17 th International Conference of Racing Analysts and Veterinarians

October 2008
ANTALYA , Turkey

Special Lecture (Monday 13 th October)

20 th Century : Chemical Doping
21 st Century : Cell Doping

Professor Gerard DINE
Ecole Centrale de Paris (France)

Scientific doping : the rational

Aerobic network

➔Transport, disponibility, use of O2

- Specific training
- Training period in altitude
- Hypobaric room
- Hypoxic tent

- Blood transfusions
- Stimulation of erythropoïesis : EPO, ...
- Artificial blood: PFC, reticulated Hb
- Dissociation of oxy-haemoglobin
- Microcellular action: target therapies indeed gene therapies

Anaerobic network

➔Strenght, power, speed

➔Resistance to acidosis

- Specific training
- Improved capture by muscle of aminoacids : specialized nutrition
- Optimum immediate energetic reserve: creatine, carnitine, …

- Anabolics : natural or synthesis androgens
  : growth factors and hormones
  : myostatin inhibitors
- Métabolic agents : insulin, etc…
- Target therapies, cell therapies indeed gene therapies

The biotechnological revolution

➔Genomics and Proteomics

In 2008 : 107 marketed »biodrugs » in EU.

Sporting effective misuse for most of them.

Present development of new efficient and doubtful approaches in term of toxicologic control (urine and hair).

Some examples : New EPO : Dynepo (2002)
: Bio similar EPO (5 European MAA in 2007)
: Synthetic EPO : Hématide in phase III (2006)
: Modulators of EPO : FG-2216 in phase I (oral taking)
: New growth hormones (nasal spray)
: Bio similar growth hormones (1 European MAA in 2006)
: Muscle growth factors : IGF1and bFGF (1996)
: Myostatin inhibitors : AMG745 in phase I
: Activators of fat's consumption :GW501516 in phase II

The reverse side of decor

Fig. 1

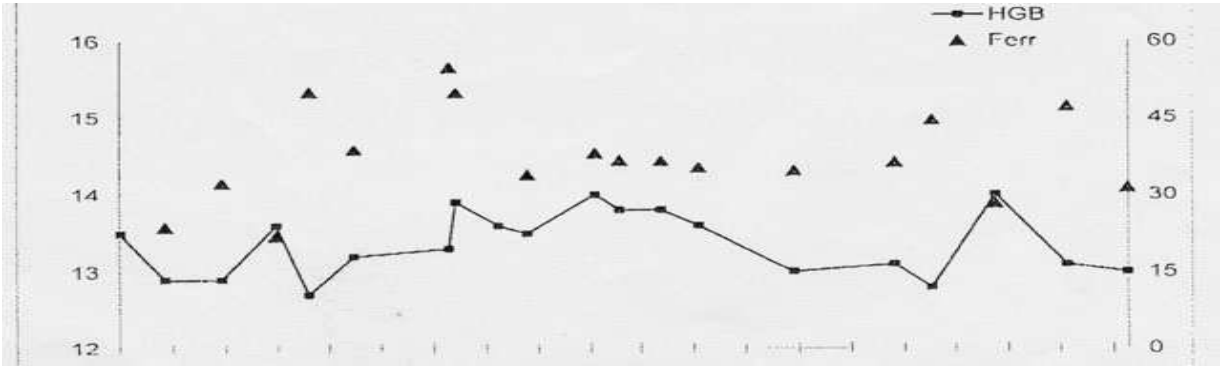


Fig. 2

	juil-07	02/10/2007	30/01/2008	29/02/2008	21/03/2008
NGR	4,35	4,67	4,69	4,28	4,63
Hb	15,2	15,5	15,5	14,8	15,5
Hte	46%	47	46	43	46
VGM	92	96	98	**101**	**101**
TCMH	33,4	33,1	33	34,5	33,4
CCMH	32,9	32,8	33,4	34,2	33,1
Réticulocytes	82000	88000	80000	**107000**	78000
Ferritine	61	30	85	53	44
RsTF µg/l		1,68	1,89	**4,6 mg/l**	2,01
Protides	81	67	77	71	81
Na+	142	142	142	140	145
IGF-1 ng/ml	305	353	315	328	363
EPO	10,4			**23,2**	3

Fig. 3

		02/06/03	**30/07/03**	**20/08/03**	**17/09/03**
LH	1 - 9 UI/L	0,1	5,6	< 0,1	2,4
Testosterone	2,9 – 15,1µg/L	4,8	1,2	3,3	1,5
IGF-1	82 – 221µg/L	218	178	410	194

Biological blood passport
A tool of early detection allowing the tracability of training and the sanction by No Start

Réf. : Dine G, Van Lierde F, Rehn Y. Biological profile to detect erythropoietin use in healthy sport performers. Haematologica 1999; 84 (EHA abstract) 199

Réf. : Dine G, Genty V, Van Lierde F. Biological profile usefulness to prevent rh EPO misuse in sport people. Hematology Journal 2002; 3 (EHA abstract) 381

➔Deposit INPI at 30/07/98 with the number 20556 (blood handling by EPO)

➔Deposit INPI at 25/05/99 with the number 44952 (hormonal profile concerning the use of GH)

Routine–use for the account of FFR, FFS, FFT and LNR since 2000

Necessary tool which is not an universal panacea

The confusion face up to doping

➔A non-adapted regulation to the doping's reality (thieves don't have scientific advance)

➔Biological tools : urines, blood, hairs and passport (necessary co-ordination)

➔Controls throughout the season : programed, unexpected and targeted (training, event, post event)

➔Dilemma for doctors submitted to sport authorities (no real indépendance)

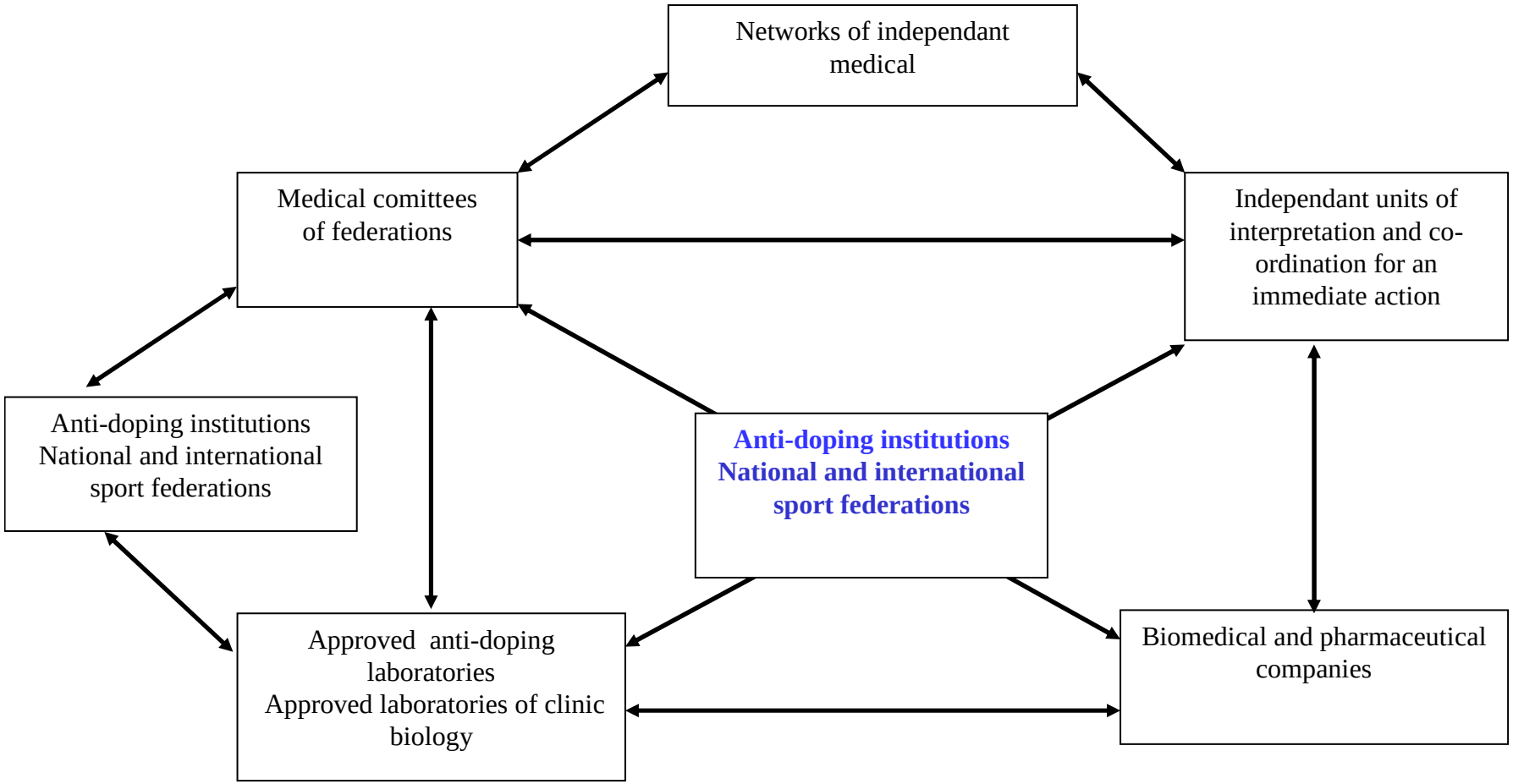
➔No incrimination for key decision makers of sport (employers responsability)

