



**STEM CELLS: WHAT FUTURE FOR THERAPY?  
scientific aspects and bioethical problems**

**FINAL PROGRAMME**

**THURSDAY SEPTEMBER 14<sup>TH</sup>**

16:30 Opening of the Congress

**Presentation of the Conference**

Gian Luigi Gigli, Past President, World Federation of Catholic Medical Associations

**Opening remarks**

H.E. Elio Sgreccia, President, Pontifical Academy for Life

José Maria Simon de Castellvi, President, World Federation of Catholic Medical Associations

Jean Marie Le Méné, President, Fondation Jérôme Lejeune

**17.00 1<sup>th</sup> SESSION: GENERAL ASPECTS**

Prentice DA. *Historical development of research on stem cells*  
(Georgetown, VA, USA)

Vescovi A. *Biology and physiology of stem cells*  
(Milan, Italy)

Silburn P. *Embryonic and non-embryonic stem cells*  
(Brisbane, Queensland, Australia)

Huriet C. *Stem cells: economic interests and political implications*  
(Paris, France)

Discussion



**STEM CELLS: WHAT FUTURE FOR THERAPY?  
scientific aspects and bioethical problems**

**FRIDAY SEPTEMBER 15<sup>TH</sup>**

**8.00 FREE COMMUNICATIONS (Session 1)**

Mauceri J. *Embryology is Teleology*  
(Kingston, NY, USA)

Vout B. *Ethical and anthropological significance of forming disabled human embryos or human-animal hybrid embryos as a source of human embryonic stem cells* (Melbourne, Victoria, Australia)

Di Pietro ML, Romano L. *Cryopreserved embryos and ESCs: viability, death and duration of storage. Biomedical and ethical problems*  
(Rome and Naples, Italy)

Silva F, Pau KYF, Izadyar F, Slepko N, Sayre C. *Therapeutic reprogramming of the post-natal germ-line*  
(Irvine, CA, USA)

Neri D. *Patenting human embryonic stem cells*  
(Messina, Italy)

Doerflinger RM. *The problem of deception in stem cell research*  
(Washington, DC, USA)

Discussion

**9:20 2<sup>nd</sup> SESSION: CLINICAL APPLICATIONS I**

Strauer BE. *Therapeutic applications of stem cells in the cardiologic field*  
(Düsseldorf, Germany)

Hess DC. *Stem cells and neurological diseases*  
(Augusta, GA, USA)

Lima C. *Possible uses of stem cells in the lesions of the spinal cord*  
(Lisbon, Portugal)



**STEM CELLS: WHAT FUTURE FOR THERAPY?  
scientific aspects and bioethical problems**

Discussion

**11.00 Coffee break**

Mackay-Sim A. *Stem cells and genetic disease*  
(Nathan, Queensland, Australia)

McGuckin CP. *Potential for access to embryonic-like cells from human umbilical cord blood*  
(Newcastle upon Tyne, UK)

Mancuso S. *The regenerative medicine in prenatal life: the engraftment after in utero stem cell transplantation via intracelomic route*  
(Rome, Italy)

**13.00 Lunch Break**

**14.30 FREE COMMUNICATIONS (Session 2)**

Sotomayor G, Moldenhaver S, Sotomayor S, Packer K. *Ethical alternatives to embryonic stem cell research using umbilical cord blood and other post natal tissues*  
(Atlanta, GA, USA)

Forraz N, Baradez MO, McGuckin C. *Cord blood multi lineage progenitor cell line: an ethical and practical stem cell source for research and development*  
(Newcastle upon Tyne, UK)

Parolini O. *Fetal membranes from human term placenta: a source of progenitor/stem cells*  
(Brescia, Italy)

Discussion



**STEM CELLS: WHAT FUTURE FOR THERAPY?  
scientific aspects and bioethical problems**

**15.15 3<sup>rd</sup> SESSION: CLINICAL APPLICATIONS II**

Habib NA. *Possible use of stem cells in regenerative medicine*  
(London, UK)

De Luca M. *Epithelial stem cells and regenerative medicine*  
(Venice and Modena, Italy)

Sherley JL. *Validating stem cell technologies*  
(Cambridge, MA, USA)

Discussion

**16.50 Coffee break**

**17:10 4<sup>th</sup> SESSION: ALTERNATIVE PROPOSALS**

Condic ML. *Embryonic stem cells without embryos?*  
(Salt Lake City, UT, USA)

Brevini TAL, Gandolfi F. *Establishment and characterization of pluripotent cell line from human parthenotes*  
(Milan, Italy)

Takahashi K and Yamanaka K. *Identification of factors that generate pluripotent stem cells from fibroblast culture*  
(Kyoto, Japan)

Discussion



**STEM CELLS: WHAT FUTURE FOR THERAPY?  
scientific aspects and bioethical problems**

**18:45 FREE COMMUNICATIONS (Session 3)**

Hess DC, Yasuhara T, Hara K, Maki M, Matsukawa N, Yu G, Xu L, Mays RW, Deans RJ, Carroll JE, Borlongan CV. *Minimally invasive intravenous delivery of human bone marrow-derived multipotent adult progenitor cells leads to engraftment and host cell loss reduction in the ischemic brain, and stable behavioral recovery in experimental stroke*  
(Augusta, GA and Cleveland, OH, USA)

Quaini F, Fagnoni F, Frati C, Graiani G, Cavalli S, Lagrasta C, Lazzaretti M, Quaini E, Musso E, Squarcia U. *Searching for different sources of adult stem cells to repair the heart: the cardiogenic potential of human myocardial and bone marrow stromal cells*  
(Parma, Brescia and Pavia, Italy)

Discussion



**STEM CELLS: WHAT FUTURE FOR THERAPY?  
scientific aspects and bioethical problems**

**SATURDAY SEPTEMBER 16<sup>TH</sup>**

**8:15 5<sup>th</sup> SESSION: ANTHROPOLOGICAL AND ETHICAL ASPECTS**

Carrasco I. *General ethical principles on the use of "adult" stem cells*  
(Valencia, Spain)

Faggioni M. *Anthropological-ethical reflections on production and use of "embryonic" stem cells*  
(Rome, Italy)

Discussion

Test of evaluation for CME credits

10:00 Departure to the Papal residence in Castelgandolfo (by bus)

**12:00 SPECIAL AUDIENCE OF HIS HOLINESS BENEDICT XVI**  
(reserved to the invited speakers and registered participants)

13.00 End of Congress



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

### POSTER SESSION

**Posters will be displayed during the entire congress in the area of the coffee breaks.**

**Authors will be present for personal discussion during coffee breaks.**

Bosch Barrera J, Villarreal MA, Argemí Ballbé JA. *Undergraduate bioethical training on stem cells at university*  
(Pamplona and Barcelona, Spain, Roma, Italy)

Baylis F, Mélançon MJ. *Canadian guidelines for human embryonic stem cell research: shifting tides from frozen to fresh embryos*  
(Halifax, Nova Scotia and Chicoutimi, Québec, Canada)

Caso C. *Beyond stem cells: experience of a general practitioner*  
(Salerno, Italy)

Ferreira AT, Oshiro, MEM, Paredes-Gamero, EJ. *Role of intracellular Ca<sup>2+</sup> in cytokine signaling in mice long-term bone marrow cultures*  
(São Paulo, SP, Brazil)

Gérman Zurriarain R. *Embryonic stem cells: biomedical ethics and economic interests*  
(Logroño, Spain)

Huarte J, Lang M, Suarez A. *Embryo-free methods to generate human pluripotent stem cells, and genomic anomalies directly inhibiting the appearance of neural activity (DIANA anomalies)*  
(Geneva and Zurich, Switzerland)

Kukura JW, Friedman G. *Promoting cord blood donation*  
(Princeton and Livingston, NJ, USA)

Lucena E, Lucena C, Andersson K, Esteban C, Mojica S, Englund M, Lucena M, Davila A, Emanuelsson K, Hyllner J. *Establishment and characterization of the first latin-american embryonic stem cell line*  
(Bogotá, Colombia)

Markeljevic J. *The dignity of human life and stem cell research*  
(Zagreb, Croatia)

Mélançon MJ, Baylis F. *Canadian updated guidelines for human pluripotent stem cell research (june 28, 2006): continuities and discontinuities*  
(Chicoutimi, Québec and Halifax, Nova Scotia, Canada)



**STEM CELLS: WHAT FUTURE FOR THERAPY?  
scientific aspects and bioethical problems**

Perrella G, Brusini P, Spelat R, Hossain P, Hopkinson A, Dua HS. *Expression of haematopoietic stem cell markers, CD133 and CD34 on human corneal keratocytes*

(Udine, Italy, and Southampton and Nottingham, UK)

Pisu S, Caocci G, Fadda G, Castello G. *Reflections about relationships between stem cells and embryonic individuality*

(Cagliari, Italy)

Planinc-Peraica A. *Ethical problems in bone marrow transplantation in adult patients with haematological malignancies*

(Zagreb, Croatia)

Sipr K. *Czech paradox: successful adult stem cell therapy and the recent passing of the embryonic stem cell law*

(Olomouc, Czech Republic)

Suwancharas T. *Bioethics issues in autologous stem cell renal transplantation in Thailand*

(Srinakharinwirot, Thailand)



**STEM CELLS: WHAT FUTURE FOR THERAPY?**  
scientific aspects and bioethical problems

**THURSDAY SEPTEMBER 14<sup>TH</sup>**  
**5:00 p.m.**

**1<sup>TH</sup> SESSION: GENERAL ASPECTS**



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

**Prentice DA**

### ***HISTORICAL DEVELOPMENT OF RESEARCH ON STEM CELLS***

(Georgetown, VA, USA)

The history of stem cells can be traced through several phases, starting first with our awareness of their existence and function, through our ability to culture stem cells in the laboratory for study, to a re-discovery of their function, and to their application in medical therapies. Recognition of the existence of stem cells traces back at least to the mid-20th century, and possibly earlier.

Experimental work with embryonic stem cells grew out of studies with teratocarcinomas, starting in the 1950's. The first successful culture of mouse embryonic stem cells was in 1981, while human embryonic stem cells were first grown in the laboratory in 1998.

There is as yet no clinical application of embryonic stem cells, owing to the lack of demonstration of safety and efficacy even in animal models. Adult stem cells from bone marrow were first successfully cultured in the 1960's. Clinical application of adult stem cells began early, with the first bone marrow transplant in the 1960's and the first cord blood transplant in the 1980's. The potential for applications beyond hematopoietic reconstitution, however, was not realized until recently, with the discovery that some adult stem cells can generate cell types beyond their tissue of origin. The current explosion of interest in stem cells and these paradigm-changing discoveries have unlocked a host of possibilities for understanding basic biology and development, studying disease mechanisms, and an increasing potential for stem cells to treat human maladies.



**STEM CELLS: WHAT FUTURE FOR THERAPY?  
scientific aspects and bioethical problems**

**Vescovi A**

***BIOLOGY AND PHYSIOLOGY OF STEM CELLS***

(Milan, Italy)

Abstract not received in time.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

**Silburn P**

### ***EMBRYONIC AND NON-EMBRYONIC STEM CELLS***

(Brisbane, Queensland, Australia)

It has become increasingly apparent that there are multiple sources of mammalian stem cells and that these can be obtained at various stages of life. It is generally accepted that these cells may provide fundamental information on developmental biology and aid biomedical research.

One of the major goals in stem cell biomedical research is the generation of patient specific stem cells. It can be argued that these cells will provide information on the multiple diverse phenotypes within a disease process occurring in individuals and enhance assessment of pharmaceutical agent development, cellular transplantation and regenerative strategies. Major scientific problems exist such as generation of adequate cell numbers, cell genotypic and phenotypic stability, renewability, transplant integration and avoidance of immune rejection.

This overview will focus on the relative capabilities of embryonic and non-embryonic stem cells to achieve these goals. With the increasing capacity of scientific technology it is becoming increasingly important to assess the relative benefits and risks of embarking upon research involving stem cells from all stages of life. It is hoped that scientific facts will provide a basis for rational decision making regarding the use of stem cells and allocation of resources to enhance the study of human diseases.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

Huriet C

### **STEM CELLS : ECONOMIC INTERESTS AND POLITICAL IMPLICATIONS**

Sénateur honoraire, Vice-président du Comité International de Bioéthique de l'Unesco, Président de l'Institut Curie de Paris, France

La médecine régénérative suscite des espoirs considérables et représente un marché à l'échelon planétaire qui attire les investisseurs. Mais les intérêts économiques ne coïncident pas nécessairement avec les intérêts de santé publique ni avec le respect des valeurs éthiques.

Le financement des investissements fait appel à des fonds privés et à des financements publics qui font l'objet de débats dans des instances démocratiques. Outre les intérêts économiques, les cellules souches comportent aussi des implications politiques.

I°) **Les enjeux économiques** se comptent en milliards de dollars, et les modèles économiques sont variables, allant des grandes firmes pharmaceutiques aux sociétés biotech, et aux banques de sang de cordon ombilical qui connaissent depuis quelque temps un regain d'actualité.

