistema inmune

Por día se producen 100.000.000.000 de neutrófilos
(Cartwright, Blood, 1964, 24:780)

>1.000.000 nacen/seg
5-10X durante una infección

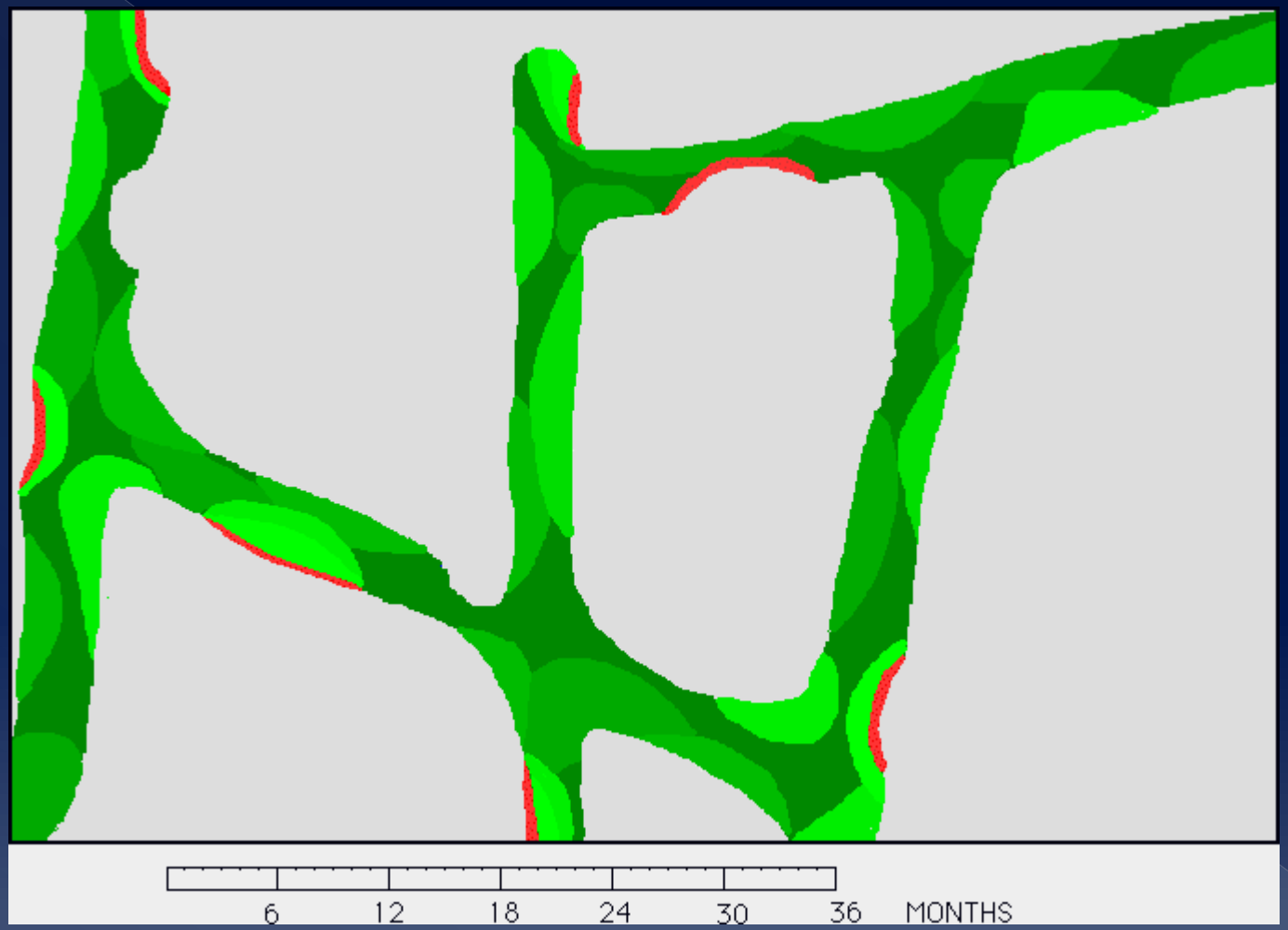




Organismo con alta capacidad de cambio

Recambio óseo

Organismo con alta capacidad de cambio



Verde claro: hueso nuevo

Verde oscuro: hueso viejo

El 10% de los huesos se recambia por año

(Manolagas et al, Endocr. Rev. 2000 21: 115-137)

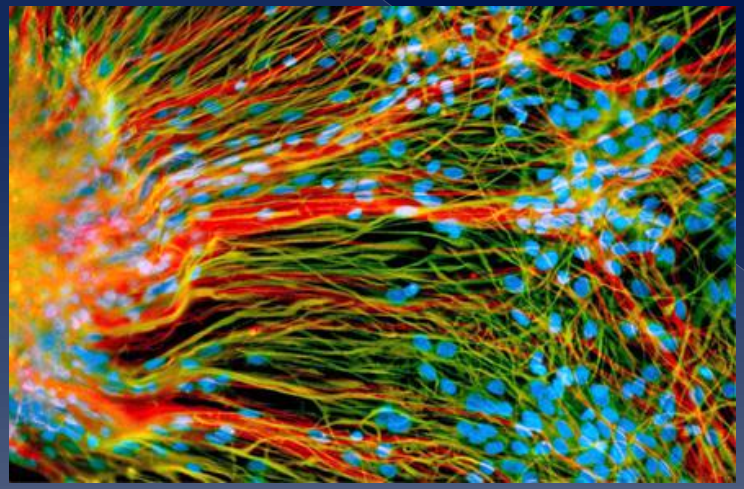
Organismos con capacidad de cambio extrema

Nadia Rosenthal-_Howard Hughes Medical Institute

Células madre

Células con capacidad de:

- autoperpetuarse (prolongada o ilimitada)
- diferenciarse a distintos tipos celulares



Pregunta básica

Qué célula madre utilizar?

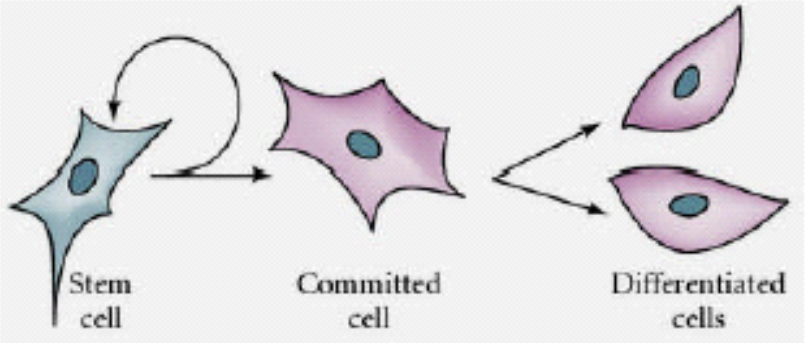
Células madre

Células con capacidad de:

- autoperpetuarse (prolongada o ilimitada)
- diferenciarse a distintos tipos celulares

Tipos:

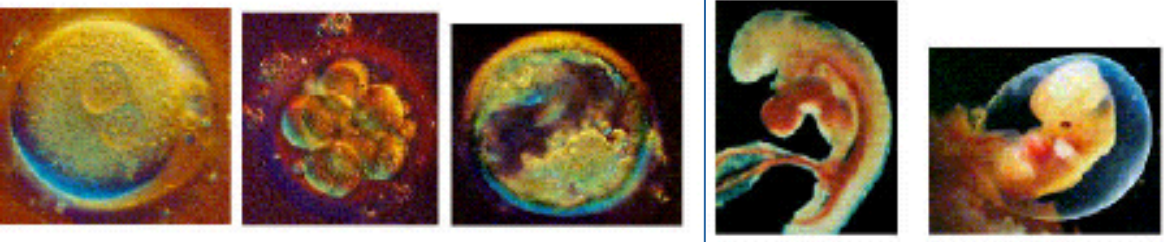
Embrionarias
Adultas
hematopoyéticas
epiteliales
músculo cardíaco
hígado
páncreas
sistema nervioso
Reprogramadas



Stem Cells

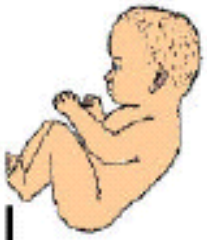
Human Developmental Continuum

14 days



Single-cell Embryo | 3-day Embryo | 5-7 day Embryo | 4-week Embryo | 6-week Embryo

56 days



Infant

Adult

Teratocarcinoma (germ cell tumor)

Embryonic Stem (ES) cells
Totipotent

Embryonic Germ (EG) cells
(primordial germ cells)
Pluripotent

Fetal Tissue Stem cells
Pluripotent or Multipotent

"Adult" Stem cells
Pluripotent or Multipotent

Cord Blood Stem cells
Placental Stem cells
Pluripotent or Multipotent

Embryonal Carcinoma (EC) cells
Pluripotent

Video desarrollo

Células madre embrionarias

Células madre

Embrionarias multipotentes

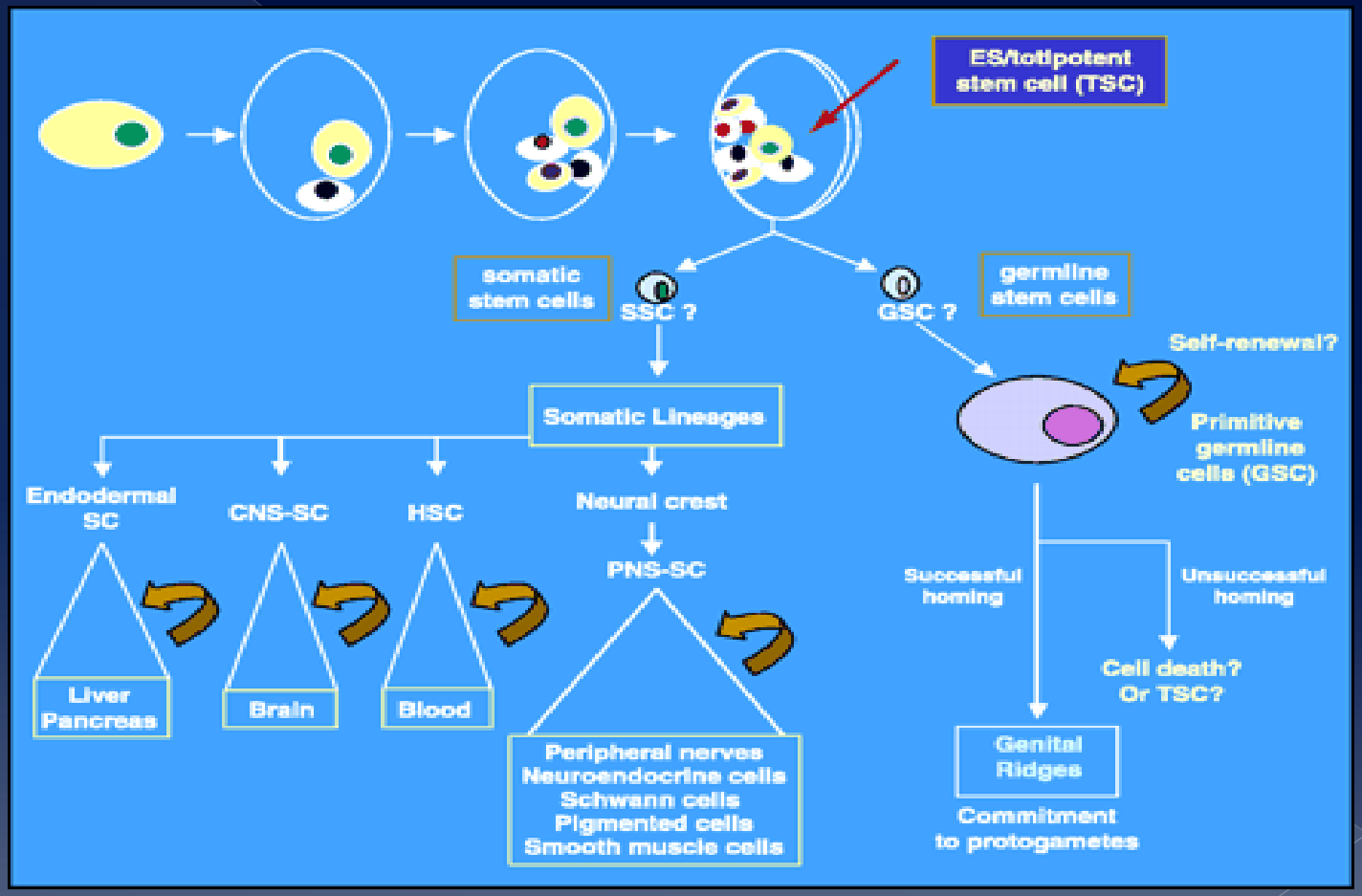
Derivan del embrión en período de preimplantación o periimplantación.
Aisladas hace 20 años.