➔ Ambiguous proximity of different actors within institutions (judge and part)

➔Evident criminal (traffic and counterfeit)

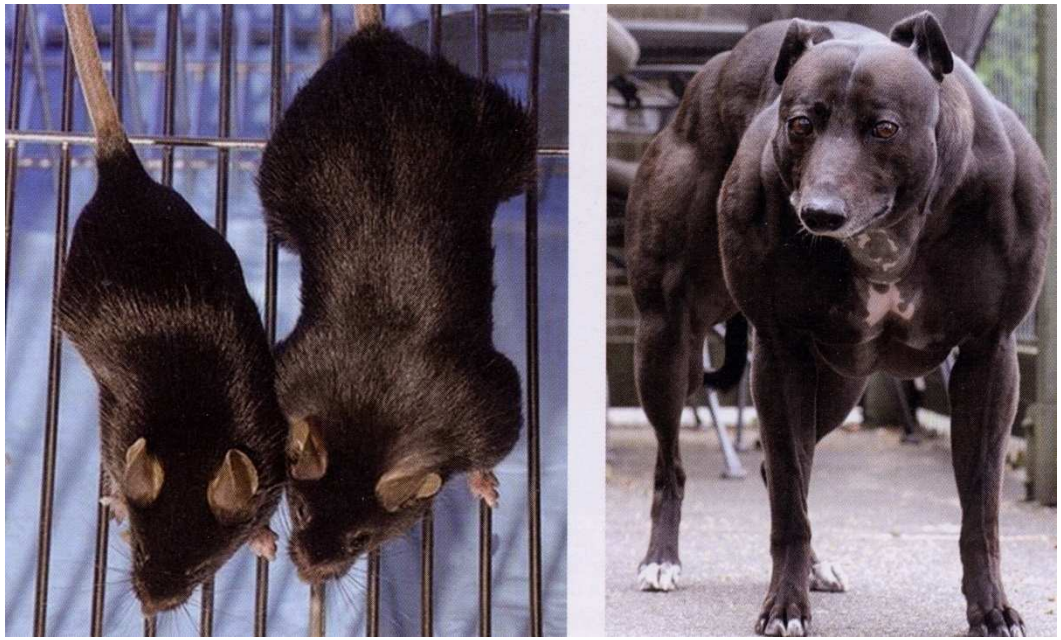
➔Question on the myth of superman (ethical débate)

Transparency and confidentiality



➔Disclosure Act

➔Actions of police, customs and justice



In 2008, the reality among our superior mammal cousins

➔Muscle transfection: IGF1, myostatin

➔Target therapies : myostatin inhibitors

➔Human natural mutations as models

➔Human tests under way : myopathy, cancerous cachexia

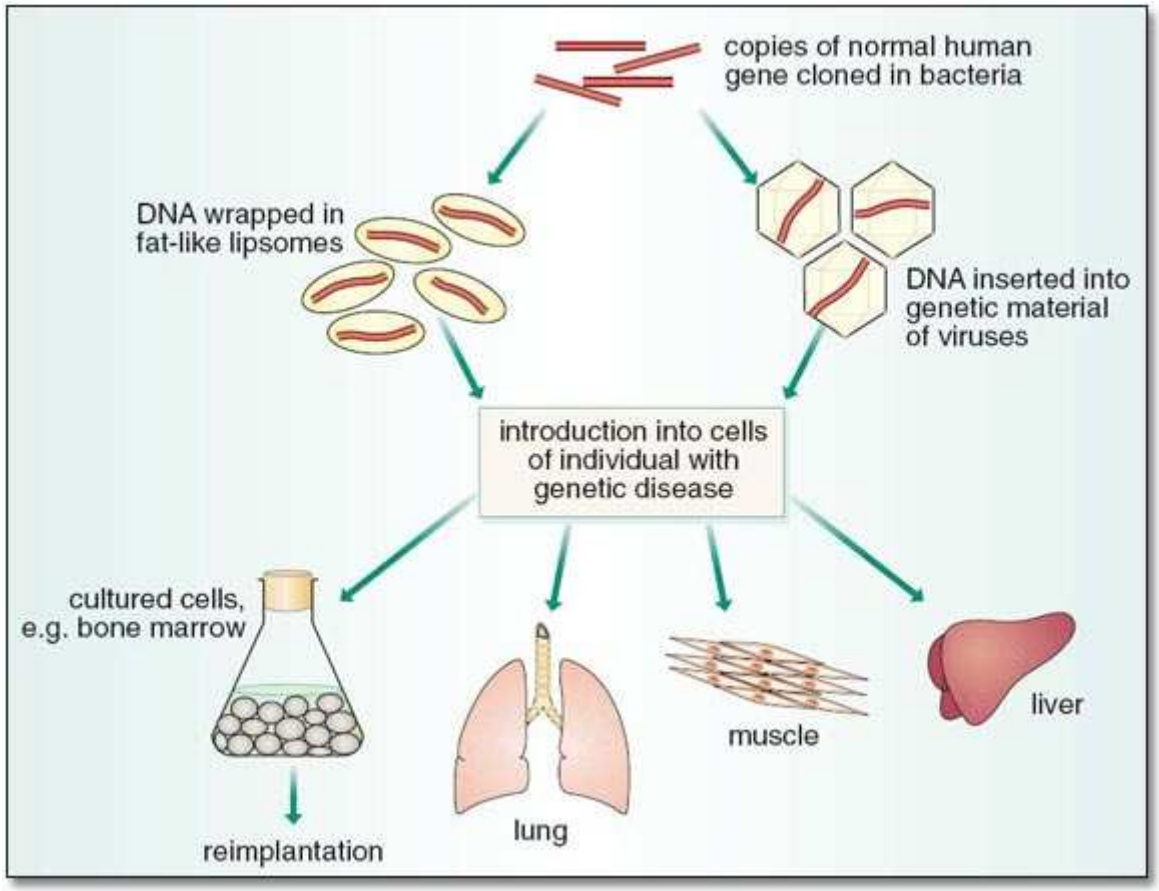

copies of normal human gene cloned in bacteria
DNA wrapped in fat-like lipsomes
DNA inserted into genetic material of viruses
introduction into cells of individual with genetic disease
cultured cells, e.g. bone marrow
reimplantation
lung
muscle
liver

Table 1 Human gene therapy studies with potential application to sport

Model	Status
Muscular dystrophy	Using animals with muscular dystrophy caused by mutations in the dystrophin gene, it is possible with gene therapy to inject into muscle a functional dystrophin gene.[4] The effects observed include a reduction in contraction induced injury, and an increase in muscle bulk.
Muscular atrophy	The National Aeronautics and Space Administration (NASA) in the United States has shown that space travel can produce skeletal muscle atrophy. Experimental studies are now underway to determine the preventive effects of *IGF1* in a retroviral vector given regularly by intramuscular injection.[5]
Rheumatoid arthritis	Phase I studies show little toxicity when inflammatory molecules such as interleukin 1 are inhibited by intra-articular injection of gene therapy products.[6]

Tendinous gene therapy

Transfection of tenocytes producing growth factors in order to accelerate healing (IGF1 et b-FGF)

Controlled animal models

Marathon mice (1)

➔Continuous expression without aerobic training of PPAR-delta protein after muscle gene transfection

➔Consumption of fat to the detriment of sugar

➔Increasing of type I muscular fibres

➔Controlled animal models

➔Y Wang & al, Plos Biology, 294, 2004

Activators of fat's consumption

Stimulation of the activity of PPAR-delta protein by GW501516 (GSK) molecule

→ phase I and II in the treatment against cholesterol

→ increasing of muscle fibres of type 1 (PPAR-delta transgenic mice)

Marathon mice (2)

➔Mice without HIF1 α gene

➔Permanent aerobic conditions in muscle

➔Decreasing of lactic acid in muscle

➔Not controlled animal models → after 4 days : muscle damages (free radicals)

➔S Mason & al, Plos Biology, 288, 2004

Speedy Gonzales

- ➔Surexpression of PEPCK-C gene in muscle of transgenic mice
- ➔Increasing of VO_2 max and decreasing of lactic acid in muscle
- ➔Increasing of aerobic and anaerobic performances
- ➔Controlled animal models for sport conditions but particular behaviour (hyper activity)
- ➔R Hanson & al, Journal of biological chemistry, 35, 2007