II°) Contrairement aux **financements** publics, les données concernant les financements privés des investissements sont incertaines. Les financements publics diffèrent selon les états. Depuis quelques années, les débats dont ils font l'objet dans les assemblées parlementaires sont de plus en plus fréquents et animés. Ils présentent une grande sensibilité aux évolutions politiques, d'autant que les opinions publiques, sensibilisées par les médias, se sentent de plus en plus concernées.

III°) Les prises de position **politiques** sont caractérisées par une très grande diversité, y compris au niveau des états - tels que les États-Unis -, ou les communautés - telles que la Communauté européenne -, et par des changements rapides. Diversité et fluctuations reflètent les différences culturelles, les courants de pensée, ainsi que les convictions religieuses qui prédominent dans les différents pays. Elles traduisent aussi l'embarras profond des décideurs qui, par conviction, défendent le respect de la vie dès son origine, mais sont sensibles à la détresse des malades dont le seul espoir réside dans des progrès de la médecine grâce à l'utilisation du vivant.



**STEM CELLS: WHAT FUTURE FOR THERAPY?**  
scientific aspects and bioethical problems

**FRIDAY SEPTEMBER 15<sup>TH</sup>**  
**8:00 a.m.**

**FREE COMMUNICATIONS (Session 1)**



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

**Mauceri J**

### ***EMBRYOLOGY IS TELEOLOGY***

UN Delegate of FIAMC, Cephas Institute, [www.cephasinstitute.org](http://www.cephasinstitute.org)  
(Kingston, NY, USA)  
(e-mail: [drjosephmauceri@msn.com](mailto:drjosephmauceri@msn.com); [info@cephasinstitute.org](mailto:info@cephasinstitute.org))

We simply plead for the felicitous collaboration between philosophy and science. The embryo is its own witness to the "Architect of Life", it issues out of His plan within its own entelechin, the form and goal of its existence. Each and every embryo comes out of the unceasing stream of "living waters" going back to Genesis and forward to Revelation on its vector of aspiration toward eternal life, all human life continuously homogeneous with itself.

Why then, this new thralldom? What is so important that we would render this new life "non-enabled"? We say that any manipulation of the embryo or its precursors, beginning with IVF and oocyte, the woman's donation, is the breach of the integrity of the "life giving waters", from nuclear transfer and oocyte reprogramming to blastomere isolation, or any future techniques which in any way disable, divest or divert the embryo from its "future before" and the teleological order. We must likewise respect the oocyte as it holds, since Genesis, an aspect of the life giving powers, not a therapeutic power, but an ontological power.

Proceed instead, with prudential care, to these possibilities":

- 1) Blastemas, acting ab initio
- 2) Primordial cancer "stem" cells
- 3) Telomeres and stem cells, cancer and longevity
- 4) Bioscaffolding tissue regeneration
- 5) Gene therapy
- 6) VSELs - very small embryo-like cord cells
- 7) Placental immunology and cellular plasticity - the unknown riches of the placenta



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

### Vout B

#### ***ETHICAL AND ANTHROPOLOGICAL SIGNIFICANCE OF FORM DISABLED HUMAN EMBRYOS OR HUMAN-ANIMAL HYBRID EMBRYOS AS A SOURCE OF HUMAN EMBRYONIC STEM CELLS***

John Paul II Institute for the Study of Marriage and Family, Melbourne, Australia,  
Life Office, Catholic Archdiocese of Sydney  
(e-mail: [brigid.vout@sydney.catholic.org.au](mailto:brigid.vout@sydney.catholic.org.au))

This paper considers two proposals which would use cloning technology to generate pluripotent stem cells: altered nuclear transfer involving human somatic cells and human oocytes, and nuclear or altered nuclear transfer using human somatic cells and animal oocytes. Altered nuclear transfer (ANT) is a broad conceptual proposal for producing pluripotent stem cells without creating and destroying human embryos. It has already generated rigorous debate among scientists, ethicists, philosophers and theologians. Good people disagree about whether proposals involving ANT with human somatic cells and human ova would directly create a pluripotent cell or a seriously defective human embryo. This paper broadly reviews this debate, but also asks whether these procedures would be, in the first place, an appropriate use of the human generative capacity of oocytes. While there has been some discussion of ethical issues surrounding the process of oocyte donation by women, the prior question of whether it is ever appropriate to use oocytes for non-procreative purposes is one which has been largely overlooked. If there is a morally strong argument against the use of human oocytes, a further question arises, namely, "if not human oocytes, why not use animal oocytes?" To this end, the second part of this paper considers the anthropological and ethical significance of creating animal-human hybrid embryos by conventional and altered nuclear transfer using human somatic cells and the oocytes of animals.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

Di Pietro ML\*, Romano L\*\*

### ***CRYOPRESERVED EMBRYOS AND ESCs: VIABILITY, DEATH AND DURATION OF STORAGE. BIOMEDICAL AND ETHICAL PROBLEMS***

I\* School of Medicine "A. Gemelli", Catholic University of the Sacred Heart, Rome – Italy

(e-mail: [mldipietro@rm.unicatt.it](mailto:mldipietro@rm.unicatt.it))

\*\* School of Medicine, University of Naples "Federico II", Naples - Italy

(e-mail: [lucioromano@libero.it](mailto:lucioromano@libero.it))

#### **Purpose**

ESC (Embryonic Stem Cells) are derived from cryopreserved human embryos by a process that necessarily destroys them. A proposal for not destroying human embryos is to remove material for ESCs derivation from "non viable embryos". Are "non viable" embryos dead? Is it possible to determine "organismic death" for human embryos without destroy them? There is a date in which cryopreserved human embryos are surely dead?

#### **Methods**

We analyse some studies about criteria for embryonic death, methods of cryopreservation, criteria for the evaluation of the embryo-quality after thawing, implantation and pregnancy rate after cryopreservation.

#### **Results**

A "non viable embryo" is not a "dead embryo" because a "viable embryo" only is regarded acting as implantation and pregnancy development. An embryo is defined "dead" when there is "the irreversible loss of capacity for continued an integrated cellular division, growth and differentiation" (Lanry DW et al. *Rgenerative Med* 2006; 1: 367-371). In this approach there are two unsolved problems: 1. the death of an embryonic organism as "a whole" isn't the same thing as death of its constituent blastomeres (a totipotent blastomere is capable of growing into a complete human being); 2. because it is impossible to know in advance which embryos will not divide and which will continue to divide, what happens of no dead embryos?

It is impossible to fix a date in which cryopreserved human embryos are surely dead too, because it depends on the methods of cryopreservation and quality of embryo before and after thawing. Some case reports on survival of human embryos after long-term storage moreover are published: live birth from a zygote cryopreserved for 8 years (Go KJ et al. *Hum Reprod* 1998; 13: 2970-2971); full term delivery following cryopreservation of human embryo for 7.5 years (Ben-Ozer S et al. *Hum Reprod* 1999;14:1650-1652); birth of a healthy baby after transfer of



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

embryos that were cryopreserved for 8.9 years (Quintas CJ et al. *Fert Steril* 2002;77:1074-1076); twin delivery following 12 years of human embryo cryopreservation. and the authors “confirms the finding that the duration of the storage does not appear to adversely affect the survival of frozen embryos” (Revel A et al. *Hum Reprod* 2004;19:328-329). Some cryobiologists have showed, in frozen mice treated with radiation that simulated 2000-year storage, no detectable effect on their viability (Glenister PH et al. *Mamm Genome* 2000;11:565-571).

### **Conclusion**

Based on these data, we think that this proposal isn't actually acceptable and believe that it merits further experimental research involving no human embryos.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

Silva F, Pau KYF, Izadyar F, Slepko N, Sayre C

### ***THERAPEUTIC REPROGRAMMING OF THE POST-NATAL GERM-LINE***

PrimeCell Therapeutics LLC, Irvine, CA, USA

(e-mail: [fsilva@primegenbiotech.com](mailto:fsilva@primegenbiotech.com))

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

#### **Purpose**

Recent studies have demonstrated that post-natal germ cells in the mouse have the ability to regain pluripotency via culture induced reprogramming. This flexibility that seems to be unique to the germ-line in being able to regain pluripotency has significant implications for generating an autologous pluripotent stem cell without the use of an embryo. Using our murine germ cell culture induced reprogramming method as a model, we report for the first time the culture induced reprogramming of human germ cells to exhibit pluripotency.

#### **Methods**

Human testicular material isolated from men ranging between the ages of 26-51 years old were subjected to enzymatic digestion, followed by a differential adhesion step to enrich for germ cells. Culture induced reprogramming was performed using culture methods established in our murine model with a combination of several growth factors. Reprogramming was determined by the up-regulation of OCT4 a transcription factor associated with pluripotency, not found in post-natal tissues.

#### **Results**

Up-regulation of OCT4 was observed in 7 of 11 samples that underwent culture induced reprogramming. Reprogrammed cells were expanded for 40 days and directionally differentiated to cell types derived from all three germ layers.

#### **Conclusion**

We have demonstrated that post-natal human germ cells can be isolated and reprogrammed to exhibit markers associated with pluripotency and the ability to differentiate to cell types with therapeutic value. Further research should be focused on developing this novel technology since it can potentially solve the ethical and technical issues associated with the derivation of human pluripotent stem cells for autologous cell based therapies.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

**Neri D**

### ***PATENTING HUMAN EMBRYONIC STEM CELLS***

Cattedra di Bioetica, Dipartimento di Filosofia, Università di Messina, Italy  
(e-mail: [demetrioneri@tin.it](mailto:demetrioneri@tin.it))

During 2004 the European Patent Office's departments of first instance refused "for ethical reasons" to grant patents involving embryonic stem cells. The EPO President, Mr. Alain Pompidou, declared that in this field patenting procedures are subjected to "precise ethical limits", adding that the moratorium is justified because "there are too many ethical aspects that have not been resolved at the political level". But what "ethical reasons and limits"? How much "precise"? And to be fixed by what "political level" and through what kind of procedures? In my presentation I'll try to discuss some aspects of this issue, which I consider as emblematic of a more general issue, namely the great difficulty experienced by public authorities, in democratic and pluralistic societies, when they have to regulate sensitive sectors of social activity, as advanced biomedical research, especially when - as it is very often the case - morally controversial issues are involved, issues about which there is in our societies a genuine moral disagreement. What kind of criteria should be taken into account by regulative bodies in the decision-making procedures?



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

**Doerflinger RM**

### ***THE PROBLEM OF DECEPTION IN STEM CELL RESEARCH***

Interim Executive Director, Secretariat for Pro-Life Activities, U.S. Conference of Catholic Bishops, Washington, DC, USA

In August 2006, Dr. Robert Lanza of Advanced Cell Technology (ACT) announced in the journal *Nature* that he had resolved the ethical dispute over embryonic stem cell research, creating stem cell lines using single cells obtained from 8- to 10-celled embryos without harming them. Soon his claim was proved false. Lanza's team had destroyed 16 embryos, obtaining 4-7 cells from each so they could culture the cells together to mimic the cell-to-cell signaling that promotes healthy development in intact embryos. This was something they could not do with a single isolated cell from each embryo. They solved no ethical problem, but highlighted the ethical problem of deception in science.

This is the latest in a series of deceptions. The "therapeutic cloning" hoax of Dr. Woo-Suk Hwang damaged the credibility of another major journal, *Science*. A third journal, *The New England Journal of Medicine*, admitted in July 2006 that it had misrepresented two stem cell studies as showing the promise of "nuclear transfer" (cloned) human embryos – in fact the studies used cell lines created before August 2001 from fertilized embryos. Dr. Lanza and ACT were involved in two earlier scandals: An exaggerated report of success in cloning human embryos in 2001, and a report of "embryonic stem cell" success in 2004 that in fact required growing cloned mouse embryos to the fetal stage and aborting them.

Having foolishly promised "cures" from embryonic stem cells to silence ethical objections, embryo researchers now have to exaggerate and deceive to maintain their status. The utilitarian ethic of "the end justifies the means" needed to defend embryo destruction is being applied to the ethics of truth-telling, with disastrous results for society's trust in science. The true path to progress lies in a sober and realistic account of the promise and problems of stem cell research, and in a commitment to focus on morally sound ways to realize that promise.



**STEM CELLS: WHAT FUTURE FOR THERAPY?**  
scientific aspects and bioethical problems

**FRIDAY SEPTEMBER 15<sup>TH</sup>**  
**9:20 a.m.**

**2<sup>nd</sup> SESSION: CLINICAL APPLICATIONS I**



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

**Strauer BE, Brehm M**

### ***THERAPEUTIC APPLICATIONS OF STEM CELLS IN THE CARDIOLOGIC FIELD***

Division of Cardiology, Pneumology and Angiology, Heinrich-Heine-University, Düsseldorf, Germany

Therapeutic application of bone-marrow-derived stem cells have the object to avoid the development of congestive heart failure, induced by acute myocardial infarction or ischemic coronary artery disease. Nowadays, intensive research is enforced with bone marrow-derived stem cells in acute myocardial infarction as well as in chronic ischemic heart disease by different application techniques (I) intracoronary, (II) transendocardial and (III) intramyocardial. The object is to regenerate damaged myocardium by differentiation of autologous bone marrow-derived stem cells into cardiac cells, by cytokine-induced repair of apoptotic heart muscle cells and by the accumulation of cardiac stem cells in the infarcted heart. For the therapeutic effect the proportion of different mechanisms may be responsible for the beneficial regeneration process in various heart diseases.