50% de eficiencia en dar líneas celulares
Diferenciables a neuronas, oligodendrocitos, astrocitos, islotes pancreáticos, cartílago, hueso, cardiomiocitos, cél hematopoyéticas, endoteliales y hepatocitos.

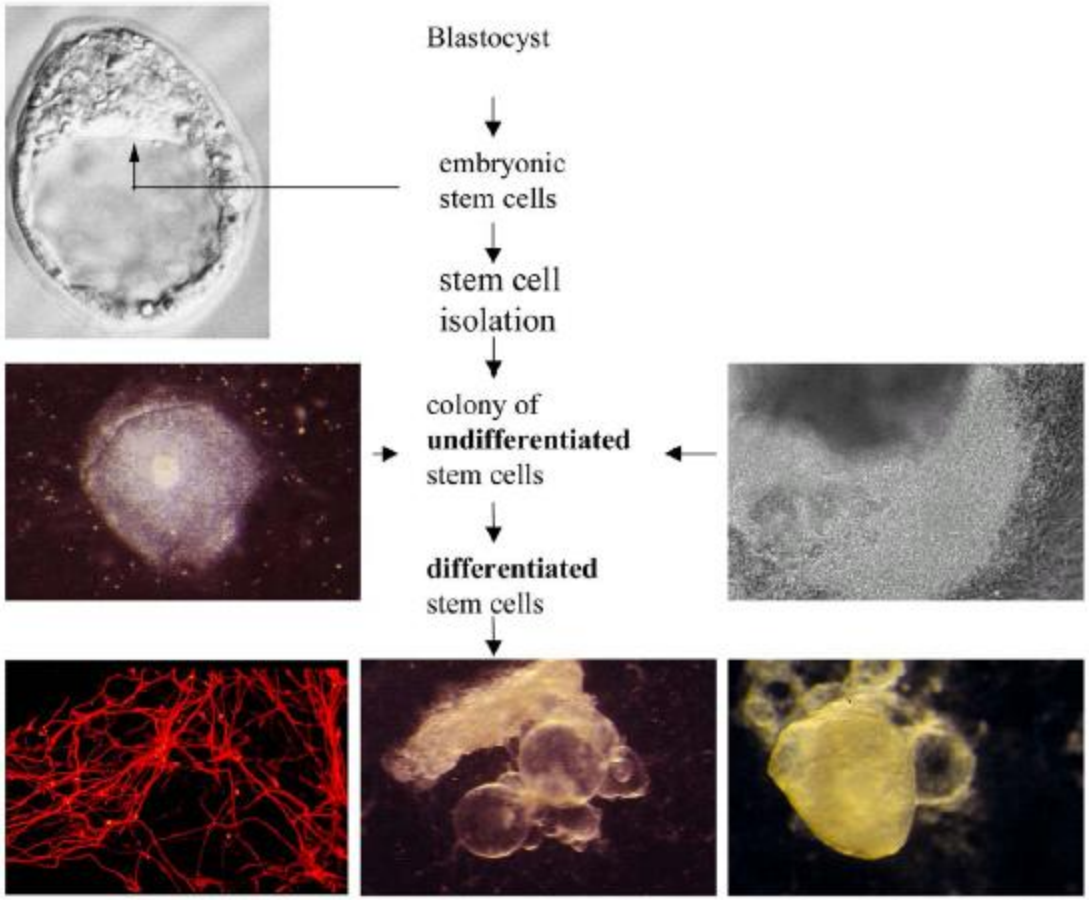
<u>Problemas técnicos</u>
feeder layer (humana)
comportamiento en cultivo muy variable
teratogénicas

Células madre embrionarias

Diferenciación



Células madre
Embrionarias multipotentes in vitro



From blastocyst, to embryonic stem cell line, to somatic cells. hES: human embryonic stem cell. Photographs D. Ward and L. Tertoolen, Hubrecht

Células madre

Utilización

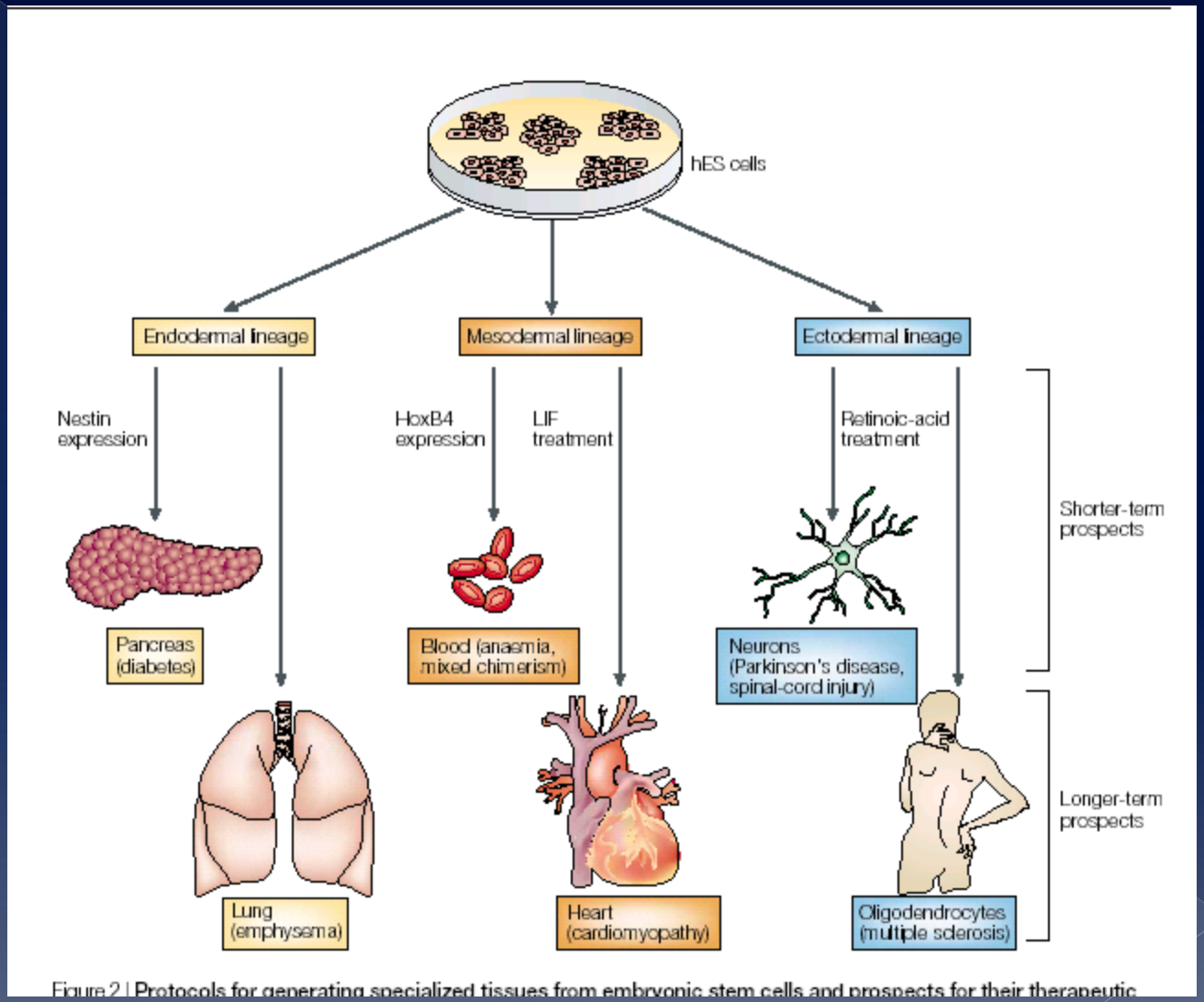
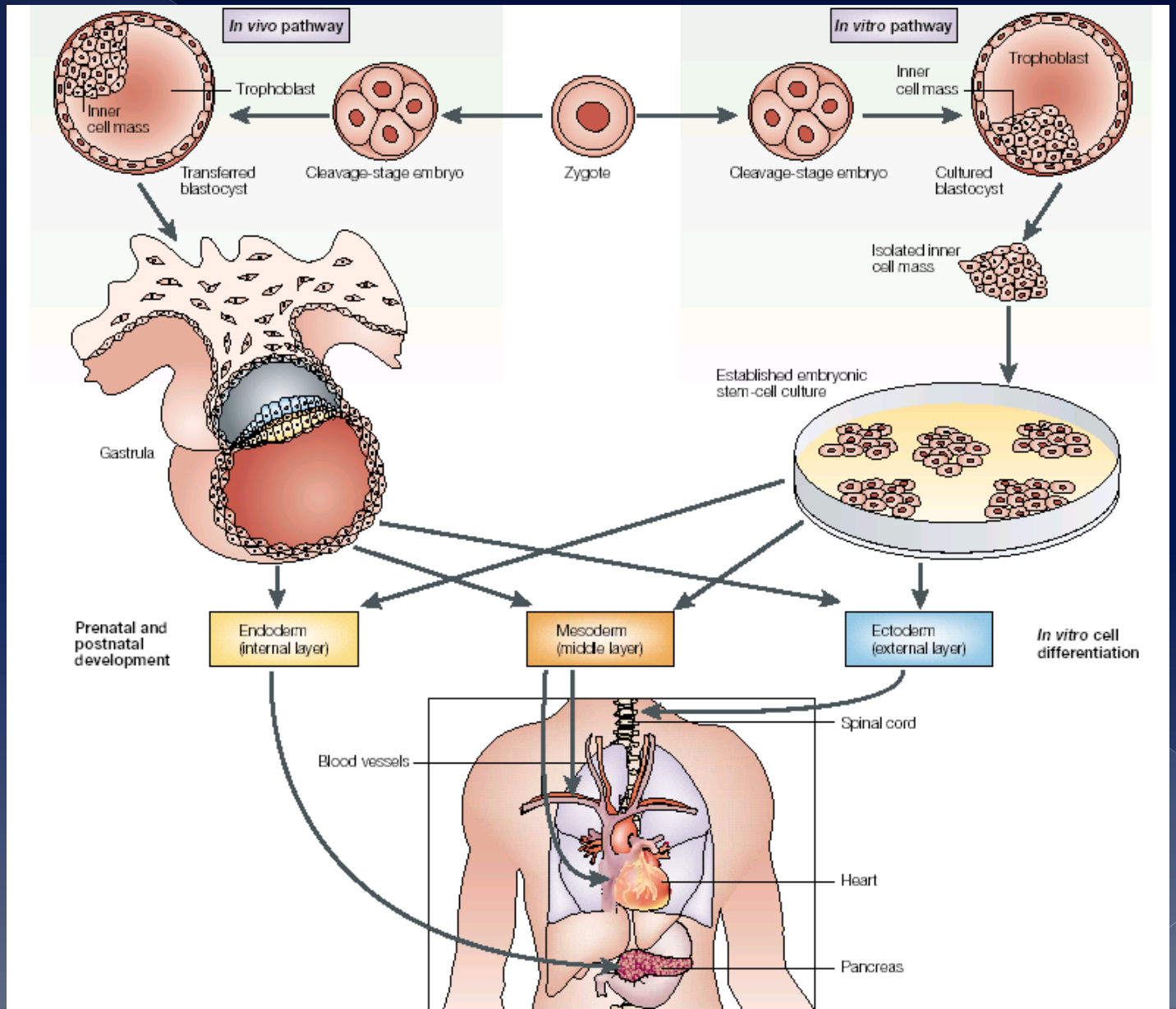


