Current Status and Future Prospects of Stem Cell Research

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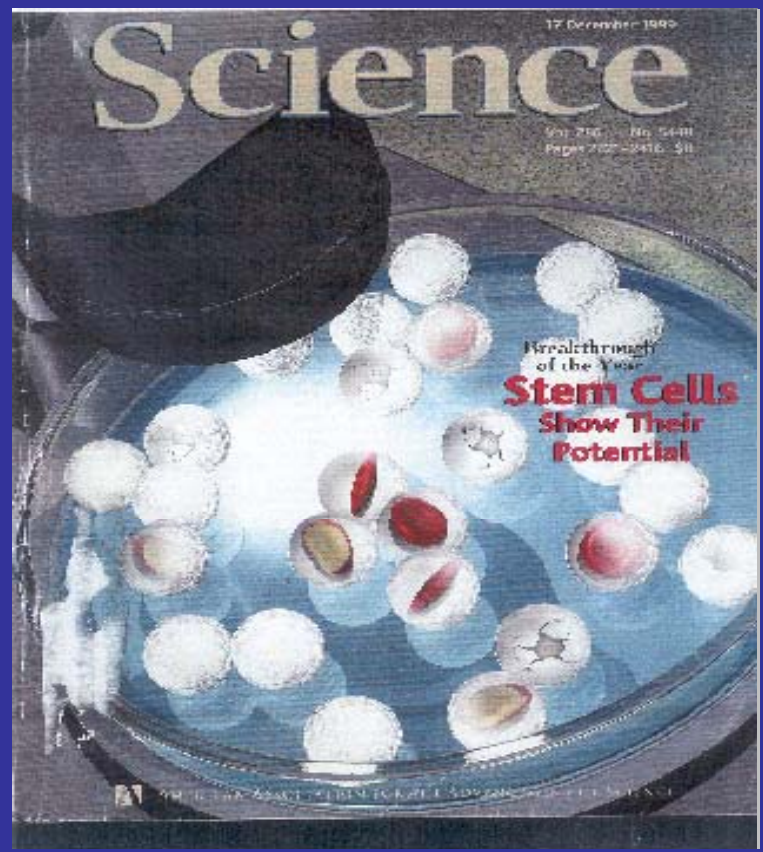
再生醫學時代的來臨???!

- 一九九九年，美國「科學」期刊選「幹細胞」為十大科學發現榜首

- 二〇〇〇年，美國「科學」期刊選「幹細胞」為「年度科學」(Science of the Year)



Definition of Stem Cells

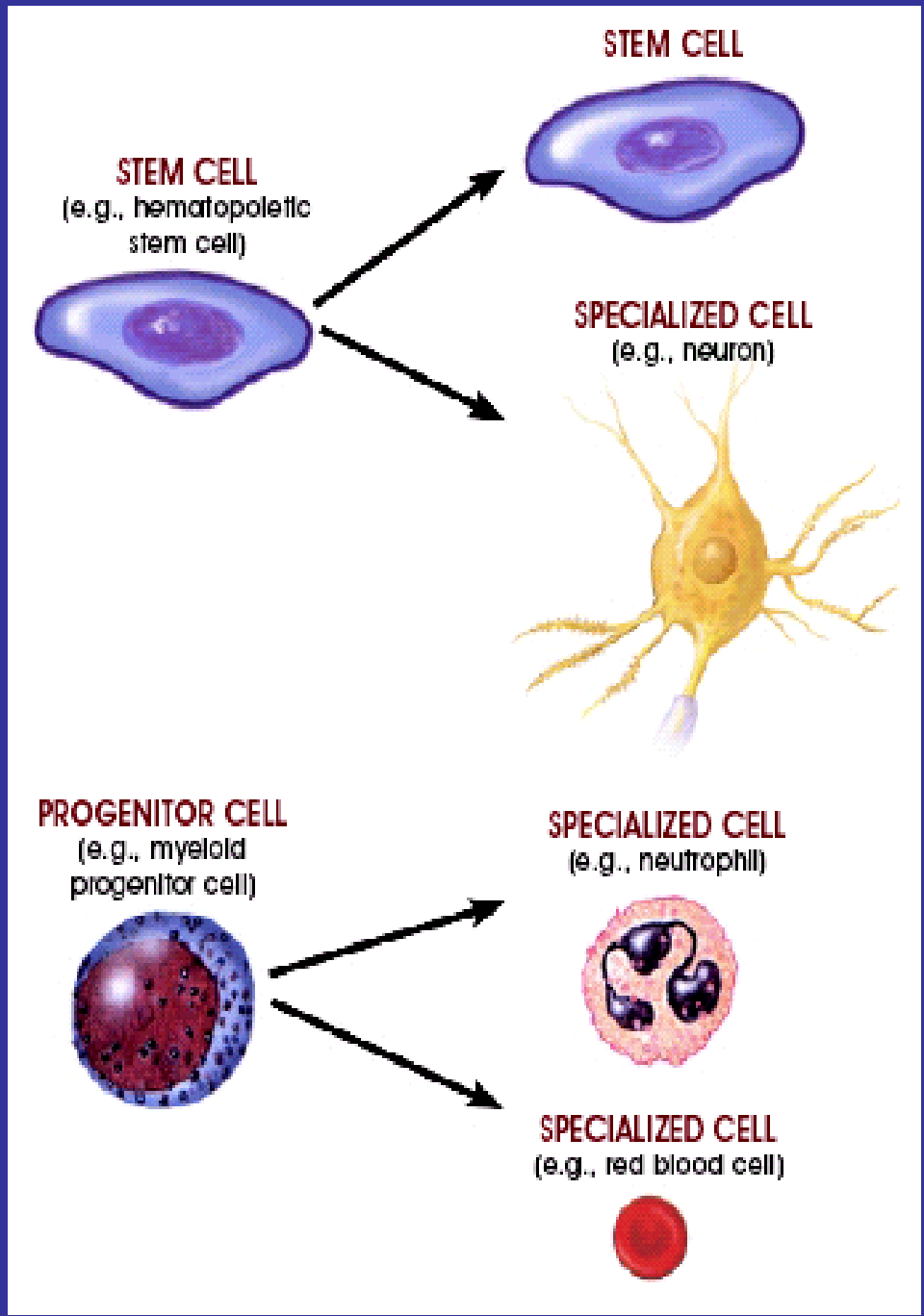
(1)self-renewal

(asymetric division and unlimited growth)

(2)proliferation

(3)differentiation

(4)functioning


STEM CELL
STEM CELL
(e.g., hematopoietic stem cell)
SPECIALIZED CELL
(e.g., neuron)
PROGENITOR CELL
(e.g., myeloid progenitor cell)
SPECIALIZED CELL
(e.g., neutrophil)
SPECIALIZED CELL
(e.g., red blood cell)

幹細胞的種類

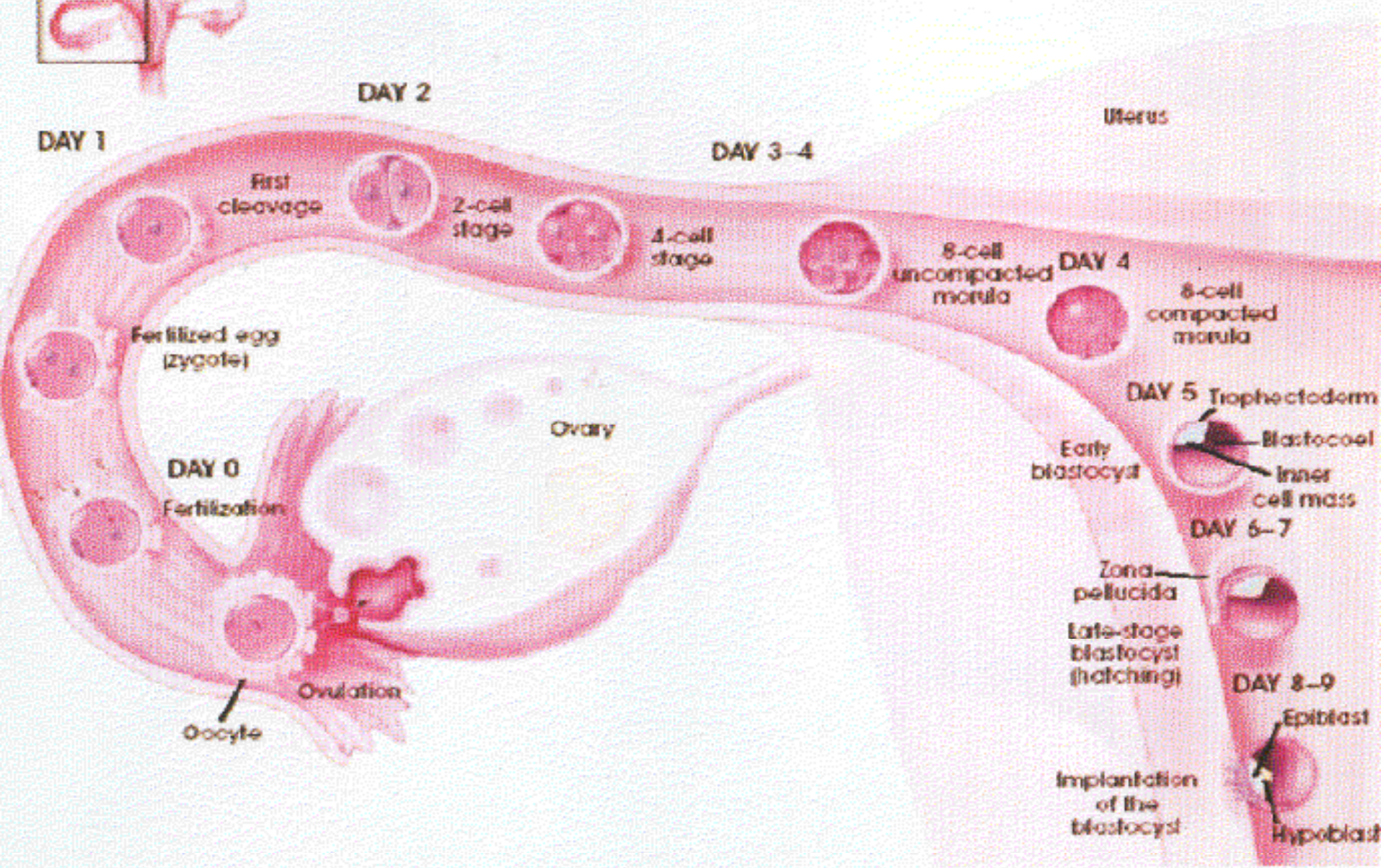
- 胚幹細胞(**Embryonic Stem Cell**)

  胚(embryo, 8週以前)胎(fetus,8週以後)人工受精的胚(胎)之內細胞團所培育出來之細胞株

- 成體幹細胞(**Adult Stem Cell**)

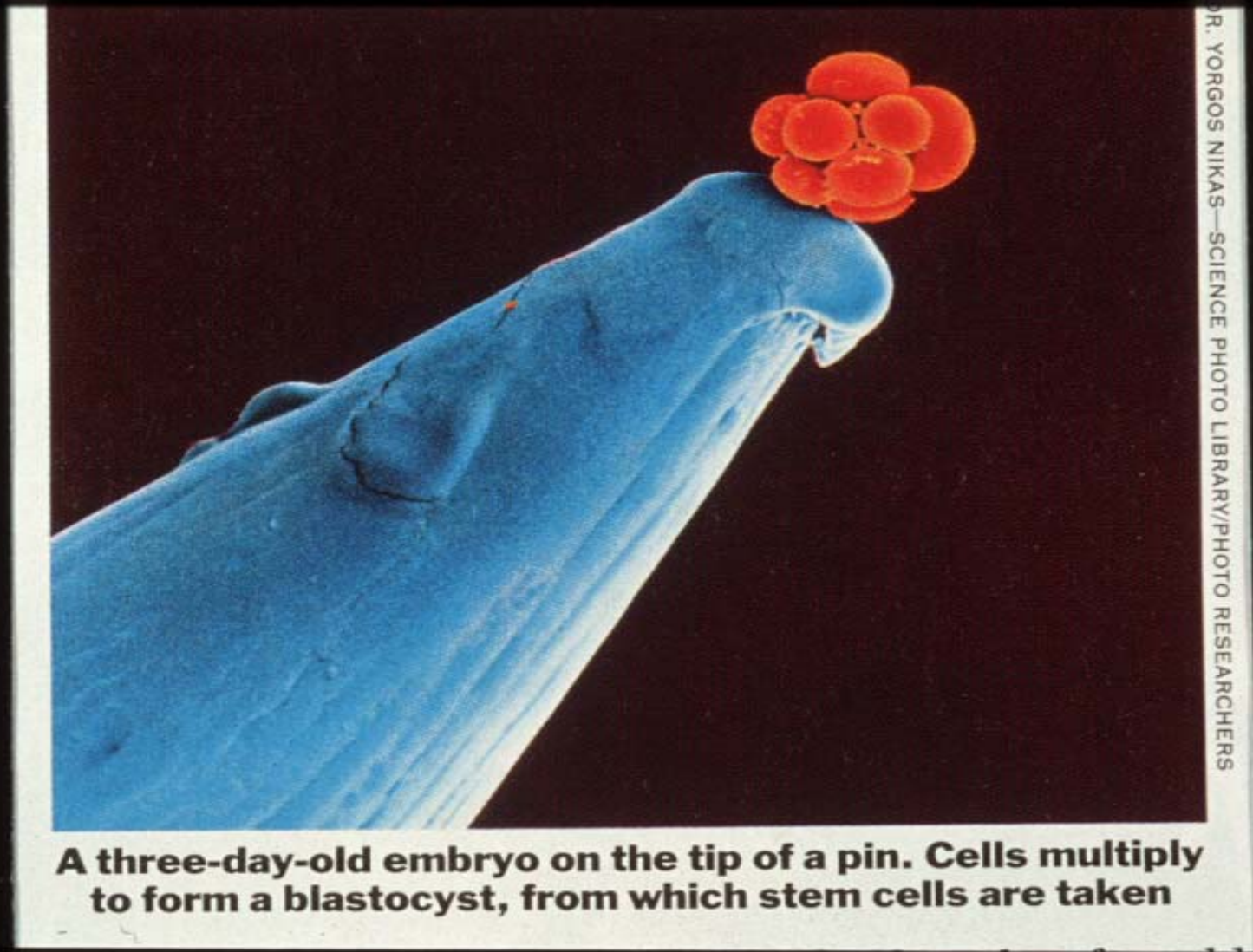
  -Hematopoietic Stem Cell

  -Somatic Stem Cell



排卵受精，到移動至子宮著床的過程

NIH 2001, June



A three-day-old embryo on the tip of a pin. Cells multiply to form a blastocyst, from which stem cells are taken