In various experimental and clinical studies the concept of myocardial regeneration after the injection of autologous bone marrow-derived stem cells in acute myocardial infarction and chronic ischemic heart disease could be documented in histological, immunohistochemical, molecularbiological experiments and already clinically. Strauer injected for the first time autologous bone marrow-derived stem cells during routine catheterization in a reopened left anterior descendent coronary artery (LAD) in a patient with anterior wall infarction (1) with a definite recovery of the anterior wall motion, anterior myocardial perfusion and the left ventricular function in March 2001 and by the use of autologous stem cells there were no ethical issues. In a controlled study, Strauer et al. could demonstrate in patients with acute myocardial infarction, treated with autologous bone marrow stem cell, an improvement of the global left ventricular function after 3 months (2). This beneficial effect continued still till 3 years after intracoronary cell injection. In patients with chronic ischemic heart disease Strauer et al. could also demonstrate an additional improvement of the global left ventricular function after intracoronary transplantation of stem cells (3). In both patient groups no complications and side effects could be documented immediately and after years (no arrhythmias).

### **Conclusions**

The old dogma, that the myocardium does not have the ability to repair itself, seems not to be supportable. Several clinical trails have demonstrated the beneficial effect of replacement therapy after stem cell transplantation in coronary



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

heart disease. The main challenge for the future in the stem cell therapy is the improvement of the functional activity, the enhancement of the proliferation rate and the augmentation of the migration capacity of the stem cells, in order to increase the clinical results and to enable elderly patients, which have a reduced stem cell reservoir, the stem cell therapy.

### References

- (1) Strauer BE, Brehm M, Zeus T, et al. Myocardial regeneration after intracoronary transplantation of human autologous stem cells following acute myocardial infarction.  
*Dtsch med Wschr.* 2001;126:932-938.
- (2) Strauer BE, Brehm M, Zeus T, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans.  
*Circulation* 2002;106:1913-1918.
- (3) Strauer BE, Brehm M, Zeus T, et al. Regeneration of human infarcted heart muscle by intracoronary autologous bone marrow cell transplantation in chronic coronary artery disease – the IACT study.  
*JACC* 2005;46:1651-1658.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

Hess DC

### **STEM CELLS AND NEUROLOGICAL DISEASES**

Medical College of Georgia, Augusta, GA, USA  
(e-mail: [dhess@mail.mcg.edu](mailto:dhess@mail.mcg.edu))

The central nervous system was once thought to be incapable of regeneration. Ramon y Cajal wrote, "In adult centres, the nerve paths are something fixed, ended, immobile. Everything may die, nothing may be regenerated." This dogma has been challenged in the last decade with studies showing new, migrating stem cells in the brain in many rodent injury models and findings of new neurons in the human hippocampus in adults. Moreover, there are reports of bone marrow-derived cells developing neuronal and vascular phenotypes and aiding in repair of injured brain. These findings have fueled excitement and interest in regenerative medicine for neurological diseases, arguably the most difficult diseases to treat.

There are a number of proposed regenerative approaches to neurological diseases. These include:

- 1.) Cell therapy approaches in which cells are delivered intracerebrally or infused by an intravenous or intra-arterial route;
- 2.) Stem cell mobilization approaches in which endogenous stem and progenitor cells are mobilized by cytokines such as G-CSF or chemokines such as SDF-1;
- 3.) Trophic and growth factor support such as delivering brain-derived neurotrophic factor (BDNF) or glial-derived neurotrophic factor (GDNF) into the brain to support injured neurons. These approaches may be used together to maximize recovery.

The cell therapy approach is broad and will need to be tailored to the specific brain disease. For example, in Parkinson's disease (PD) specific cell populations of dopaminergic neurons degenerate in the substantia nigra and other brainstem and spinal cord areas. PD is best targeted by replacing these specific populations; however, the environment of the brain will likely be hostile to these cells and it will be important to provide a more favorable environment for the graft. In stroke, on the other hand, many cell populations are damaged including neurons, astrocytes and blood vessels; the cell therapy approach will need to repair vessels (angiogenesis) and well as neurons (neurogenesis) and other supporting cell types.

While initially, it was thought that cell therapy might work by a "cell replacement" mechanism, a large body of evidence is emerging that cell therapy works by providing "trophic" or "chaperone" support to the injured tissue and brain.



## **STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems**

Providing support to blood vessels appears to be key to preserving function especially in stroke. Angiogenesis and neurogenesis are coupled in the brain. Increasing angiogenesis with adult stem cell approaches in rodent models of stroke leads to preservation of neurons and improved functional outcome.

A number of stem and progenitor cell types have been proposed as therapy for neurological disease ranging from neural stem cells to bone marrow derived stem cells to embryonic stem cells. Any cell therapy approach to neurological disease will have to be “scalable” and easily “commercialized” if it will have impact on public health. Currently, bone marrow-derived cell populations such as the marrow stromal cell, multipotential progenitor cells, and umbilical cord stem cells meet these criteria the best. Of great clinical significance, initial evidence suggests these cell types may be delivered by an allogeneic approach so strict tissue matching may not be necessary. The most immediate impact on patients will be achieved by making use of the trophic support capability of cell therapy and not a cell replacement mechanism.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

Lima C\*, Vital JP\*, Escada P\*, Ferreira H\*, Capucho C\*, Peduzzi J#.

### ***POSSIBLE USES OF STEM CELLS IN THE LESIONS OF THE SPINAL CORD***

\*Hospital de Egas Moniz, Lisbon-Portugal;  
#Wayne State University- Detroit, USA

#### **Background**

Olfactory mucosa is a readily accessible source of stem-like/progenitor and olfactory ensheathing cells for neural repair.

#### **Aim and methods**

To determine the safety and feasibility of transplanting olfactory mucosa autografts into patients with traumatically injured spinal cords, experimental animal studies and a human pilot trial were conducted. Experimental animal studies consisted of olfactory mucosa transplants in guinea pigs with subacute transected spinal cords and in rats with chronic, severe contusive spinal cord injury. In the human studies, 7 patients ranging from 18-32 years of age (ASIA class A ) were treated at 6 months-6.5 years post-injury. Olfactory mucosa autografts were transplanted into lesions ranging from 1-6 cm that were present at C3-T8 vertebral levels. Operations were performed between July 2001 and March 2003. MRI, EMG, ASIA neurological and otolaryngological evaluations were performed before and after surgery.

#### **Results**

Animal experiments demonstrated graft integration and functional improvement. In human studies, MRI studies revealed moderate to complete filling of the lesion sites. No significant adverse events were observed clinically, except for a slight sensory decrease in one patient . On a 18 months follow-up almost every patient improved in their ASIA neurological scores. Using parametric statistics, there was a significant improvement in the sensory light touch, sensory pinprick, and motor legs component of the ASIA scores using both parametric and non-parametric statistics. Olfaction returned completely within 3 months. From March 2003 to present 63 additional patients performed the surgery with variable levels of recovery and similar and/or better outcomes. Results from these patients with long follow-up are being updated.

#### **Conclusion**

This study demonstrates that olfactory mucosa autograft transplantation into the human injured spinal cord is feasible, safe, and potentially beneficial..

Long-term patient monitoring is however necessary to rule out any delayed side effects and assess any further improvements.



**STEM CELLS: WHAT FUTURE FOR THERAPY?  
scientific aspects and bioethical problems**

Also, a coupled intensive and regular rehabilitation program focused on the damaged area of the body is being set and it is considered an essential step to maximize the recovery potential of the procedure.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

**Mackay-Sim A**

### **STEM CELLS AND GENETIC DISEASE**

Eskitis Institute for Cell and Molecular Therapies, Griffith University, Brisbane, Queensland, Australia

[a.mackay-sim@griffith.edu.au](mailto:a.mackay-sim@griffith.edu.au)

For the last 40 or 50 years intravenous infusions of whole bone marrow have been used to treat patients with acute leukaemia, bone marrow aplasia and congenital immune deficiencies. The success of these treatments was due to the haematopoietic stem cell, a rare cell that has provided the model for all adult stem cell research. With the development of methods to isolate and characterise adult stem cells from various tissues, there arise new avenues for the development of new cell-based therapies for genetic diseases, and not only genetic diseases of the blood. Adult stem cells provide other avenues such as the development of cellular models of genetic disease to identify the genes and biochemical pathways involved in cellular pathology. This will be useful for developing biomarkers and diagnostic tests and for identifying new targets for drug treatments of genetic disease.

Our research concerns adult stem cells found in the olfactory mucosa, the organ of the sense of smell. This small patch of nervous tissue in the nose is easily accessible via biopsy through the external naris and contains stem cells and neural progenitor cells that continually regenerate and replace the olfactory sensory neurons whose location makes them vulnerable to death from inhaled factors such as pathogens or chemicals (Mackay-Sim and Kittel, 1991; Mackay-Sim and Chuah, 2000; Mackay-Sim, 2003). The rate of neurogenesis in the olfactory epithelium is very high and can be observed in human at all ages (Murrell et al., 1996; Feron et al., 1998). The stem cells of the human olfactory mucosa are multipotent and can be obtained from people of all ages (Murrell et al., 2005). In the olfactory epithelium they reconstitute both the neural and non-neural elements (Chen et al., 2004). In vitro these can be induced to differentiate into numerous cell types, including neurons, glia, liver, kidney, heart, skeletal muscle and fat cells (Murrell et al., 2005).

The most intuitive use for stem cells in genetic disease is for cellular "repair" of tissues affected by the genetic mutation: stem cells without the mutation would be transplanted to restore normal function. The stem cells might be derived from a non-mutated but otherwise tissue-matched donor, or they might be derived from the patient but genetically engineered. Stem cells would have the added capacity to differentiate into the desired cell type to restore normal tissue function after transplantation.



## **STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems**

One aim of our laboratory is develop stem cell therapies using autologous stem cells from a patient's own nose. We are currently undertaking a Phase I clinical trial of autologous olfactory ensheathing cell transplantation in human spinal cord injury (Feron et al., 2005). These cells are not stem cells but preclinical animal models demonstrate their effectiveness in assisting repair after spinal cord injury (Mackay-Sim, 2005). In the nose the olfactory ensheathing cells assist the regeneration and regrowth of the olfactory sensory neuron axons as they grow from the nose to the brain. Our on-going clinical trial indicates that transplantation of autologous olfactory ensheathing cells into the human spinal cord is safe at one year. We are now working on preclinical animal models of Parkinson's disease to determine the efficacy of olfactory stem cells in providing a source of dopamine after transplantation into the striatum. In our laboratory we are also exploring the potential of olfactory stem cells for transplantation after genetic manipulation. They can be transfected ex-vivo using vector carrying foreign genes such as the green fluorescent protein and they retain gene expression for many months after transplantation into the brain. These experiments suggest that olfactory stem cells will be a useful source of cells for genetic manipulation and transplantation in therapies for genetic diseases.

Another use for stem cells is to provide cellular models of disease. The great advances in understanding cancers are the result of intensive investigations of cell lines derived from different cancers. Other diseases are investigated via animal models but for the majority of diseases there are neither cellular models nor animal models. Stem cells have the potential to provide such models, especially for diseases for which the genetic contribution is unknown. For example, stem cells derived from people with amyotrophic lateral sclerosis would provide a model of this motor neuron disease if they were differentiated into motor neurons. The cellular effects of the SOD1 mutation in these patients could be identified and used to identify commonly affected pathways in other motor neuron disease patients without a known genetic mutation. Deeper understanding of the genetic and biochemical aetiologies of disease will provide new treatments, new drug targets and potential preventative strategies.

Olfactory stem cells grow in characteristic cultures, known as "neurospheres", which are tight clusters of cells containing stem cells, neural precursors and differentiating neurons and glia (Murrell et al., 2005). The stem cells comprise less than 1% of these neurosphere cells but they can be maintained in vitro for many generations. They are self-renewing and produce many millions of progeny. Olfactory neurosphere cultures can be stored frozen and recovered without apparent loss of multipotency.

In our laboratory we now have olfactory neurosphere cultures from more than 50 patients including some with Parkinson's disease, motor neuron disease, or schizophrenia. These cell lines are being developed as cellular models. For example, we are developing methods for differentiation into dopaminergic neurons for Parkinson's disease and into motor neurons for motor neuron disease. In



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

schizophrenia, a disorder of brain development, the interest is in the fundamental aspects of cellular proliferation and differentiation of stem cells. We have already shown that olfactory neurogenesis is altered in nasal biopsies in schizophrenia compared to control, with increased cell proliferation and altered gene expression (Feron et al., 1999; McCurdy et al., 2005).

There are many sources of adult stem cells. Adult stem cells are suspected in all adult tissues that can self-repair. If all adult stem cells prove to be multipotent, the exciting possibility is that there is an appropriate adult stem cell available for repair of any tissue type, with the potential for investigations of genetic diseases and their treatments. For example, bone marrow stem cells may be best for blood and bone diseases, cartilage stem cells for joints and olfactory stem cells for the nervous system, each providing “specialised tools” appropriate for the task at hand.