Figure 2 | Protocols for generating specialized tissues from embryonic stem cells and prospects for their therapeutic

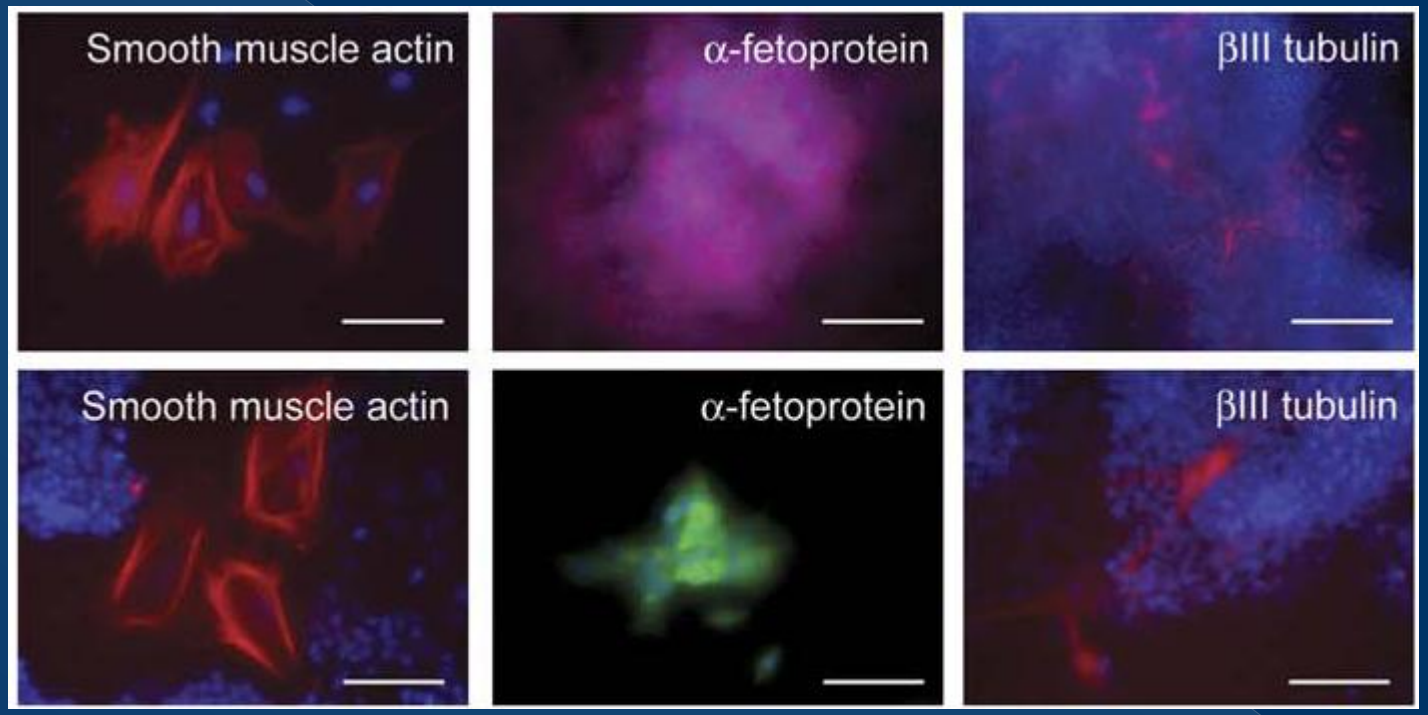
Células madre

Utilización

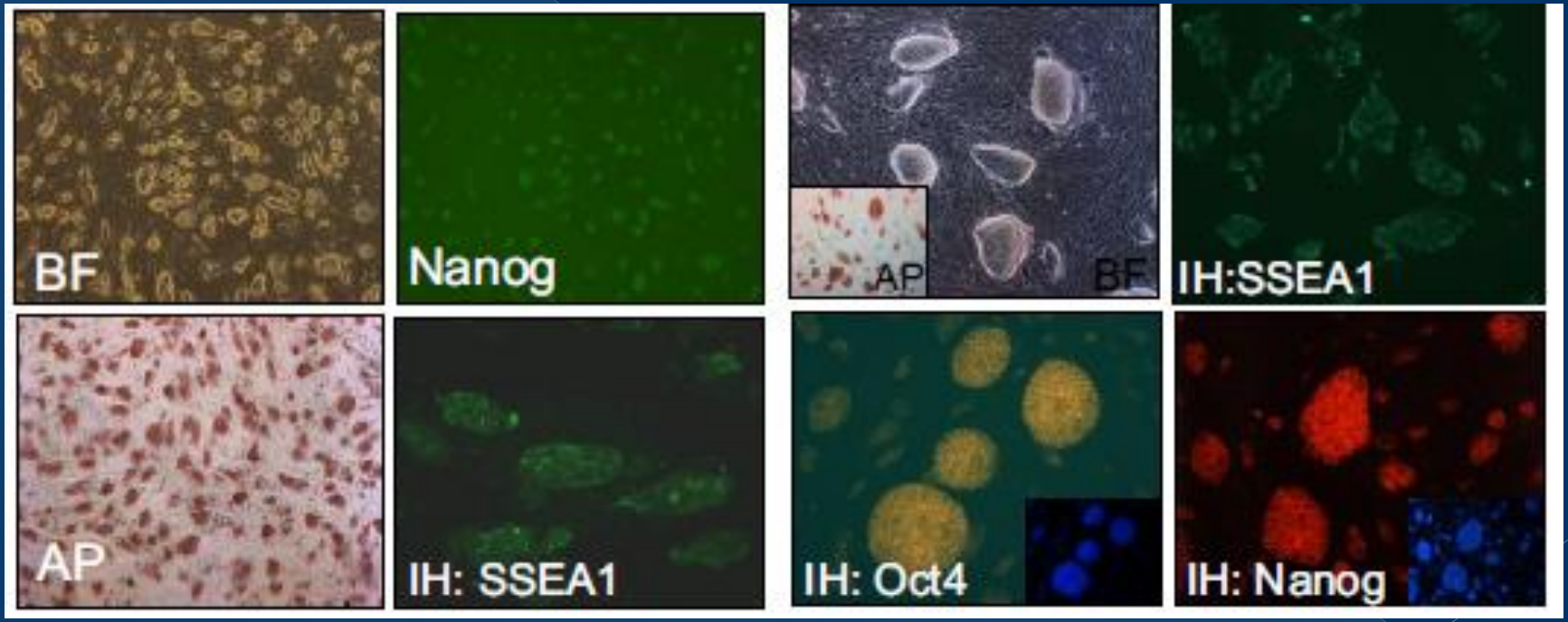


Bradley, 2002,
Nat Rev Immunol

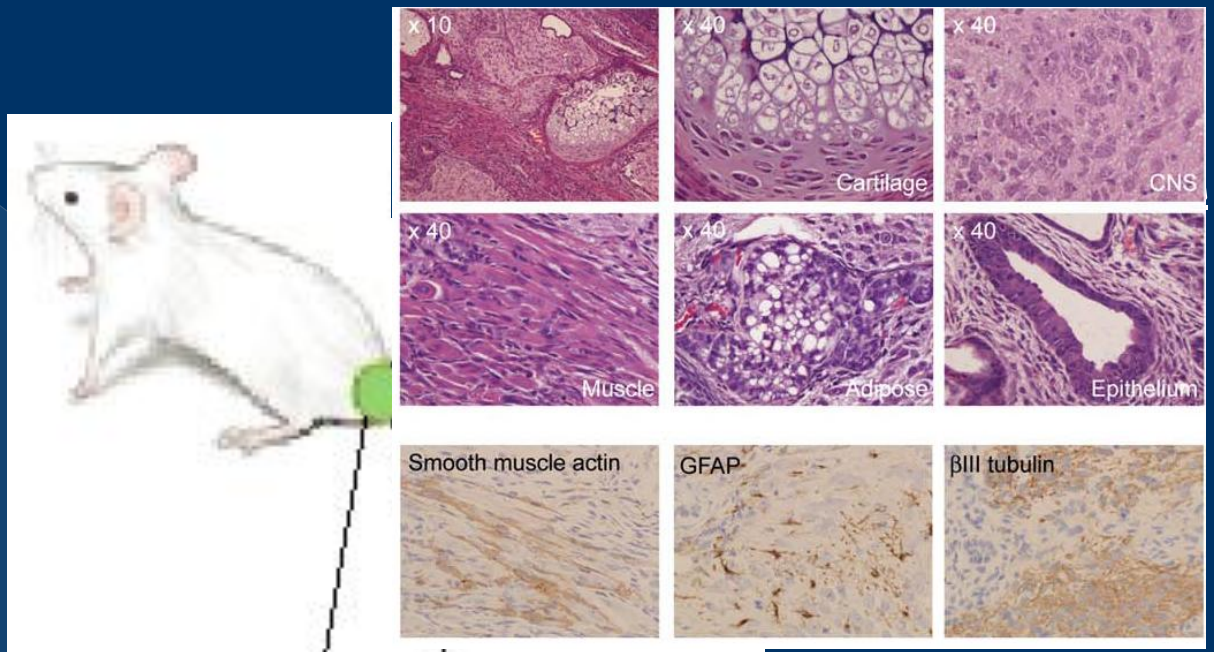
PLURIPOTENCY *in vitro* differentiation



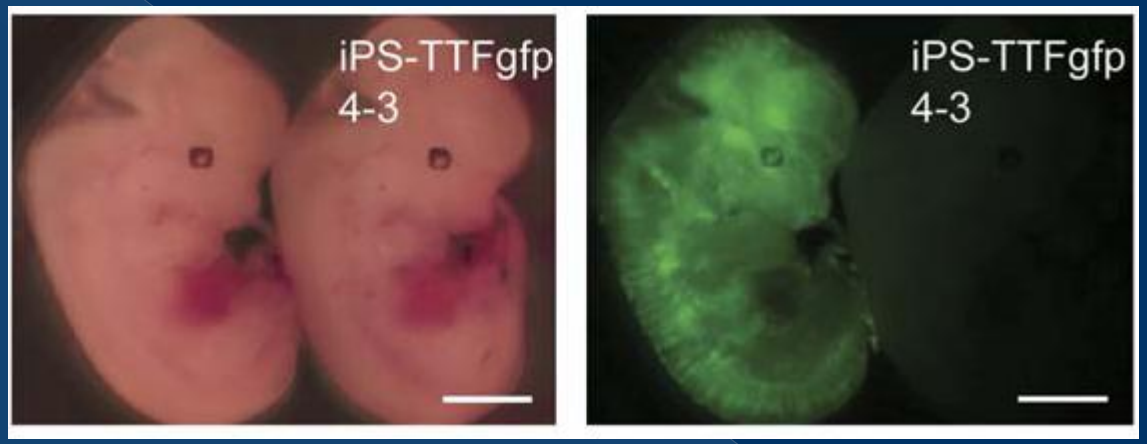
PLURIPOTENCY *in vitro*: markers

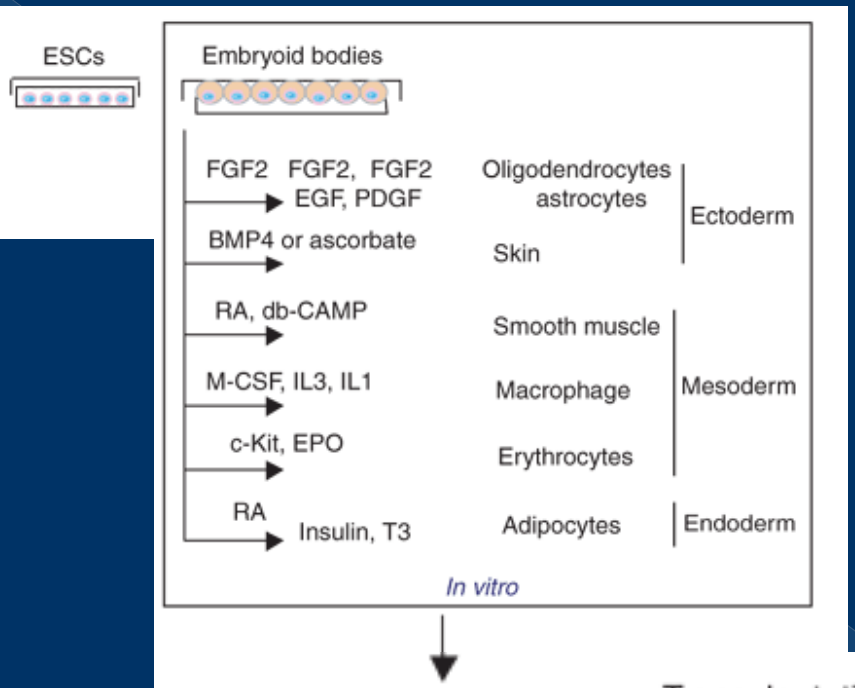


PLURIPOTENCY *in vivo*: Injection into SCID mice



PLURIPOTENCY *in vivo*: chimeras





Cell type	Transplantation into → Rodent model
GABAergic neurons	Striatum of rat model for Huntington disease[73]
Glial precursors	Brain of myelin-deficient rat (Pelizaeus-Merzbacher disease)[6]
Dopaminergic neurons	Striatum of rat model for Parkinson disease[5]
Cardiomyocytes	Myocardium of dystrophic mice[4]
Hematopoietic precursors	Irradiated mice: myeloid and lymphoid engraftment[70]
Hepatocytes	CCl4 intoxicated liver damage mice[74]
Undifferentiated ESCs	Myocardium of infarcted rats[75]