3天大的胚胎
(16個細胞) 在針尖上

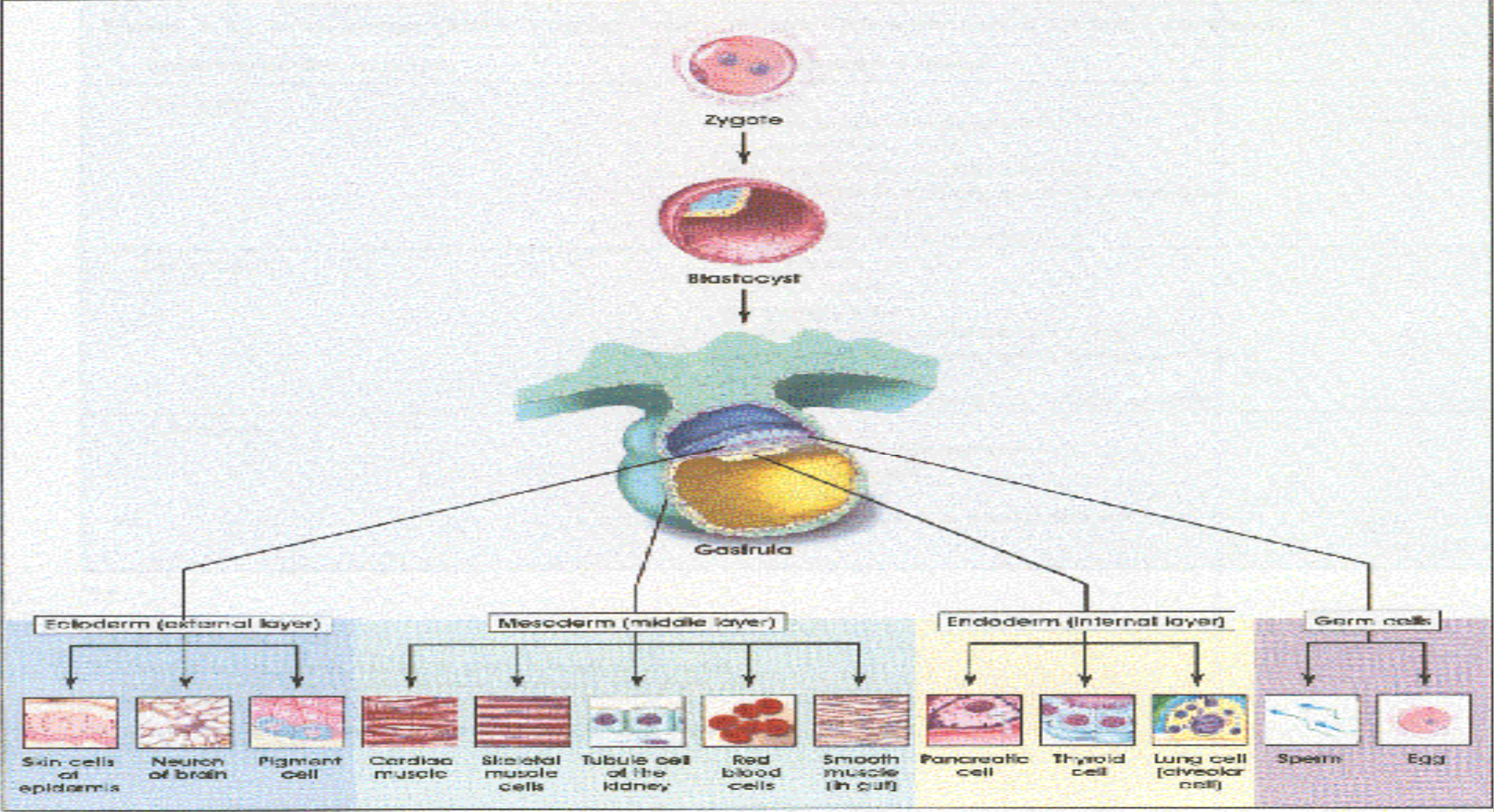
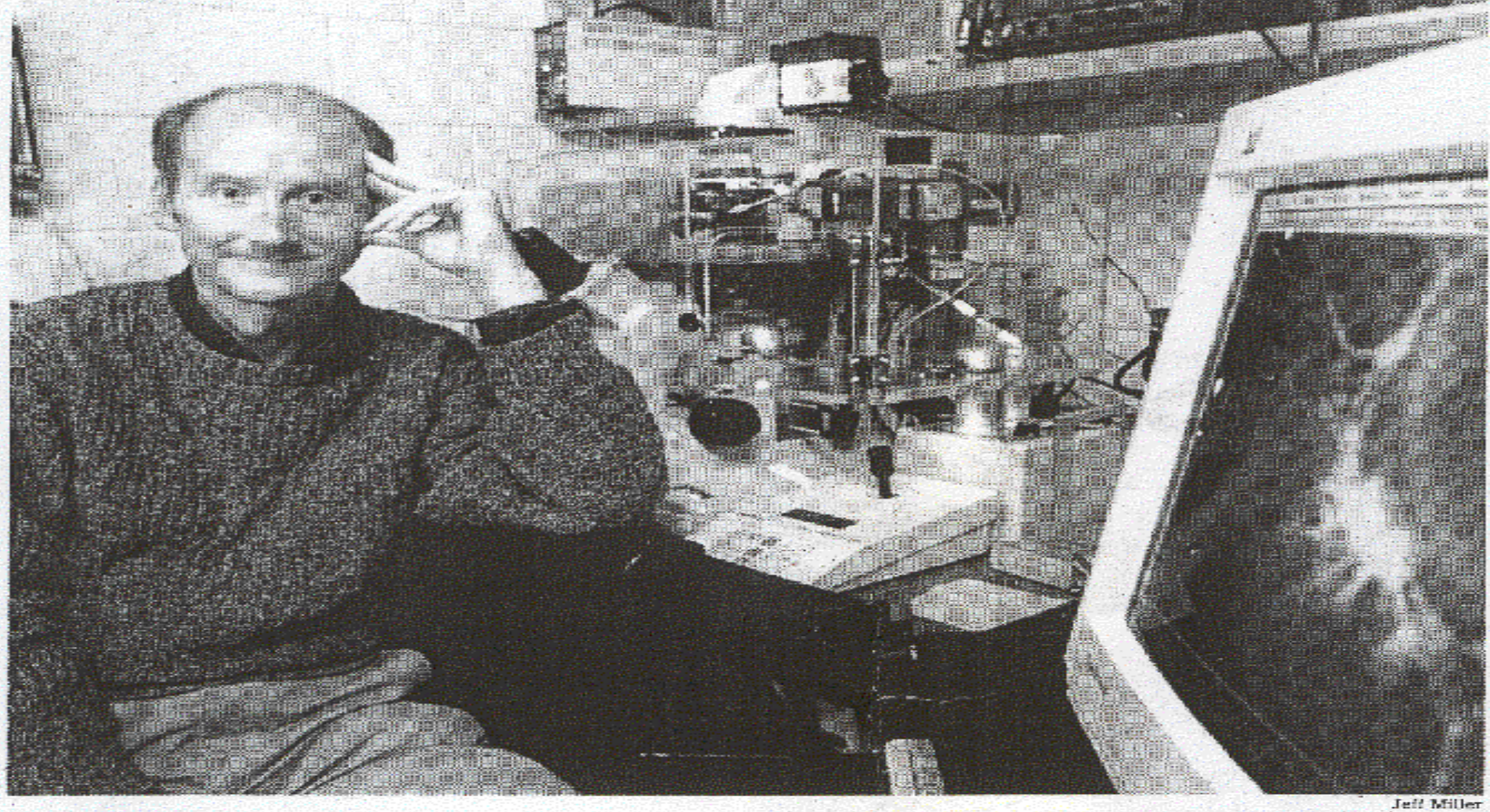


Figure 1.1. Differentiation of Human Tissues.

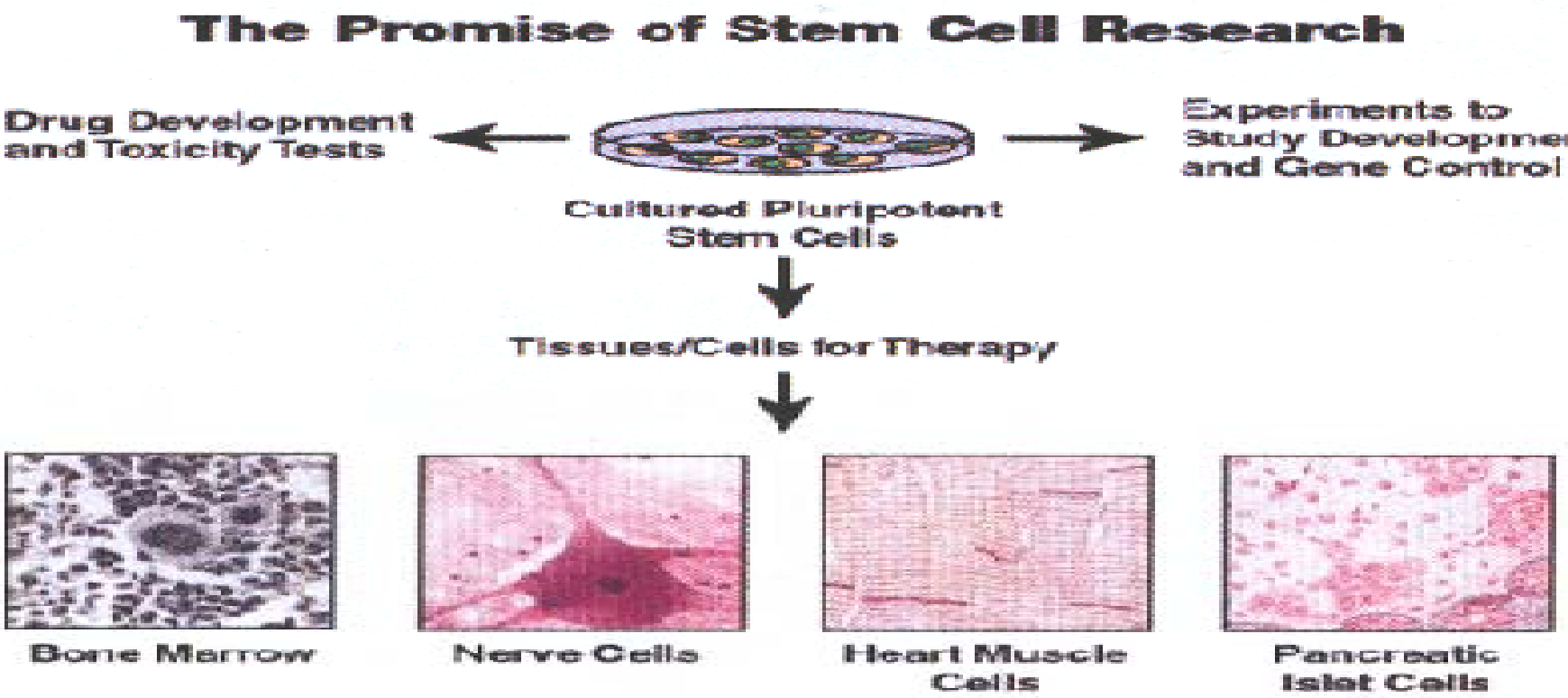
胚胎幹細胞可以分化成外胚層，
中胚層，內胚層之各種細胞，理論上可達兩百多種



Jeff Miller

James Thomson says the importance of his work on stem cells outweighs the arguments against it on ethical grounds. He was in his primate research center with a computer image of stem cell neurons, right.

第一位培養出人類胚胎幹細胞株
“James Thomson 博士”



There are several important reasons why the isolation of human pluripotent stem cells is important to science and to advances in health care.

幹細胞之研究發展方向

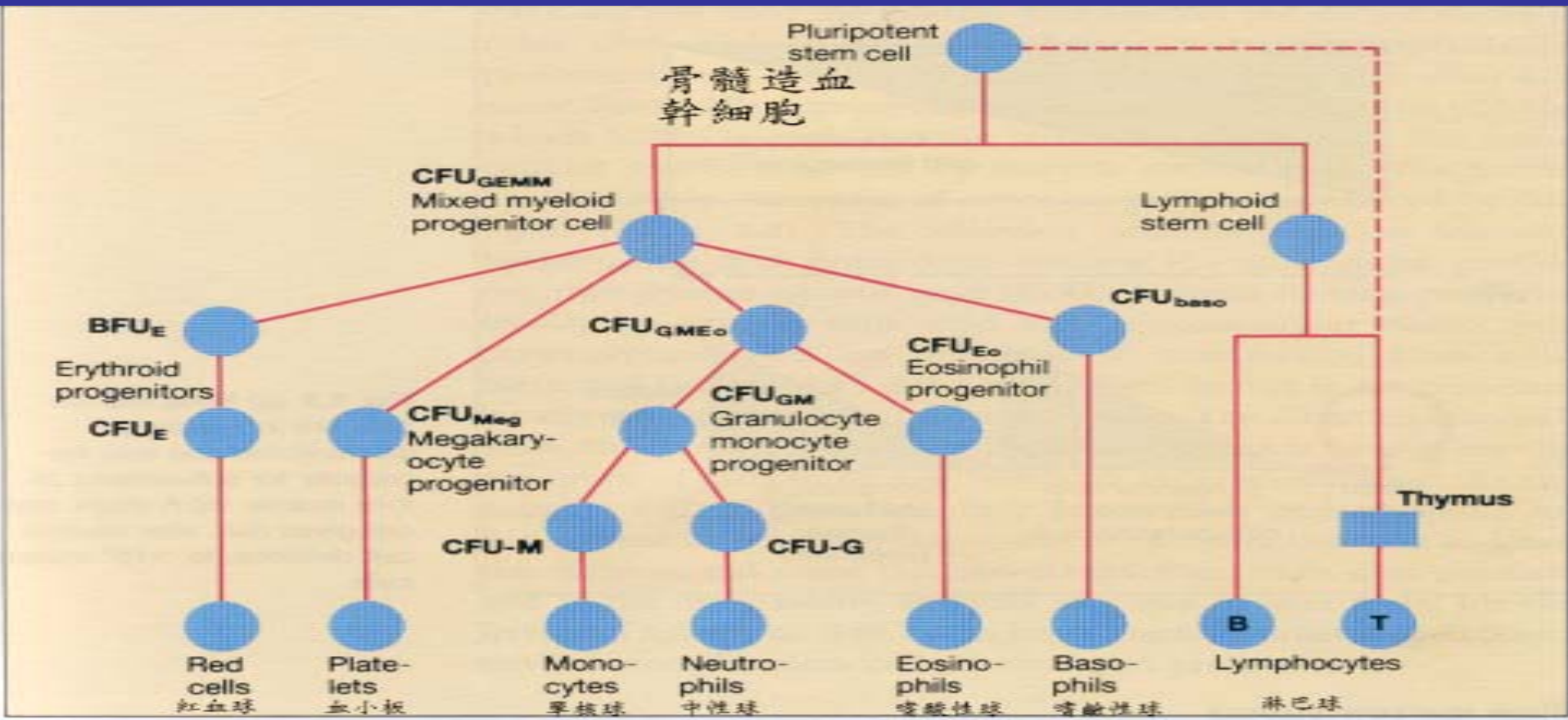
(1)藥物之效果與毒性研究

(2)組織/細胞治療 (3)基因之調控(基因治療)

Source of Adult Stem Cells

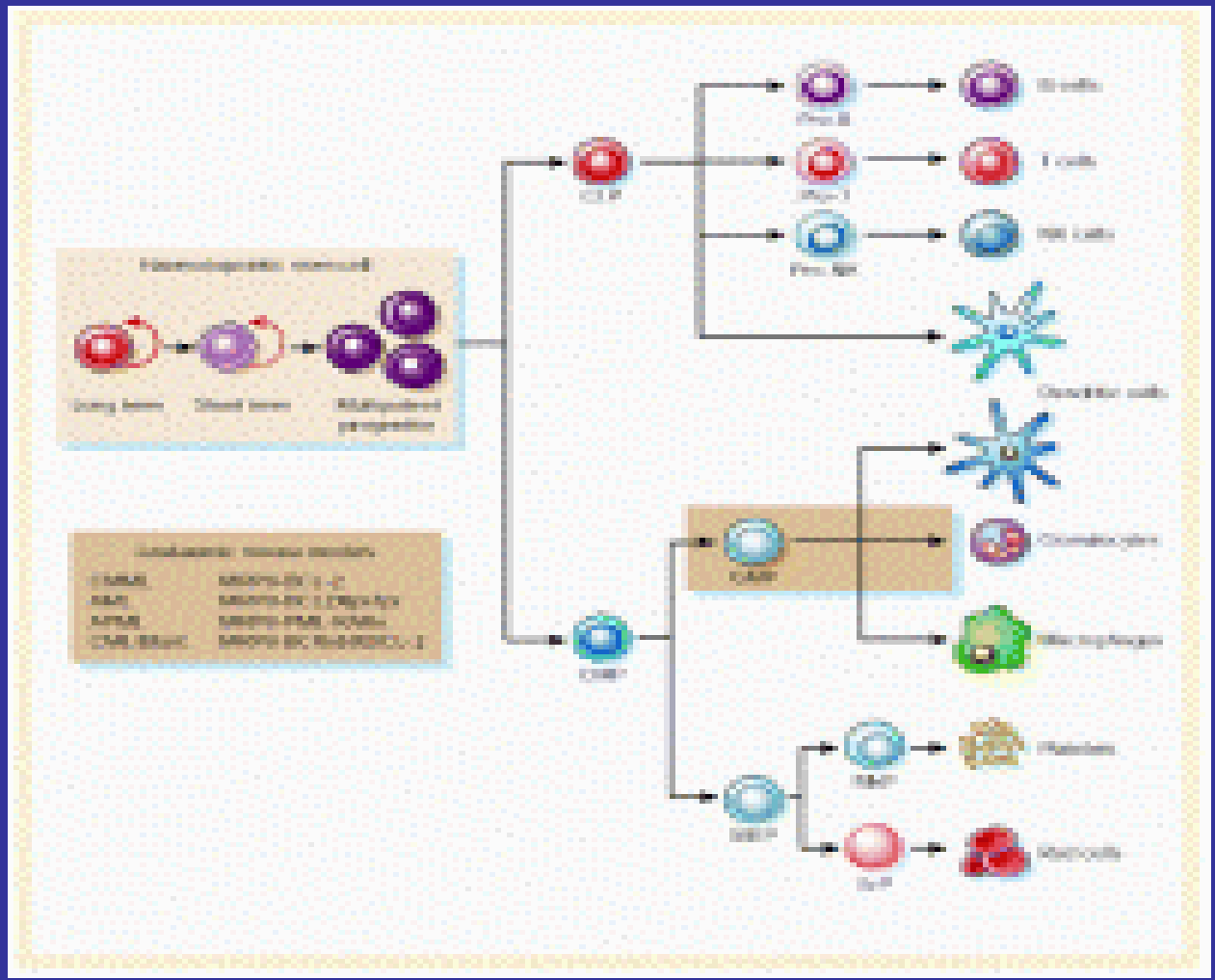
- Tissue derived stem cell (including hematopoietic stem cell of BM)
- Mesenchymal stem cells (MSC)
- Multi-potent adult progenitor cells (MAPCs)
- Plasticity (transdifferentiation of tissue - specific stem cell)

骨髓造血幹細胞



紅血球　血小板　單核球　中性球　嗜酸性球　嗜鹼性球　淋巴球

CD34 Hematopoietic Stem Cell



Proposed cell-surface markers of undifferentiated hematopoietic stem cells

Mouse	Human
$CD34^{low/-}$	$CD34^{+}$
$SCA\text{-}1^{+}$	$CD59^{+*}$
$Thy1^{+/low}$	$Thy1^{+}$
$CD38^{+}$	$CD38^{low/-}$
$C\text{-}kit^{+}$	$C\text{-}kit^{-/low}$
lin^{-*}	lin^{-**}

Resources of CD34+ cell

- Bone marrow
- Peripheral blood

  mobilization: chemotherapy
  growth factor

  collection: cell separator
- Cord blood

Clinical Use of CD34+ cell

- Allogeneic bone marrow/hematopoietic cell transplantation
- Autologous transplantation
- Selected CD34 transplantation