### References

- Chen X, Fang H, Schwob JE (2004) Multipotency of purified, transplanted globose basal cells in olfactory epithelium. *J Comp Neurol* 469:457-474.
- Feron F, Perry C, McGrath JJ, Mackay-Sim A (1998) New techniques for biopsy and culture of human olfactory epithelial neurons. *Arch Otolaryngol Head Neck Surg* 124:861-866.
- Feron F, Perry C, Hirning MH, McGrath J, Mackay-Sim A (1999) Altered adhesion, proliferation and death in neural cultures from adults with schizophrenia. *Schizophr Res* 40:211-218.
- Feron F, Perry C, Cochrane J, Licina P, Nowitzke A, Urquhart S, Geraghty T, Mackay-Sim A (2005) Autologous olfactory ensheathing cell transplantation in human spinal cord injury. *Brain*.
- Mackay-Sim A (2003) Neurogenesis in the adult olfactory neuroepithelium. In: *Handbook of Olfaction and Gustation, Second Edition* (Doty R, ed), pp 93-113. New York: Marcel Dekker.
- Mackay-Sim A (2005) Olfactory ensheathing cells and spinal cord repair. *Keio J Med* 54:8-14.
- Mackay-Sim A, Kittel P (1991) Cell dynamics in the olfactory epithelium.
- Mackay-Sim A, Chuah MI (2000) Neurotrophic factors in the primary olfactory pathway. *Prog Neurobiol* 62:527-559.
- McCurdy RD, Feron F, McGrath JJ, Mackay-Sim A (2005) Regulation of adult olfactory neurogenesis by insulin-like growth factor-I. *Eur J Neurosci* 22:1581-1588.
- Murrell W, Bushell GR, Livesey J, McGrath J, MacDonald KP, Bates PR, Mackay-Sim A (1996) Neurogenesis in adult human. *Neuroreport* 7:1189-1194.
- Murrell W, Feron F, Wetzig A, Cameron N, Splatt K, Bellette B, Bianco J, Perry C, Lee G, Mackay-Sim A (2005) Multipotent stem cells from adult olfactory mucosa. *Dev Dyn* 233:496-515.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

McGuckin CP, Forraz N

### **POTENTIAL FOR ACCESS TO EMBRYONIC-LIKE CELLS FROM HUMAN UMBILICAL CORD BLOOD**

Newcastle Centre for Cord Blood, Stem Cell Institute, Medical School, Newcastle upon Tyne, UK

[c.mcquckin@newcastle.ac.uk](mailto:c.mcquckin@newcastle.ac.uk)

All too often media attention clouds the reality that there are many types of stem cells. Embryo's, bone marrow (BM) and umbilical cord blood (UCB) are the three most used sources. However, despite what it would appear in the media, embryonic stem cells are not the most likely to yield life saving cures at this point in history. Faster routes to clinical intervention are the adult stem cells that can be sourced in bone marrow and cord blood and which are readily accessible and more ethically acceptable to the public. Both these non-embryonic sources have been able to provide sufficient numbers of cells to allow development of clinical translational protocols. BM derived cells have been used successfully in myocardial infarct therapy where relining of the endothelial tissue has allowed limited reperfusion to the damaged heart tissues. UCB has also had significant success for around 20 years in haematotransplantation. With a global human population in excess of 6 billion, UCB remains the largest untouched source of stem cells available every year. UCB also provides a distinct advantage over other adult stem cells due to the length of the telomere and also from the protected immunological status of the developing neonatal environment. The total mutation load in the UCB populations is clearly likely to be significantly less than in adult tissues.

Limited numbers of adult stem cells have often hindered their clinical development, necessitating expansion of the cells. However, historically, *ex vivo* expansion of cells whilst limiting differentiation has proven a difficult task. We have investigated some of the characteristics of available cell populations from UCB and developed these intriguing cells for tissue engineering and tissue regeneration protocols, including the use of microgravity simulated bioreactor culture to *ex vivo* expand our stem cells. We have accessed rotating culture devices originally developed with NASA to successfully expand stem cell populations of extreme immaturity prior to loading the cells onto 3-dimensional scaffolds allowing defined hepatic cell generation. Perhaps one of the most intriguing characteristics of the extremely immature cells available from UCB is that they can show an *in vitro* growth pattern similar to that of "embryoid bodies" seen in embryonic stem cell cultures.



## **STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems**

We believe the creation of clinical grade protocols for rapid harvest and processing of UCB can reveal cell populations ranging from mature cells right back to stem cell groups of extreme immaturity and useful for both diagnosis and for therapeutic development. UCB adult stem cells are an exciting therapeutic prospect and with the advent of international cord blood banking provide an increasing resource of stem cells useful for the treatment of global disease.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

**Mancuso S, Noia G**

### ***THE REGENERATIVE MEDICINE IN PRENATAL LIFE: THE ENGRAFTMENT AFTER IN UTERO STEM CELL TRANSPLANTATION VIA INTRACELOMIC ROUTE***

(Rome, Italy)

Over the last 15 years, the available models for in vivo stem cell assay have been represented by either stem cell injection in pre-irradiated NOD/SCID mice or in-utero stem cell transplantation in pre-immune sheep. In adult NOD/SCID mice or in pre-immune sheep, hematopoietic chimeras have been shown to be produced by human hematopoietic stem cell (HSC) transplantation with a degree of human cell engraftment ranging from 0.1 to 35 % depending on the number, subset and source of stem cell injected (1,2). In this context, Rio et al. recently reported that the in-utero injection of retrovirally transduced HSC into fetal mice results in the transfer and long-term expression of exogenous genes (3).

The intraperitoneal approach for in-utero transplantation in sheep model allows stem cell injection only after 55 days of gestational age (4) while the intracelomic route can be used  $\geq 35$  days onward with the theoretical advantages of an earlier stem cell injection (i.e. the low competence of the fetal immune system, the low number of fetal hematopoietic cells competing with the transplanted donor cells). In recent studies we reported for the first time that intracelomic injection of HSC into sheep fetuses at early gestational age is feasible and associated with an high degree of engraftment in hematopoietic and non-hematopoietic tissues (5,6).

In our series, 8 evaluable fetuses which received either human T cell-depleted cord blood mononuclear cells (CB-MNC) ( $50 \times 10^6$  per lamb) or CD34+ cord blood-purified cells ( $1-2 \times 10^5$  per lamb) had hematopoietic engraftment revealed by antigenic analysis of human CD45. In 6 of these fetuses chimerism was confirmed by PCR analysis for human  $\beta 2$ -microglobulin in brain, spinal cord, heart, lung and skeletal muscle, also in long-term follow-up (18 months after birth).

With this background, we planned further experiments to investigate whether the number and source of stem cells injected via intracelomic route could affect the rate of chimerism and/or the degree of engraftment in sheep model. In all, 4 sheep fetuses were in-utero transplanted with either T cell-depleted CB-MNC or fetal bone marrow (FBM) CD34+ cells. In particular, 2 fetuses received a double transplantation with a first intracelomic injection of T cell-depleted CB-MNC ( $20 \times 10^6$  and  $30 \times 10^6$  in animal N° 72491 and N° 217125 respectively), followed by a second injection of  $20 \times 10^5$  T cell-depleted CB-MNC administered intravenously 1 month after birth. Other 2 fetuses were in-utero transplanted via intracelomic route with two different amounts of FBM CD34+ cells ( $0.8 \times 10^5$  and  $2 \times 10^5$  in animal N° 217132 and N° 37783 respectively).



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

All fetuses were spontaneously delivered at term, but no human engraftment was observed at birth and/or 2 months later in animals N° 72491, 217125 and 217132. On the other hand, in animal N° 37783 RT-PCR analysis showed a positivity to human myoglobin in the liver and the positivity to both human myoglobin and troponin I in the heart.

The long term follow-up (18 months after birth) confirmed the positivity of engraftment with different degree of chimerism depending on the applied methodology.

According to our experience with the intracelomic route of administration, no engraftment can be achieved with a number of T cell-depleted CB-MNC lower than  $50 \times 10^6$ . As previously described by our group with CD34+ cord blood-purified cells (5,6), a number of CD34+ cells  $> 1 \times 10^5$  seems to be necessary to obtain a certain degree of chimerism even if using FBM-derived cells.

In conclusion, our data confirm that, after in-utero stem cell transplantation also via intracelomic route, the number, subset and source of cells injected are critical factors affecting engraftment.

### Acknowledgements

This work was partly supported by Cord Blood Stem Cell Project "Fondazione Cassa di Risparmio", Rome, Italy.

### References

- 1) Bhatia M., Wang IC, Kapp U, Bonnet D, Dick JE. Purification of primitive human hematopoietic cells capable of repopulating immune-deficient mice. *Proc Natl Acad Sci USA* 1997;94:5320-5325.
- 2) Zanjani ED, Pallavicini MG, Ascensao JL, et al. Engraftment and long-term expression of human fetal hemopoietic stem cells in sheep following transplantation in utero. *J Clin Invest* 1992;89:1178-1188.
- 3) Rio P, Martinez-Palacio J, Ramirez A, Bueren JA and Segovia JC. Efficient engraftment of in utero transplanted mice with retrovirally transduced hematopoietic stem cells. *Gene Ther* 2005;12:358-363.
- 4) Zanjani ED. The human sheep xenograft model for the study of the in vivo potential of human HSC and in utero gene transfer. *Stem Cells* 2000;18:151.
- 5) Noia G, Pierelli, Bonanno G, et al. A novel route of transplantation of human cord blood stem cells in preimmune fetal sheep: the intracelomic cavity. *Stem Cells* 2003;21:638-646.
- 6) Noia G, Pierelli L, Bonanno G et al. The intracelomic route: a new approach for in utero human cord blood stem cell transplantation. *Fetal Diagn Ther* 2004;19:13-22.



**STEM CELLS: WHAT FUTURE FOR THERAPY?**  
scientific aspects and bioethical problems

**FRIDAY SEPTEMBER 15<sup>TH</sup>**  
**2:30 p.m.**

**FREE COMMUNICATIONS (Session 2)**



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

Sotomayor G (\*, \*\*); Moldenhaver S\*\*; Sotomayor S\*\*; Packer K\*\*\*

### ***ETHICAL ALTERNATIVES TO EMBRYONIC STEM CELL RESEARCH USING UMBILICAL CORD BLOOD AND OTHER POST NATAL TISSUES***

Northside Hospital\*, Atlanta, GA;  
Babies For Life Foundation\*\*, Atlanta, GA;  
Kenyon State University\*\*\*, Ohio USA  
(e-mail: [GSotomayor@babiesforlife.org](mailto:GSotomayor@babiesforlife.org))

#### **Purpose**

Establishment of universal cord blood collection and other post natal tissues for stem cell research and transplants as an alternative to embryonic stem cell research.

Methods: A non-profit organization was developed in 2001 as an alternative to embryonic stem cell research. Recruitment of accredited blood banks, ethical research centers and a public education campaign in order prove that the main focus of stem cells should be in adult sources and not in the destruction of the human embryo. A political campaign strategy was developed that illustrated to the lawmakers the need of universal cord blood collection, establishment of state funded stem cell research labs and mandated discussion on the merits of cord blood donation with every pregnant woman. Key religious leaders, community activists, colleges, healthcare providers, bioethicists, scientists in biotechnology, television and other media have all been invited to take an active participation.

#### **Results**

The amount of awareness created and the expertise collecting thousands of units of umbilical cord blood has led to the point where multiple blood banks and research centers are now requesting every postnatal tissue possible and advance the research in stem cells. Over 65 diseases are now being treated with umbilical cord blood stem cells and new discovery of very small embryonic stem cells in the cord blood have proven the need to keep all efforts in the direction we have taken.

#### **Conclusion**

Our effort has proven that universal cord blood collection is possible and will provide the basis for future stem cell research and therapies without the need to destroy human embryos.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

**Forraz N (1), Baradez MO, McGuckin C (1)**

### ***CORD BLOOD MULTI LINEAGE PROGENITOR CELL LINE: AN ETHICAL AND PRACTICAL STEM CELL SOURCE FOR RESEARCH AND DEVELOPMENT***

(1)UK Centre for Cord Blood, University of Newcastle upon Tyne, United Kingdom  
(e-mail: [nico.forraz@newcastle.ac.uk](mailto:nico.forraz@newcastle.ac.uk))

#### **Purpose**

Using human Embryonic stem cell lines has been questioned for ethical and moral reasons but major scientific hurdles further complicate their inability to produce large quantities of homogeneous tissue, without contaminating animal feeder layers, and diversified immunological phenotypes. Human Umbilical Cord Blood (UCB), however, offers ethically sound easily accessible sources of immature stem cells for research and therapy. We have characterized the first normal UCB-derived Multi-Lineage Progenitor Cell line (MLPC) cloned by BioE Inc. (MN, USA).

#### **Methods**

High-definition microarray multiparametric gene expression analysis compared MLPCs to UCB mononucleated cells (MNCs), PrepaCyte-purified cells, CD133+ progenitor cells, lineage-restricted stem cells (Lin-) and bone marrow mesenchymal stem cells (BMMSCs) spanning a range of cell groups at various stages of differentiation.