Células madre embrionarias

Potencialidad terapéutica

Developmental Origin of a Bipotential Myocardial and Smooth Muscle Cell Precursor in the Mammalian Heart

Sean M. Wu,[1,4,9] Yuko Fujiwara,[1,3] Susan M. Cibulsky,[2] David E. Clapham,[2,3] Ching-ling Lien,[5] Thomas M. Schultheiss,[6] and Stuart H. Orkin[1,3,7,8,*]

Cell *127*, 1137–1150, December 15, 2006

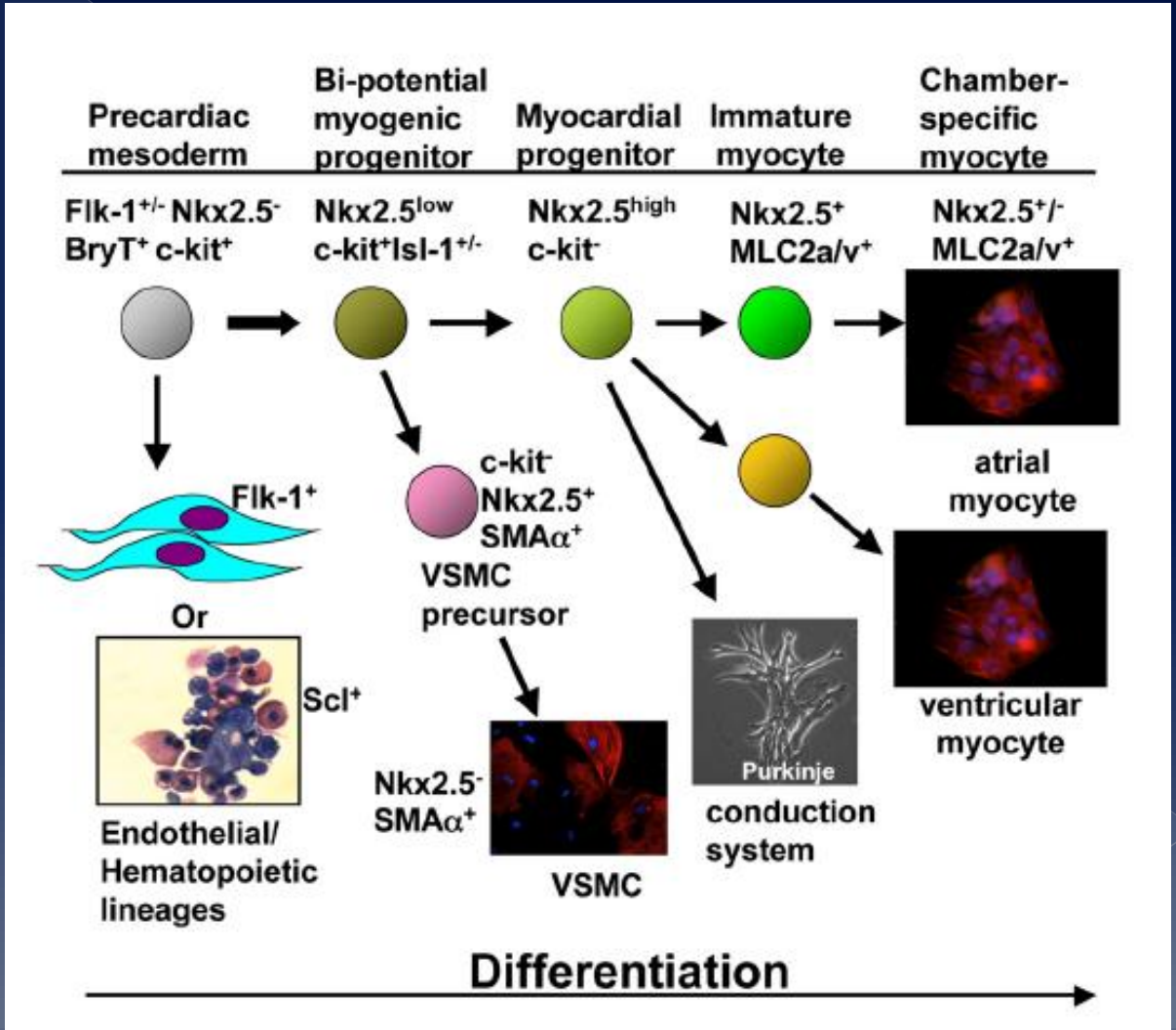
beating cardio.mov

beating cardio GFP.mov

Nr de TERAPIAS ESTABLECIDAS
CON CÉLULAS MADRE EMBRIONARIAS= 0

POR QUÉ?

Células madre embrionarias

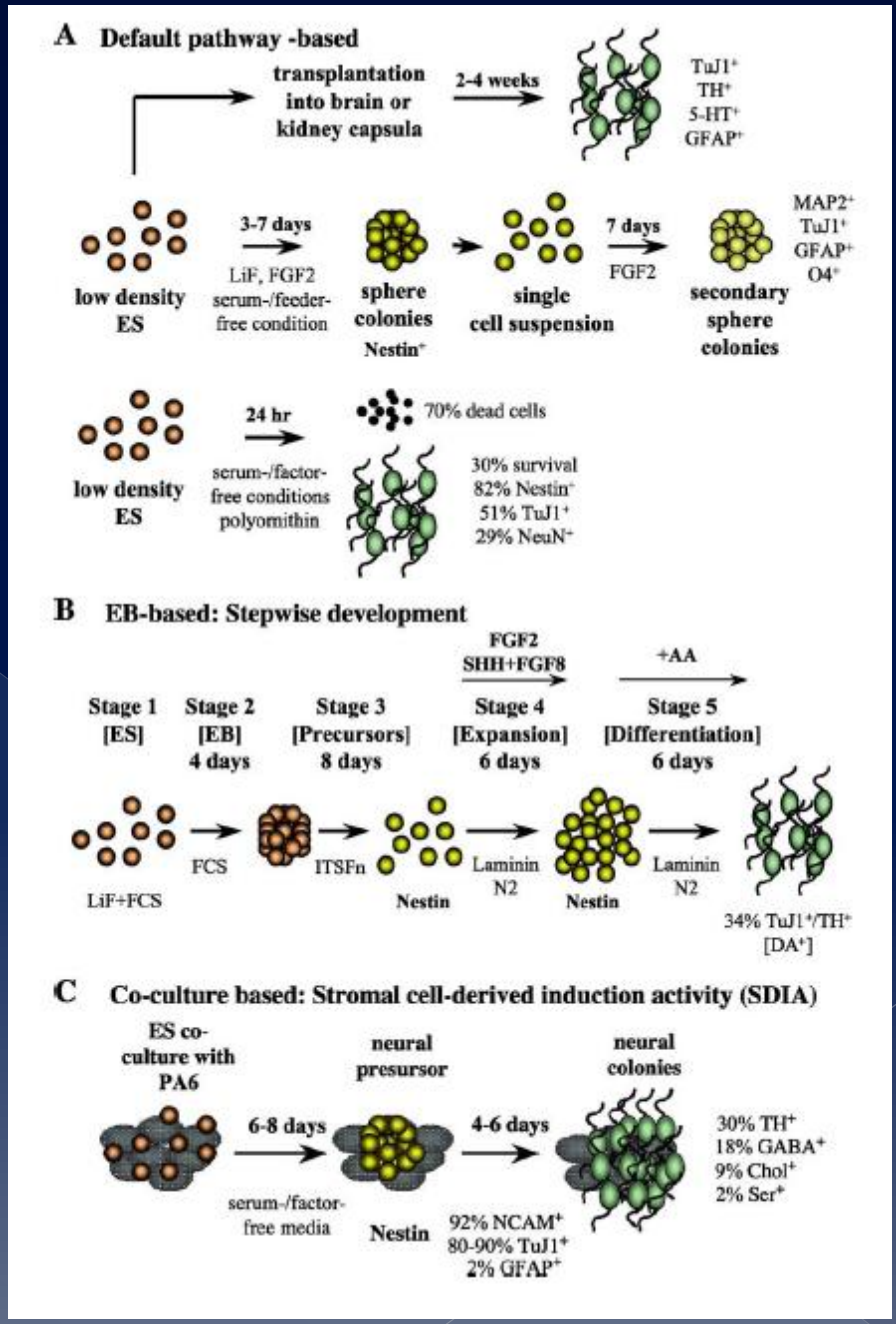


Células madre embrionarias

Problemas sin resolver

Feeder layer
Comportamiento en cultivo muy variable

Problemas éticos



Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model

Lars M. Björklund*†‡, Rosario Sánchez-Pernaute*†§, Sangmi Chung*¶, Therese Andersson*†¶, Iris Yin Ching Chen§, Kevin St. P. McNaught*†, Anna-Liisa Brownell*§, Bruce G. Jenkins§, Claes Wahlestedt||, Kwang-Soo Kim*†, and Ole Isacson*†‡**