1st BMT in Taiwan

(1)病人：張武松先生
(2)時間：1983年11月9日
(3)診斷：Cutaneous-T-cell lymphoma
(EBV associated lymphoma)
(4)疾病狀況：very refractory to C/T
(5)TBI (single fraction)
(6)骨髓收集
*Epidural anesthesia
*Non-cryopreserved BM
(7)Outcome：short-term CR
(8)Relpase in 3 months

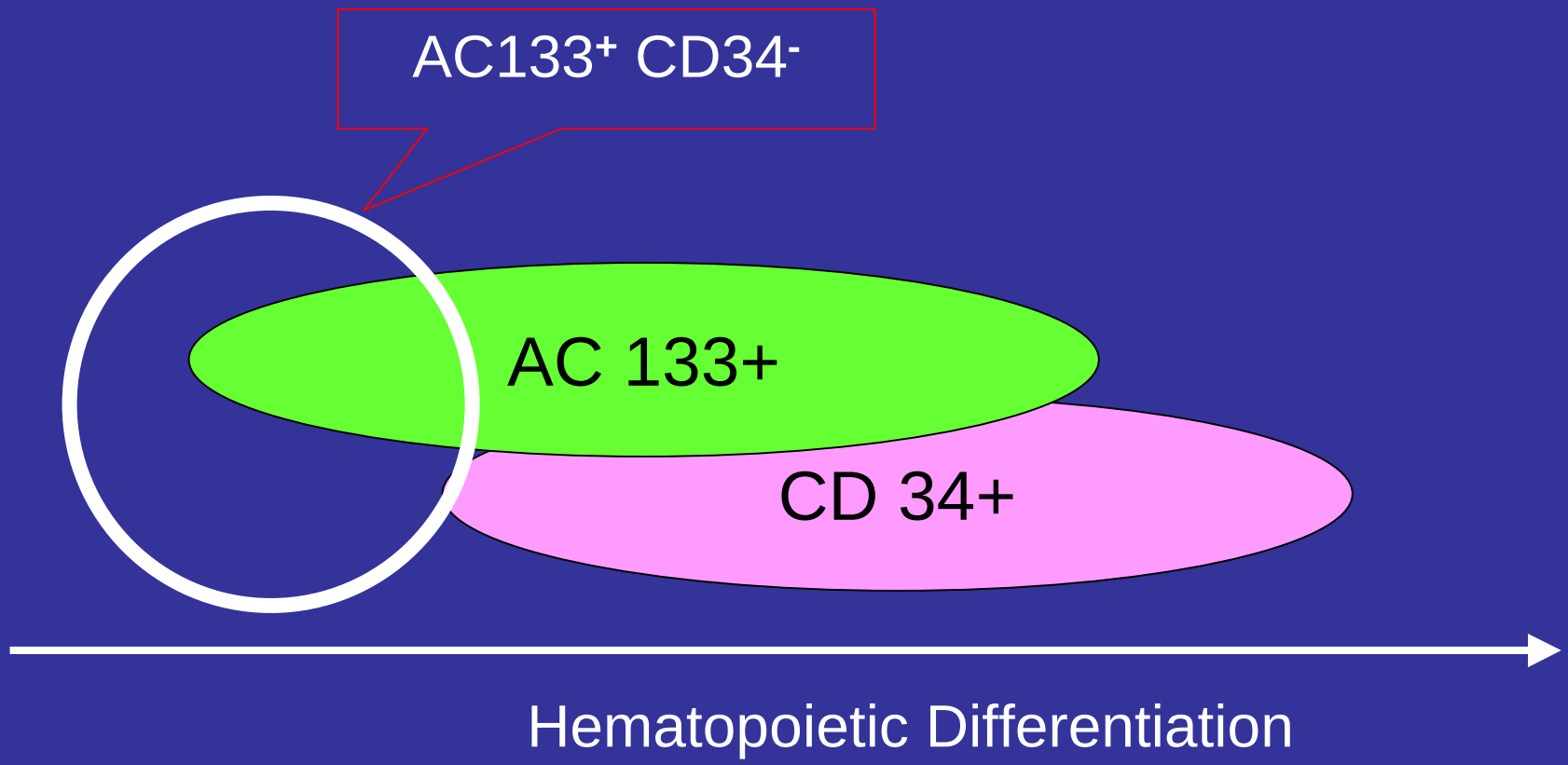
資料提供：台大醫院 王秋華醫師等

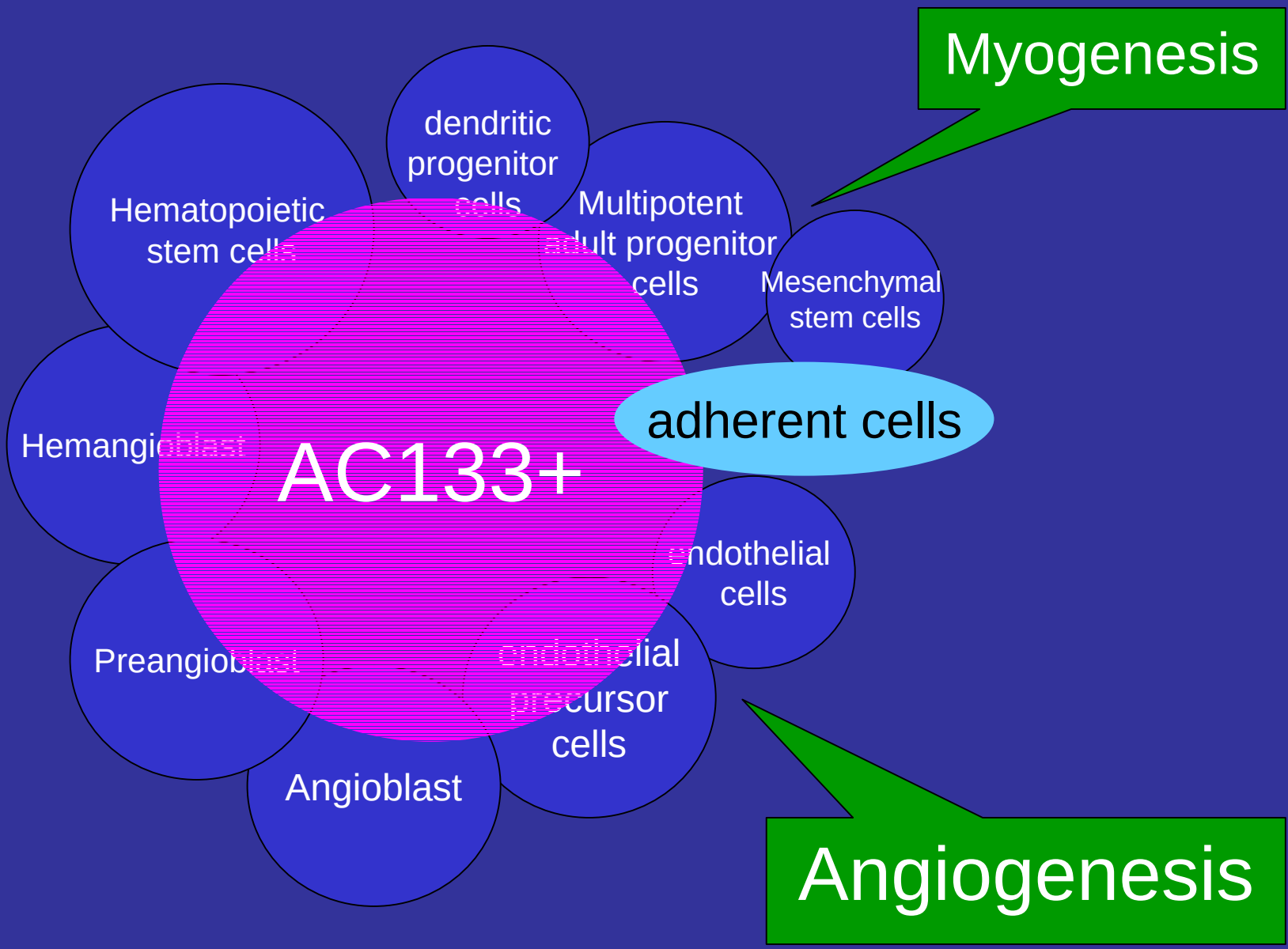
1st Allo-BMT

(1)病人：○○○先生
　捐髓者：林淑貞小姐
　時間：1984年3月9日
(2)診斷：CML
(3)併發症
　VOD
　aGVHD. GrII
　cGVHD : liver+skin
(4)意外發現
　HBsAg(+) ⟶ Anti-HBs(+)
(5)Alive and DFS x 16^{+}yrs

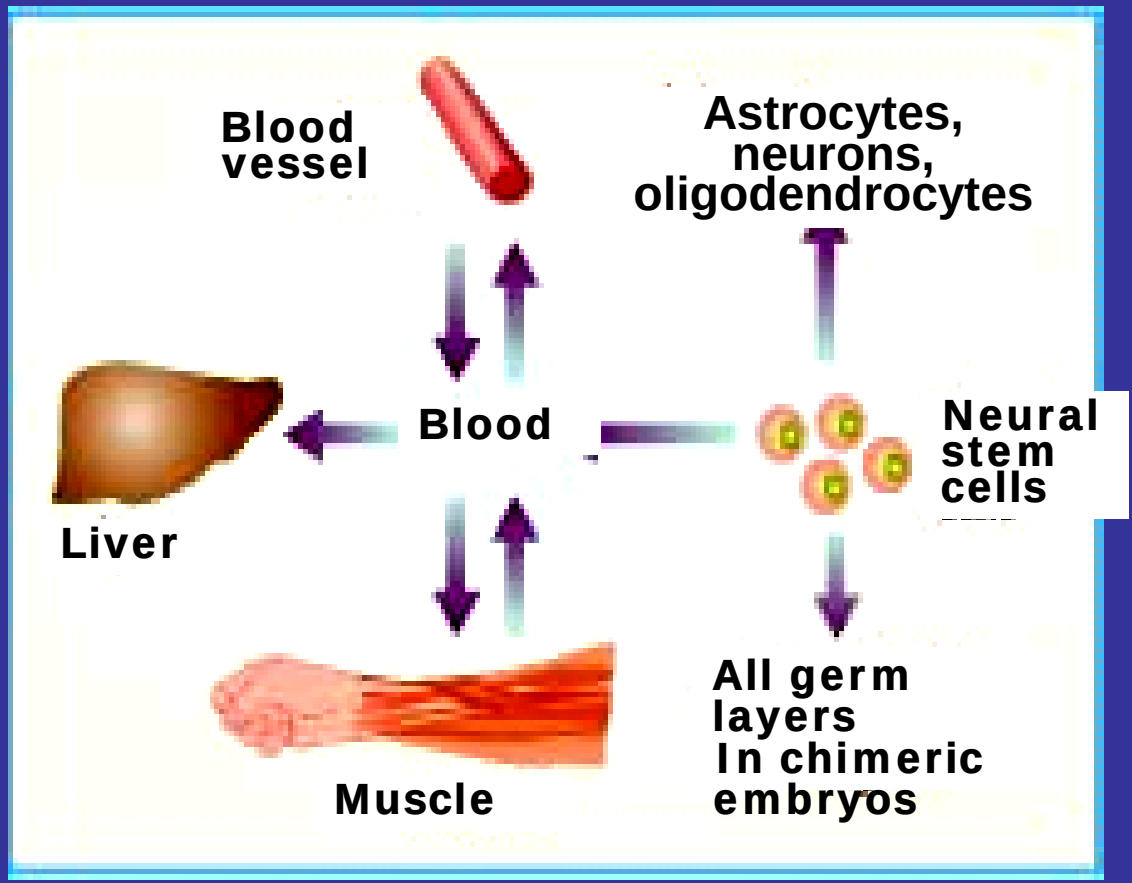
台大醫院提供

Clinically approved stem cell markers



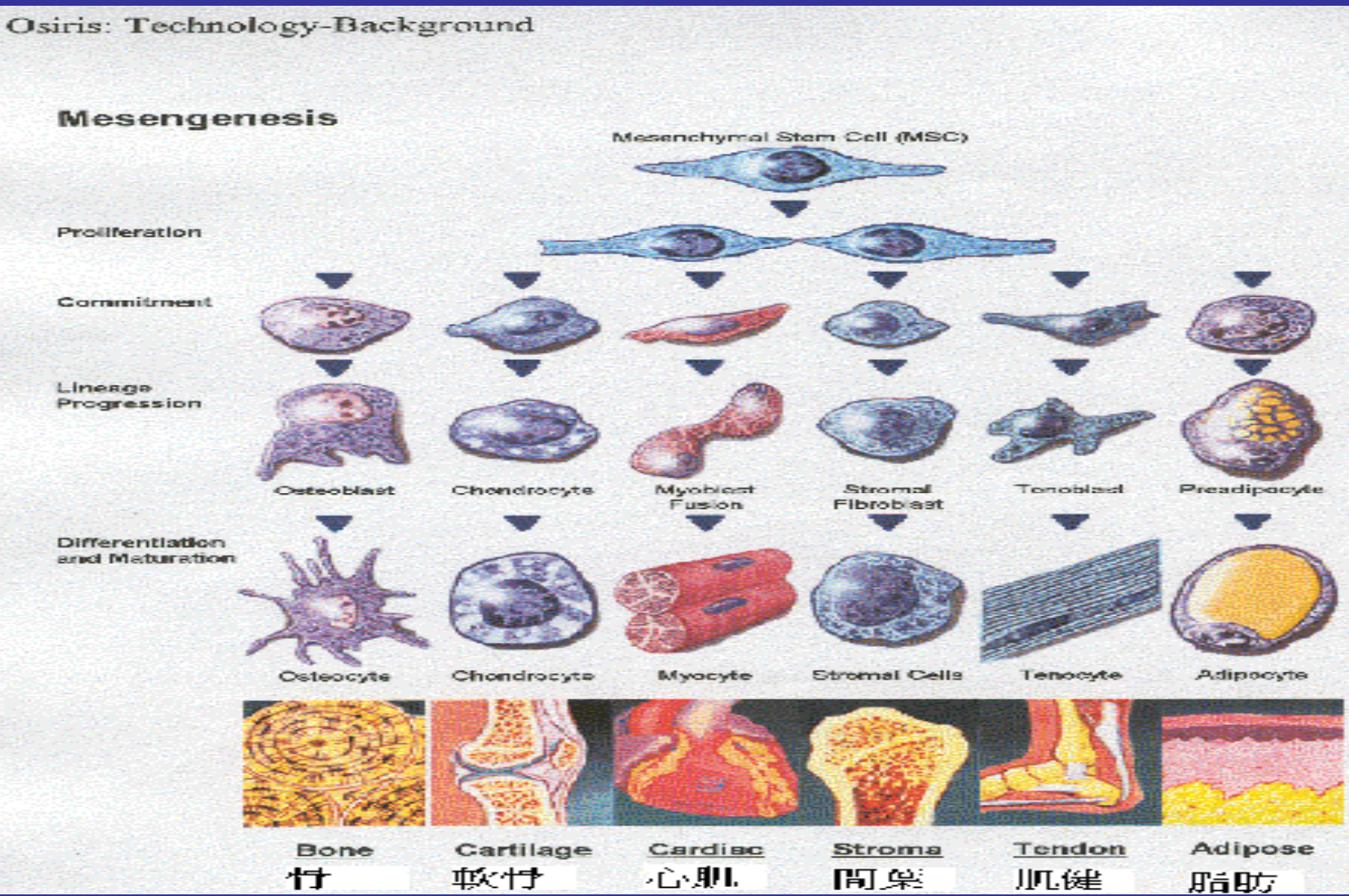

Myogenesis
dendritic progenitor cells
Hematopoietic stem cells
Multipotent adult progenitor cells
Mesenchymal stem cells
adherent cells
Hemangioblast
AC133+
Endothelial cells
Preangioblast
endothelial precursor cells
Angioblast
Angiogenesis

In vivo tissue stem cell plasticity



Eric Lagasse et al, Nature Med 6:1229, 2000

骨髄間質幹細胞


Osiris: Technology-Background
Mesengenesis
Mesenchymal Stem Cell (MSC)
Proliferation
Commitment
Lineage Progression
Osteoblast
Chondrocyte
Myoblast Fusion
Stromal Fibroblast
Tenoblast
Preadipocyte
Differentiation and Maturation
Osteocyte
Chondrocyte
Myocyte
Stromal Cells
Tenocyte
Adipocyte
Bone
Cartilage
Cardiac
Stroma
Tendon
Adipose
骨
軟骨
心肌
間葉
肌腱
脂肪

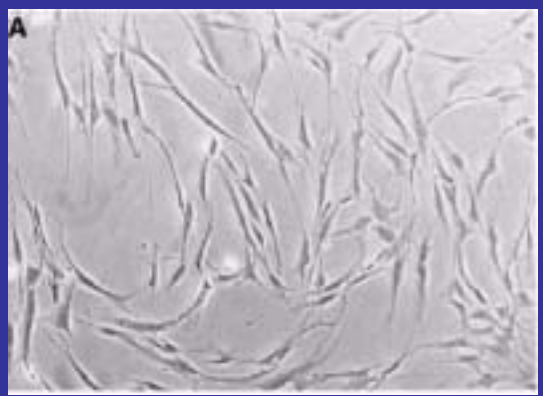
BM-derived MSC

More than 20 years ago, Friedenstein and then others grew adult stem cells from bone marrow called mesenchymal stem cells or marrow stromal cells (MSCs).