Schwarzenegger mice

➔Myocytes transfection with IGF1 gene

➔Enhancing muscle hypertrophy in resistance trained rats

➔Controlled animal models

➔HL Sweeney & al, Journal of Applied Physiology, 96, 3, 1097-1104, 2004

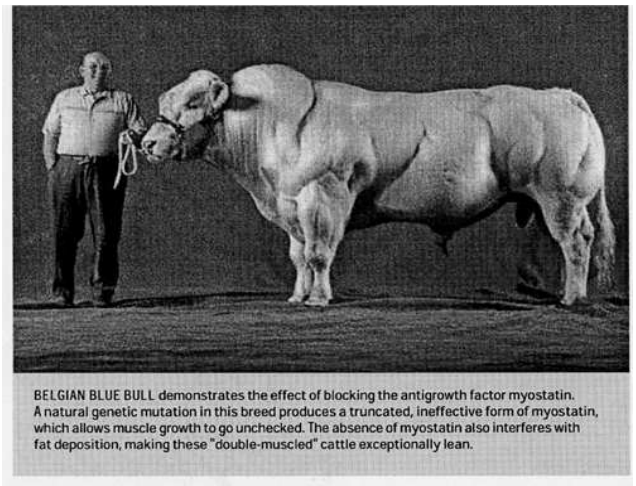
Muscle growth factors

Insulin Like Growth Factor (IGF1)	Lot of biotechnological companies	Recombinant IGF1 Phase III in muscular and tendinous healing
basic Fibroblast Growth Factor (b-FGF)	Lot of biotechnological companies	Recombinant b-FGF Phase III in muscular and tendinous healing
Platelet Derived Growth Factor (PDGF)	Lot of biotechnological companies	PDGF recombinant Phase II in muscular and tendinous healing and angiologic dévelopment
Vascular Endothelial cell Growth Factor (VEGF)	Lot of biotechnological companies	Recombinant VEGF Phase II in muscular and tendinous healing and angiologic dévelopment

Belgian Blue Bull
« double muscled «

➔Truncated ineffective form of myostatin

➔Increasing of muscle growth and decreasing of fat deposition



BELGIAN BLUE BULL demonstrates the effect of blocking the antigrowth factor myostatin. A natural genetic mutation in this breed produces a truncated, ineffective form of myostatin, which allows muscle growth to go unchecked. The absence of myostatin also interferes with fat deposition, making these "double-muscled" cattle exceptionally lean.

➔Similar natural human mutation : M Schueke & al, NEJM, 350, 2004

Mighty mice

➔Transgenic mice with a truncated form of myostatin

➔Increasing muscle mass and strenght

➔Controlled animal models

➔ST Lee & al, PNAS, 98, 2002

« Myostatin inhibition : therapeutic target for the treatment of muscle disorders »

Myostatin inhibitors (1)

➔Myostatin monoclonal antibodies

➔Myostatin receptor-blocking monoclonal antibodies

➔Myostatin receptor-blocking fusion protein

Clinical studies : phases I, II, III

Myostatin inhibitors (2)

AMG 745 (Amgen)

➔Myostatin peptibody
(fusion protein of Ig and antagonist)

➔Cancer cachexia

Clinical studies : phases I, II

Other muscle gene therapies

➔Active form of calcineurin : increasing slow muscle fibers

➔Activating 2B myosin gene dormant in humans : change slow fiber to fast

« Unlike systemic drugs gene therapy allows key muscle subgroups to be targeted based on the biomechanics of a given sport »

EPO gene therapies

Introduction of gene encoding EPO in muscle cells of mice or monkeys.

Important increasing of hematocrits > 80%

Not controlled animal models (macaques)

➔ autoimmune response to transgenic EPO and severe anemia

Negative unexpected side effects

Natural somatic mutations of genes involved in the regulation of erythropoiesis

Gene therapy models

➔ Eero Mantyranta : truncated EPO receptor (SH-PTP1 enzyme mutation)

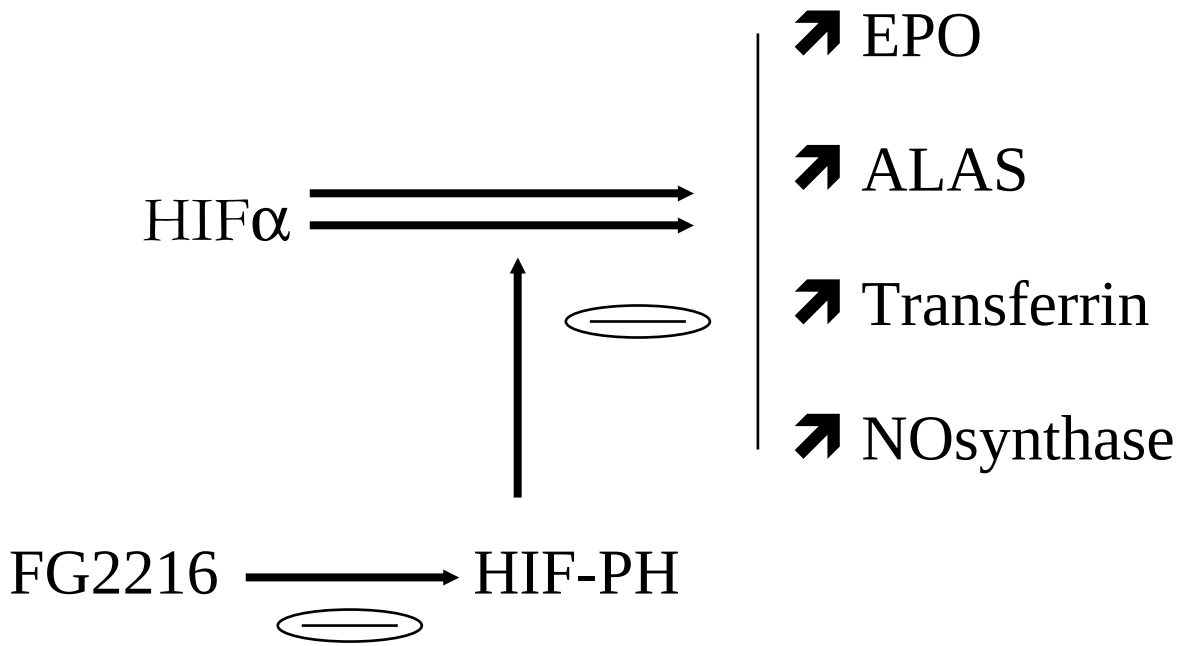
➔ Rutger Beke : mutated PHD2 protein (hydroxylating of HIF-1 α less efficient)