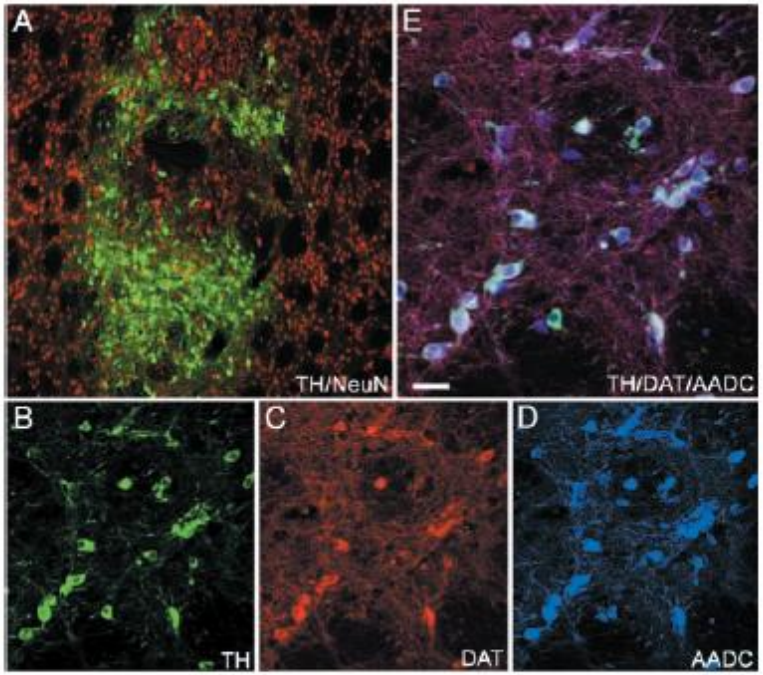
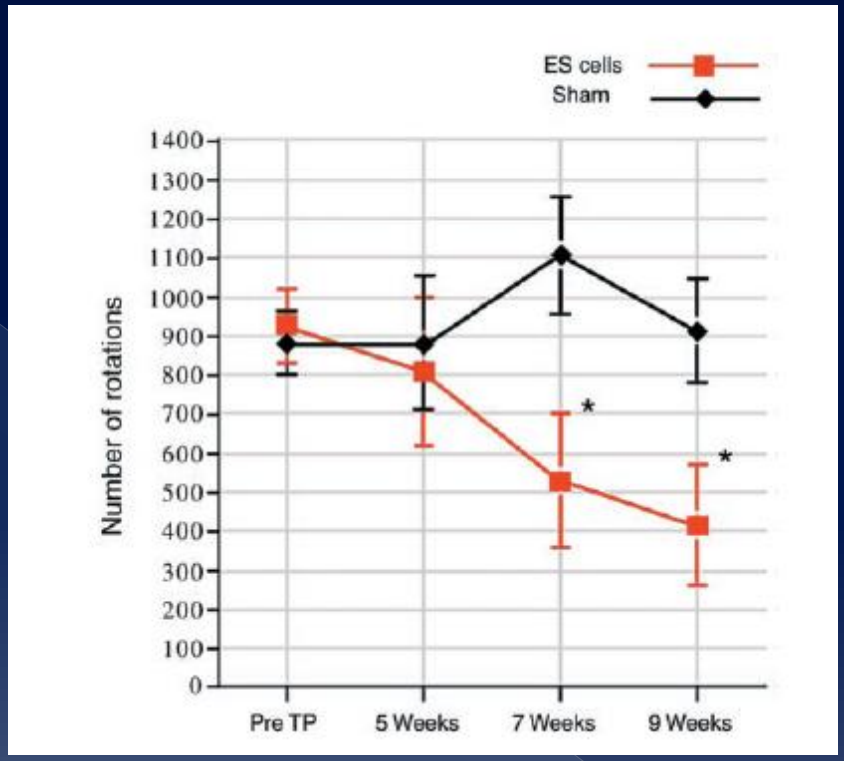


Fig. 1. Immunohistochemical staining of a graft 16 weeks after implantation of a low concentration (1,000–2,000 cells per μl) of D3 ES cells into adult 6-OHDA lesioned striatum. Numerous TH-positive neurons were found within the graft (*A* and *B*, green). All TH-positive profiles coexpressed the neuronal marker NeuN (*A*, red). TH (*B*) also was coexpressed with DAT (*C*, red) and AADC (*D*, blue), demonstrated by white triple labeling (*E*). (Scale bars: *A*, 150 μm; *B–D*, 50 μm; *E*, 25 μm.)

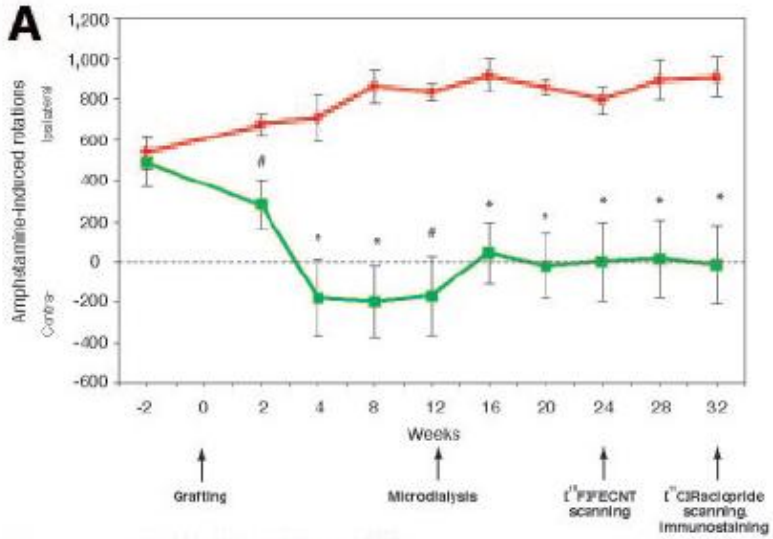


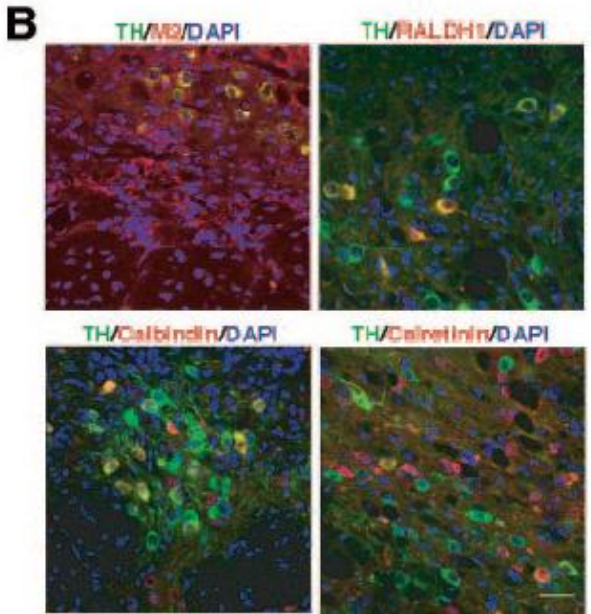
25% de las ratas con teratomas

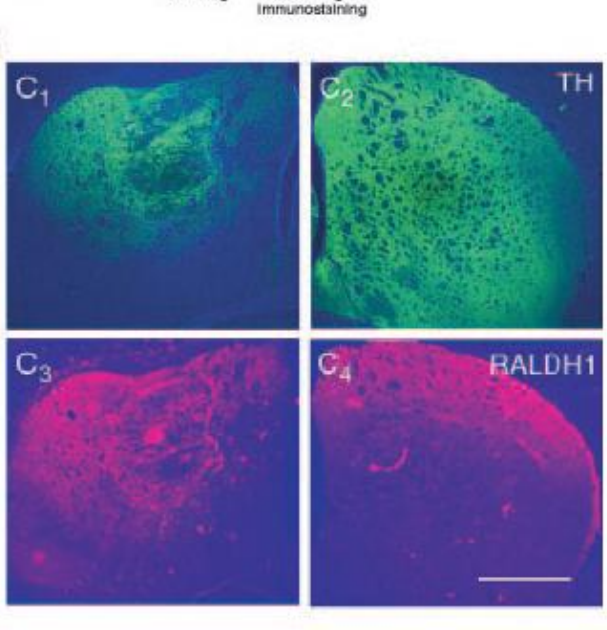
STEM CELLS®
EMBRYONIC STEM CELLS

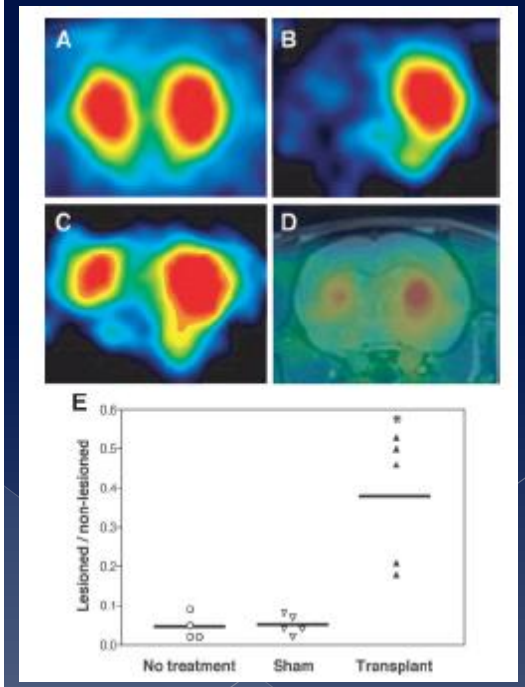
Persistent Dopamine Functions of Neurons Derived from Embryonic Stem Cells in a Rodent Model of Parkinson Disease

...GHBI,[b]
...EIDEL,[c]
...BERT B. INNIS,[b]









STEM CELLS 2007;25:918–928

Células madre embrionales

Ventaja

Alta plasticidad: fuente potencial de cualquier célula

Desventajas

Feeder layer
Comportamiento en cultivo muy variable.
Tumorigénicas
Respuesta inmunológica al trasplante

Debate ético: fuente celular

Células madre

Células con capacidad de:

- autoperpetuarse (prolongada o ilimitada)
- diferenciarse a distintos tipos celulares

Tipos:

Embrionarias
Adultas
hematopoyéticas
epiteliales
músculo cardíaco
hígado
páncreas
sistema nervioso
Reprogramadas