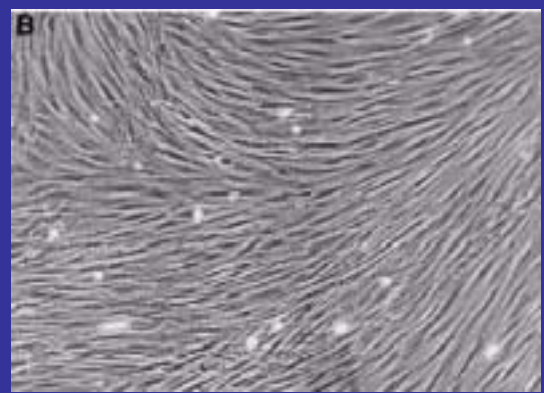
Friedenstein A. :Stromal mechanisms of bone marrow: cloning *in vitro* and retransplantation *in vivo*.
In: Thienfelder S, Rodt H, Kolb HJ (eds). *Immunology Of Bone Marrow Transplantation*. Berlin: Springer Verlag, 1980, 19-20.

Function:

1. In vivo: Support HSC growth
   in vitro: Feeder layer of HSC growth (in LTC-IC assay etc.)

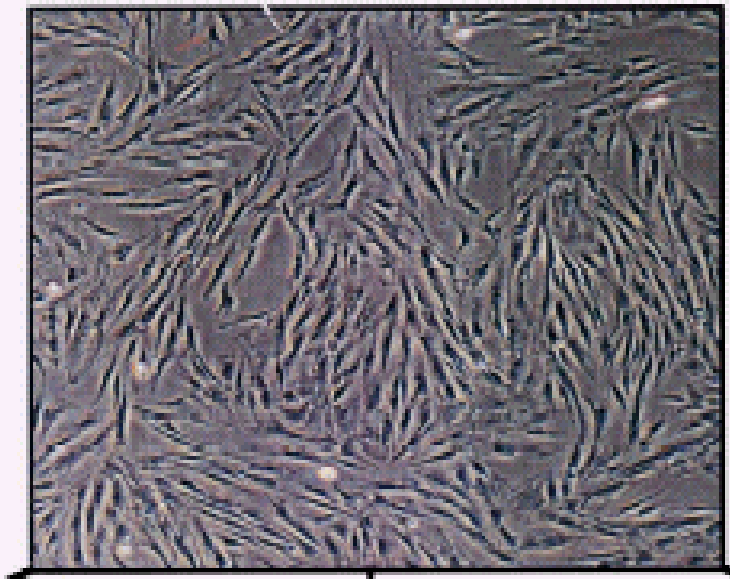


7 days BM culture

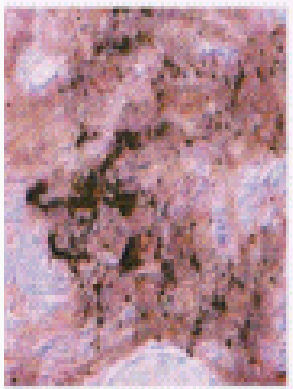


21 days BM culture

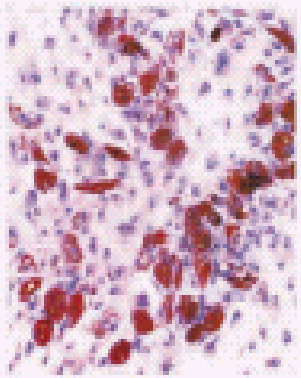
Human MSCs -- Mesenchymal Differentiation in vitro



Culture expanded human MSCs



osteogenesis



adipogenesis

chondrogenesis

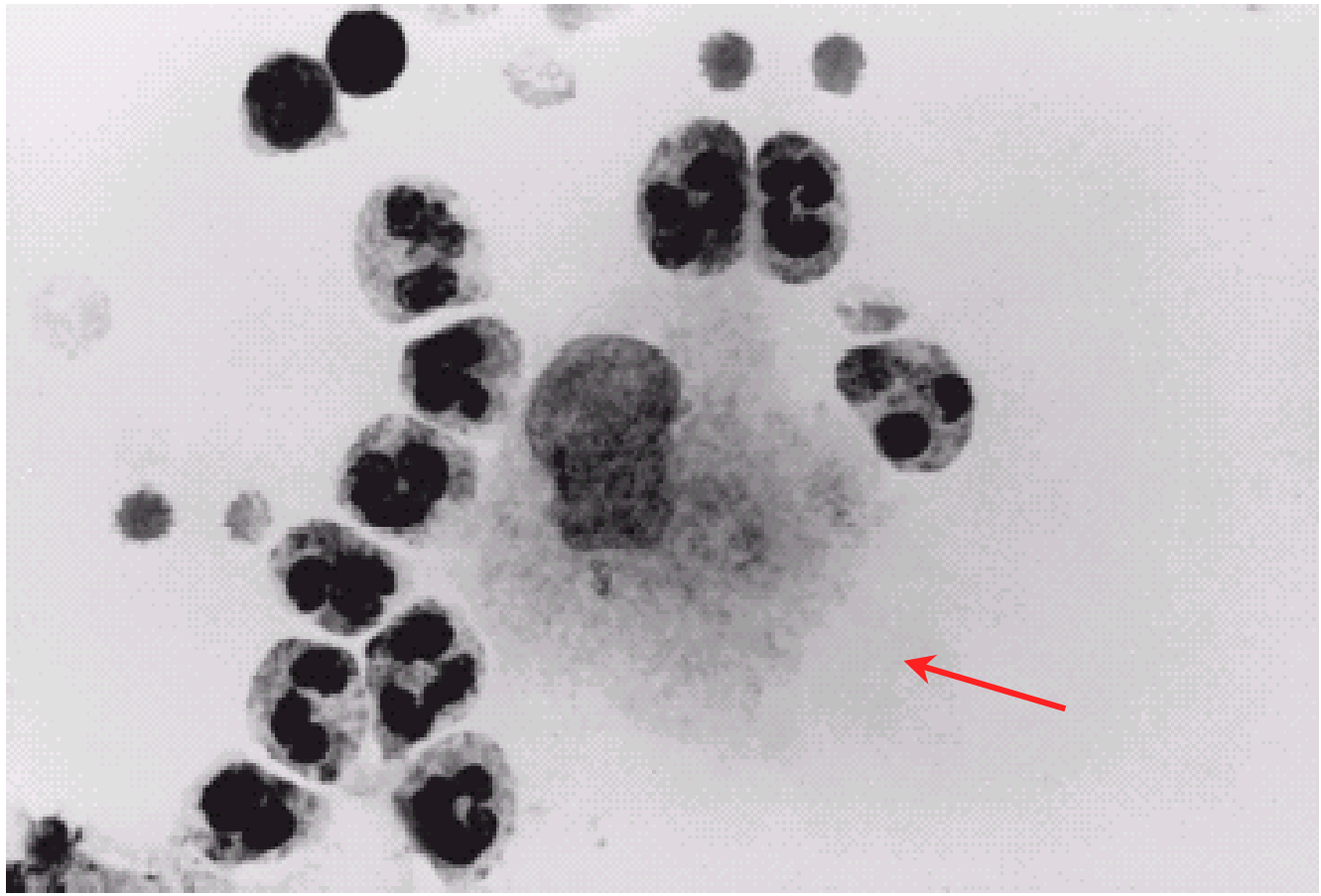


Fig 5. Photomicrograph of a detached MSC (magnification ×100). After detachment, MSCs were mixed ex vivo with peripheral-blood mononuclear cells for direct comparison of size. Cytospin preparation (1,000 rpm) was made and a representative MSC was photographed along with admixed neutrophils.

Surface Phenotype of Human MSC

Surface marker	Expression
Growth factor receptor	
IL-1R (CD121)	+
IL-2R (CD25)	-
IL-3R (CD123)	+
Transferrin receptor (CD71)	+
SCF-R (CD117)	±
G-CSF-R (CD114)	-
PDGF-R	±
EGF-R	±
Hematopoietic markers	
CD1a	-
CD11b	-
CD14	-
CD34	-
CD45	-
CD133	-

Surface Phenotype of Human MSC

Surface marker	Expression
Adhesion molecules	
ALCAM (CD166)	+
ICAM-1 (CD54)	+
ICAM-2 (CD102)	+
ICAM-3 (CD50)	±
L-Selectin (CD62L)	+
E-Selectin (CD62E)	-
PECAM (CD31)	±
VCAM (CD106)	+
Hyaluronate receptor (CD44)	+
Integrins	
VLA- α 1 (CD49a)	+
VLA- α 2 (CD49b)	+
VLA- α 3 (CD49c)	+
VLA- α 4 (CD49d)	-
VLA- α 5 (CD49e)	+
VLA- β (CD29)	+
β 4 integrin (CD104)	+

Surface Phenotype of Human MSC

Surface marker	Expression
Other markers	
Thy-1 (CD90)	±
Endogin (CD1055)	+
SH-3	**+**
SH-4	**+**
B7-1 (CD80)	-
B7-2 (CD86)	-

+ indicates routinely positive in all studies

± indicates variably expressed

- indicates lack of expression

Steven M. Devine

Journal of Cellular Biochemistry Supplement 38:73-79 (2002)

Mesenchymal Stem Cells in Bone Marrow

Ages	Estimated Frequency
Newborn	1: 10,000
Teenage	1: 100,000
50 y/o	1: 400,000
80 y/o	1: 1~2,000,000

Isolation of Multipotent Adult Progenitor Cells (MAPC)

Catherline Verfaillie, Univ. Minnesota

Bone marrow mononuclear cells

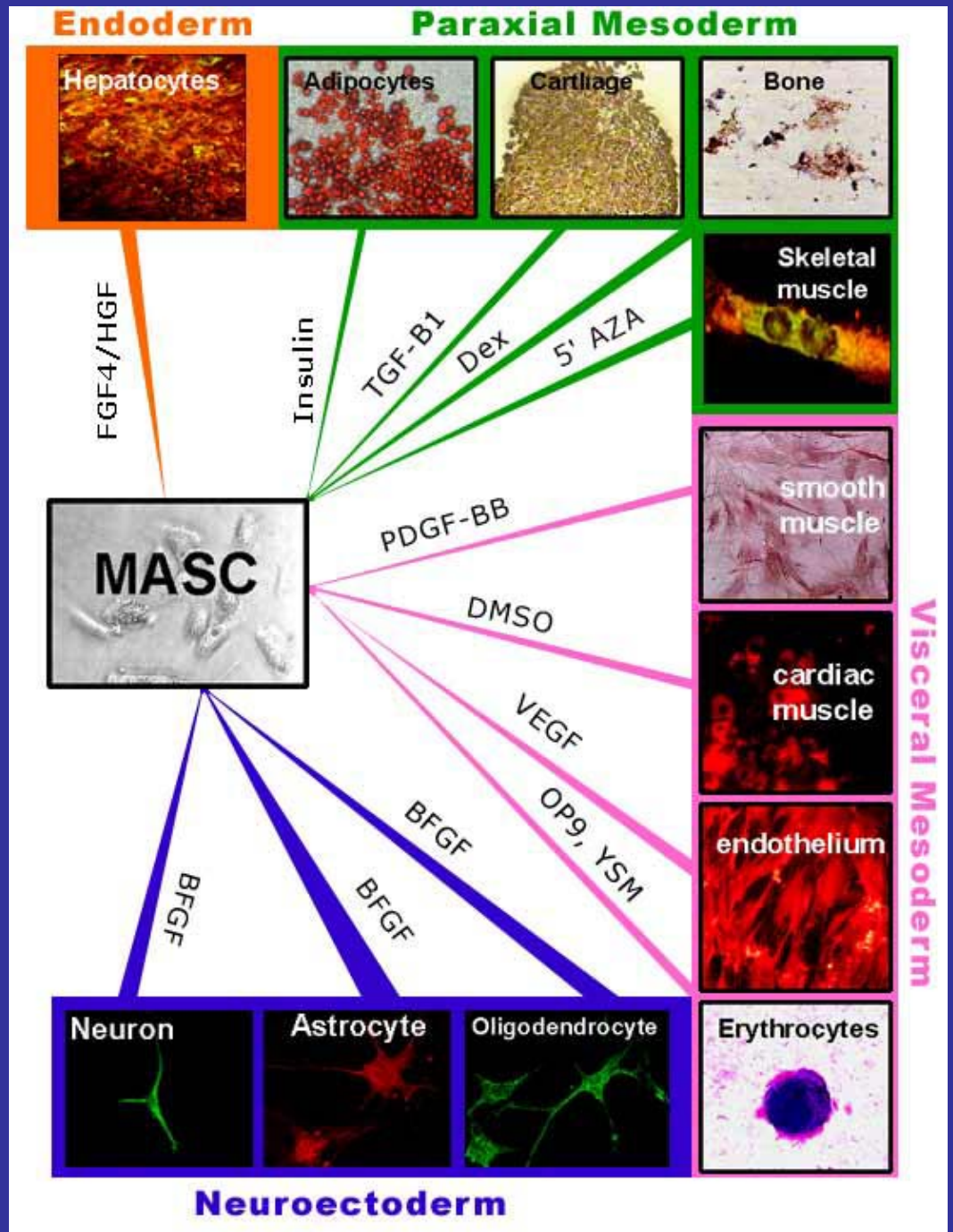
$CD45^-$,$Gly\text{-}A^-$ Cells, 5×10^3 / cm^2

Fibronectin-coated Plastic dish

DMEM, MCDB, 2% FCS
PDGF-BB, EGF, Ascorbic a.
BSA, ITS, Linolic acid, Dexa

Maintain at < 50% confluence
cell doubling time 48~60 hrs (20-25代)

Catherine Verfaillie
Director
Stem cell institute,
Prof. Medicine
Mdical school,
Univ. Minnesota



以「細胞治療」代替「器官移植」

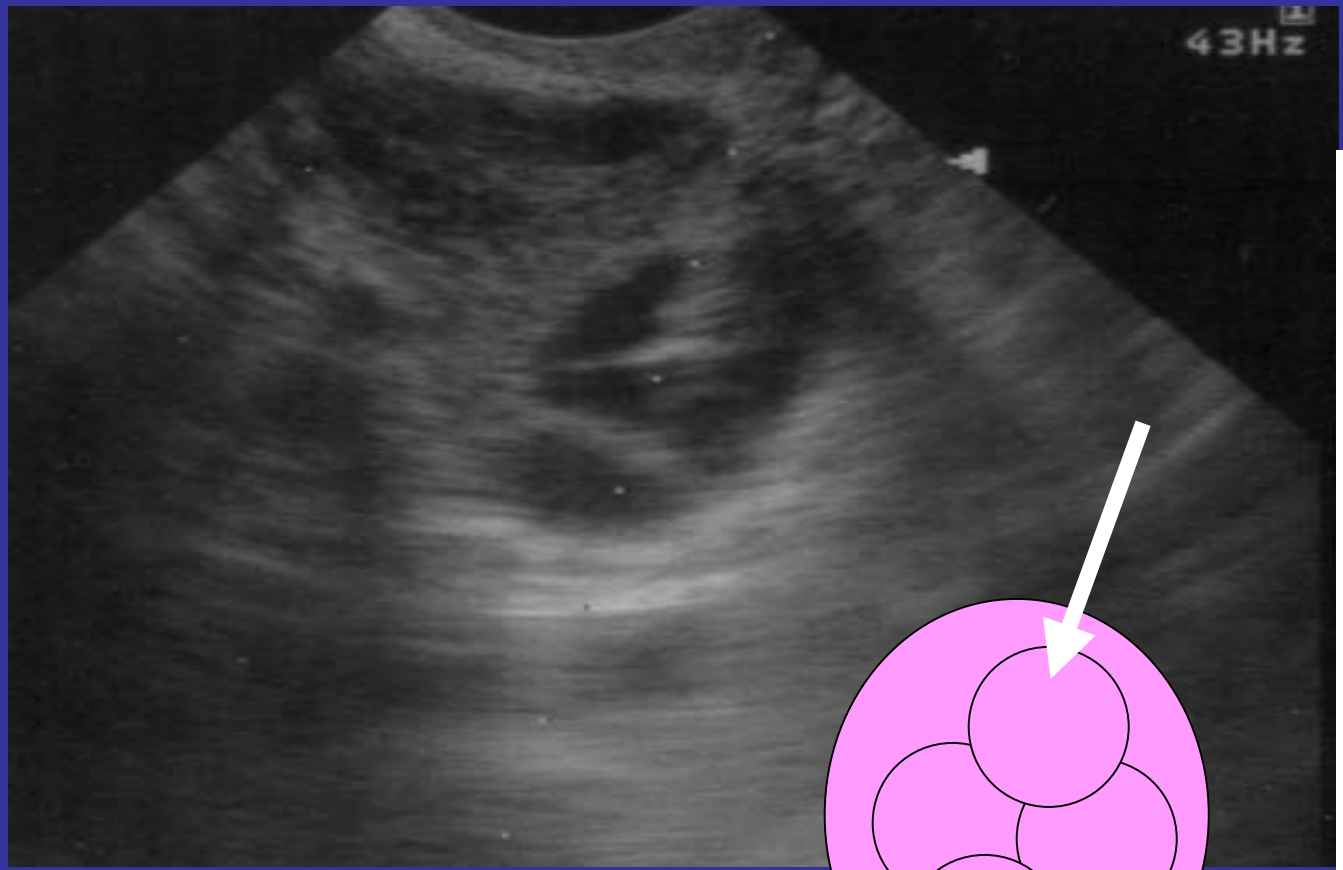
- 科學家們認為，幹細胞可望用來治療各種有關骨質生成的疾病、神經受傷、退化性神經疾患、心肌梗塞、心臟衰竭、先天性肌肉生成不良、肝臟細胞壞死、糖尿病等等。