#### **Results**

MLPCs phenotypic profile was significantly different (>1.4 fold down- or up-regulated) by 631 genes to other cell groups. Compared to mature cell populations (MNCs/PrepaCyte), MLPCs downregulated 65 genes associated with active protein synthesis (e.g. ribosomal sub-units), 18 genes linked with phosphate metabolism (kinases and phosphatases), 123 genes regulating proliferation and cell cycling (cyclins, cyclin-dependent kinases, check point proteins); reflecting a high degree of stemness, immaturity and quiescence. Comparison to stem/progenitor cell subsets (CD133+/Lin-) highlighted MLPCs differentiation multipotential: down-regulation of 12 different clusters of differentiation surface marker genes (e.g. epithelium / endothelium) but up-regulation of 80 nucleic acid-binding/transcription factor genes regulating tissue differentiation from all three germ layers.

#### **Conclusion**

Comparative multiparametric microarray analysis characterised UCB MLPC to have a homogeneous, primitive and uncommitted phenotype with high potential for multi-lineage tissue differentiation.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

**Parolini O**

### ***FETAL MEMBRANES FROM HUMAN TERM PLACENTA: A SOURCE OF PROGENITOR/STEM CELLS***

Centro di Ricerca E. Menni, Fondazione Poliambulanza, Brescia, Italy  
(e-mail: [ornella.parolini@tin.it](mailto:ornella.parolini@tin.it))

#### **Purpose**

Even though stem cells are at the center of many studies, the search continues for sources of these cells that do not pose ethical problems, are easily accessible and high yielding.

We have been focusing our attention on fetal membranes (amnion and chorion) of human term placenta to explore the possibility that they contain cells with progenitor characteristics.

#### **Methods**

Human term placenta were obtained after maternal informed consent. The amnion and chorion were then manually separated and enzymatically digested in order to obtain mesenchymal cells. Phenotypic analysis, immunological tests and differentiation studies were performed as previously reported (Bailo et al. Transplantation, 2004, 78: 1439-1448; Parolini et al. J.Rep.Med Endoc. 2006, 3: 117-126).

#### **Results**

In their undifferentiated state, cells isolated from the stromal regions of amnion and chorion exhibit a mesenchymal stem cell-like phenotype (CD105+, CD29+, CD44+, CD54+, CD90+, CD73+, CD34-, CD45-). After *in vitro* culture, they undergo osteogenic, chondrogenic and adipogenic differentiation. Additionally, these cells can successfully engraft in xenogeneic animal models, while their *in vivo* regeneration capacity is at the centre of ongoing studies.

Furthermore, placenta-derived mesenchymal cells do not induce allogeneic or xenogeneic lymphocyte proliferation responses, and they exhibit immunoregulatory properties as demonstrated by the suppression of allogeneic T cell response *in vitro*. Interestingly, amniotic mesenchymal cells retain their immunosuppressive properties after culturing.

#### **Conclusions**

Evidence of stem/progenitor phenotype, together with their lack of immunogenicity, make cells derived from amniotic and chorionic fetal membranes an extremely attractive alternative source of progenitors for cell therapy and tissue engineering.



**STEM CELLS: WHAT FUTURE FOR THERAPY?**  
scientific aspects and bioethical problems

**FRIDAY SEPTEMBER 15<sup>TH</sup>**  
**3:15 p.m.**

**3<sup>rd</sup> SESSION: CLINICAL APPLICATIONS II**



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

**Habib NA, Levicar N, Pai M, Nicholls J, Gordon M**

### ***POSSIBLE USE OF STEM CELLS IN REGENERATIVE MEDICINE***

Department of Surgery and Haematology, Imperial College London, UK

Liver transplantation is the only current therapeutic modality for liver failure, but it is available to only a small proportion of patients due to the shortage of organ donors. Adult stem therapy could solve the problem of degenerative disorders, including liver disease, in which organ transplantation is inappropriate or there is a shortage of organ donors. The view is predicated upon the evidence that stem cells, particularly those in haematopoietic tissue, have the ability to develop into endodermal, mesodermal and ectodermal cell types.

Several sources of stem cells have been proposed as sources for cell therapy. Embryonic stem cells are the most potent, but may be tumorigenic when transplanted *in vivo* and their use is beset by ethical issues. Adult stem cells may be found in any tissues, but haematopoietic tissue is most accessible. From laboratory studies we identified a CD34<sup>+</sup> subpopulation with a morphology associated to that of primitive stem cells. We used this population for a phase I safety and toxicity study of cell therapy in patients with liver disease.

Five patients were recruited with Ethical Committee approval and were administered granulocyte colony stimulating factor for five days to mobilise the CD34<sup>+</sup> cells into the circulation. Following a leukapheresis procedure CD34<sup>+</sup> cells were selected and re-infused into the patient via either the portal vein or hepatic artery. No complications or specific side effects related to the procedure were observed. Three of the five patients showed an improvement in serum bilirubin and four of five in serum albumin. The best two responders had primary sclerosing cholangitis and one of these two has had improved liver function for more than one year.

As these results were encouraging permission was given to initiate a phase I/II clinical study in a larger group of patients with liver disease. Recruitment for this study is ongoing.



**STEM CELLS: WHAT FUTURE FOR THERAPY?  
scientific aspects and bioethical problems**

**De Luca M**

***Epithelial stem cells and regenerative medicine***

(Venice and Modena, Italy)

Abstract not received in time.



## **STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems**

**Sherley JL**

### ***VALIDATING STEM CELL TECHNOLOGIES***

Division of Biological Engineering, Massachusetts Institute of Technology, USA

Whether intentional or unavoidable, misinformation erodes the legitimacy of any public debate. Since the start of the human embryonic stem cell debate, in the United States, misinformation about the nature of human embryos, the availability of human embryos for research, and the potential for using them to develop new medical therapies has been widespread and persistent. Basic facts, well understood by physicians and biologists, have been so misstated and misrepresented in the news media and political speeches that the general public has been put in a state of constant uncertainty. Many people are not sure whether they should be in awe or in fear of new stem cell technologies. Although misinformation is difficult to reform, the solution to the present troubling condition is better education in the form of diligent, honest, and complete scientific disclosure by responsible scientists and physicians; and more care given to accurate reporting by news media. Several key aspects of newly emerging embryonic and non-embryonic stem cell technologies will be defined and discussed as they relate to the debate over the use of human embryos for medical research. An important topic for consideration will be how to disclose with clarity the scientific basis for human embryonic life. Thereafter, failings in proposed technologies for developing new therapies with human embryonic stem cells, that have been grossly under-reported, will be examined. Finally, properties of adult stem cells will be presented in contradistinction to embryonic stem cells, both in terms of adult stem cells as a scientifically better alternative to embryonic stem cells and in terms of the technological challenges that must be overcome to realize the potential of adult stem cells for new medical therapies.



**STEM CELLS: WHAT FUTURE FOR THERAPY?**  
scientific aspects and bioethical problems

**FRIDAY SEPTEMBER 15<sup>TH</sup>**  
**5:10 p.m.**

**4<sup>th</sup> SESSION: ALTERNATIVE PROPOSALS**



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

**Condic ML**

### ***EMBRYONIC STEM CELLS WITHOUT EMBRYOS?***

Salt Lake City, UT, USA

Several proposals have been made regarding possible alternative methods of obtaining pluripotent stem cells without destroying human embryos. One such alternative method has been termed Altered Nuclear Transfer, or ANT. ANT is a broad conceptual proposal, not linked to any specific molecular manipulation. In the most general sense, ANT proposes to alter the DNA of a mature, somatic cell and/or the cytoplasm of an oocyte such that when the somatic nucleus is transferred into an enucleated oocyte, it *does not* form a zygote, but rather forms a different kind of cell all together: A cell that generates pluripotent stem cells *without* being an embryo.

Thus far, two somewhat different methods for achieving the goals of ANT have been suggested; ANT in conjunction with Oocyte Assisted Reprogramming (ANT-OAR) and ANT in conjunction with gene deletion. Both forms of ANT raise the serious question of how the products of an ANT manipulation will be evaluated, specifically; Is it possible to know with confidence that ANT manipulations have indeed generated a non-embryo, rather than merely a damaged or defective embryo?

Here I will address the general question of how embryos are distinguished from both non-embryos and from defective embryos using scientific criteria. I will propose that a necessary and sufficient condition for an entity to be considered an embryo is that it exhibits global coordination of parts for the sake of the entity as a whole. Damaged or disabled embryos show agenesis of parts or malformation of parts against a background of global coordination. In contrast, non-embryos may be capable of generating multiple (or even *all*) cell types of the mature body, and yet fail to integrate these cells into a global program of coordinated development. Thus, global organization is the criteria by which non-embryos are distinguished from defective embryos, regardless of the severity of the defect or the developmental stage at which the defect is first manifest.

This analysis will be applied to the two forms of ANT proposed thus far; ANT-OAR and ANT *via* gene deletion. Specifically, I will address the question of how much (if any) organized behavior can be observed in a entity generated by ANT before the entity must be considered an embryo (albeit, a defective or disabled embryo). The importance of the first globally coordinated event in human development, the segregation of cells into the trophoblast and inner cell mass (ICM) lineages, will be discussed in the context of possible ANT manipulations. I will present recent scientific evidence that suggests trophoblast-ICM formation critically requires an interaction between two transcription factors (Cdx2 and Oct3/4), and consider



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

possible molecular approaches to generating pluripotent stem cells *via* ANT in light of this evidence.

Finally, I will propose that formation of trophoblast-ICM *via* Cdx2-Oct3/4 mutual cross-repression is both the earliest act of the embryo and one of the *definitive, intrinsic powers of the embryo*, such that in the absence of this power, an entity is not and cannot be an embryo. Thus, I will argue, formation of trophoblast-ICM lineages is both necessary and sufficient criterion for distinguishing whether ANT has generated an embryo or a non-embryonic entity.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

Brevini<sup>a</sup> TAL, Tosetti<sup>a</sup> V, Antonini<sup>a</sup> S, Paffoni<sup>b</sup> A, Crestan<sup>a</sup> M, Ragni<sup>b</sup> G, Gandolfi<sup>a</sup> F

### ***ESTABLISHMENT AND CHARACTERIZATION OF PLURIPOTENT CELL LINE FROM HUMAN PARTHENOTES***

<sup>a</sup>Centre for Stem Cell Research, University of Milan, Milan, Italy;

<sup>b</sup>Infertility Unit, Department of Obstetrics, Gynaecology and Neonatology, Ospedale Maggiore Policlinico, Mangiagalli and Regina Elena, Milan, Italy  
(e-mail: [tiziana.brevini@unimi.it](mailto:tiziana.brevini@unimi.it))

Human embryonic stem cells hold exciting promises for research and are of great interest to medicine but, at the same time, raise great ethical concerns since their derivation involves the destruction of a viable embryo. A possible alternative is to derive stem cells from parthenotes, embryo-like structures that can be obtained from eggs without fertilization. The development of mammalian parthenotes is limited but it is long enough to derive stable cell lines, as previously shown in mice and non human primates. Recent experiments in our laboratory were aimed to verify whether human parthenotes could be a source of pluripotent cell lines.

In Italy no more than 3 embryos per cycle can be obtained therefore, in our Unit, patients undergoing an intracytoplasmic sperm injection (ICSI) procedure from whom we retrieve more than 3 good quality oocytes are routinely offered the opportunity to cryopreserve supernumerary eggs. Patients refusing this possibility were offered to participate to the present study. Approval for the study was obtained by the local institution review board and all participating women gave informed consent.

One hour after removal of cumulus cells, oocytes were sequentially exposed to 5  $\mu$ M ionomycin in IVF medium (Vitrolife Sweden AB, Sweden) for 5 minutes at 37 °C, 6% CO<sub>2</sub> in the dark, washed twice and incubated in 2 mM 6-DMAP in medium G1 (Vitrolife Sweden AB, Sweden) for 3 hours at 37 °C, 6% CO<sub>2</sub>. Oocytes were then washed three times in fresh G1 medium, placed separately in 40  $\mu$ l microdrops of the same medium under mineral oil and cultured in the standard conditions (37 °C, 6% CO<sub>2</sub>). Parthenotes were washed twice and kept in culture in fresh medium (G1) for 3 days followed by further 2 days of culture in G2 medium (Vitrolife Sweden AB, Sweden). Blastocyst formation was observed on day 5 (118-120 h post activation).

Inner cell masses were isolated by microsurgical procedures and plated on mitomycin-C inactivated STO mouse fibroblast feeder layers. Cells were cultured in 5% CO<sub>2</sub> at 37°C in low glucose DMEM/F10 nutrient mix, supplemented with 1000 IU/ml of recombinant LIF, 15 ng/ml human recombinant basic FGF, 10% Knockout serum replacer and 5% FBS. Two cell lines have been derived which showed expression of surface markers (SSEA-4, alkaline phosphatase, TRA-1-81,



## **STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems**

TRA-2-54) and transcription factors (Oct-4, Nanog, Rex-1) that are associated with an undifferentiated state and are characteristic of stem cell self-renewal. At present, cells have been cultured for up to 46 passages and both lines survived freeze and thaw cycles.

Under appropriate conditions these cells formed embryoid bodies and differentiated into derivatives of all three germ layers as indicated by immunohistochemical and/or RT-PCR screening for interferon- $\gamma$ , BMP-4, neurofilament-H,  $\alpha$ -amilase 2,  $\beta$ -tubulin III, cytokeratin-17, desmin, and vimentin. Moreover when cells were cultured in specific medium, the formation of different cell types of the neural lineage was observed.