Natural increasing of red cells

Controlled animal models : transgenic mice with these 2 mutated genes

HIF – PH inhibitor

FibroGen	FG-2216 (oral taking)	Phase I



Stem cells

- **Embryonic stem cells**

  10 days embryo

  → blastocyst : inner cell mass
- **Embryonic germ cells**

  5 - 9 weeks fœtus
- **Cord blood stem cells**

  → bone marrow graft
- **Adult stem cells**

  - bone marrow stem cells

  - mesenchymal stem cells

  - other tissue stem cells

  → muscle, skin, CNS, liver, kidney, pancreas, heart, fat

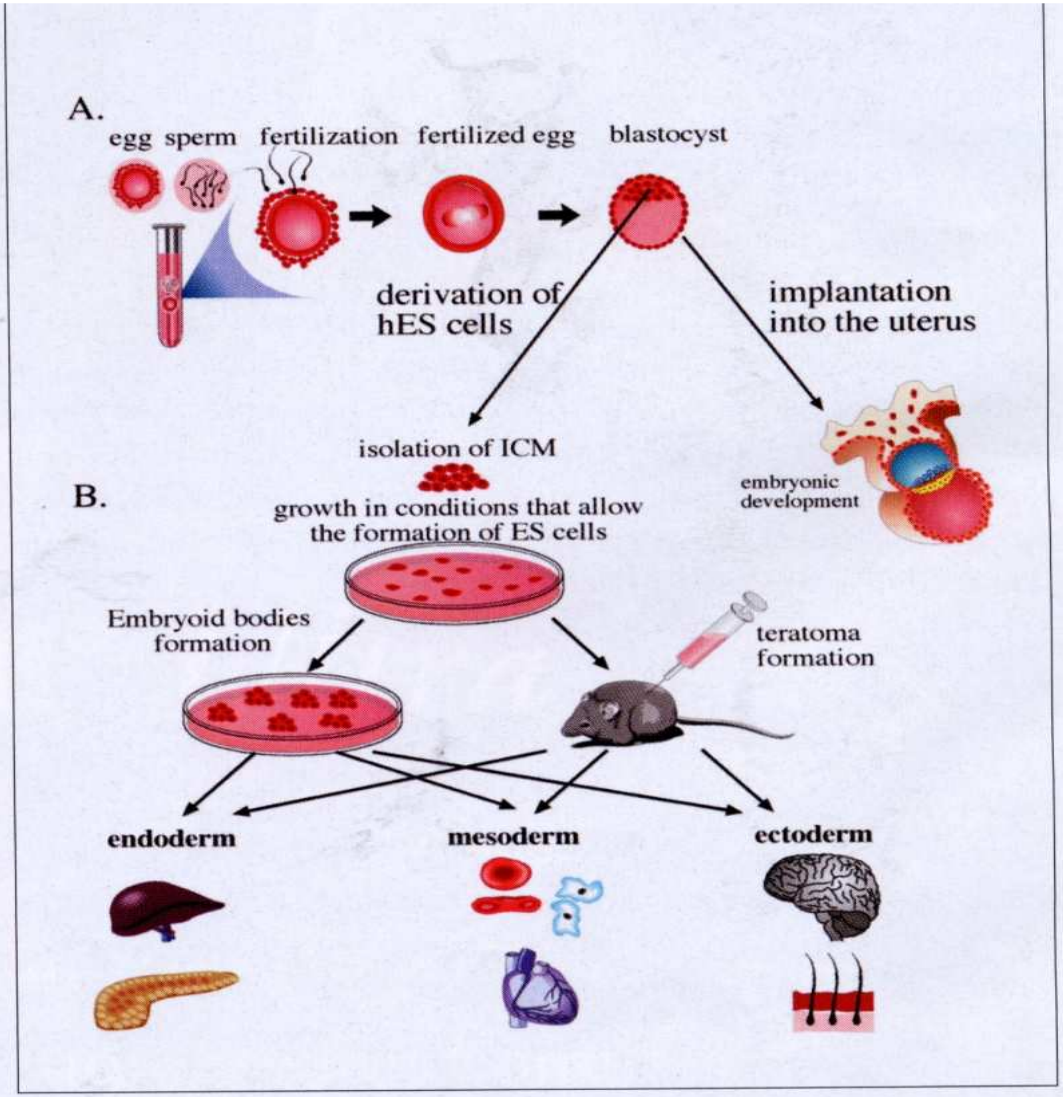
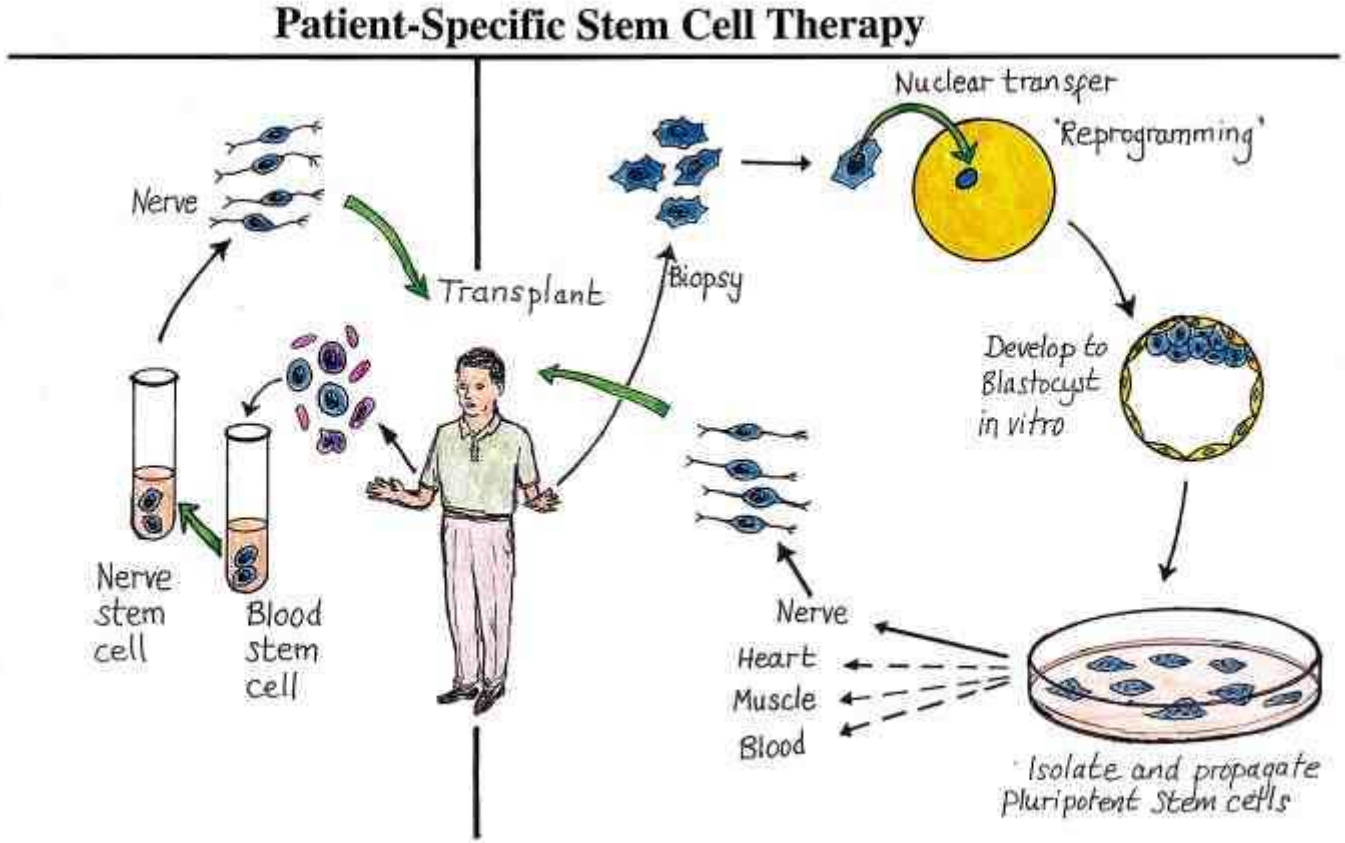


Figure 1. Derivation and differentiation of human ES cells. A. The main source for HESCs is the ICM of surplus blastocytes from IVF that do not undergo implantation into the uterus. B. In vitro and in vivo differentiation of HESCs into cell types from the three embryonic germ layers. Specific differentiation can be achieved by addition of appropriate growth factors to the media or by controlling the expression of master genes.


Patient-Specific Stem Cell Therapy
Nuclear transfer
'Reprogramming'
Nerve
Transplant
Biopsy
Develop to Blastocyst in vitro
Nerve stem cell
Blood stem cell
Nerve
Heart
Muscle
Blood
Isolate and propagate Pluripotent Stem cells

Cell therapies for sporting use

♦ Cartilage

Controlled culture of chondrocytes (ASC)

> industrial phase : Genzyme

♦ Tendon

Culture of tenocytes in progress (ASC)

> phase III

♦ Muscle

Culture of myocytes in progress (ASC, mesenchymal SC and ESC)

> phases I and II in myocardial implantation

> phase I and II in muscular repairing

« Multiple Origin of stem cells : ASC, cord blood SC, bone marrow SC, mesenchymal SC and ESC »

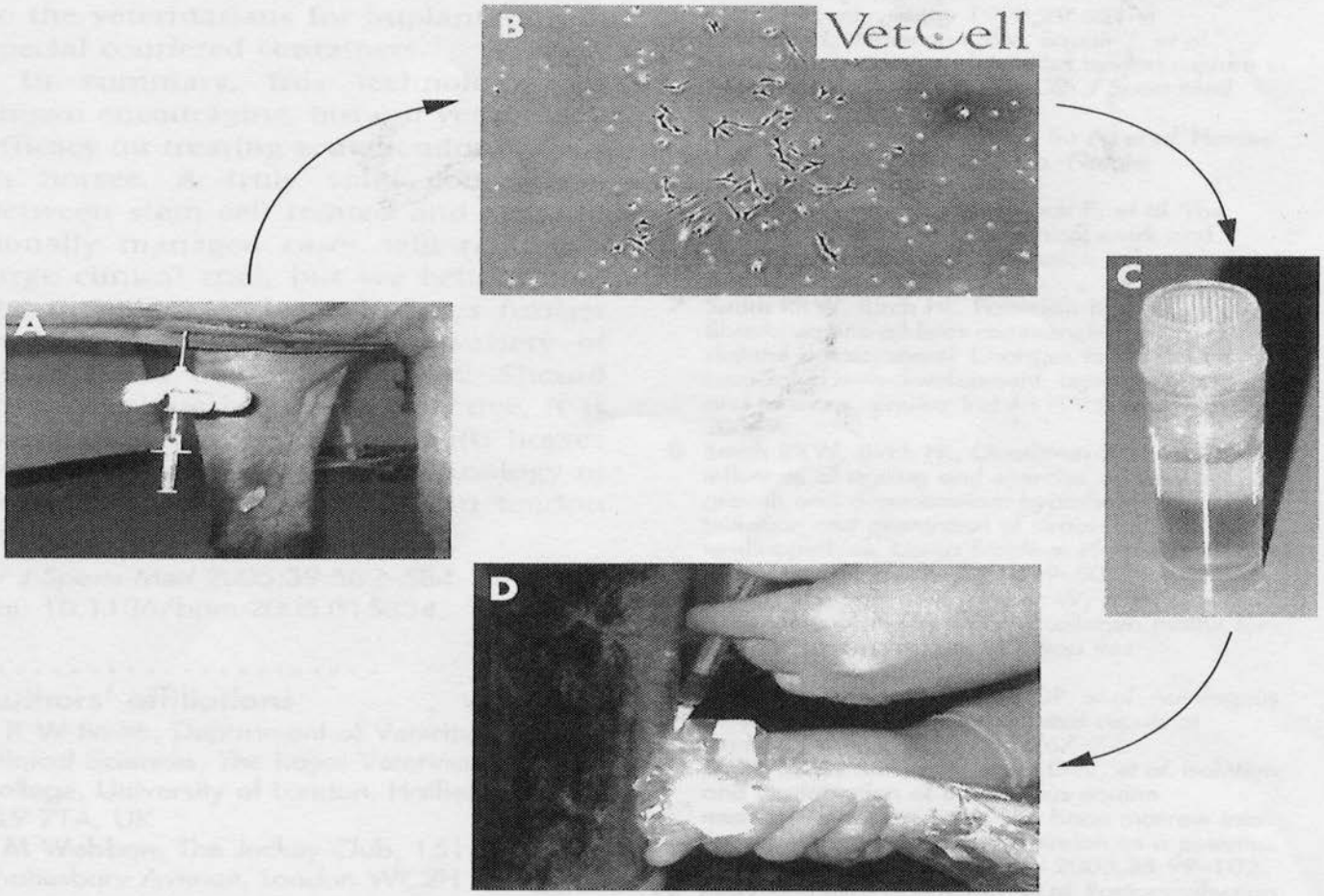


Figure 2 The stem cell based approach to the treatment of equine superficial digital flexor tendinopathy. (A) Aspiration of bone marrow from the sternum of the standing horse. (B) Recovery and expansion of mesenchymal stem cells (bone marrow stromal cells) by VetCell Bioscience Ltd. (C) Resuspension of $>4 \times 10^6$ stem cells in citrated bone marrow supernatant. (D) Sterile implantation of the cells into the central core lesion under ultrasonographically guided injection.