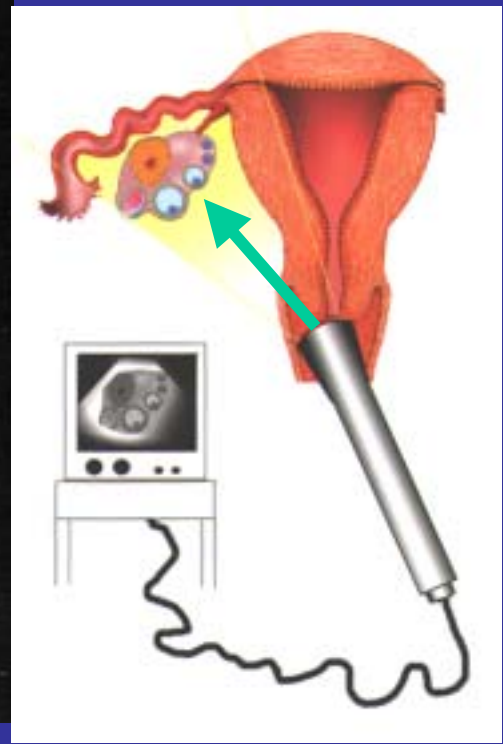
- 預計在未來五到十年中，會對人類的醫療保健，產生革命性的影響。

胚胎幹細胞

- 「胚胎幹細胞」的運用，因為有宗教、倫理、法律上的種種正反兩極的意見，一直佔領著新聞版面。二○○一年八月九日，美國布希總統宣佈有條件支持有關胚胎幹細胞的研究，同時也開啟了這一領域的科學競賽。

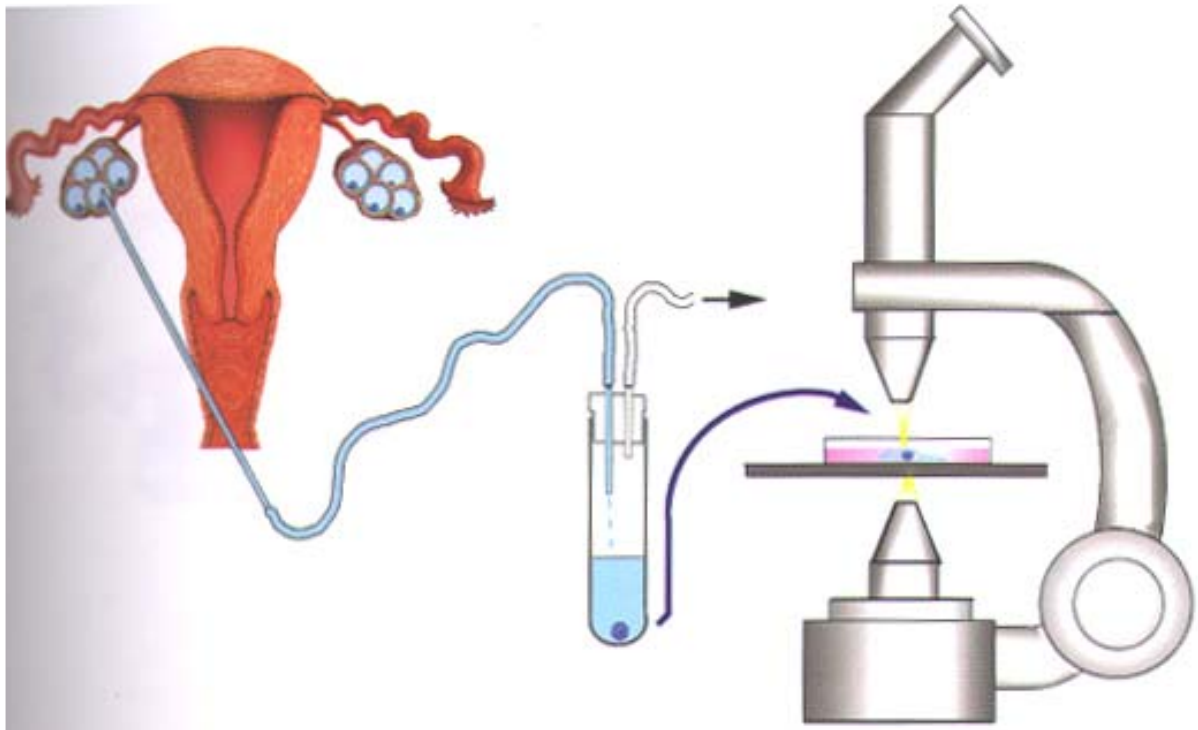
- 擁護胚胎幹細胞的科學家們認為惟有胚胎幹細胞才能分化出各種我們所需要的細胞,在細胞數目方面也較容易達到臨床治療所須之量,只要能夠克服免疫排斥作用,就是未來「再生醫學」的希望所寄。





經陰道取卵

取卵後顕微鏡観察



ARTにおける卵胞刺激の際に、血中エストラジオール測定や超音波による卵胞計測が用いられます。主席卵胞が17mmを越えるか、他の卵胞が14mm以上に成長し、エストラジオールが14mm以上の卵胞1個当たり200pg/ml以上あればHCGの投与時期とする施設もあります。

IVF (in vitro fertilization)
(體外受精;試管嬰兒)

Figure 1.4 Mature oocytes and spermatozoa, the beginnings of a new life

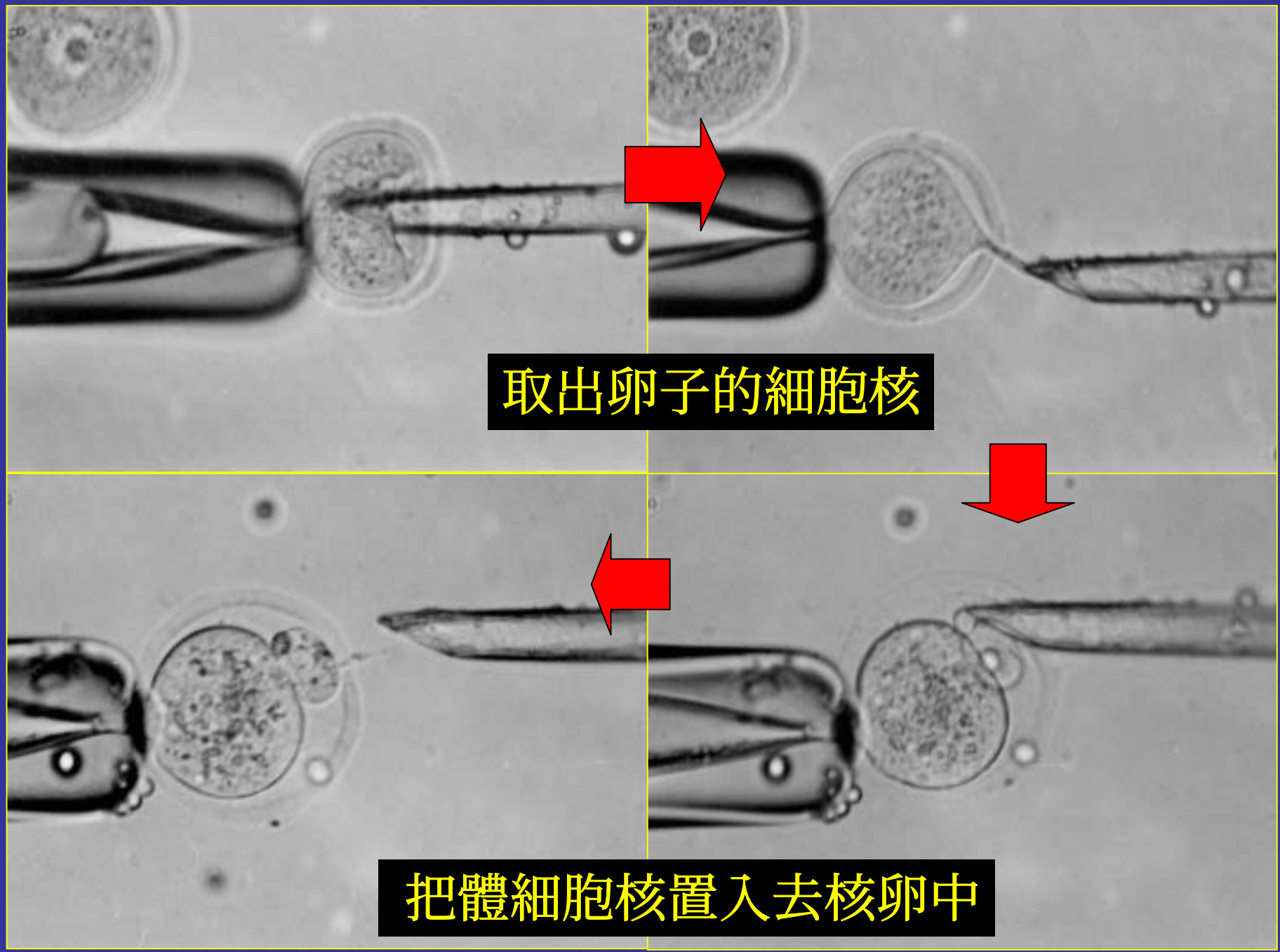
胚胎 (embryo)

Figure 5.4 Two-cell conceptus with asymmetrical division. One blastomere is larger than the other, very likely the result of recent or impending cleavage. Faint nuclei can be visualized in each blastomere, particularly in the blastomere on the right

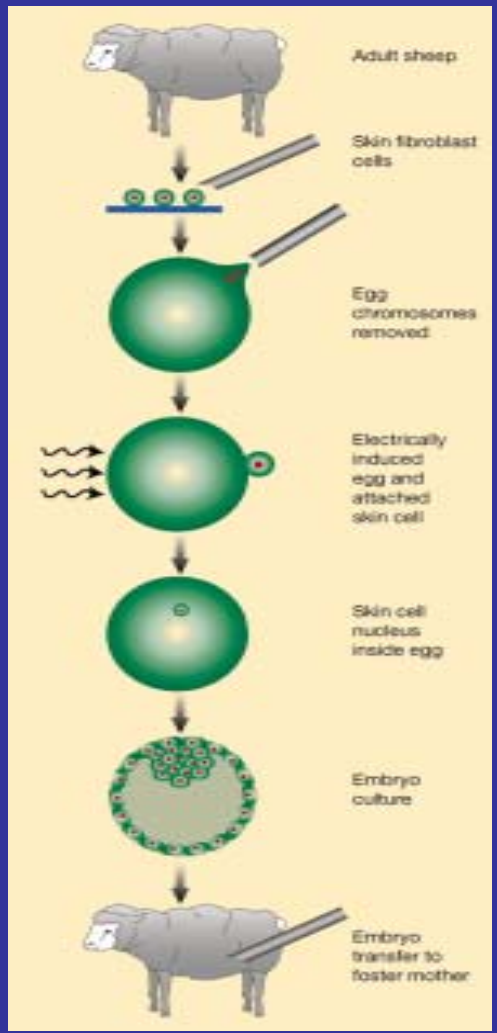
Figure 5.5 Three-cell conceptus with one larger and two smaller blastomeres. Both smaller blastomeres have nuclei whereas the larger blastomere displays only a faint central nuclear remnant

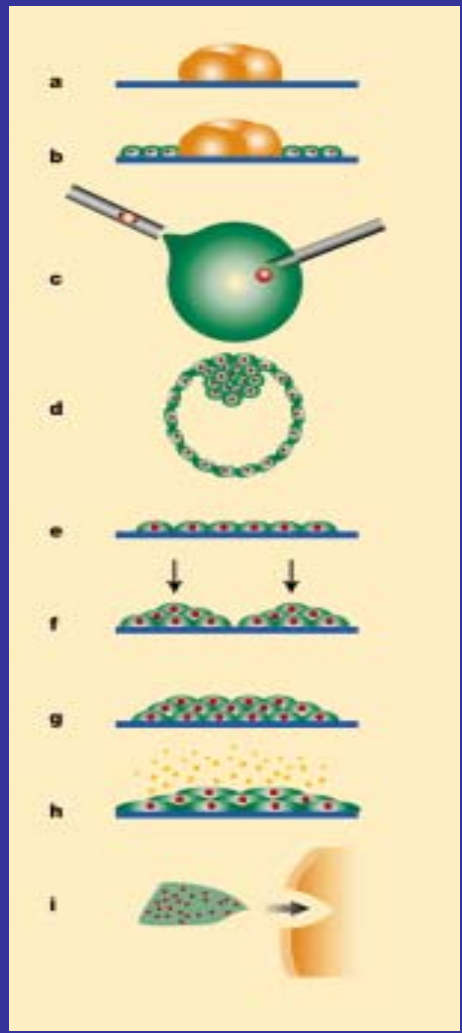
Figure 5.6 Three-cell conceptus with blastomeres that are more equal in size compared with those in Figure 5.5. Two upper blastomeres are the result of division of a single superior blastomere

Figure 5.7 Typical four-cell conceptus showing two blastomeres on one plane of focus and a further two in alternate apposition on another plane

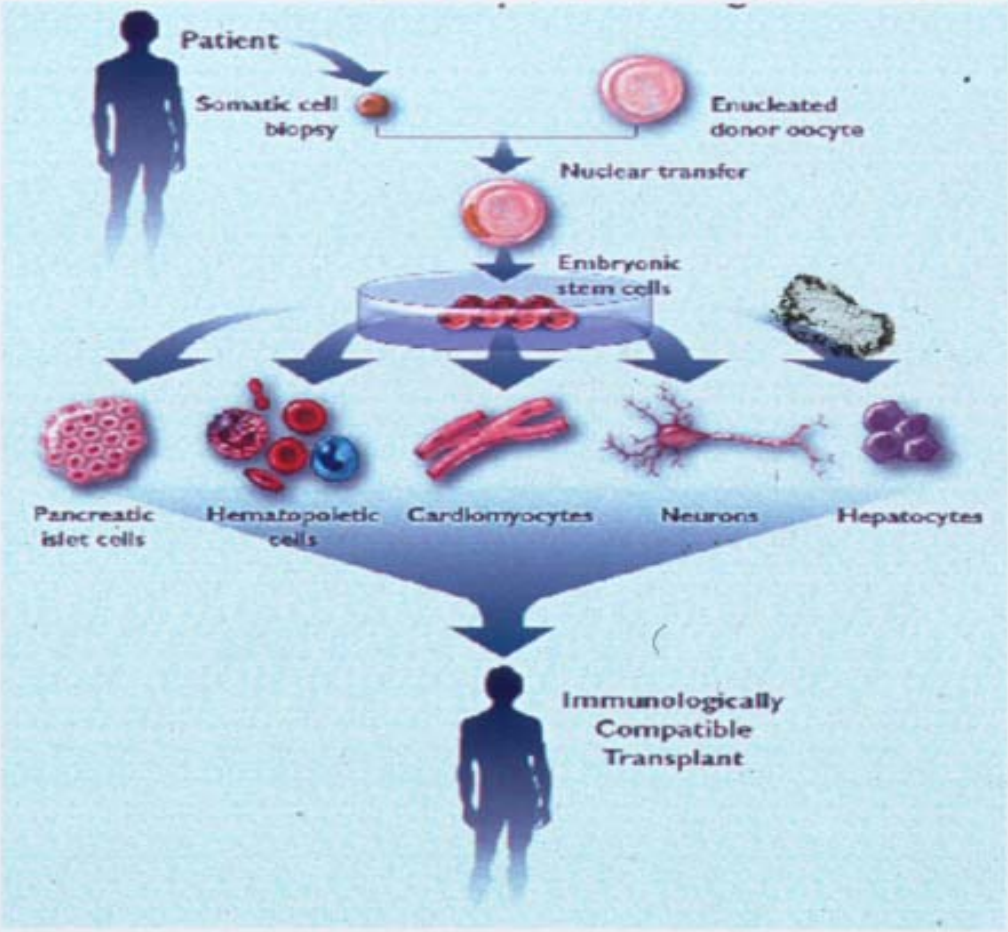

取出卵子的細胞核
把體細胞核置入去核卵中

Reproductive Cloning vs. Therapeutic cloning





Therapeutic cloning using nuclear transfer techniques



治療性複製(細胞複製)

Scientists Create Human Embryo for Its Stem Cells, Igniting Debate on Medical Ethics

Continued From Page A1

University in Baltimore. "You will hear none of the scientists who are involved in this work talk about making embryos to destroy them in any way. We don't think it's necessary."

Experts in academia and industry said that the experiment might be whether to make an exception to that ban so that taxpayers could finance studies on cells derived from frozen embryos that would otherwise be discarded. The experiment by the Jones Institute, which is affiliated with the Eastern Virginia Medical School, would fall outside such an exception, because no federal money was involved.

cells would be of any greater therapeutic benefit than those already in existence.

"We haven't asked that scientific question," he said.

The scientists retrieved 162 eggs from the donors; when those eggs were fertilized in the laboratory with the sperm, 118 embryos resulted. Of these, 50 grew for six days to become

What some see as promising research others see as ghoulish.

07/06

A18 YNE THE NEW

HE NEW YORK TIMES **NATIONAL** *FRIDAY, APRIL 27, 2001*

Stem-Cell Advances Are Likely to Heighten Ethics Debate

Continued From Page A1

few days old at the stage when it is a barely visible, hollow sphere of cells, are expected to play a central role in regenerative medicine since these powerful cells give rise to all the tissues of the adult body.

Probably as a defense against can-

sight. "Our magnificent biomedical research enterprise won't be working in this area, Dr. Goldstein said. "We think that is very unfortunate."