Células madre hematopoyéticas adultas

Células madre hematopoyéticas

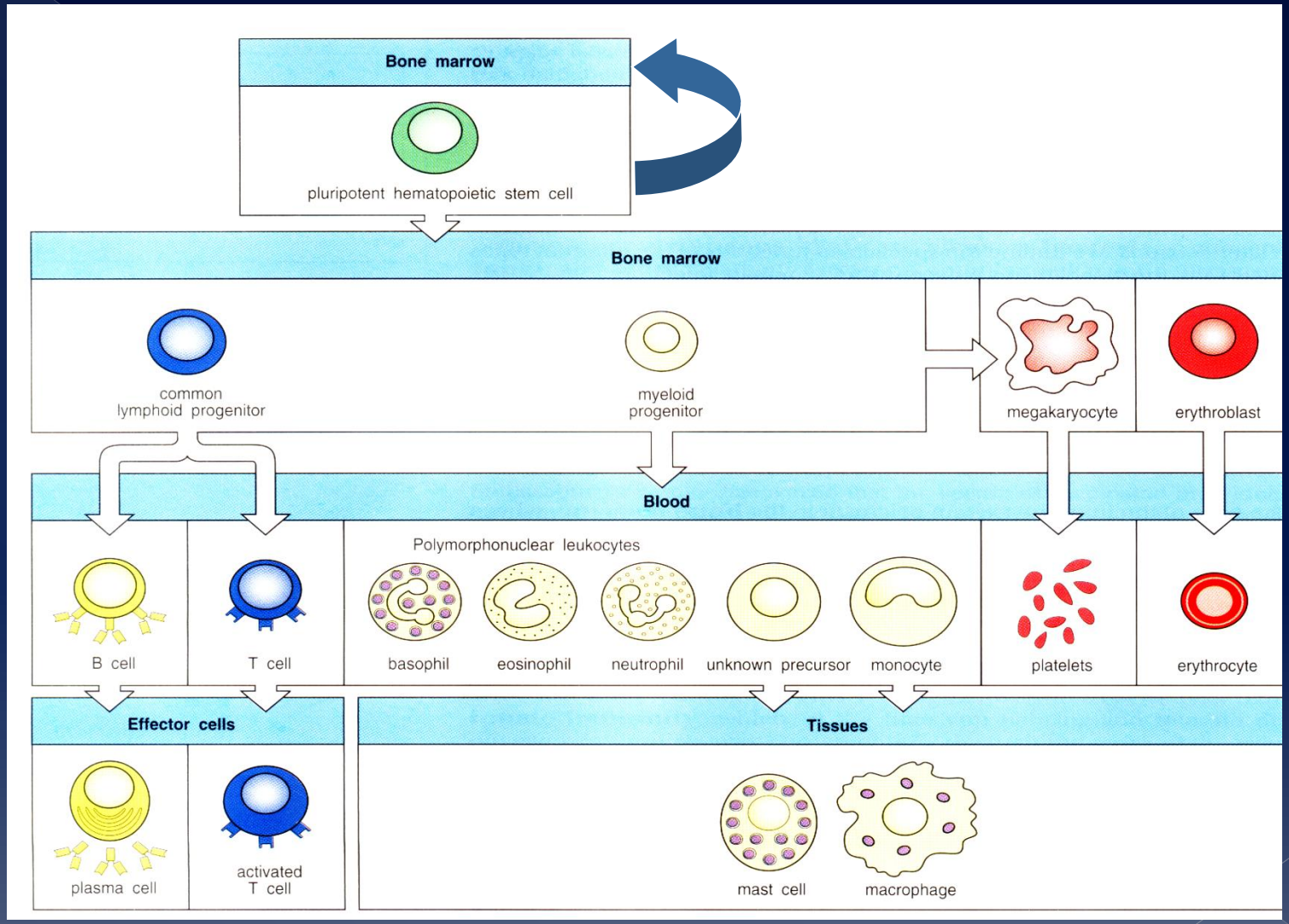
1945. Irradiación y reconstitución

1961 Identificación de BMSC del bazo

1980s. Anticuerpos monoclonales y FACS: CD34 (AC133)

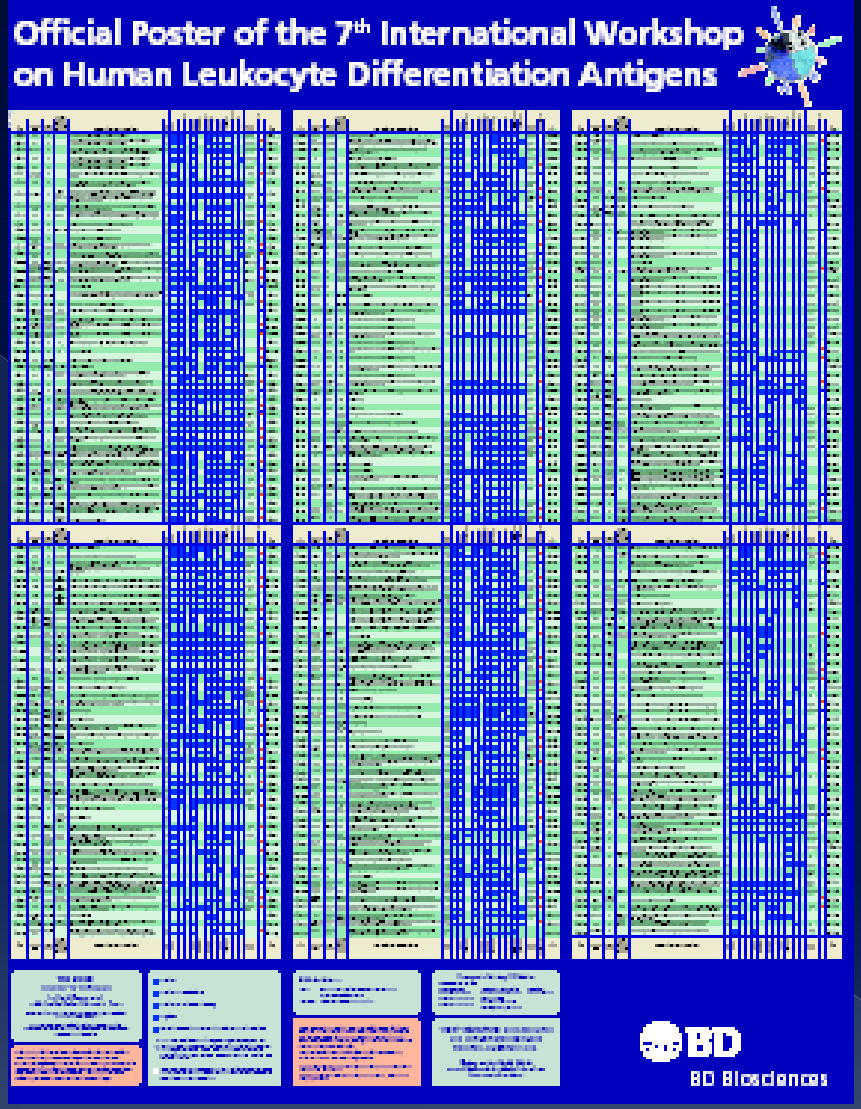
Hígado fetal>Bazo fetal> BM

Células madre hematopoyéticas adultas

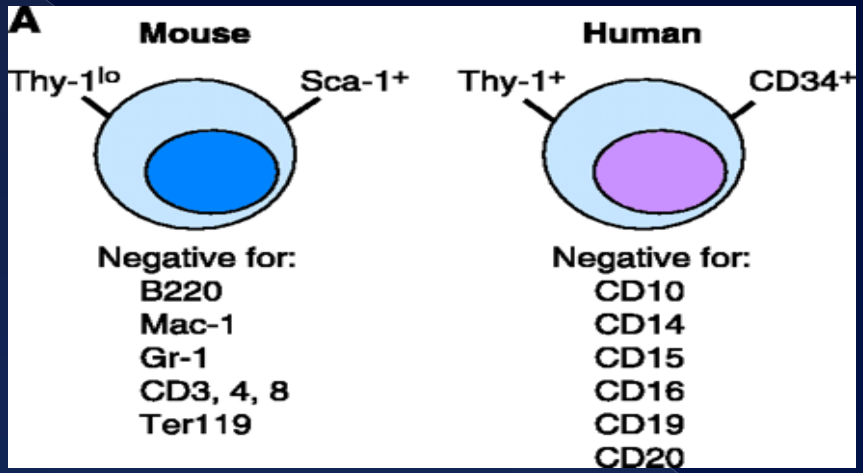


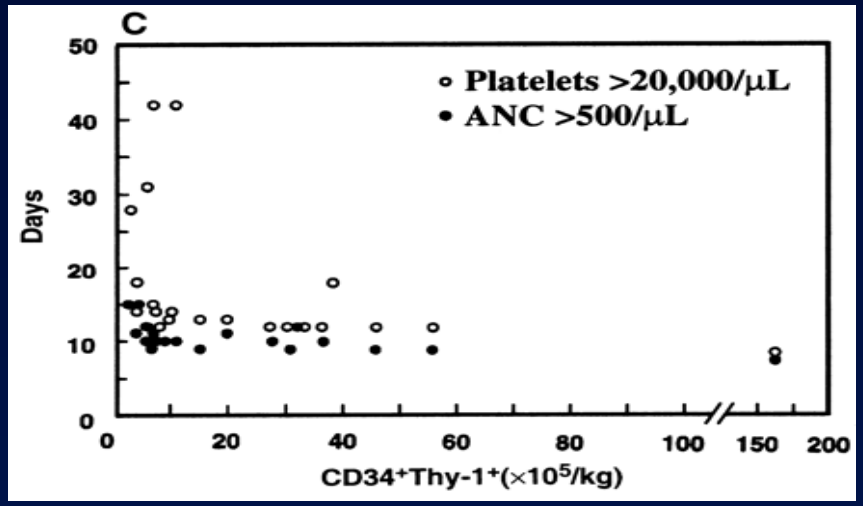
De-diferenciación no ha sido detectada

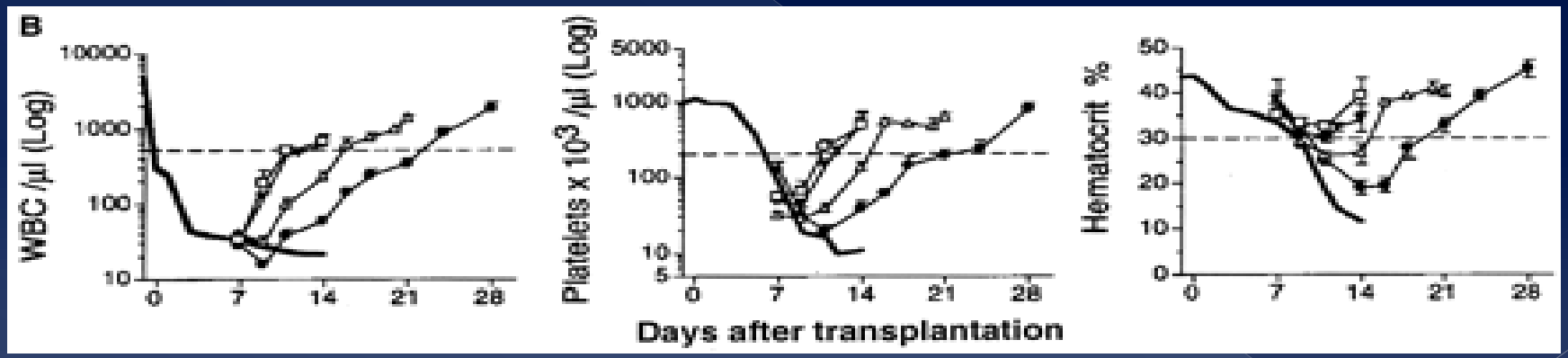
4 destinos: renovación, diferenciación, apoptosis, migración



CD	Alternative Name	HLDA Section	Ligand/ receptor/ substrate/ associated molecule	Description and Function	MW (kda)	T Cell	B Cell	Dendritic Cell	NK Cell	Stem Cell/ Precursor	Macrophage/ Monocyte	Granulocyte	Platelet	Erythrocyte	Endothelial Cell	Epithelial Cell	Gene Locus	Available at BD	CD
			receptor	from CD45 or CD148.	250	+	+				+	+	+	–					
CD246	ALK	T	Tyrosine kinase R	Expressed in T-cell lymphoma subtype; suggested role in cellular proliferation, apoptosis and embryonic neural differentiation.	200										+		2p23		CD246
CD247	Zeta Chain	T		Essential signal sub-unit of activating receptor on T and NK cells.		+			+								2p23		CD247





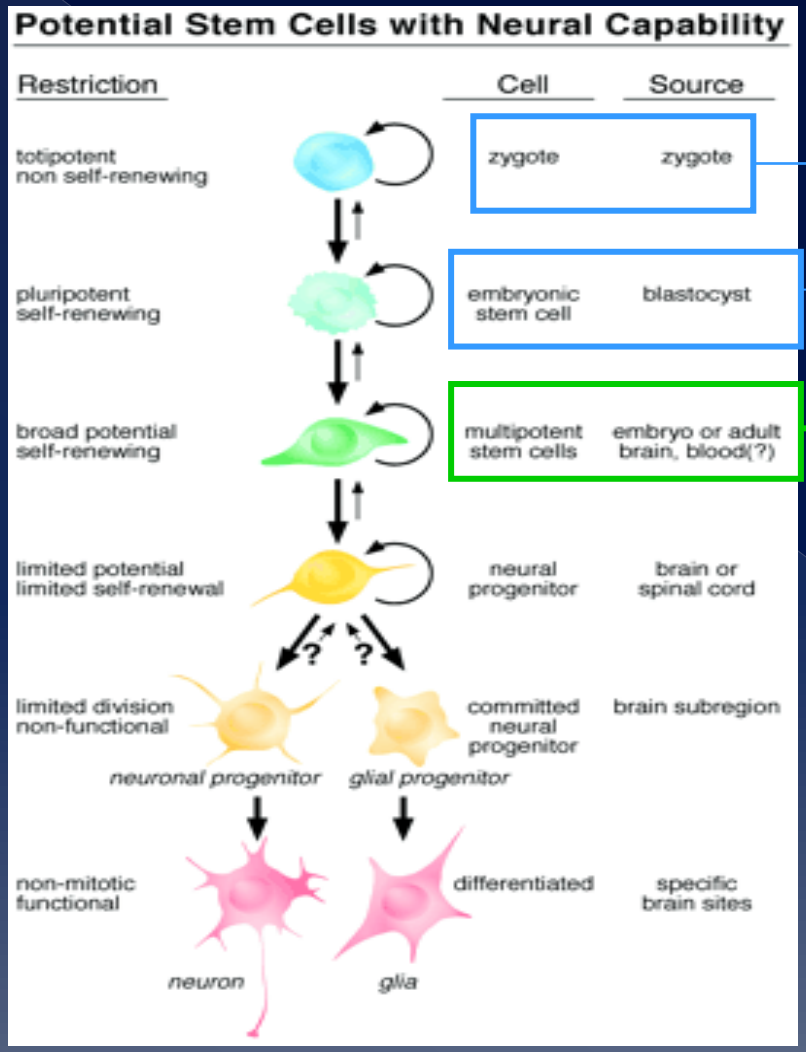


Reconstitución: transplante de médula ósea.