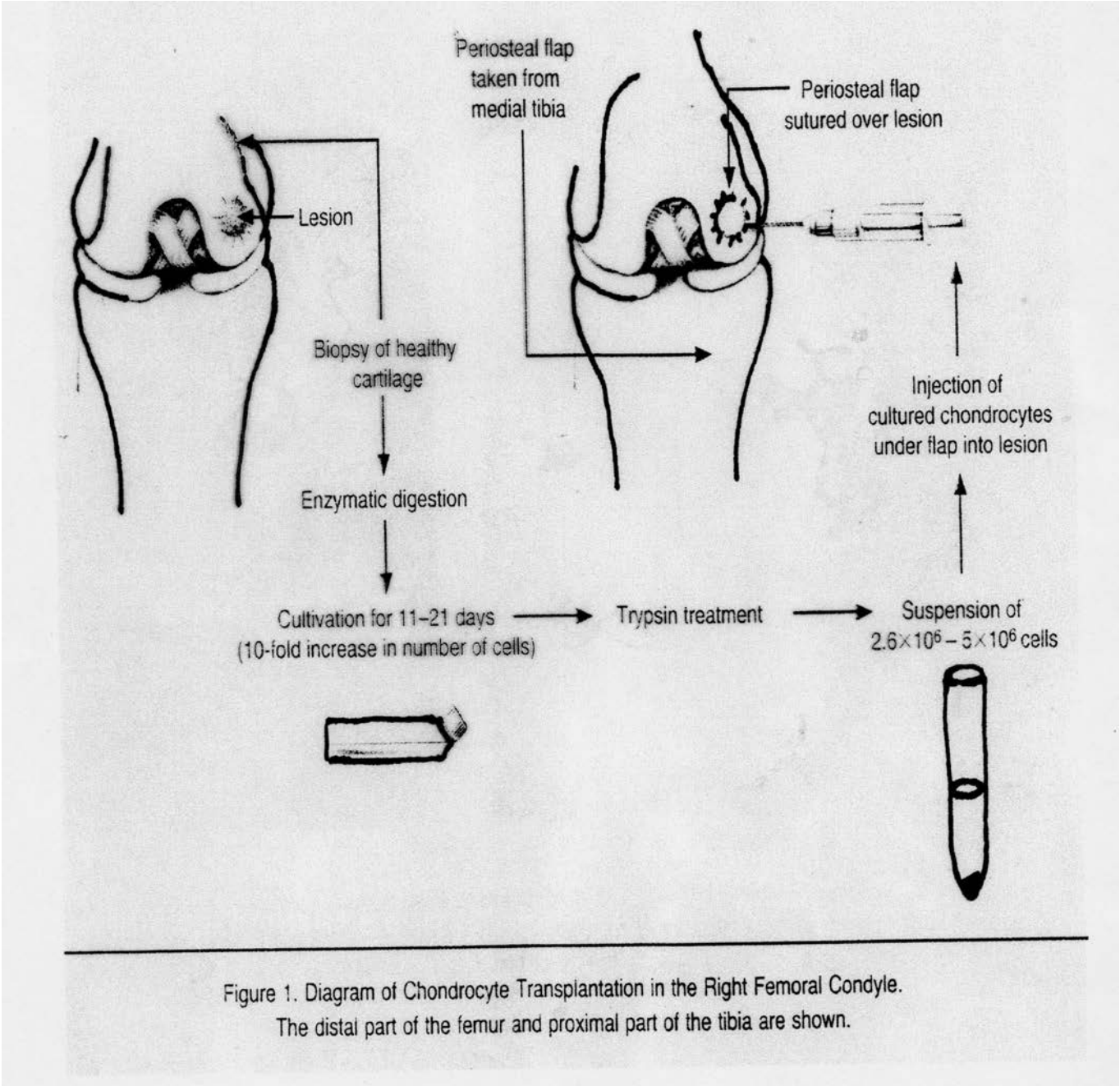
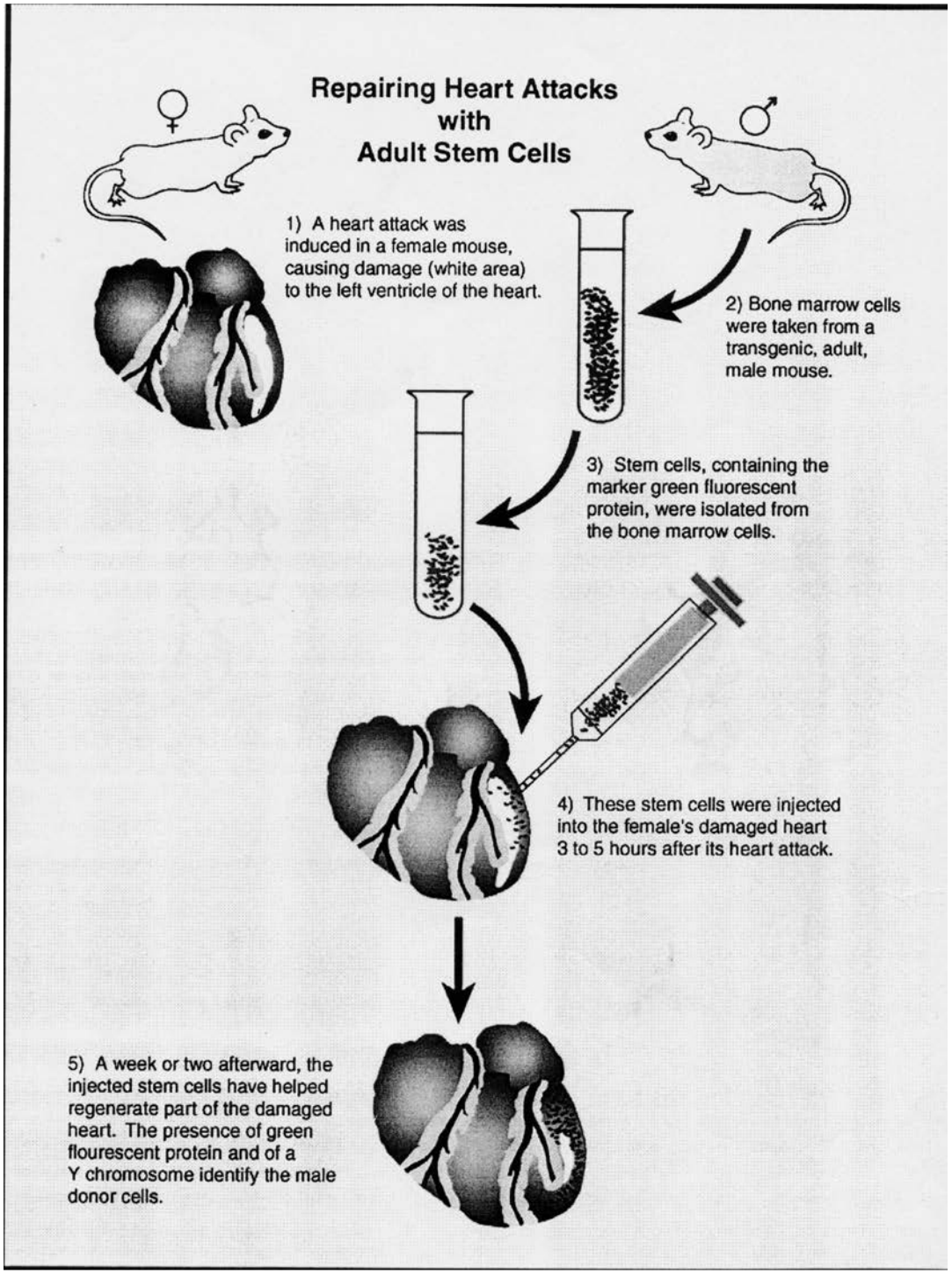
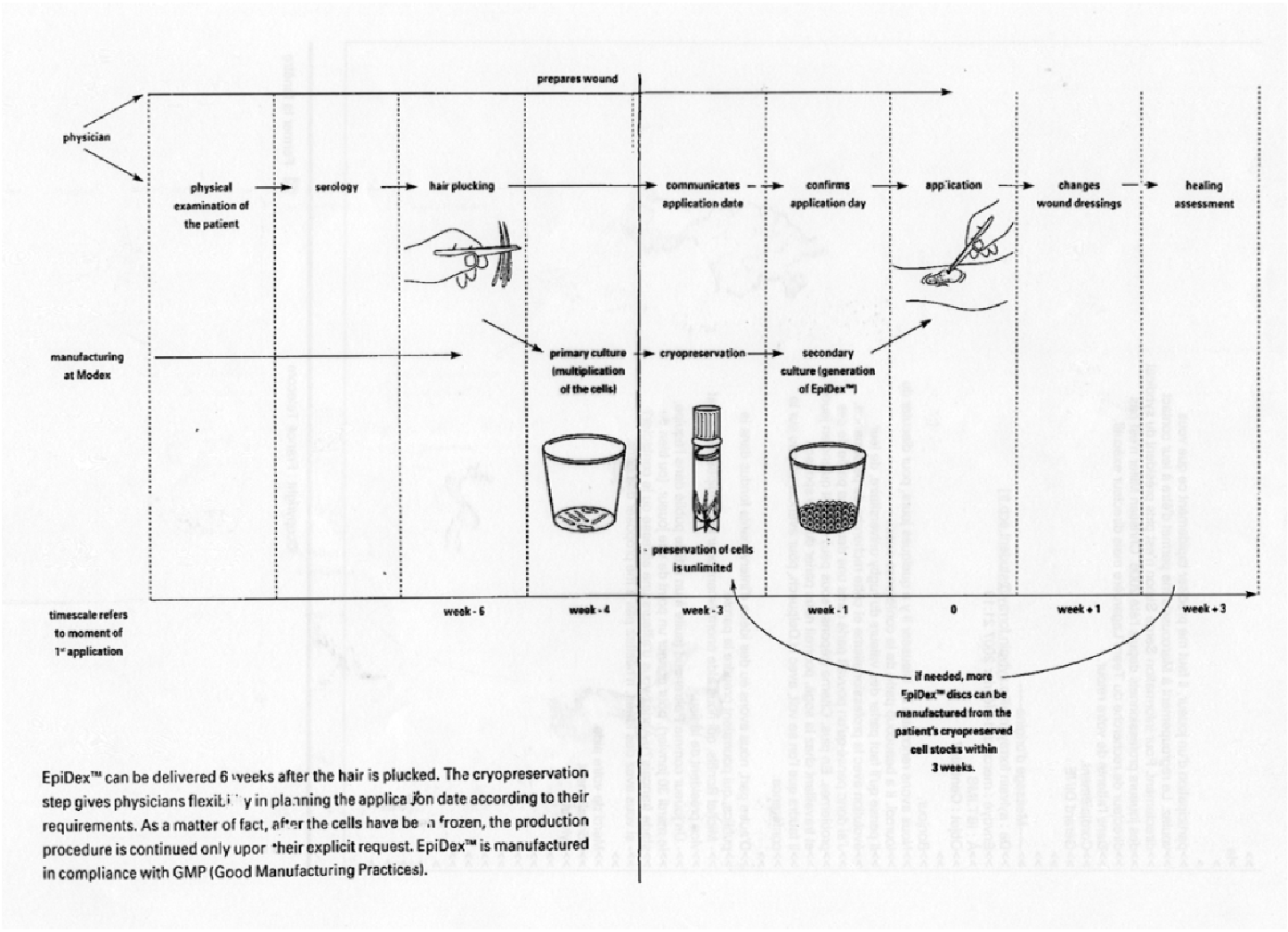
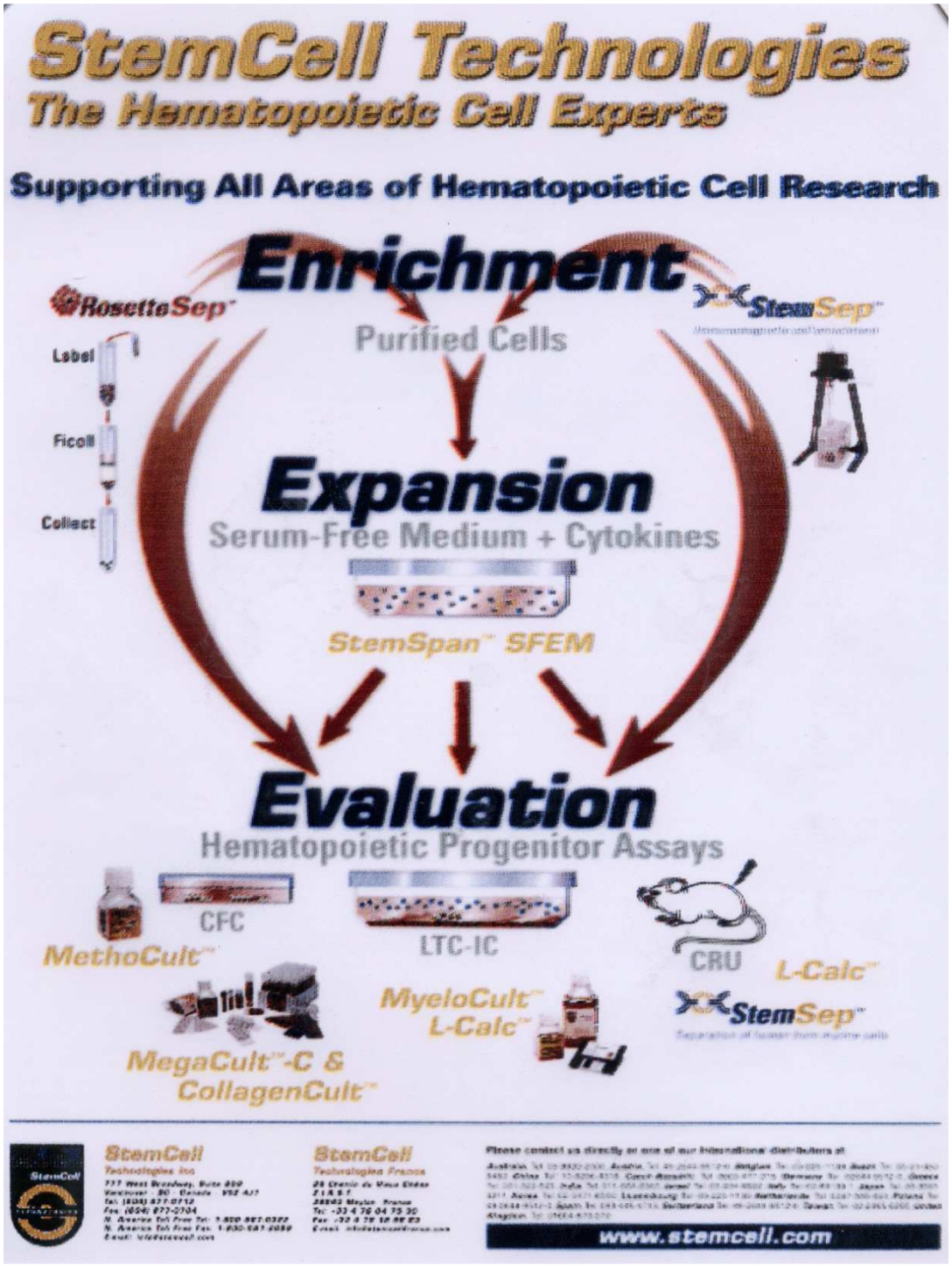