Our data show that it is possible to derive cell lines from human parthenotes that display many characteristics of biparental embryonic stem cells.

The ability of these cells to form teratomas is currently being tested by injection into the rear leg muscle of SCID beige male mice.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

**Takahashi K, Yamanaka S**

### ***IDENTIFICATION OF FACTORS THAT GENERATE PLURIPOTENT STEM CELLS FROM FIBROBLAST CULTURE***

Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan

Embryonic stem (ES) cells, which are derived from the inner cell mass of mammalian blastocysts, have the ability to grow indefinitely while maintaining pluripotency, the ability to differentiate into cells of all three germ layers. These properties have raised the hope that ES cells might be used to treat a host of degenerative diseases, such as Parkinson's disease, spinal cord injury, and diabetes. However, clinical application of human ES cells faces ethical difficulties regarding use of human embryos, as well as the problem of tissue rejection following implantation in patients. One way to circumvent these issues is the generation of pluripotent cells directly from somatic cells. A critical step toward this goal is the identification of factors that are capable of converting somatic cells back into an embryonic state.

We hypothesized that factors that played important roles in the maintenance of pluripotency of ES cells also played pivotal roles in induction of nuclear reprogramming. Long-term maintenance of pluripotency in ES cells requires transcription factors specifically expressed in pluripotent cells (e.g. Oct3/4, Sox2 and Nanog), and activation of tumor-related genes that are widely expressed. These factors are good candidates for reprogramming factors. To examine the reprogramming activity of the candidates, we have developed a system in which nuclear reprogramming can be detected as marker gene expression. In this system, we utilized Fbx15 that is specifically expressed in ES cells and early embryos, but is dispensable for self-renewal of ES cells and development. We inserted the  $\beta$ geo cassette (a fusion of  $\beta$ -galactosidase and the neomycin resistant gene) into the mouse Fbx15 genes by homologous recombination. Fbx15 is specifically expressed in mouse ES cells and early embryos, but is dispensable for the maintenance of pluripotency and mouse development. ES cells homozygous for  $\beta$ geo knock-in (Fbx15 $\beta$ geo/ $\beta$ geo) were resistant to an extremely high concentration of G418 (up to 12 mg/ml), whereas somatic cells derived from Fbx15 $\beta$ geo/ $\beta$ geo mice were sensitive to the selection. We expected that even partial reprogramming of somatic cells would result in resistance to G418 of normal concentration (0.3 mg/ml).

We introduced the candidate genes into Fbx15 $\beta$ geo/ $\beta$ geo mouse embryonic fibroblasts (MEF) by retrovirus-mediated transfection and culture them in ES cell medium containing G418. With any single factor, we did not obtain G418-resistance colonies. However, by combining several factors, we obtained multiple G418-



## **STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems**

resistant colonies. These cells showed morphology and proliferation similar to ES cells. Furthermore, when transplanted into nude mice, these ES-like cells produced teratomas containing various tissues of the three germ layers. These data demonstrated that pluripotent cells can be generated from MEF culture with a few defined factors.



**STEM CELLS: WHAT FUTURE FOR THERAPY?**  
scientific aspects and bioethical problems

**FRIDAY SEPTEMBER 15<sup>TH</sup>**  
**6:45 p.m.**

**FREE COMMUNICATIONS (Session 3)**



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

Hess DC 1,2; Yasuhara T 2; Hara K 2; Maki M 2; Matsukawa N 2; Yu G 2; Xu L 2; Mays RW 3; Deans RJ 3; Carroll JE 1,2; Borlongan CV 1,2

### ***MINIMALLY INVASIVE INTRAVENOUS DELIVERY OF HUMAN BONE MARROW-DERIVED MULTIPOTENT ADULT PROGENITOR CELLS LEADS TO ENGRAFTMENT AND HOST CELL LOSS REDUCTION IN THE ISCHEMIC BRAIN, AND STABLE BEHAVIORAL RECOVERY IN EXPERIMENTAL STROKE***

1 Department of Neurology, Medical College of Georgia, Augusta, GA, USA; 2 Research & Affiliations Service Line, Augusta VAMC, GA, USA; 3 Regenerative Medicine, Athersys, Inc., Cleveland, OH, USA  
(e-mail: [dhess@mail.mcg.edu](mailto:dhess@mail.mcg.edu))

#### **Introduction**

In an effort to determine the clinical potential of transplanting human bone marrow-derived multipotent adult progenitor cells (MAPC) in ischemic stroke, in the present study we examined the efficacy of intravenous administration of 1 million MAPC at different time points after stroke injury. MAPC represent a well characterized and GMP-processed stem cell type providing a clinical grade and scalable off-the-shelf product.

#### **Methods**

Adult Sprague-Dawley rats received ligation of the distal middle cerebral artery, and at day 1, 2 or 7 underwent intravenous (IV) infusion of 1 million MAPC. A control group of stroke animals received non-viable MAPC IV at day 7 after ischemic injury. The animals did not receive immunosuppression. Performance in routine stroke behavioral tests was monitored up to 56 days post-transplantation, thereafter histological analysis of grafts was performed.

#### **Results**

MAPC transplantation, regardless of timing at 1, 2 or 7 days post injury, reduced stroke-induced motor and neurological impairments compared to ischemic adult animals that received the control grafts, which was apparent as early as 7 days post-transplantation and sustained up to 56 days post-transplantation. Preliminary histological data revealed that the peripherally delivered MAPC successfully migrated into the ischemic penumbra. Although graft survival was 1%-2% consisting mostly of undifferentiated MAPC, the cell loss in the ischemic penumbra was significantly reduced by the grafts, especially when transplantation was initiated at day 1 post-stroke. In addition, cultured MAPC secrete VEGF within reported therapeutically effective dose range.



## **STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems**

### **Conclusion**

The minimally invasive IV transplantation of MAPC led to stable behavioral recovery in ischemic stroke animals. The synergistic effects of graft survival and trophic factor secretion possibly mediated this therapeutic outcome. The extended therapeutic window with MAPC transplantation, as compared to the current 3-hour tPA treatment, is a significant clinical advance in stroke therapy and supports our goal of proceeding with allogeneic intravenous transplantation of MAPC in patients that suffer ischemic stroke.

Supported by Athersys, Cleveland, Ohio, USA



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

Quaini F <sup>\*</sup>, Fagnoni F <sup>¥</sup>, Frati C <sup>\$</sup>, Graiani G <sup>\$</sup>, Cavalli S <sup>\$</sup>, Lagrasta C <sup>\$</sup>,  
Lazzaretti M <sup>\$</sup>, Quaini E <sup>#</sup>, Musso E <sup>§</sup>, Squarcia U <sup>#</sup>

### ***SEARCHING FOR DIFFERENT SOURCES OF ADULT STEM CELLS TO REPAIR THE HEART: THE CARIOGENIC POTENTIAL OF HUMAN MYOCARDIAL AND BONE MARROW STROMAL CELLS***

\*Dept of Internal Medicine, \$ Pathology, § Physiology, #Pediatric Cardiology, °Cardiac Stem Cell Center (CISTAC)- University of Parma; #Dept of Cardiac Surgery, Poliambulanza, Brescia; ¥ Experimental Oncology, S. Maugeri Foundation, Pavia  
(e-mail: [federico.quaini@unipr.it](mailto:federico.quaini@unipr.it))

The bone marrow is the predominant regenerative approach to reconstitute the damaged myocardium by means of adult stem cells. However, the heart possesses a resident population of stem cells that may represent an efficient therapeutic strategy. Aim of the present study was to isolate human myocardial cells and to compare their in vitro biological properties with marrow stromal cells. For this purpose, on patients undergoing cardiac surgery, cell cultures from myocardial fragments (H) and from the bone marrow (BM) were concomitantly obtained. Although the stem cell antigen c-kit was present in 1% of both cultures, nearly 80% of H cells were committed to cardiogenic fate. By FACS analysis and immunocytochemistry the mesenchymal phenotype of cultured H and BM cells was apparent by a similar expression of surface antigens. When receptors for HGF, VEGF and EGF were evaluated, cells positive for c-met, Flk1 and c-erbB2, respectively were found in both cultures. The differentiation throughout the three major myocardial compartments was present in both cell cultures in serum free condition and after dexamethasone exposure. However, H cells were more capable to generate cardiomyocytes whereas formation of vascular cells was higher in BM cells. Moreover, 1:1 co-cultures of female H and male BM cells enhanced the differentiating ability of both cultures as by FISH cardiomyocytes, endothelial cells and smooth muscle cells carrying XX or XY chromosomes were quantified. In summary, resident cardiac progenitor and BM cells possess different and synergistic cardiogenic potential both representing a suitable autologous cell therapy to repair the heart.



**STEM CELLS: WHAT FUTURE FOR THERAPY?**  
scientific aspects and bioethical problems

**SATURDAY SEPTEMBER 16<sup>TH</sup>**  
**8:15 a.m.**

**5<sup>th</sup> SESSION:**  
**ANTHROPOLOGICAL AND ETHICAL ASPECTS**



**STEM CELLS: WHAT FUTURE FOR THERAPY?  
scientific aspects and bioethical problems**

**Carrasco I**

***General ethical principles on the use of “adult” stem cells***

(Valencia, Spain)

Abstract not received in time.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

**Faggioni M**

### ***Anthropological-ethical reflections on production and use of “embryonic” stem cells***

Bioethics Professor, Member of the Academy for Life, Rome, Italy

There are several sources of stem cells for research and for therapy: from adult and foetal organisms, from umbilical cord blood, from early stage embryos. The biological characteristics and the potentialities of these kinds of stem cells are partly different and are the object of an intense study on the part of scientists. From the point of view of ethics the most difficult problems come from embryo-deriving stem cells.

At the basis of everything is the problem of the value of the life of the early stage human embryo and of the respect to which it is entitled. It is not acceptable to deliberately destroy an embryo for a scope that may be good, nor is it decent to produce human embryos for research and therapy, even if the procedures involved do not encompass their destruction. The same respect must be given to embryos created for non reproductive cloning.

In many countries there is an ethical and political debate under way on the use of embryos derived from artificial fecundation techniques, be they surplus embryos or non transferable embryos. No matter what we think of the production outside the body of embryos and of their freezing, there is the problem of what thousands of cryoconserved embryos which are being abandoned will become. Once we exclude any cooperation to their production, we may set forth the hypothesis that they are used as a source of stem cells, as long as we can make sure with rigorous criteria that they are not vital, which is different from being not installed and from the impossibility of a complete development.

A situation creating several ethical dilemmas concerns the morals of the use of stem cells legally produced in some countries and exported to other countries where the production of surplus embryos or embryo manipulation are prohibited. Since it is known that their production involved the destruction of embryos, we are asking ourselves whether the use of those stem cells is not a form of unlawful cooperation.

Trying to overcome the ethical obstacles, studies are being carried out to try and obtain cells with the desired characteristics without having to manipulate and destroy human embryos.

In the midst of sensationalistic news which were then proved wrong and put back in their right perspective, some proposals seem technically feasible and morally acceptable, or, at least, they deserve to be honestly taken into consideration.

Some researchers were able to obtain parthenotes from human eggs and to derive stem cells from them. The discussion on the nature of the parthenote from the biological and ethical point of view is under way: some think that the parthenote is a real embryo since it develops as normal embryos; others maintain that the parthenote, since it doesn't have the potentiality to develop beyond the first stages, is to be considered a pseudo-embryo.

Other groups proposed the transfer in enucleated oocytes of altered nuclei (ANT) through the silencing of some genes. A variant proposes we should manipulate the oocyte which



## **STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems**

will receive the transferred nucleus (OAR). As in the above case, we ask ourselves if the biological entity thus produced would be radically different from a human embryo obtained by cloning with normal nuclei transfer or with the transfer in non reprogrammed oocytes.

Recently some researchers managed to reprogram adult mouse fibroblasts transferring in them, with retroviral transduction, specific factors. In this way we can have embryo type pluripotent stem cells without passing through the formation of embryos.

All the above eventually leads to the wider question of the relationship between the reasons of science and the reasons of ethics and questions us on the role and aims of science within a complex society.



**STEM CELLS: WHAT FUTURE FOR THERAPY?  
scientific aspects and bioethical problems**

**POSTERS**



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

**Bosch Barrera J (1,2,3), Villarreal MA (2,3,4), Argemí Ballbé JA (2,3,5)**

### ***UNDERGRADUATE BIOETHICAL TRAINING ON STEM CELLS AT UNIVERSITY***

- (1) Clínica Universitaria de Navarra, University of Navarra, Pamplona, Spain;
- (2) ABEM, Associació Bioètica d'Estudiants de Medicina, Barcelona, Spain; ACEB,
- (3) Associació Catalana d'Estudis Bioètics, Barcelona, Spain;
- (4) Hospital Universitari Germans Trias i Pujol, Badalona; Spain;
- (5) Università Campus Biomedico di Roma, Italy

#### **Purpose**

To report our experience in promoting education on Bioethics among undergraduate medical students focussing on issues like the stem cells debate.