Abortion opponents object to the fact that embryonic stem cells are derived by destroying embryos and advocate using a different class of stem cell, known as adult stem cells, which are found in various adult tissues. But adult stem cells are less versatile; none can yet be converted to the tissues needed to treat diabetes and Parkinson's.

Today's other report proves that therapeutic cloning works in mice, though the researchers have yet to inject the dopamine-producing cells they created into the brains of mice with Parkinson's disease to see if symptoms are relieved. The creation of mouse embryonic stem cells from mouse skin cells was carried out by Teruhiko Wakayama and colleagues in the Rockefeller University laboratory of Dr. Peter Mombaerts. Lorenz Studer at Sloan-Kettering inverted the embryonic cells into skin cells.

But to take therapeutic cloning from mice to people will not be simple. The mouse skin cell is converted to embryonic form by removing its nucleus and inserting it into a mouse

Grants for Stem Cell Work Are Delayed

By NICHOLAS WADE

In a possible political setback for stem cell research, the National Institutes of Health has been told to postpone the first meeting of a committee to review grant applications for research on human embryonic stem cells.

The N.I.H. was told by its parent agency, the Department of Health and Human Services, to put off the meeting until the department has completed a review of the legal basis for research with the cells, which hold promise of new therapies because they can generate all the tissues and organs of the body.

certain whether the move is the simple administrative step the department suggests, or a first step toward banning the research altogether.

If the administration had done nothing, the N.I.H. would have proceeded to review grant applications and finance those that were successful, under the course set by the Clinton administration.

Analyzing the meaning of a postponed meeting.

ionic stem cell research if he had wished to do so. That he has not "is a good sign and shows the administration understands that this is a complex issue," said Lawrence Soler, a chairman of the Coalition for the Advancement of Medical Research.

Congress has forbidden the use of federal funds for any research in which a human embryo is destroyed. Under the Clinton administration, the department's general counsel, Harriet Rabb, ruled that federal funds could be used to do research on the cells, but not to derive them. It is this ruling that is under review.

The cells are derived from embryos created in fertility clinics but not needed by the parents. Because the

The New York Times

Founded in 1851

ADOLPH S. OCHS, *Publisher 1896-1935*
ARTHUR HAYS SULZBERGER, *Publisher 1935-1961*
ORVIL E. DRYFOOS, *Publisher 1961-1963*
ARTHUR OCHS SULZBERGER, *Publisher 1963-1992*

Stem Cell Politics

The Bush administration is divided over whether to bar federal funding for research on stem cells derived from human embryos. President Bush is said to be listening carefully to the opinion of Roman Catholics and other religious groups that such research destroys human life. On the other hand, most scientists and many conservatives in the administration and Congress take the more sensible view that these microscopic clumps of cells are not the same as a fetus and that research on them can bring major medical breakthroughs. Whatever Mr. Bush decides, he will be sending an important signal on whether his political ties to religious conservatives or his social and scientific

womb does. He an personal experienc real "pro-life" posi tifically sound rese

In the last few reportedly stepped mise allowing some stances that would opponents of abort large numbers la Tuesday that he wo after what his aides the moral, religious

These aides in the decision without is nevertheless tro an obvious factor leaders in the Hou block the research, chief political adv conservatives, is p

In his campaig the Clinton admini overreliance on p

Stem Cell Debate Revives an Old Ideological Battle

By Ruth J. Katz

NEW HAVEN

As the debate over whether to approve stem cell

us, is tantamount to destroying human life.

To the president's father, this argument must sound familiar. In 1989, the subject was federal funding for research that involved fetal tissue

tion. A core question framed the debate then, as it does today: On what basis will the biomedical research agenda of this country be set — scientific merit or political controversy?

Henry A. Waxman, a Democrat,

tissue study might save a third child, still unborn but already diagnosed with the same inevitably fatal condition. People with Parkinson's disease and parents of diabetic children told stories of suffering that they believed might eventually be eased

and they continue to do so. Federal involvement has also ensured that the research is monitored and that donors provide adequate informed consent.

The stem cell research that is the subject of today's controversy will im-

ow quickly the re- ve results, but the d with the research in the treatment of mer's, cancer and the Clinton administ- ing federal funding rovided they were you derived from in be discarded, any-

胚胎幹細胞之爭議:政治,宗教,倫理

論定有難命救命毀 胞細幹胎胚

生新獲它因望寄 者患疾痼分部 同不仍兒胎與 養培內室驗實

驗檢理倫格嚴受接 胞細幹胎胚

想理學醫壞破能可責譴者對反 益權者卵精贈及顧已認者持支

生命之開始如何算??
人可以扮演神嗎??

Biotechnology in Singapore

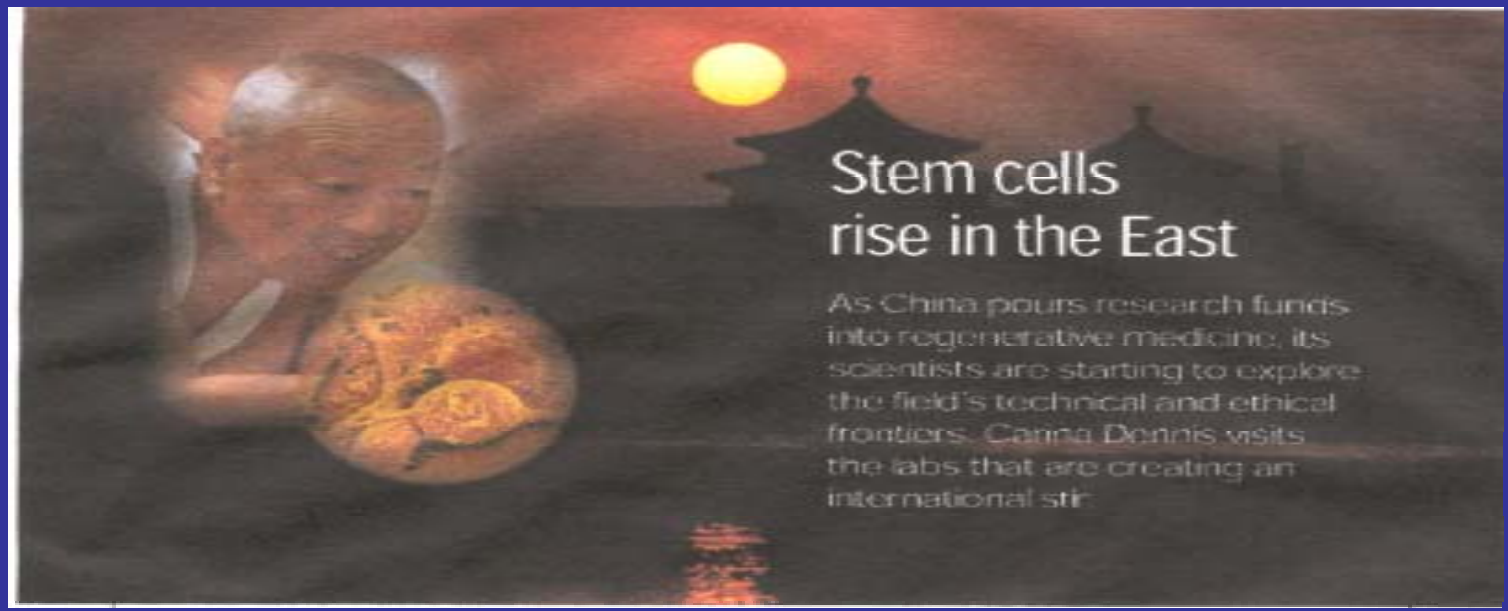
Send in the clones

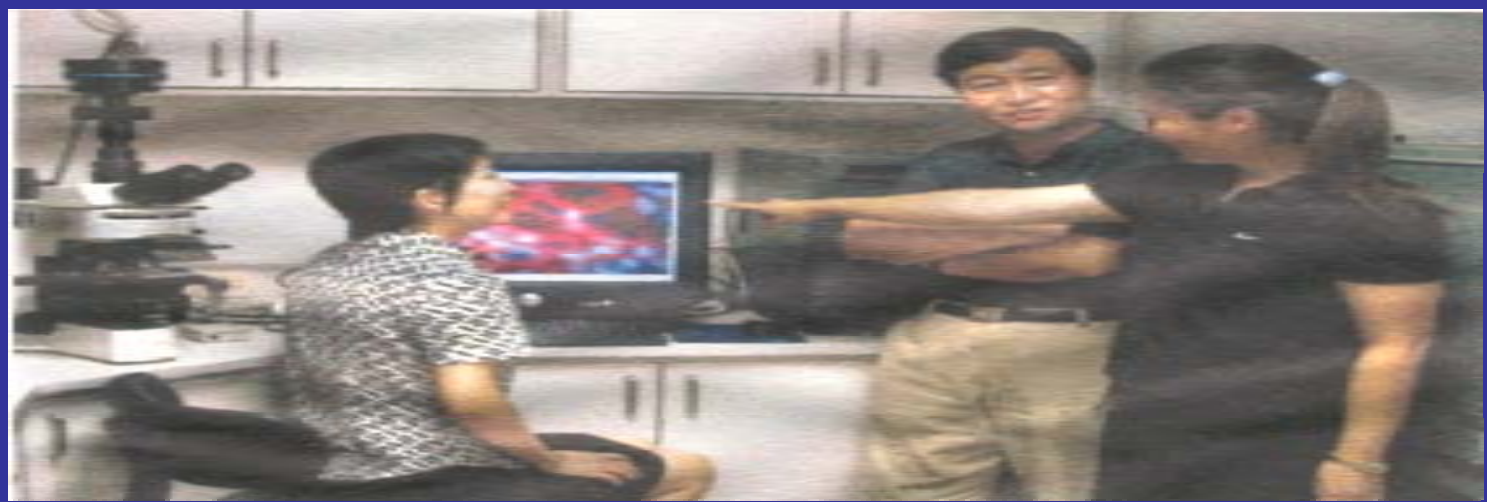
The Economist August 24th 2002

SINGAPORE

Will a relaxed attitude to regulation make Singapore the stem-cell-research capital of the world?

新加坡在政府以「法規開放」來製造研究的大環境，再碰上新加坡幣三十億（合台幣六〇〇億）的大手筆建造一流設備，成立一流學院，建立跨國合作，聘來世界各國頂級研究團隊，一年之內，把新加坡脫胎換骨變成「經濟學人」雜誌所稱的「世界幹細胞研究之都」





幹細胞研究在中國----

自然期刊　2002/9/26

NIH Human Embryonic Stem Cell Registry
美國衛生研究院　幹細胞登記

公司/機構	細胞株數
美國 BresaGen, Inc., Athens, Georgia	4
美國 CyThera, Inc., San Diego, California	9
澳洲 **ES Cell International, Melboume, Australia**	6
美國 **Geron Corporation,Menlo Park, California**	7
瑞典 Göteborg **University. Göteborg, Swedem**	19
瑞典 Karolinska Institute, Stockholm, Sweden	6
南韓 **Maria biotech Co. Lte.- Maria Infertility Hospital Medical Institute, seoul, Korea**	3
南韓 **MizMedi Hospital – Seoul National University, Seoul, Korea**	1
印度 **National Centre for biological Sciences/ Tata Institute of fundamental research, Bangalore, India**	3
南韓 **Pochon ChA University, Seoul, Korea**	2
印度 Reliance Life Sciences, Mumbai, India	7
以色列 Technion University,- Haifa, Israel	4
美國 University of California, San Francisco	2
美國 Wisconsin Alumni Research Foundation	5

排斥作用(II)
(胚胎幹細胞之最大技術問題)

(三)如何解決胎胎幹細胞衍生細胞植入體內之排斥問題

(1)以核轉移技術(nuclear transfer)

問題：失敗率高

常出現基因異常

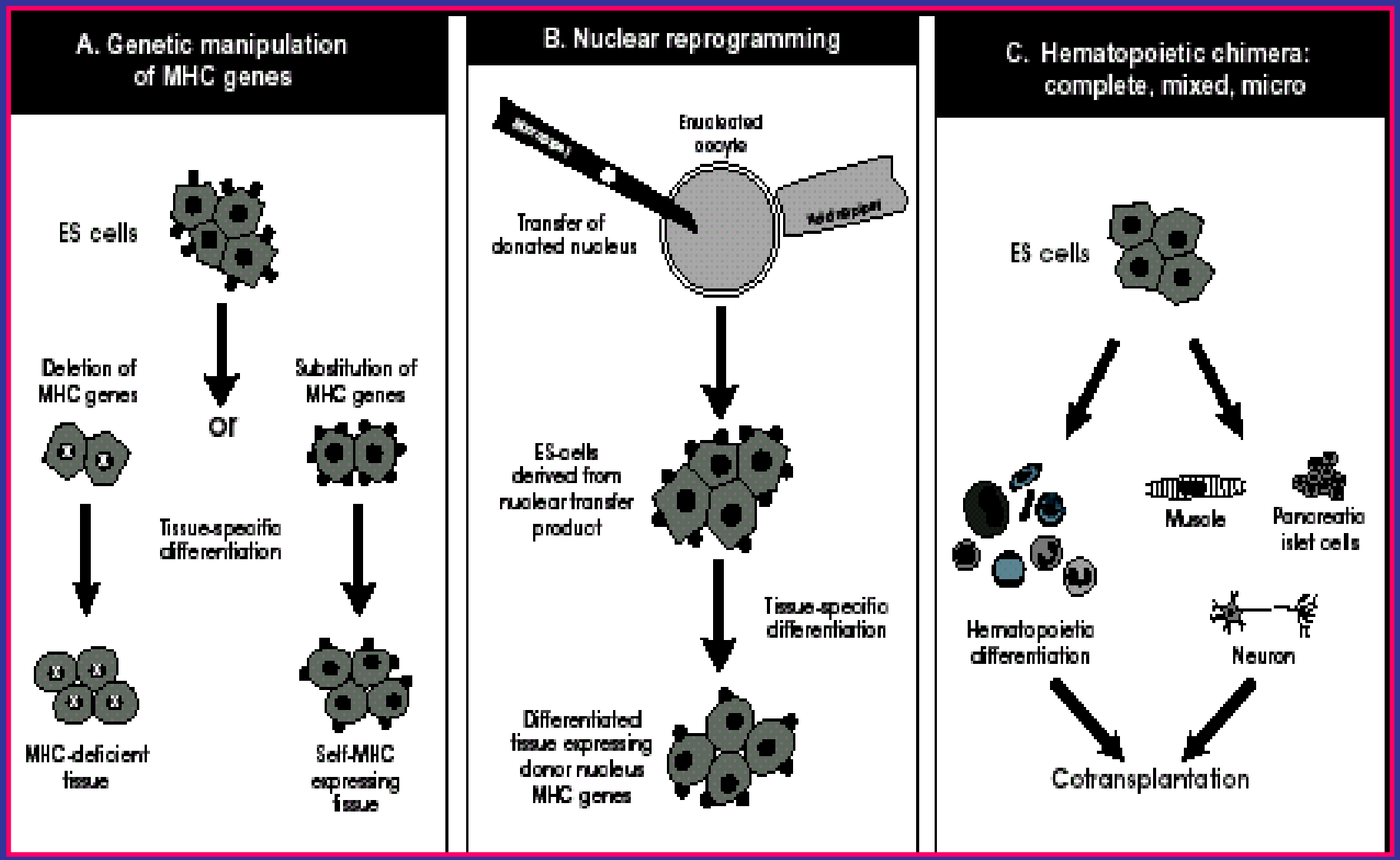
(2)將基因剔除(gene knock-out)或置換(substitution)

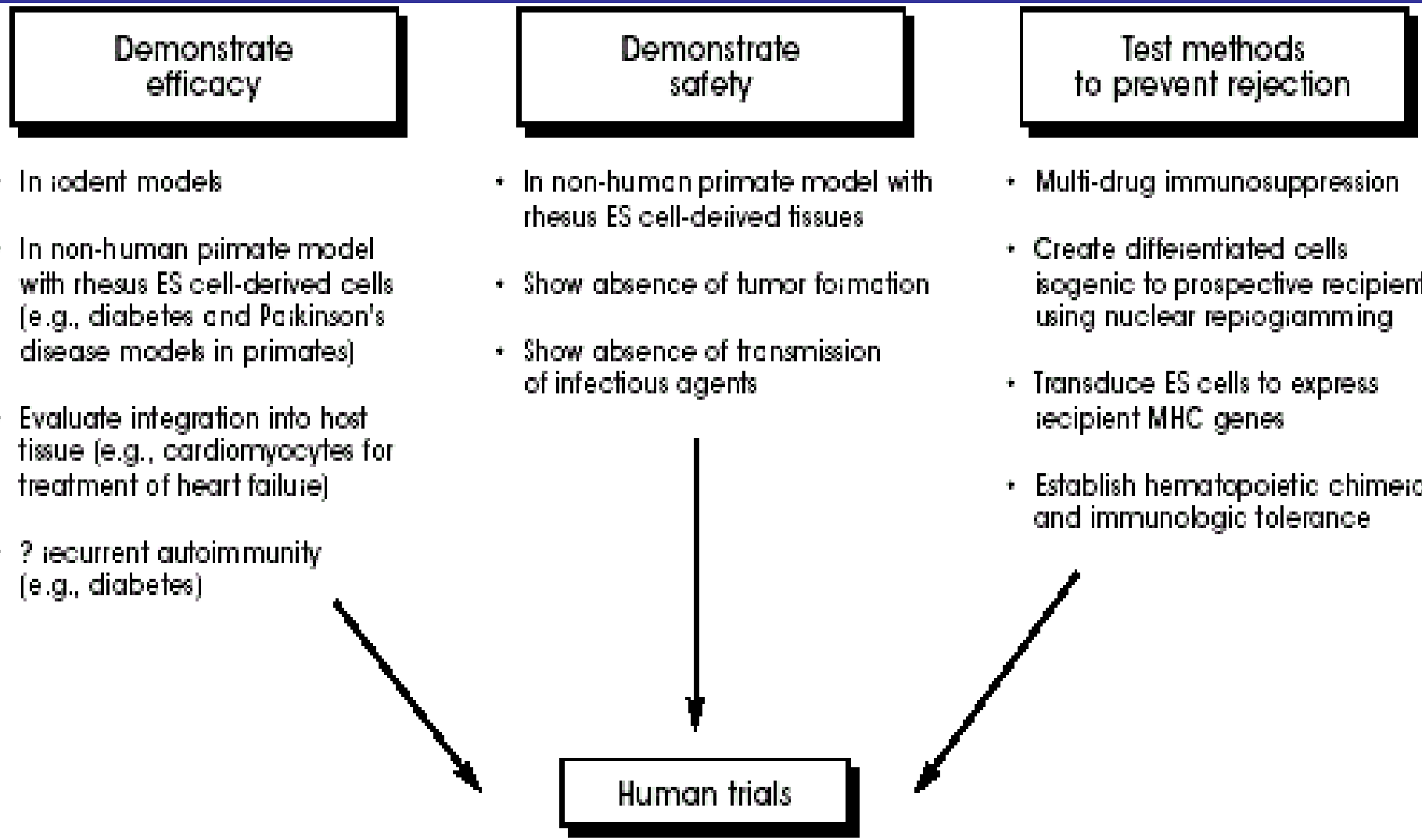
問題：不能解決 minor histocompatibility antigens