Figure 1. Diagram of Chondrocyte Transplantation in the Right Femoral Condyle.
The distal part of the femur and proximal part of the tibia are shown.


Repairing Heart Attacks
with
Adult Stem Cells
1) A heart attack was induced in a female mouse, causing damage (white area) to the left ventricle of the heart.
2) Bone marrow cells were taken from a transgenic, adult, male mouse.
3) Stem cells, containing the marker green fluorescent protein, were isolated from the bone marrow cells.
4) These stem cells were injected into the female's damaged heart 3 to 5 hours after its heart attack.
5) A week or two afterward, the injected stem cells have helped regenerate part of the damaged heart. The presence of green flourescent protein and of a Y chromosome identify the male donor cells.



EpiDex™ can be delivered 6 weeks after the hair is plucked. The cryopreservation step gives physicians flexibility in planning the application date according to their requirements. As a matter of fact, after the cells have been frozen, the production procedure is continued only upon their explicit request. EpiDex™ is manufactured in compliance with GMP (Good Manufacturing Practices).


StemCell Technologies
The Hematopoietic Cell Experts
Supporting All Areas of Hematopoietic Cell Research
Enrichment
RosetteSep
StemSep
Label
Ficoll
Collect
Purified Cells
Expansion
Serum-Free Medium + Cytokines
StemSpan SFEM
Evaluation
Hematopoietic Progenitor Assays
CFC
MethoCult
LTC-IC
CRU
L-Calc
StemSep
MyeloCult
L-Calc
MegaCult-C &
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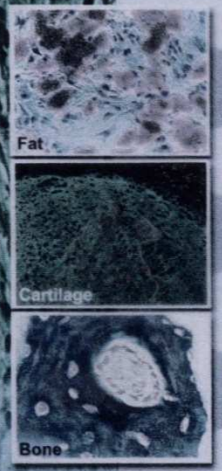
Now Available, Exclusively From POIETICS™

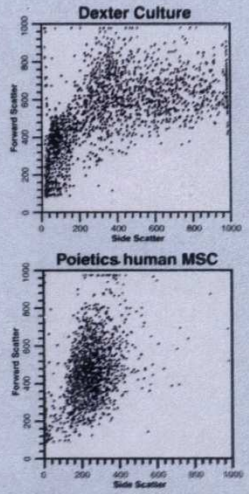
Normal Human
Mesenchymal Stem Cells

Standardized Cell Culture System containing Cells and Medium
Quality Tested and Guaranteed



QC Tested for:

- Adipogenic Lineage as indicated by Neutral Lipid
- Chondrogenic Lineage as indicated by Collagen Type II
- Osteogenic Lineage as indicated by Calcium Mineral

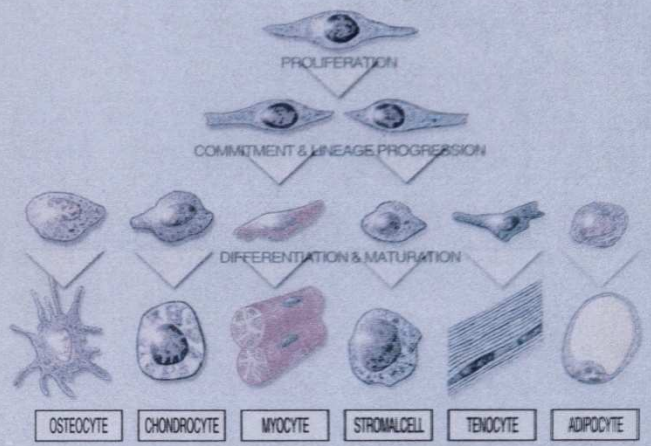


Poietics human MSCs are more homogeneous than a Dexter Culture

Specifications and Characterization:

- Cells cryopreserved in passage two
- Recover 750,000 cells/amp
- Flow cytometric analysis of surface antigens:
  Positive for:
  SH2; SH3; CD29; CD44
  Negative for:
  CD14; CD34
- Cells test negative for HIV-1, hepatitis B & C, mycoplasma, bacteria, yeast and fungi
- Performance guaranteed using Poietics Mesenchymal Stem Cell Growth Medium (MSCGM™)

* Not for use in humans.
For research use only.



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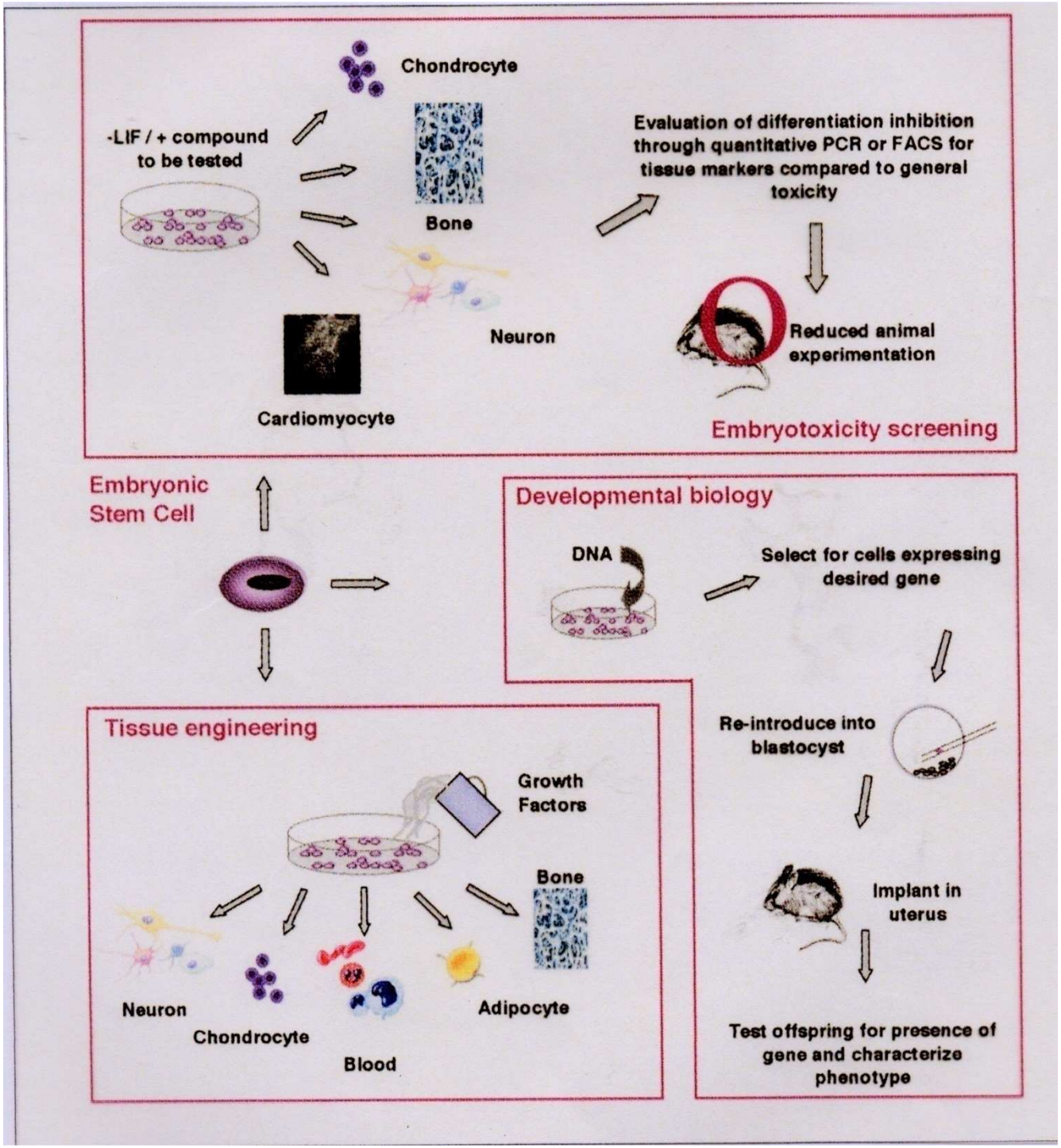
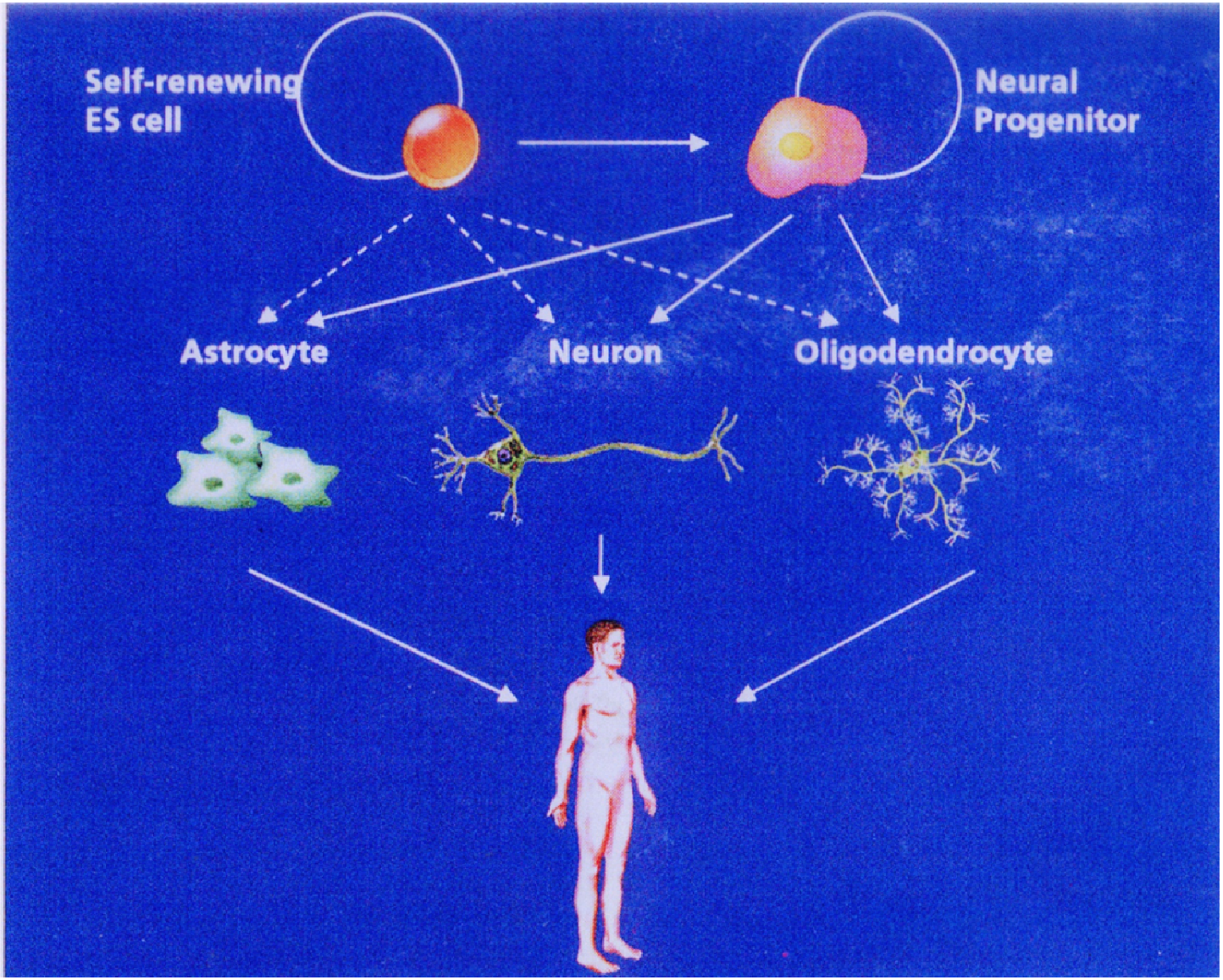
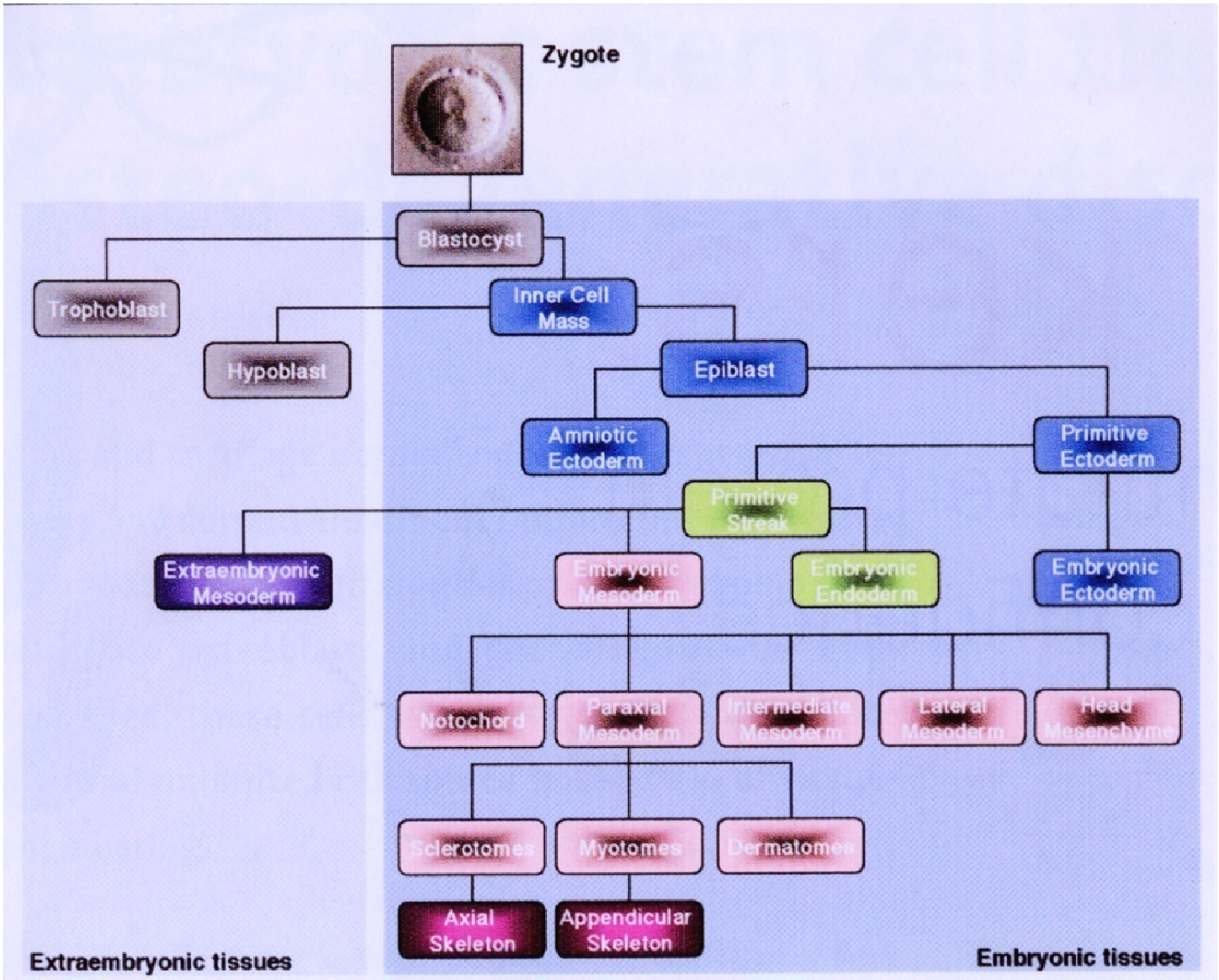
BioWhittaker UK: Tel: 0118 979 5234 Email: laspinwall@biowhittaker.co.uk