#### **Methods**

A course on Bioethics ("Human Dignity and Medical Practice") was organized by the bioethical society of medical students at the University of Barcelona (ABEM) under the sponsorship of ACEB. This was a non-compulsory subject offered to all undergraduate medical students since 2003 on. Each lecture consisted of two parts: first, a topic was covered by the professor during 45 minutes; after that, students were encouraged to debate the issue in an argumentative way. Finally, opinions were summarized and a set of conclusions was listed. A bioethical website was created and maintained by the students (<http://www.infoabem.org>).

#### **Results**

More than 200 medical students have attended the lectures during the four editions. Courses have been highly scored among students. Many participants joined ABEM and helped to set up the future courses and to update the website. 87% were very grateful to this 2-credit course. The students referred a significant increase in their knowledge on scientific and ethical problems on regenerative medicine.

#### **Conclusions**

New ethical questions are emerging and the undergraduate students demand a more comprehensive and accurate answers. Our experience shows the efficacy of a direct implication of medical students in the process of the design and execution of bioethical education. This particular experience can be useful to other university settings. More extensive education on bioethical and scientific problems of stem cells is needed at Medical Schools.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

Baylis F\*, Mélançon MJ\*\*

### **CANADIAN GUIDELINES FOR HUMAN EMBRYONIC STEM CELL RESEARCH: SHIFTING TIDES FROM FROZEN TO FRESH EMBRYOS**

\*Dalhousie University, Halifax, Nova Scotia, Canada;

\*\*Université du Québec à Chicoutimi, Québec, Canada

In 2002, the Canadian Institutes of Health Research (CIHR) released its guidelines *Human Pluripotent Stem Cell Research: Guidelines for CIHR Funded Research* (<http://www.cihr-irsc.gc.ca/e/28216.html>). Since then, the *Guidelines* have twice been amended – 2005 (<http://www.cihr-irsc.gc.ca/e/28216.html>); 2006 (<http://www.cihr-irsc.gc.ca/e/31488.html>).

The 2002 *Guidelines* limited embryonic stem cell research to embryos remaining after infertility treatment and it was assumed that the research would involve unused **frozen** embryos no longer wanted for transfer in a subsequent cycle. With the *Updated Guidelines* there is explicit permission to use **fresh** embryos for research.

This presentation by members of the *Ad hoc* Working Group responsible for the 2002 *Guidelines*, reviews the ethical arguments against the research use of **fresh** healthy embryos, as contrasted with the research use of **frozen** embryos or fresh embryos not eligible for transfer or freezing.

Donating fresh healthy embryos to stem cell research,

- (i) **is not in the interest of women** as this potentially increases the physical, psychological, social and economic harms associated with IVF.<sup>1</sup>
- (ii) **undermines free and informed choice**, as most women and couples with frozen embryos designated for future research typically change their mind (71%).<sup>2</sup> Time for “sober second thought” is eliminated.
- (iii) **creates an incentive for stem cell researchers to pressure IVF clinicians** into making extra embryos so that some will be available for research; and



**STEM CELLS: WHAT FUTURE FOR THERAPY?  
scientific aspects and bioethical problems**

- (iv) **increases the risk of coercion and exploitation** of women<sup>3</sup> especially if IVF clinicians (in turn) pressure women to create more embryos than will be transferred or frozen.<sup>4</sup>

These arguments clearly warrant a return to the 2002 Guidelines (as happened in 2006 with the rules for informed consent) (<http://www.cihr-irsc.gc.ca/e/31594.html>)



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

### Caso C

#### ***BEYOND STEM CELLS: EXPERIENCE OF A GENERAL PRACTICIONER***

G.P. and member of the Ethical Committee ASL Salerno, Italy  
(e-mail: [caso.corrado@libero.it](mailto:caso.corrado@libero.it))

As a general practitioner, I often wonder about the induced expectations placed upon people like my patients from scientific research declaring with great sensationalism that everything is achievable. At the same time, I am daily confronted with hagiographic research that claims that the right to use any kind of tool, even the eventual suppression of the fetus, in order to clone organs and apparatus depriving the fetus of its right to life. Sometimes, two-faced research on one hand way communicated via mass media, legitimating through reason general interest, at the same time suspect of being covert and mysterious. Together, seek to in this blatant conflict, to exercise instrumental social influencing power regarding life and its presumed universal value. Medicine unfortunately as lost, due to the explosion in the last twenty years of the access of information, its primary focus that of concentrating on man. Here, We are dealing with apposing fronts, often in contrast seeking to persuade the legitimation of cloning. More and more experts are trying to rehabilitate research that besides determining finding risk factors, could eventually lead to the total redesigning of life as we know it. More importantly, this power, in the hands of a few, would risk modifying genetics and familiarity forever, in the hope of "improving" life. Given this reality, the centrality of man in medicine would be sacrificed. That is, this devastating research would eliminate what it means to be man: values.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

Ferreira AT, Oshiro MEM, Paredes-Gamero EJ

### ***ROLE OF INTRACELULAR $Ca^{2+}$ IN CYTOKINE SIGNALING IN MICE LONG-TERM BONE MARROW CULTURES***

UNIFESP-EPM, São Paulo-SP, Brazil  
(e-mail: [alice@biofis.epm.br](mailto:alice@biofis.epm.br))

#### **Purpose**

The aim of this work was to determine the role of intracellular  $Ca^{2+}$  ( $[Ca^{2+}]_i$ ) in the proliferation and differentiation of primitive hematopoietic cells when they are stimulated by cytokines in mice long-term bone marrow cultures (LTBMC).

#### **Methods**

LTBMC of mice C57BL6 were used. To measure  $[Ca^{2+}]_i$  in cobblestone areas the fluophore fluo-3 and the confocal microscopy (CM) were used.

Differentiation of hematopoietic cells was measured by flow cytometry.

#### **Results**

Stromal and hematopoietic cells were responsive to the cytokines (interleukins: IL-3, IL-6, IL-7; colony stimulate factors: G-CSF, M-CSF, GM-CSF, stem cell factor and erythropoietin). Cytokines promoted localized  $[Ca^{2+}]_i$  increases, cellular migration  $Ca^{2+}$  wave through the gap-junctions (GJ); the presence of connexin-43 was shown by CM in LTBMC. The GJ blocker, carboxolone (30  $\mu$ M), inhibited  $Ca^{2+}$  increase IL-3 and GM-CSF dependent. IL-3 (50 ng/ml) and GM-CSF (50 ng/ml) promoted increase of active proteins PLC, PKC and CaMKII.

Blockers of  $Ca^{2+}$ -signaling such as PLC inhibitors (U73122 e Neomicine), IP<sub>3</sub> receptor inhibitor (2APB), CaMKII inhibitor (KN620 and  $Ca^{2+}$  chelator (EGTA) were able to decrease proliferation when stimulated by IL-3 and GM-CSF. However, these blockers were not able to inhibit the differentiation since the c-kit e Sca1 expressions decreased.

#### **Conclusion**

Our data suggest the redundancy of  $Ca^{2+}$ -signaling in cytokines pathway, and the participation of GJ in this process.

The  $Ca^{2+}$  play an important role in proliferation, but not in the differentiation of LTBMC when were stimulated with IL-3 or GM-CSF.

This study was supported by FAPESP.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

**Gérman Zurriarán R**

### ***EMBRYONIC STEM CELLS: BIOMEDICAL ETHICS AND ECONOMIC INTERESTS***

University of La Rioja, Logroño, Spain

#### **Purpose**

Ethical judgment is intrinsic to biomedical research as a human activity. The production of embryos and research with embryonic stem cells cannot be justified for the sake of a "scientific and technological progress" that leaves all ethics a side. The enormous "curative" potential of this research with human embryos is transmitted to the public through strong media pressure, due to economic and commercial interests of important biotechnological companies.

#### **Methods**

This paper is developed from a descriptive and interdisciplinary study of key ethical concepts in this subject.

#### **Results**

1. Research with human embryos (which implies their death) cannot be based on ethical intentions.
2. Economic interests play a role in this type of research.
3. The lack of accuracy and truthfulness regarding the "therapeutic achievements" of embryonic stem cell research points to the manipulation of this information.

#### **Conclusions**

1. The technique employed to obtain embryonic cells, which in turn are used to treat diseases, requires the death of human embryos. Therefore, such research cannot claim to have ethical intentions. It does not pursue a therapeutic aim for the embryo. On the contrary, it results in its death.
2. The commercialisation of human embryos is nothing other than the final expression of the "logic of production" to which human beings are subjected from the moment they are created in artificial insemination.



**STEM CELLS: WHAT FUTURE FOR THERAPY?  
scientific aspects and bioethical problems**

**Huarte J\*§, Lang M §, Suarez A §**

***EMBRYO-FREE METHODS TO GENERATE HUMAN PLURIPOTENT STEM CELLS, AND GENOMIC ANOMALIES DIRECTLY INHIBITING THE APPEARANCE OF NEURAL ACTIVITY (DIANA ANOMALIES)***

\*§ Corresponding member of the Pontifical Academy for Life. University of Geneva Medical School, Geneva, Switzerland

§ Swiss Society of Bioethics, + The Institute for Interdisciplinary Studies, Zürich, Switzerland

(e-mail: [joachim.huarte@medecine.unige.ch](mailto:joachim.huarte@medecine.unige.ch); [suarez@leman.ch](mailto:suarez@leman.ch); [mlang@access.unizh.ch](mailto:mlang@access.unizh.ch))

Ethical concerns are stimulating the search for alternative methods to obtain pluripotent stem cells, without destroying human embryos. The supporters of these methods stress the importance of ensuring that the biological entities used in these alternative methods are not "disabled or sick human embryos". In this article we argue that biological entities bearing anomalies or alterations that directly inhibit the appearance of neural activity (DIANA anomalies) share the moral status of human organisms fulfilling the clinical criteria of brain death. By contrast, in absence of DIANA anomalies one cannot deny the moral status of a person.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

**Kukura JW, \*Friedman G**

### ***PROMOTING CORD BLOOD DONATION***

Catholic HealthCare Partnership of New Jersey, Princeton, NJ,  
\*New Jersey Stem Cell Research & Education Foundation, Livingston, NJ, USA  
(e-mail: [kukuraj@chcpnj.org](mailto:kukuraj@chcpnj.org) and [stemcellmd@hotmail.com](mailto:stemcellmd@hotmail.com))

#### **Purpose**

To create a common ground of dialogue among government, clinical, and religious leaders and to state clearly the Catholic Church's endorsement of adult stem cell usage and research.

#### **Method**

The following case study:

The Catholic HealthCare Partnership of New Jersey (representing 14 Catholic hospitals) and the New Jersey Catholic Conference (representing the 5 dioceses of NJ) publicly committed the Catholic hospitals of the state and the resources of the dioceses to support and facilitate the donation of umbilical cord and placenta blood to the state's public storage program. The secular and religious media reporting of this public commitment (see attached) has led to an accurate understanding of the Catholic Church's endorsement of adult stem cell usage in present clinical practice and adult stem cell research which shows great promise. At the same time, interest in the public commitment has provided the opportunity to present Catholic opposition to embryonic stem cell research based on the Church's theological teaching and clinical evidence of the dangers involved in following the embryonic pathway (e.g. the development of tumors).

#### **Results**

Catholic leaders are now welcomed into friendly dialogue and cooperation as the future of stem cell research in New Jersey is forged. Catholic faithful have a new understanding of the Church's teaching in regard.

#### **Conclusions**

A common ground of dialogue has been created. Catholic hospitals are seen as actively supportive of stem cell research as they encourage and support public donation of umbilical cord and placenta blood. Catholics see their Church (through the Catholic hospitals) actively involved in stem cell research (adult) which has already born good clinical results and has bright future promise.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

Lucena E, Lucena, C, Andersson K, Esteban C, Mojica S, Englund M, Lucena M, Davila A, Emanuelsson K, Hyllner J

### ***ESTABLISHMENT AND CHARACTERIZATION OF THE FIRST LATIN AMERICAN EMBRYONIC STEM CELL LINE***

Fertility and Sterility Colombian Center – CECOLFES Calle; Bogotá, Colombia.  
South America  
(e-mail: [cecolfes@colomsat.net.co](mailto:cecolfes@colomsat.net.co))

#### **Purpose**

The Colombian IVF center, Cecolfes has together with Cellartis AB established the first human embryonic stem (hES) cell line in Latin America.

#### **Materials and Methods**

The hES cell line has been derived from IVF surplus blastocysts with informed donor consent. Donated embryos were cultured to the age of 5-7 days at the Cecolfes laboratory.

Immunosurgery was performed as described in the mouse and humans.

Blastocysts that had not hatched and with intact zona pellucidae were treated in Pronase (Sigma-Aldrich:1mg/ml). All treated blastocysts were placed on a layer of mytomicin inactivated mouse embryos fibroblasts (MEFs) with hESC medium for six days. The expanded colonies were mechanically dispersed into small clumps, and cultured on fresh MEF layers. hESCs colonies were passaged by mechanical dissociation and removed to fresh feeder layers.

#### **Results**

A total of 43 embryos were cultured. Of these, 30 reached the blastocyst stage at 5-6 days (69.7%). Most of them were graded 3AA, according to Gardner's classification.