不保證沒有其他基因異常

Genetic Manipulation of Embryonic Stem Cells

Prevention of Graft Rejection




Demonstrate efficacy
In rodent models
In non-human primate model with rhesus ES cell-derived cells (e.g., diabetes and Parkinson's disease models in primates)
Evaluate integration into host tissue (e.g., cardiomyocytes for treatment of heart failure)
? recurrent autoimmunity (e.g., diabetes)
Demonstrate safety
In non-human primate model with rhesus ES cell-derived tissues
Show absence of tumor formation
Show absence of transmission of infectious agents
Test methods to prevent rejection
Multi-drug immunosuppression
Create differentiated cells isogenic to prospective recipient using nuclear reprogramming
Transduce ES cells to express recipient MHC genes
Establish hematopoietic chimeria and immunologic tolerance
Human trials

成體幹細胞

(1)骨髓/周邊血/臍帶血
(2)肝臟幹細胞
(3)胰臟幹細胞(內分泌、胰島幹細胞)
(4)軟組織幹細胞
(5)肌肉幹細胞
(6)眼角膜幹細胞
(7)腸道幹細胞
(8)神經幹細胞
(9)……(族繁不及備載)
*組織成體幹細胞癌化論

Table 1. Adult Human Stem Cells and Their Primary Direction of Differentiation.

Cell Type	Tissue-Specific Location	Cells or Tissues Produced
Hematopoietic stem cells	Bone marrow, peripheral blood	Bone marrow and blood lymphohematopoietic cells
Mesenchymal stem cells	Bone marrow, peripheral blood	Bone, cartilage, tendon, adipose tissue, muscle, marrow stroma, neural cells
Neural stem cells	Ependymal cells, astrocytes (subventricular zone) of the central nervous system	Neurons, astrocytes, oligodendrocytes
Hepatic stem cells	In or near the terminal bile ductules (canals of Hering)	Oval cells that subsequently generate hepatocytes and ductular cells
Pancreatic stem cells	Intraislet, nestin-positive cells, oval cells, duct cells	Beta cells
Skeletal-muscle stem cells or satellite cells	Muscle fibers	Skeletal muscle fibers
Stem cells of the skin (keratinocytes)	Basal layer of the epidermis, bulge zone of the hair follicles	Epidermis, hair follicles
Epithelial stem cells of the lung	Tracheal basal and mucus-secreting cells, bronchiolar Clara cells, alveolar type II pneumocyte	Mucous and ciliated cells, type I and II pneumocytes
Stem cells of the intestinal epithelium	Epithelial cells located around the base of each crypt	Paneth's cells, brush-border enterocytes, mucus-secreting goblet cells, enteroendocrine cells of the villi

由骨髓移植到成體幹細胞移植

- 骨髓移植

  骨髓生病，不能以藥物治癒 → 將骨髓全部殺光 → 打入捐髓者正常骨髓 → 骨髓細胞再生/恢復

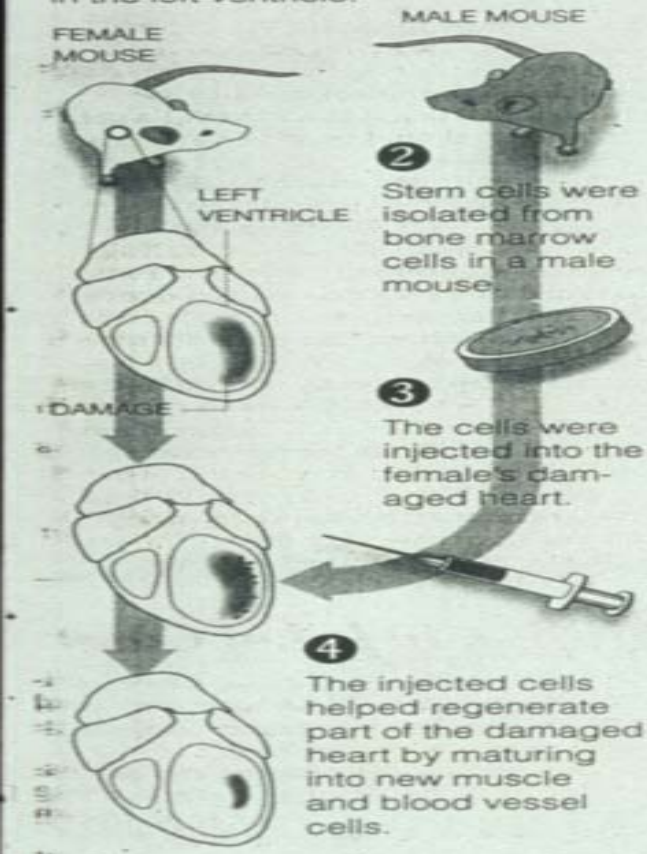
- 幹細胞移植（修補醫學或再生醫學） →

  各器官之疾病(細胞受損) → 以心肌梗塞為例 → 取出自體骨髓內成體幹細胞(實驗室培養？)打入心肌內,加速心肌恢復

With Its Own Cells

In three new studies, scientists report having repaired damaged heart tissue in animals with stem cells taken from bone marrow.

ONE STUDY: SUCCESS IN MICE



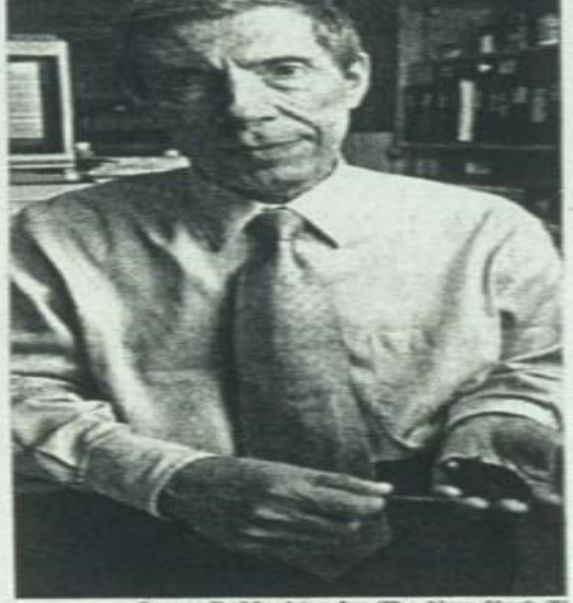
the same way in people, the researchers say, people suffering a heart attack would be treated by having cells extracted from their bone marrow. The cells would be sorted and amplified, then injected either directly into the heart, or maybe just into the bloodstream, from which they would home in on damaged heart tissue and on the enlarged heart muscle cells that soon grow around it.

It may even prove possible, though this concept has not yet been tested, to do no more than inject a heart attack patient with a cytokine, a natural protein that stimulates the bone marrow's stem cells to proliferate. The cells would home in on damaged heart tissue, and repair it. Biologists say it is too early to know if the blood-forming stem cells of the bone marrow are also the heart's own stem cells, which researchers have been seeking in vain for years, or if their remarkable ability to repair the heart is just a general property of stem cells.

Stem cells are unspecialized cells that can turn into the mature cells of the body while replenishing their own numbers so as to remain a constant source of new cells. The cells involved in the new reports are called adult stem cells, and differ from the controversial embryonic stem cells that generate the fetus and adult organism.

The new results all depend on the recent finding that the stem cells of the bone marrow are far more versatile than supposed and can generate other tissues besides the red and white blood cells, their best-known function. It seems that the cells are a kind of universal clay, so responsive to local cues that if placed in the heart they will develop into heart tissue instead of blood cells.

In one of the new reports, pub-



Susan B. Markisz for The New York Ti

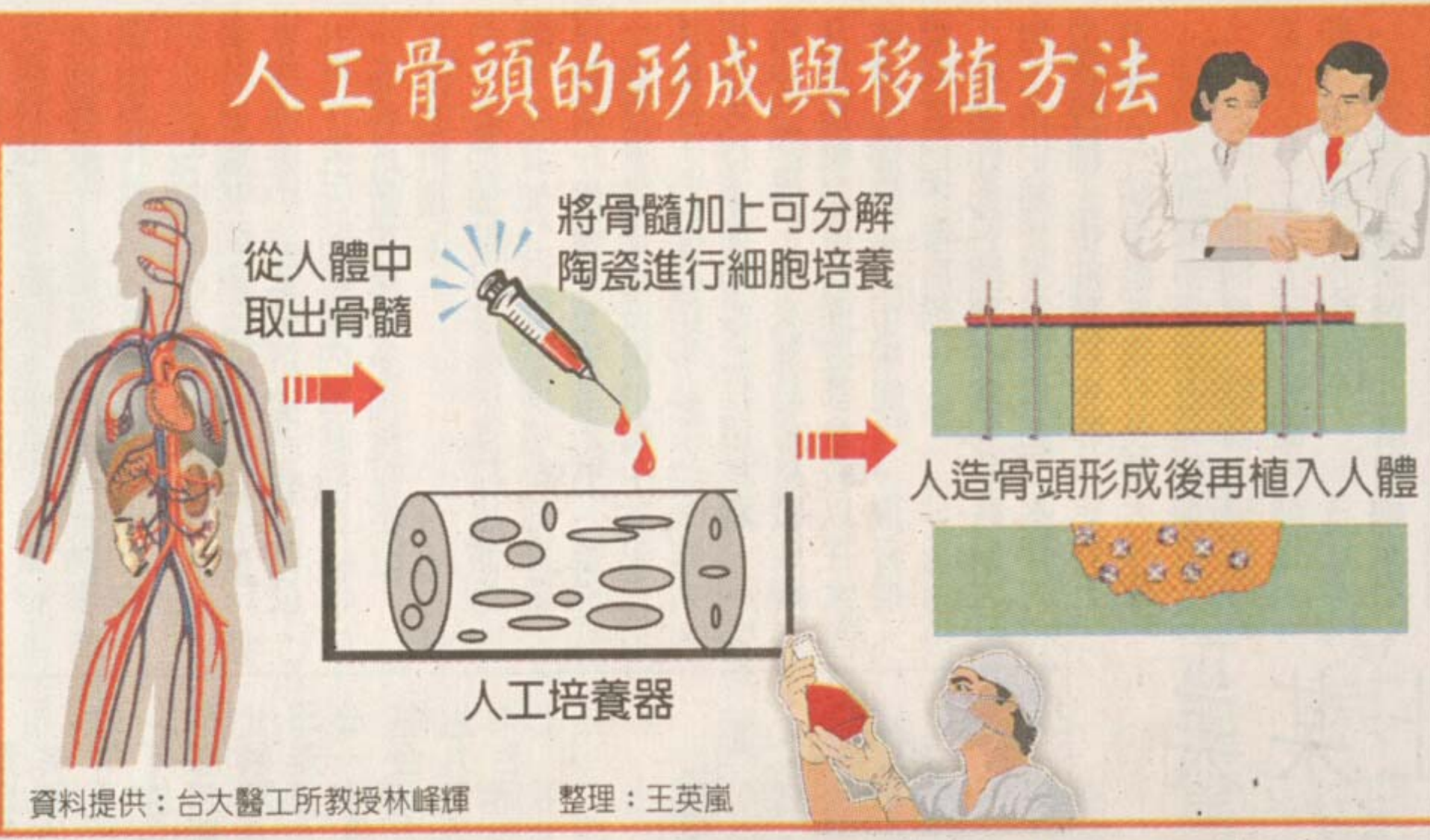
Dr. Piero Anversa of the N York Medical College in Valha used mice in his heart repair stu

lated a special kind of stem cell fr human bone marrow. The ce which they call angioblasts, ar subset of the blood-forming st cells that make the red and wh blood cells. Angioblasts generate cells of the fine blood vessels. though their existence had been ferred, they had not been isola before, Dr. Itescu said.

After a heart attack, caused blockage of one of the heart's c arteries, the muscle cells that w deprived of oxygen die off and cells around them grow four or f times larger to compensate for damaged tissue. That is why m

打入骨髓間質細胞 來治療心肌梗塞

(2001.3.31 紐約時報)



打入骨髓間質細胞來生成骨頭或軟骨
(中國時報)

細胞治療之可能對象

1.肝臟:威爾遜氏症,猛爆性肝炎,肝硬化?

2.心臟:心肌梗塞,心衰竭

3.血液:間質造細胞與造血幹細胞移植並用

4.眼睛:人工網膜

5.胰臟:糖尿病

6.神經:退化疾病,脊髓損傷

成體幹細胞

- 在另一面，骨髓之「成體幹細胞」因為取得方便，與目前骨髓移植之過程較類似，較無倫理與法律上的爭議，因此，在實驗室研究，動物實驗以及臨床試驗上，皆在悄悄而快速進展之中。

- 「骨髓幹細胞」之運用由過去的「骨髓移植」，變成更進一步的「修補醫學」的運用。

- 這一派的擁護者認為，既然成體幹細胞與胚胎幹細胞一樣，都可以分化出各種組織型態，使用成體幹細胞就行了，何必去使用有倫理、道德、法律爭議的胚胎幹細胞。

- 但也有人認為，成體幹細胞也許真能分化成不同組織的細胞，但「量」是否足夠臨床使用，是一個很大的問題。

Medical Progress: Adult Stem Cells for Tissue Repair—A New Therapeutic Concept?

Korbling & Estrov, NEJM 2003/8/7;349(6):570-83

Preclinical *in vivo* studies

From BM

1. **Marked unselected cells→conditioning treatment of animals**
   - **See transdifferentiation but origin of SC is obscure**
2. **More specific methods**
   a. **Purified cells with specific marker**
   b. **Limiting-dilution technique**

From PB

- **Most are mobilized s/p cytokine**
  - **Cardiac studies: neovascularization, myocyte replacement**
  - **Most rat/mice models OK, primate not**

Clinical *in vivo* studies

- 1999: MSCs in allogeneic sex-mismatched BMT improved osteogenesis imperfecta [Nat Med]
- 2000: donor-derived hepatocytes after BMT [2 groups: Hepatology, Nature]
- 2001: endothelial cell chimerism after renal transplantation [Lancet]
- 2002:
  - Liver-tissue chimerism after liver transplantation [Hepatology]
  - donor-origin epithelial cells [Nat Med], hepatocytes, endothelial cells [NEJM], cardiomyocyte [Circ] after BMT
  - Chimerism after heart transplantation [NEJM]
- 2003: donor-origin neurons after BMT [PNAS * 2]

Table 2. Potential Clinical Applications of Hematopoietic Tissue–Derived Adult Stem Cells for Tissue Repair or Replacement.*

Disease or Category	Model	Stem-Cell Source	Route of Cell Application	Outcome	Study
Myocardial infarction	Experimental BMT	Purified bone marrow–derived hematopoietic stem cells	Intracardial	Reduction of infarcted area, improved cardiac hemodynamics	Orlic et al.[15]
	Experimental BMT	Purified bone marrow–derived hematopoietic stem cells	Intravenous	Generation of donor-derived cardiomyocytes and endothelial cells	Jackson et al.[16]
	G-CSF–induced experimental stem-cell mobilization	Peripheral-blood stem cells	NA	Decrease in infarct size and mortality, increase in ejection fraction, improvement in hemodynamics	Orlic et al.[25]
	Experimental G-CSF–mobilized peripheral-blood SCT	Mobilized and purified human peripheral-blood–derived angioblasts	Intravenous	Stimulation of neovascularization and angiogenesis in infarcted region	Kocher et al.[24]
	Clinical BMT (autologous)	Bone marrow cells	Intracoronary	Decreased infarct size, improved ventricular function and myocardial perfusion†	Strauer et al.[43]
	Clinical BMT (autologous)	Purified bone marrow–derived hematopoietic stem cells	Intramyocardial	Enhanced left ventricular function, improved infarct tissue perfusion‡	Stamm et al.[44]
	Clinical BMT (autologous)	Bone marrow or peripheral-blood cells	Intracoronary infusion	Improved left ventricular ejection fraction, improved regional wall motion in infarct zone†	Assmus et al.[45]

Table 2. Potential Clinical Applications of Hematopoietic Tissue–Derived Adult Stem Cells for Tissue Repair or Replacement.*

Disease or Category	Model	Stem-Cell Source	Route of Cell Application	Outcome	Study
Osteogenesis imperfecta	Clinical BMT (allogeneic)	Bone marrow cells	Intravenous	Increased total-body bone mineral content	Horwitz et al.[27]
Tyrosinemia type I (liver)	Experimental BMT	Purified bone marrow–derived hematopoietic stem cells	Intravenous	Correction of metabolic liver disease	Lagasse et al.[13]
Hepatitis B or C	Experimental BMT or peripheral-blood SCT	Interferon-β–transfected bone marrow–derived or peripheral-blood–derived stem cells	Intravenous	Profoundly reduced viremia in vivo	Eto and Takahashi[41]
Liver cirrhosis	Experimental BMT or peripheral-blood SCT	HGF-transfected bone marrow–derived or peripheral-blood–derived stem cells	Intravenous	Inhibition of fibrinogenesis and apoptosis, resolution of hepatic fibrosis	Ueki et al.[42]
Ischemic heart disease	Clinical BMT (autologous)	Bone marrow cells	Intramyocardial	Improved myocardial perfusion and function	Tse et al.[46]
	Clinical BMT (autologous)	Bone marrow cells	Intramyocardial	Improved myocardial perfusion and left ventricular function	Perin et al.[47]
Impaired cardiac angiogenic function associated with aging	Experimental BMT	Bone marrow cells	Intravenous	Improvement of aging-impaired cardiac angiogenic function	Edelberg et al.[48]
Chronic limb ischemia	Clinical BMT (autologous)	Bone marrow cells	Intramuscular injection in ischemic leg	Improvement in ankle–brachial index, pain at rest, and pain-free walking time	Tateishi-Yuyama et al.[49]
Ischemic vascular disease	GM-CSF–induced experimental stem-cell mobilization	Peripheral-blood–derived endothelial progenitor cells	NA	Improved neovascularization of ischemic tissues	Takahashi et al.[23]

Table 2. (Continued.)