Weissman, Science 2000

Células madre neurales adultas

Células madre neurales adultas



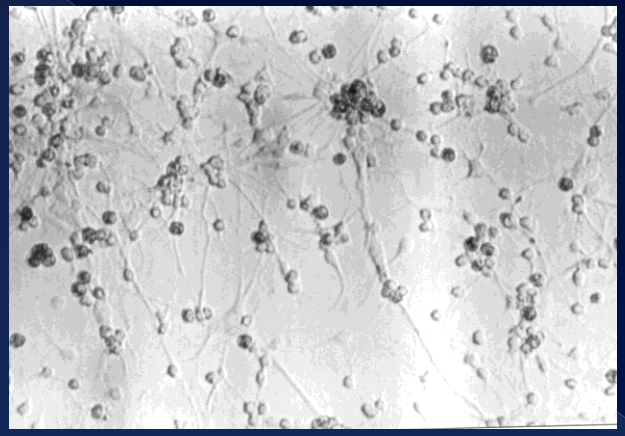
New organism

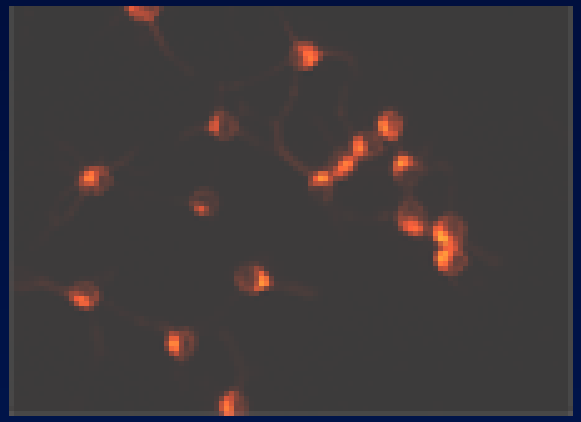
New cells/tissues

Actual Marker: nestin
expressed in:
skeletal muscle progenitor cells,
gastrointestinal and other tumors,
liver cells,
pancreatic progenitor cells,
endothelial cells,
adrenal gland cells
AC133

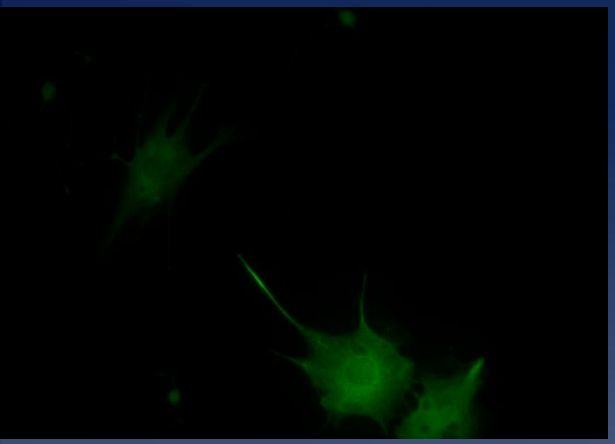
Neuronal markers: NeuN, b3-tubulin, PSA-NCAM, etc.
Glial markers: GFAP, O4, ED-1, etc

Células madre neurales adultas

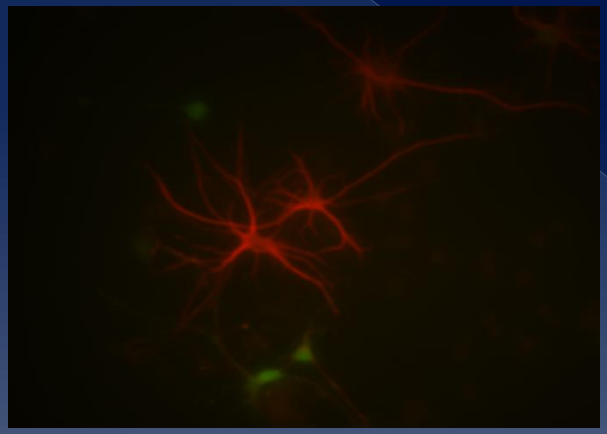




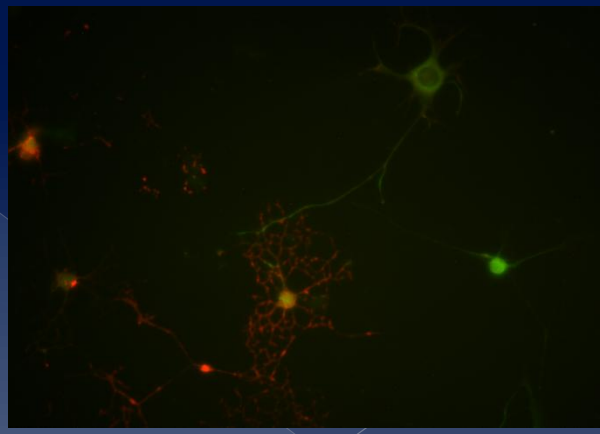
Nestina: rojo



GFAP: verde

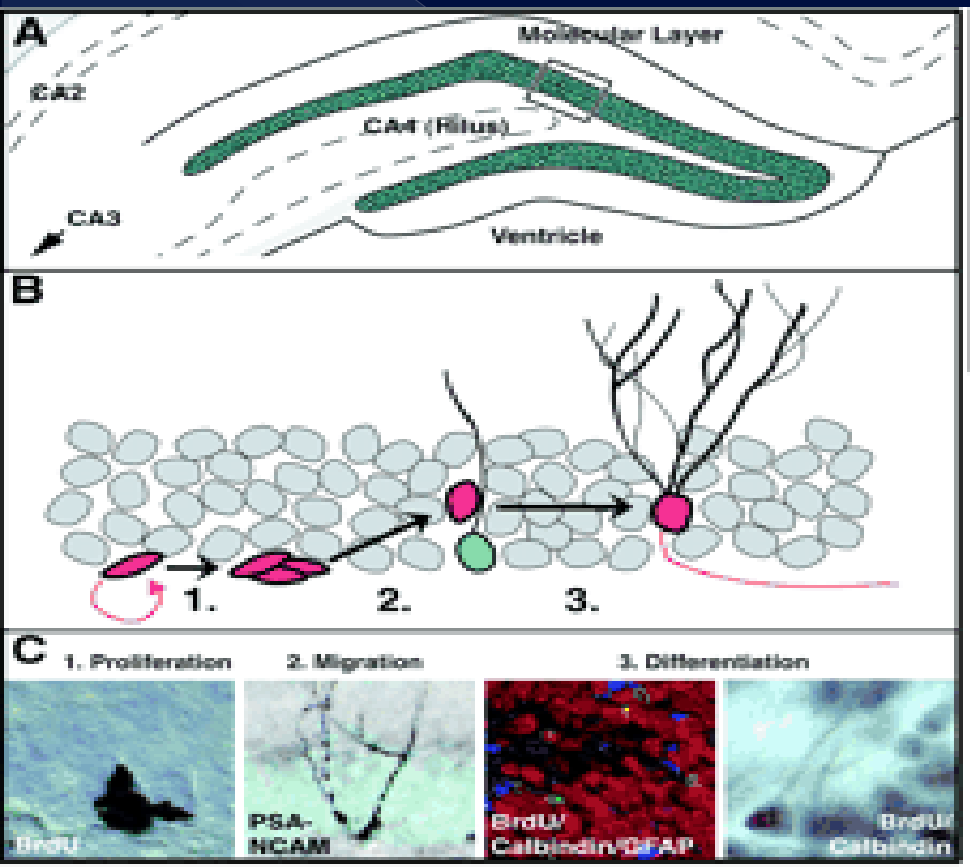


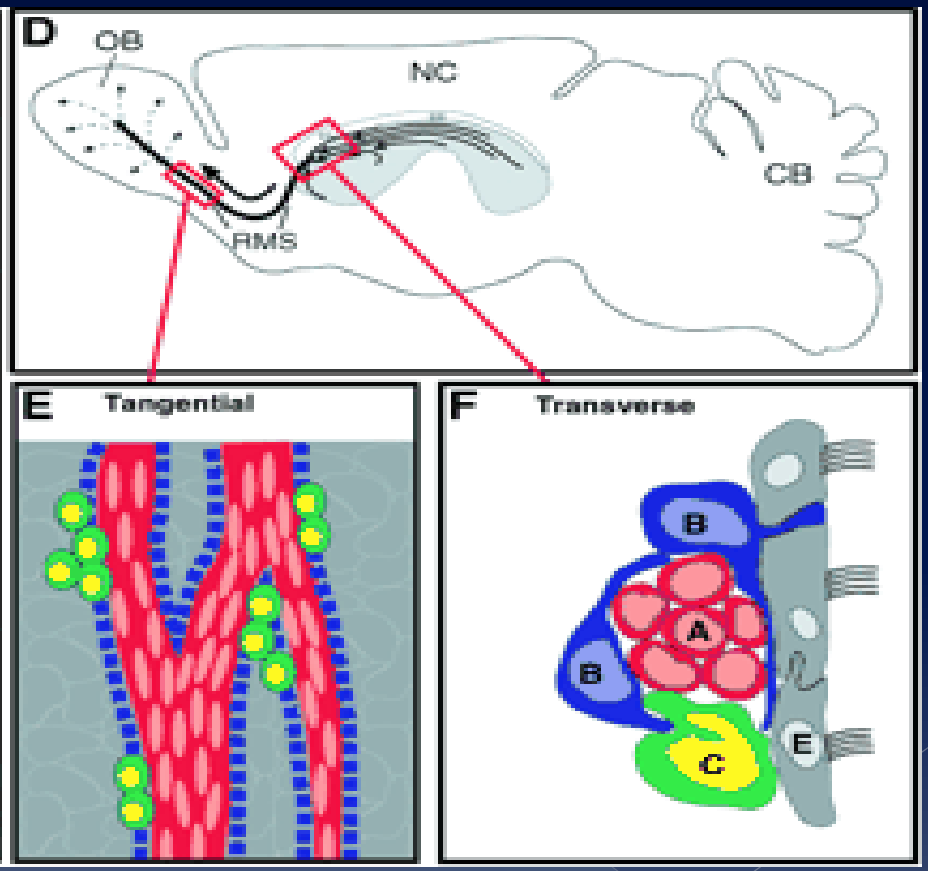
BIII-tubulina: rojo
Vimentina: verde



RIP: rojo
Vimentina: verde

Células madre neurales adultas





1400 BrdU+/-700 neuronas c/día.
6% del DG

Gage, 2000, Science

Las células madre neurales adultas y el ambiente

MARCADORES MOLECULARES
MORFOLOGÍA

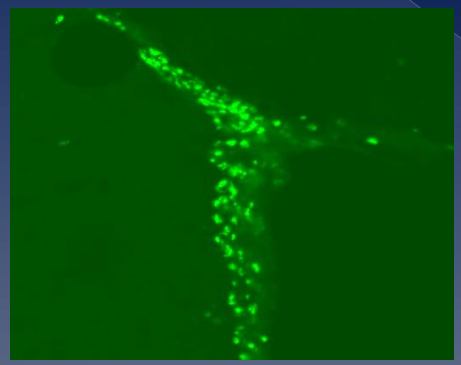
Marcadores de división celular:

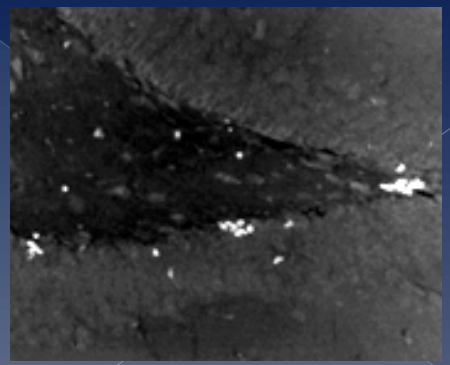
BrdU
Timidina
PCNA
Ki-67

Marcadores de stem/neuronas inmaduras:

GFAP/Nestina
Tuj
PSA-NCAM
Calbindina
Doublecortin

Célula nueva





Influencia del ambiente sobre la diferenciación

TABLE 1 Distribution of grafted cells

(*a*) Regional distribution of AHPs in olfactory bulb

	SEZ	Granule cell layer	Glomeruli	Total
1 week	258* (73%)	67 (19%)	29 (8%)	354 (100%)
8 weeks	83 (9%)	611 (69%)	201 (22%)	895 (100%)

(*b*) Phenotypic distribution of AHPs in all grafted areas

	Olfactory bulb			Hippocampus			Cerebellum	
	Glomeruli	Granule cell layer	SEZ	Granule cell layer	Area CA1	Area CA3	Granule cell layer	Purkinje cell layer
$BrdU^+$	203†	204	205	102	105	101	100	50
TH^+	7%‡	0%	0%	0%	0%	0%	0%	0%
$Calb^+$	10%	0%	2%	48%	3%	4%	0%	0%
$BrdU^+$	201†	202	204	100	103	102	102	60
$NeuN^+$	0%‡	16%	0%	35%	0%	0%	0%	0%
$GFAP^+$	21%	25%	29%	4%	34%	31%	28%	17%

a, Grafted cells at one and eight weeks after implantation into the rostral tip of RMP were counted from BrdU-immunoreactive sections in the same olfactory bulb regions used for data collection in *b* (one section per animal; five animals per time point). *b*, Eight weeks post-grafting, sections including olfactory bulb, hippocampus and cerebellum were triple-labelled with neuronal markers (tyrosine hydroxylase, TH; calbindin, Calb; and NeuN) and an astrocytic marker (GFAP) and analysed by confocal microscopy. BrdU-immunoreactive cells were counted in regions outside the injection site in each target zone. Because of the high abundance of BrdU-positive cells within neuronal layers, an upper limit of 200 sampled cells was imposed. SEZ, subependymal zone.

* The total number of $BrdU^+$ cells counted per area.

† The total number of $BrdU^+$ cells counted per area.

‡ Percentage of cells double-labelled for BrdU and the indicated marker.

Suhonen, JO, et al., Nature, 1996, 383:625

EL sitio de trasplante determina el fenotipo

Influencia del ambiente sobre la diferencia ción

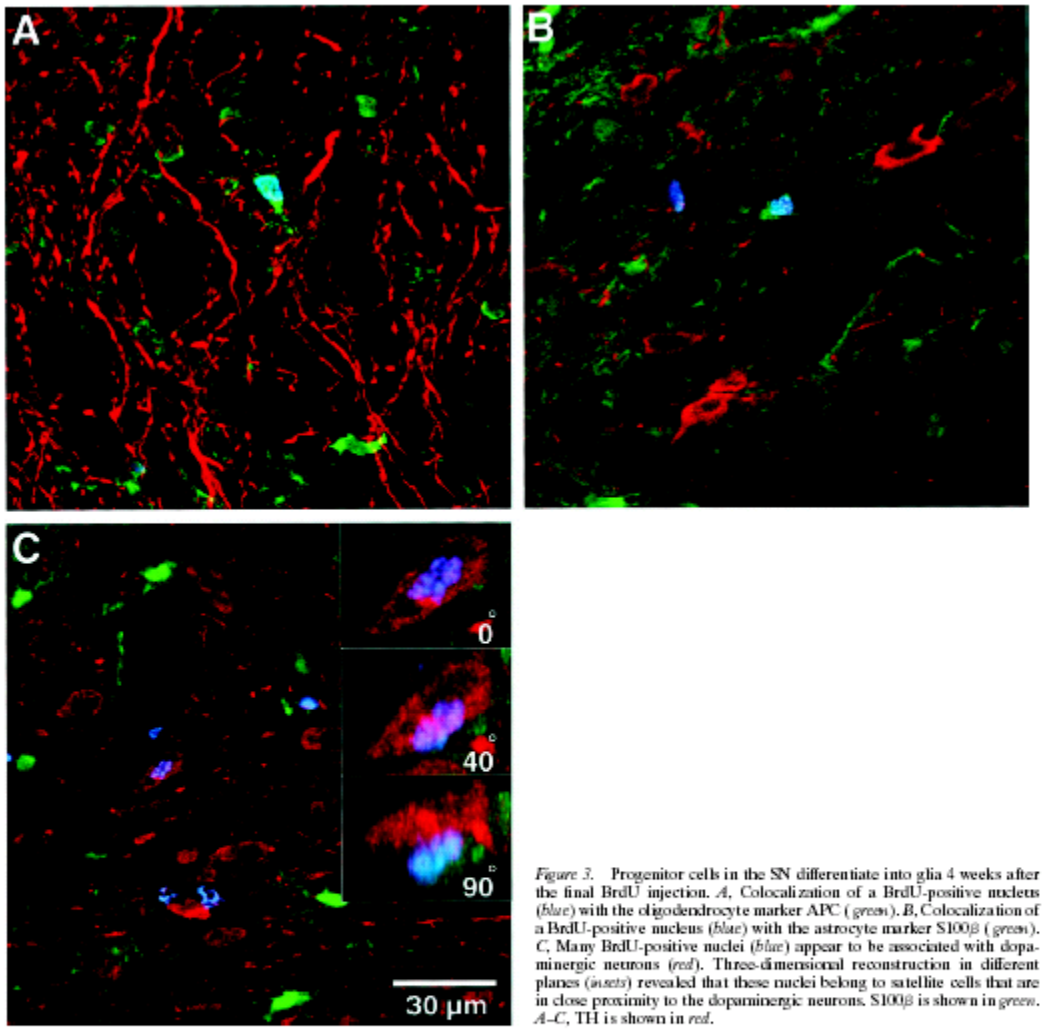


Figure 3. Progenitor cells in the SN differentiate into glia 4 weeks after the final BrdU injection. *A*, Colocalization of a BrdU-positive nucleus (*blue*) with the oligodendrocyte marker APC (*green*). *B*, Colocalization of a BrdU-positive nucleus (*blue*) with the astrocyte marker S100β (*green*). *C*, Many BrdU-positive nuclei (*blue*) appear to be associated with dopaminergic neurons (*red*). Three-dimensional reconstruction in different planes (*insets*) revealed that these nuclei belong to satellite cells that are in close proximity to the dopaminergic neurons. S100β is shown in *green*. *A–C*, TH is shown in *red*.

Table 1. Expression of glial and neuronal markers by SN progenitor cells under proliferating conditions and after differentiation

	Nestin	A2B5	NG2	GFAP	RIP	β-tubulin III
FGF2 proliferation	82.5 ± 5.7%	12.1 ± 1.9%	11.36 ± 2.0%	0.5 ± 1.8%	0%	4.3 ± 1.8%
FGF8 proliferation	77.2 ± 9.3%	1.5 ± 0.5%	16.02 ± 1.7%	0.4 ± 0.1%	0%	2.9 ± 1.3%
FGF2 differentiation	25.2 ± 5.3%	0.1%	3.1 ± 0.4%	5.9 ± 1.6%	2.2 ± 1.1%	17.1 ± 1.6%
FGF8 differentiation	22.9 ± 3.8%	0.8 ± 0.5%	2.7 ± 0.8%	16.6 ± 2.6%	1.9 ± 0.6%	17.9 ± 3.4%

SN-derived progenitor cells were propagated in the presence of FGF2 or FGF8 for 7 d and then differentiated in the presence of retinoic acid and FBS for 7 d. Lineage analysis was performed by immunofluorescent staining for lineage-associated markers: nestin (multipotent progenitors), A2B5 and NG2 (glial progenitor cells), GFAP (astrocytes), RIP (oligodendrocytes), and β-tubulin III (neurons).

A: BrdU= verde, NG2= azul, TH= rojo B: BrdU= azul, nestina= verde 10 días

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Las células madre de la Sn no se diferencian a neuronas in vivo, pero si pueden hacerlo in vitro

Potencial neurogénico: OK
Potencial stem: OK
Ambiente neurogénico: NO

Influencia del ambiente sobre la diferenciación

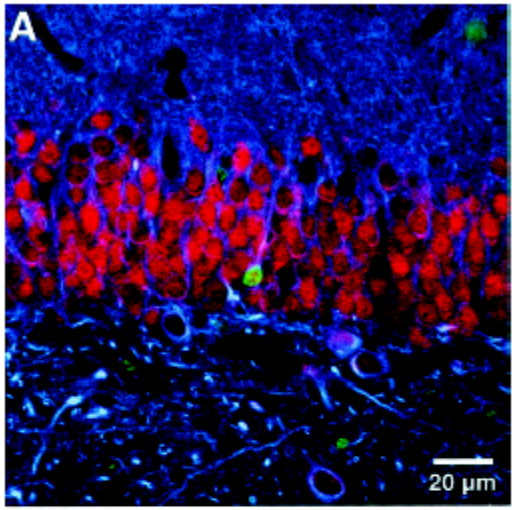

Figure 7. In vivo neuronal differentiation potential of SN progenitor cells. *A*, BrdU-labeled SN progenitor cells (*green*) differentiate into NeuN (*red*)/β-tubulin III (*blue*)-positive neurons after transplantation to the hippocampus, demonstrating that SN progenitor cells can differentiate into neurons *in vivo*. *B*, In contrast, SN progenitor cells transplanted back to the SN do not differentiate into neurons, but display an NG2-positive glial progenitor phenotype (*red*), suggesting that the SN environment is not permissive for neuronal differentiation. BrdU is shown in *green*; TH is shown in *blue*.

Cel madre de la SN en hipocampo= neuronas

BrdU: verde
NeuN: rojo
bIII: azul

Cel madre de la Sn en SN: oligos

NG-2 rojo

Células madre adultas

Ventajas

Larga experiencia en células madre hematopoyéticas
Sin evidencias de tumorigenicidad
Sin debate ético

Rechazo poco probable (autotrasplante)

Desventajas

Menor plasticidad- *específicas de linaje*

Baja homogeneidad de la muestra
Dificil acceso (neurales)
Baja eficiencia de diferenciación (neurales)