Chondrocyte
-LIF / + compound to be tested
Bone
Neuron
Cardiomyocyte
Evaluation of differentiation inhibition through quantitative PCR or FACS for tissue markers compared to general toxicity
Reduced animal experimentation
Embryotoxicity screening
Embryonic Stem Cell
Developmental biology
DNA
Select for cells expressing desired gene
Re-introduce into blastocyst
Implant in uterus
Test offspring for presence of gene and characterize phenotype
Tissue engineering
Growth Factors
Bone
Neuron
Chondrocyte
Blood
Adipocyte

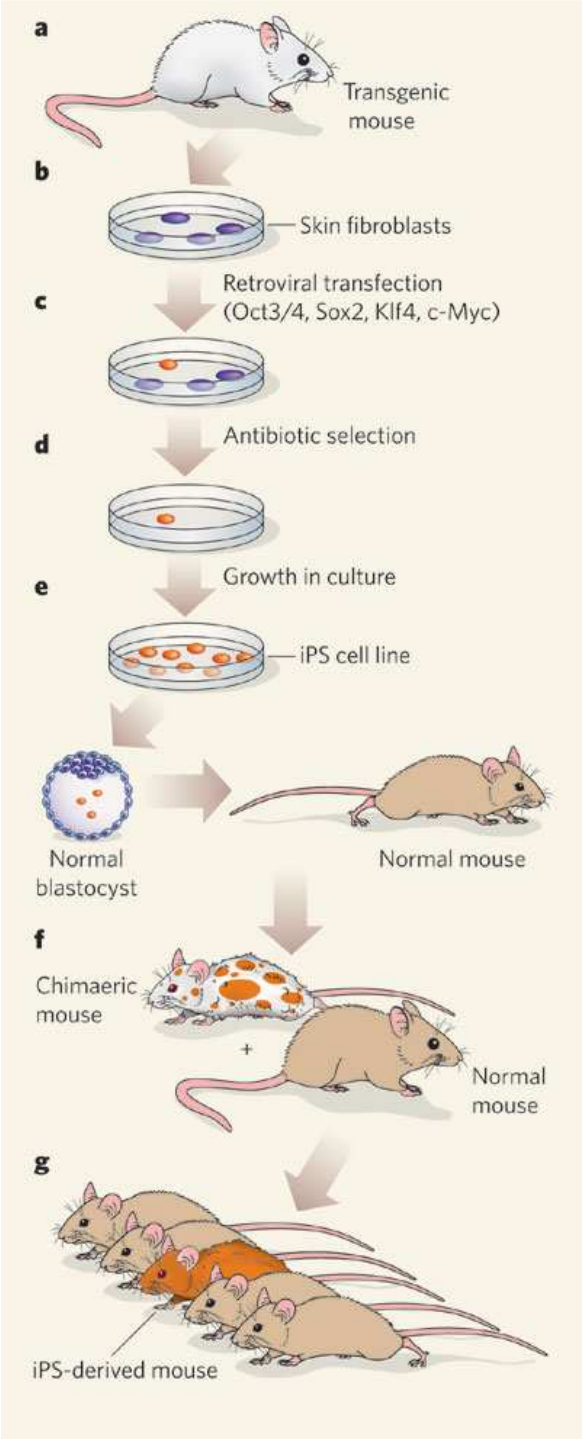

Self-renewing
ES cell
Neural
Progenitor
Astrocyte
Neuron
Oligodendrocyte


Zygote
Blastocyst
Trophoblast
Inner Cell Mass
Hypoblast
Epiblast
Amniotic Ectoderm
Primitive Ectoderm
Primitive Streak
Extraembryonic Mesoderm
Embryonic Mesoderm
Embryonic Endoderm
Embryonic Ectoderm
Notochord
Paraxial Mesoderm
Intermediate Mesoderm
Lateral Mesoderm
Head Mesenchyme
Sclerotomes
Myotomes
Dermatomes
Axial Skeleton
Appendicular Skeleton
Extraembryonic tissues
Embryonic tissues

Induced Pluripotent Stem Cells (IPS)

Takahashi K, Yamanaka S. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. Cell 2006; 126 : 663-76

Takahashi K, Tanabe K, Ohnuki M et al. Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. Cell 2007; 131 : 861-72


a
Transgenic mouse
b
Skin fibroblasts
c
Retroviral transfection (Oct3/4, Sox2, Klf4, c-Myc)
d
Antibiotic selection
e
Growth in culture
iPS cell line
Normal blastocyst
Normal mouse
f
Chimaeric mouse
+
Normal mouse
g
iPS-derived mouse

Gene and cell therapy in sport

➔Gene and cell doping

« non therapeutic use of cells, genes, genetic elements that have the capacity to enhance athletic performance »

WADA. World anti-doping code. 2005

➔Gene and cell in sport injuries

« treatment of various sports-related injuries including muscle, ligament, tendon, cartilage and bone »

What exactly is non therapeutic?

Natural or Transgenic Advantage

➔ 90 human genes or chromosomal locations associated with athletic peformance

- maximizing oxygen uptake
- heart efficiency
- power output
- endurance
- other traits

➔ Scanning athletes' genomes to discover super champions

➔ Transfer of a « sport » gene to build super champions

Gene and cell therapy in sport

« Repairing or Doping »

- Animal models ➔ human athletes
- Gene and cell doping to improve athletic performance of human athletes
- In animal sports gene and cell doping may applied too