Disease Category	Model	Stem-Cell Source	Route of Cell Application	Outcome	Study
Ischemic retinopathy	Experimental BMT	Purified bone marrow–derived hematopoietic stem cells and endothelial progenitor cells	Intravitreal	Improved retinal angiogenesis	Otani et al.[50]
	Experimental BMT	Single hematopoietic stem cells	Intravenous	Induction of retinal neovascularization	Grant et al.[19]
Duchenne's muscular dystrophy	Experimental BMT	Purified bone marrow–derived hematopoietic stem cells	Intravenous	Partial restoration of dystrophin expression in the affected muscle	Gussoni et al.[12]
	Clinical BMT (allogeneic)	Bone marrow cells	Intravenous	Partial restoration of dystrophin expression in the affected muscle	Gussoni et al.[51]
Lung diseases with extensive alveolar damage	Experimental BMT	Single hematopoietic stem cells	Intravenous	Generation of alveolar type II pneumocytes	Krause et al.[18]
Renal diseases involving glomerular mesangial tissue	Experimental BMT	Clonal population of cells derived from a single hematopoietic stem cell	Intravenous	Generation of glomerular mesangial cells	Masuya et al.[20]

Table 2. (Continued.)

Disease Category	Model	Stem-Cell Source	Route of Cell Application	Outcome	Study
Neurodegenerative diseases	Experimental BMT	Bone marrow cells	Intraperitoneal	Generation of cells expressing neuronal markers	Mezey et al.[9]
	Experimental BMT	Bone marrow cells	Intravenous	Generation of cells expressing neuronal markers	Brazelton et al.[10]
	Clinical BMT (allogeneic)	Bone marrow cells	Intravenous	Generation of cells expressing neuronal markers	Mezey et al.[32]
	Clinical BMT (allogeneic)	Bone marrow cells	Intravenous	Possible formation of Purkinje neurons	Weimann et al.[33]
Middle-cerebral-artery occlusion (stroke)	Experimental cord-blood transplantation	Stem cells derived from umbilical-cord blood	Intravenous	Improved functional recovery from neurologic deficit	Chen et al.[52]

Translational Study in Stem Cell Therapy

- Preclinical *in vivo* studies fail to show similar findings in large animals, including non-human primates
- No confirmation on validity of many clinical results as of yet
- Limitations in ID of chimeric cells

Stem Cell Therapy in Myocardial Ischemia
Cell Types

	myogenesis	angiogenesis
(I)Skeletal Myoblasts/Cardimyocytes	(+)	(-)
(II)Bone Marrow Stem Cells		
(1)unselected mononuclear cells	?	?
(2)CD34+ / CD133+ cells	?	(+)
(3)mesenchymal stem cells	(+)	(-)
(4)MAPCs	(+)	(+)

Myocardial Infarction as a Model of Adult Stem Cell Therapy (I)

(I) Cells

Resources	cell separation	culture	core-lab
1.BM mononuclear cells	-	-	no
2.BM CD34+ / 133 +cells	clinimax	-	?
3.PB CD34+ / 133+ cells	+leucopheresis	-	?
4.BM CD34- cells	clinimax	short-term	?
5.BM mesenchymal SCs	clinimax	long-term (Osiris)	yes
6.BM MAPCs	clinimax	long-term (Athersys)	yes

Myocardial Infarction as a Model of Adult Stem Cell Therapy (II)

(II) Cell Administration

1. thoracotomy and trans-epicardial injection
2. Intra-coronary infusion
3. electric mechanical mapping and injection
4. systemic injection

Human Studies

Author	Cell	Administration	Results
(1)Stamm *(Lancet)*	BM/AC133	epicardial (+bypass surgery)	Good(6pts)
(2)Tse *(Lancet)*	BM/MNCs	endocardial	Good(8pts)
(3)Assmus. *(Circulation)*	BM/PB/CD34+	intracoronary	Good(20pts)
(4)Osiris	allo/MSCs	epicardial	Good (multi-ctrs)
(5)Galinanes. *(Am Heart Ass)*	auto/BM	?	Good(14pts)

Cell Number

Group	Cell	Resources	Cell expansion	Cell No.
Stamm et al	AC 133	BM 100-200ml	none	$1\text{-}2x10^6$
Tse et al	MNCs	BM 40ml	none	$1\text{-}2x10^7$
Assmus et al	CD34	PB 250ml	x3d	$7.35±7.31x10^6$
Osiris	MSC	BM 100ml	x3wk	10^8

Cell Number: how many we need?

Heart: 250 gm

Size of infarction: 10-25 gm

AC 133 cells infused: 0.001 gm ($1x10^6$)

CD34 cell infused: 0.01 gm ($1x10^7$)

MNCs infused: 0.01-2 gm ($1\text{-}2x10^7$)

MSC infused: 0.1 gm ($1x10^8$)

Cell Expansion

(1)CD34/CD133: Many studies are undergoing in European countries.

(2)Mesenchymal stem cells : Well-established by Osiris.

(3)MAPCs: Athersys is now developing.

Cell Expansion vs. No Expansion

(1)Advantage of Expansion

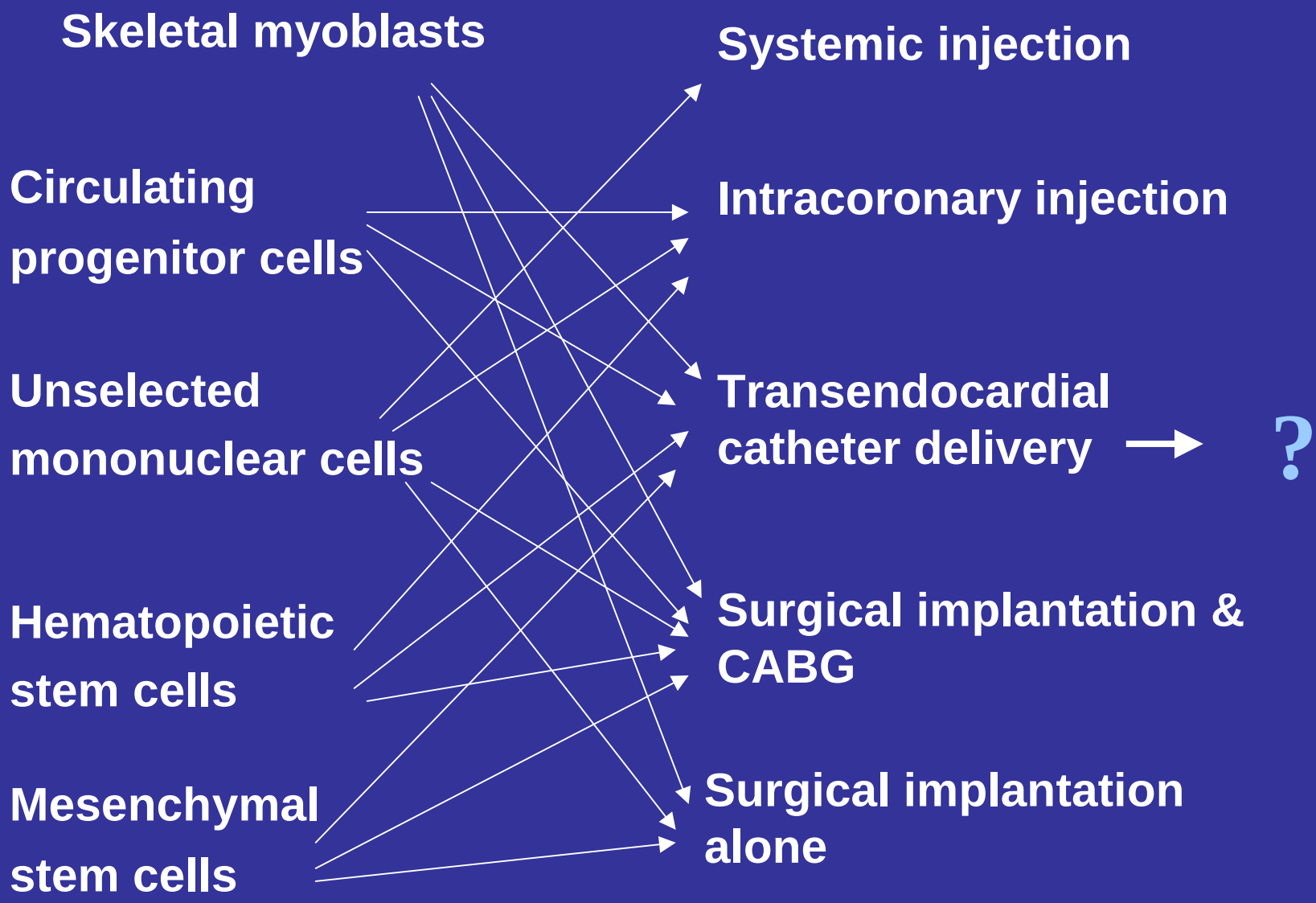
- Cell number can be more sufficient

(2)Disadvantage of Expansion

- GTP needed
- Problem of contamination
- Side-effect from growth factors
- Cost jumps
  - GTP
  - Culture
  - Growth factors
  - Man powers

(3)Clinical outcome is truly better?

Clinical Autologous Cell Transplantation



Requirements to Conduct a Good Clinical Trial in Cell Therapy

1. Multi-Center study: to obtain a significant patient number
2. Core lab(s): GTP lab, Bioreactors (?)
   quality control
   money saving
3. Research wards

Reports which question plasticity

- 2 reports in 2002 (both in Science)
  - Castro et al: no donor-derived neurons seen after BMT in mice
    - Purified and unfractionated BM used
  - Wagers (Weissman) et al: single HSC transplantation in mice
- Replies from 3 groups: sensitivity/specificity depends on experimental system

Cell Fusion

- Nature 2002 & 2003 [3 articles]: BMT-derived hepatocyte←cell fusion (used DNA ploidy as proof)
  - However, hepatocytes can be polyploid
  - Numerous other studies show diploid donor-derived cells in host solid organs
- Fusion is likely 1 of several mechanisms for incorporating donor cells
  - May be an ongoing physiologic repair mechanism
  - Data from liver models may not be applicable to other solid-organ tissues
- Requires rigorous criteria to distinguish between transdifferentiation vs cell fusion

Stem Cell Competition
幹細胞之競爭
(「科學」期刊：2002．7．4)

- 胚胎幹細胞
  - 如何克服「免疫」之障礙
  - 有倫理、法律、宗教之爭
  - 癌化現象？
  - 體外培養出來的細胞不等於體內培養之細胞
- 成體幹細胞
  - 較無倫理或法律問題問題
  - 如何克服「細胞數目」

What about embryonic stem cells?

- Superior in its differentiation ability after *ex vivo* expansion when compared to *in vivo* differentiation ability of HSCs
- Not superior to MAPC since formation of teratomas is a major concern

Conclusion

- HSCs:
  - Distributors of hematopoietic progenitor cells
  - Systemic supplier of progenitor cells for homeostasis of solid organs
- Need to learn how to direct circulating blood SCs to areas of injured or diseased organs
- Obstacles remain, including misinterpretation of data

ELSI：
倫理、法律、社會面的衝擊
(ETHICAL) (LEGAL) (SOCIAL)　(IMPACT)

- 倫理:(1)在胚胎幹細胞方面,東西方之倫理觀、宗教觀之異同

- 法律:(1)法律影響實驗室研究,研究成果又影響法律之落實施行
  (2)如全民健保將來是否給付再生醫學之治療模式

- 社會:(1)再生醫學對社會的衝擊
  (2)老年社會之考量

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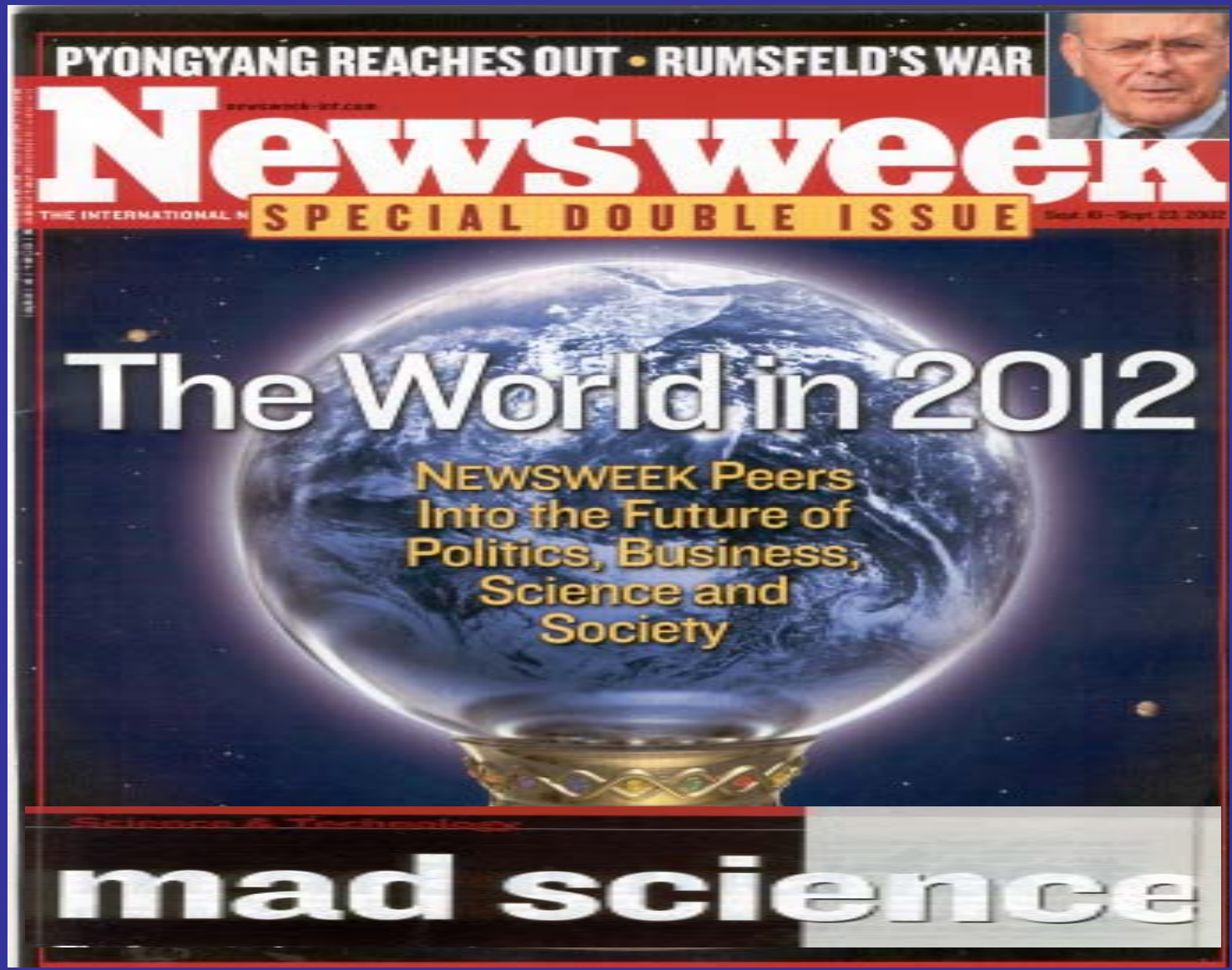
(1)集中在幹細胞生理之研究
(2)組織工程之參與
(3)與奈米科技之結合
(4)ELSI之討論
(5)甚少談及臨床
(6)Cancer Stem Cell 之觀念



Moron Laboratory (流氓實驗室)