One cell line was obtained, cecol-14. Immunocharacterization was performed on undifferentiated colonies with commonly used markers indicative of pluripotent hESC, Oct-4, SSEA1, SSEA-3, SSEA-4, TRA-1-60, and TRA-1-81.

A normal chromosomal set up for chromosomes X, Y, 13,18, and 21 was shown with FISH analysed with Vysis multivision.

Pluripotency tests in vitro and in vivo are in progress.

#### **Conclusion**

Taken together the results indicate Cecol-14 to be normal pluripotent hESC line. A bank of the Latin American cell lines has been prepared at Cecolfes.



**STEM CELLS: WHAT FUTURE FOR THERAPY?  
scientific aspects and bioethical problems**

**NOTE OF THE ORGANIZERS:**

The abstract by Lucena and coworkers, is published here as a free communication, but does not reflect at all the ethical views of the organizers of this Congress.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

**Markeljevic J**

### ***THE DIGNITY OF HUMAN LIFE AND STEM CELL RESEARCH***

University Hospital Rebro, Department of Internal Medicine, Clinical Centre Zagreb, Croatia

(e-mail: [jasenka@mef.hr](mailto:jasenka@mef.hr))

#### **Purpose**

The dignity of human life and controversies dealing with adult versus embryonic stem cell research (SCR) in relation to the Message of John Paul II for the 2001 World Day of Patients, whose theme was the “civilisation of love” in this millennium, as well as in connection with Benedict XVI’s Encyclical “God is love”, are analysed.

#### **Methods**

In order to draw attention to the domination of ethical relativism in the health care system today, religious, cultural and economic features of the society; power of bio-politics; scientific praxis created by the cost/benefit dynamics; language engineering; ethical, moral principles and the professional and scientific achievements of all the participants within the health system are considered.

#### **Results**

Ethical aspects of adult versus embryonic SCR are analysed within the context of “culture life” and “culture of death”; holistic versus mechanicalistic and «to be» versus «to have» point of view.

#### **Conclusion**

In order to change the dominant paradigm of our civilisation based on the ethical relativism, the dignity of life should be absolutely protected. It can be done on two complementary levels. On the individual level, concentrating on the personal engagement of all the participants in the health care system and society, as well as on the promotion of bioethical aspects of SCR considering the dignity of life. On the level of political, social and educational institutions, it should concentrate on the development of sensibility, knowledge and responsibility of people in charge of evolution of consciousness about human values.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

Mélançon MJ\*, Baylis F\*\*

### **CANADIAN UPDATED GUIDELINES FOR HUMAN PLURIPOTENT STEM CELL RESEARCH (JUNE 28, 2006): CONTINUITIES AND DISCONTINUITIES**

\*Université du Québec à Chicoutimi, Québec, Canada;

\*\*Dalhousie University, Halifax, Nova Scotia, Canada

Canada is a democratic and pluralistic country with people from various cultural and religious backgrounds. This diversity informs policy-making in the area of stem cell research. In March 2002, the Canadian Institutes of Health Research (CIHR) released its *Guidelines Human Pluripotent Stem Cell Research: Guidelines for CIHR Funded Research* (March 4, 2002), twice amended by the Stem Cell Oversight Committee on June 7, 2005 and June 28, 2006 (<http://www.cihr-irsc.gc.ca/e/31488.html>). In between, on March 29, 2004, the *Assisted Human Reproduction Act* received Royal Assent; this legislation also constrains embryo research in Canada.

#### **Purpose**

In this presentation, we outline: (1) the guiding ethical principles in the CIHR *Guidelines*; (2) the areas of research consistent with these principles; and (3) the substantive differences between the 2002 and 2006 *Guidelines*.

#### **Methods**

A comparative method was used to identify the differences between the 2002 and 2006 *Guidelines*.

#### **Results**

While there are differences in wording, there is significant continuity between the CIHR policy documents and agreement with the national legislation. For example, the creation of research embryos is prohibited in all versions of the *Guidelines* and in law, as is human cloning. There are, however, a few interesting discontinuities that warrant special attention. For example, the 2005 Update explicitly endorses the use of fresh embryos for research, where previously the focus was on frozen embryos.\* The 2006 Update introduces a new guiding principle, that of “cultural integrity”.

#### **Conclusion**

In this fast-paced complex area of science it is important to track policy-changes and their underlying ethical justifications.

\* (See Baylis’s & Mélançon’s other presentation)



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

Perrella G 1, Brusini P 2, Spelat R 1, Hossain P 3, Hopkinson A 4, Dua HS 4

### ***EXPRESSION OF HAEMATOPOIETIC STEM CELL MARKERS, CD133 AND CD34 ON HUMAN CORNEAL KERATOCYTES***

1. Department of Experimental and Clinical Pathology and Medicine University of Udine, Italy,
2. Department of Ophthalmology–General.Hospital of Udine, Italy,
3. Department of Ophthalmology, University of Southampton, UK,
4. Division of Ophthalmology and Visual Sciences, Larry A Donoso Laboratory for Eye research, University of Nottingham, UK  
(e-mail: [perrella@uniud.it](mailto:perrella@uniud.it))

#### **Purpose**

To study the expression of CD133 and CD34 antigens on cultured human keratocytes over time.

#### **Methods**

Primary cultures of human corneal cells were obtained from explants derived from cadaver eye donors. The cultures were sorted for CD133+ and CD34+ cells using magnetic beads. Both the primary cultures and secondary passages of sorted cells were analyzed by flow cytometry for expression of the same antigens over time. Growth curves with and without EGF and Western blot analysis for PCNA were also carried out.

#### **Results**

Times to confluence of primary and secondary CD34 sorted cells are related to donor sex, age and to one another. Percentage of CD34+ and CD133+ cells decreases with increasing time to confluence in PHC and with increasing duration of growth in sorted cultures. The CD133 sorted culture is composed by two subpopulations: CD133+/CD34+ and CD133+/CD34-. Following growth of the sorted populations, the ratio between total CD133+ cells and CD133+/CD34+ remains constant. Cultured cells showed a significantly higher growth following EGF stimulation. PCNA was present in both CD34 positive and negative cells.

#### **Conclusion**

Human corneal keratocytes express the haematopoietic stem cell markers CD133 and CD34. This expression decreases with time in culture with the majority but not all cells losing expression. Based on these markers, the corneal stroma reveals a heterogeneous population of cells. Expression or downregulation of expression of these molecules could represent different stages in the differentiation/function of these cells.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

Pisu S<sup>i</sup>, Caocci G<sup>ii</sup>, Fadda G<sup>iii</sup>, Castello G<sup>iv</sup>

### **REFLECTIONS ABOUT RELATIONSHIPS BETWEEN STEM CELLS AND EMBRYONIC INDIVIDUALITY**

<sup>i</sup>Professore di Bioetica – Università di Cagliari;

<sup>ii</sup> Centro Trapianti Midollo Osseo – Ospedale Binaghi – ASL 8 – Cagliari;

<sup>iii</sup> Servizio Immunoematologia – Azienda Ospedaliera “Brotzu” – Cagliari,

<sup>iv</sup> Clinica Urologica – Università di Cagliari  
(Cagliari, Italy)

For many centuries a halo of darkness has surrounded the mystery of the human life beginning in the maternal womb. Only recently, bio-medical technologies have been allowing a progressively and clarifying light on the human organism since its beginning. Is it possible to assign to a microscopic creature the same status of an already born child? Is the embryo a human individual, a person? In order to answer to these relevant questions, the bioethics and the law cannot ignore latest acquirements of stem cells research. The term “precocious embryo” designates the first stage of human organism development that starts from the fertilization until the fourteenth day. In this earlier stage, some researchers would not talk about embryo or “precocious embryo” because the absence of a phenotypic expression in spite of a human genotype. They would define this stage as a “pre-embryo”: a cellular pile not organized in human form, a biological material also useful for experimentations, without moral restrictions. Furthermore each of embryonic cells, at the beginning of their development, have the possibility to become a whole organism or a particular tissue of the body. Then it is very hard to spike about individuality and identity at this stage of development. Instead, we believe that, if the most important characteristics to define a person are individuality and identity. Then, to better understand which is the true nature of the “precocious embryo”, it is necessary to find a biological definition of the term individuality. An organism can remain an individual with its identity in spite of being split; the identity of an organism remains itself, undergoing not only the different phases of development but also all the continuous cellular and biochemical changes that incessantly act on the same identical body. Embryonic totipotent stem cells are only in potency a whole organism: they could become individuals capable of life and self-regulation, only after an eventual separation from the embryonal body. Moreover, recent findings on the somatic mapping of the egg cell have shown that at the level of the zygote already exists an animal pole and a vegetative pole, and that the first division splits these poles in two sister cells. Thus, the study of this early differentiation process could shine on a new, but maybe, not unexpected event: a constant and continuous determination of each embryonal and post-embryonal cellular element, that could allow to recognize the subsistence of a new human being in the single cell embryo.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

**Planinc-Peraica A**

### ***ETHICAL PROBLEMS IN BONE MARROW TRANSPLANTATION IN ADULT PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES***

University Hospital "Merkur", Zagreb, Croatia  
(e-mail: [danijel.planinc@zg.htnet.hr](mailto:danijel.planinc@zg.htnet.hr))

In the field of bone marrow and peripheral blood stem cell transplantation ethical problems are less pronounced than in solid organ transplantation. Bone marrow and peripheral stem cell transplantation can be undertaken in patients with lymphoproliferative disease (non-Hodgkin's lymphoma, Hodgkin's disease, multiple myeloma) in first remission or later depending on the nature of haematological neoplastic disease.

The purpose of this work is to review the literature on this topic and to discuss own experiences. Ethical problems may arise during the treatment of patients who are candidates for transplantation of autologous bone marrow/peripheral stem cells (PBSCT) or allogeneic bone marrow (ABMT). Candidates for autologous PBSCT are usually highly motivated for this treatment believing it crucial to be cured. But what to do when the patient is in bad physical condition that impedes this procedure?

Other problems arise in ABMT. Sometimes one sibling doesn't want to be bone marrow donor. In such situation the dilemma is whether perform HLA-match testing or not?

The review of literature on ethical issues in bone marrow and peripheral blood stem cell transplantation showed that in contrast to high number of bone marrow transplantation performed each year in Europe and all round the world, the number of articles on this topic is relatively small. The great number of these articles deals with umbilical cord blood and bone marrow transplantation in children, and possible risk of G-CSF stimulation in healthy donor of peripheral stem cells.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

Sipr K

### ***CZECH PARADOX: SUCCESSFUL ADULT STEM CELL THERAPY AND THE RECENT PASSING OF THE EMBRYONIC STEM CELL LAW***

Palacky University Faculty of Medicine, Olomouc, Czech Republic

Research in adult stem cell use is being carried out at three University research institutions in the Czech Republic, namely, Faculties of Medicine in Prague, Hradec Kralove and Brno. At the First Medical Department of the University Hospital in Brno-Pekarska, adult stem cells gained from the bone medulla are given to patients with myocardial infarction (autolog transplantation). Proven improvement in the function of the myocardium has been achieved and the treatment has been shown to be quite safe (Meluzin J et al. *Cor et Vasa* 2004;46:537-543).

On the other hand, the Parliament of the Czech Republic approved a bill in April 2006 allowing experiments on human embryos, in spite of the warnings of a group of Czech scientists-university teachers and against the written protest expressed in a petition supported with more than 70.000 signatures of Czech citizens. The debates in Parliament were preceded by a vigorous support for the bill by the mass media. Unfortunately, statements of those media which maintain a positive attitude towards the Catholic Church were hesitant and issued late. Regrettably, the first Czech human embryonic stem cell lines were established even before the bill was passed (*praeter legem*). "Spare" frozen human embryos produced by in vitro fertilization were used. In the stage of expanded blast cysts, embryo blasts (inner cell masses) were isolated with the help of immunosurgery and placed on a feeder layer of mouse embryonic fibroblast. From the total number of 98 embryos used, seven human embryonic stem cell lines were derived. Progressive karyotypic changes were noticed in one of them (*Scripta Bioethica* 2004; 4:66).

The Czech experience confirms an inadequate knowledge of the moral aspects of the human stem cells issue both in the general public and amongst believers, including catholic journalists.



## STEM CELLS: WHAT FUTURE FOR THERAPY? scientific aspects and bioethical problems

**Suwancharas T**

### ***BIOETHICS ISSUES IN AUTOLOGOUS STEM CELL RENAL TRANSPLANTATION IN THAILAND***

Faculty of Medicine, Srinakharinwirot University, Thailand  
(e-mail: [th\\_suwancharas@yahoo.com](mailto:th_suwancharas@yahoo.com), [stdave@hotmail.com](mailto:stdave@hotmail.com))

Stem cells are noted for their ability to self-renew and differentiate into a variety of cell types. Some stem cells, described as totipotent cells, have tremendous capacity to self-renew and differentiate. Embryonic stem cells have pluripotent capacity, able to form tissues of all 3 germ layers but unable to form an entire live being. Research with embryonic stem cells has enabled investigators to make substantial gains in developmental biology, therapeutic tissue engineering, and reproductive cloning. However, with these remarkable opportunities many ethical challenges arise, which are largely based on concerns for safety, efficacy, resource allocation, and methods of harvesting stem cells. The purpose of this research is to discuss the moral and legal status of human autologous stem cell renal transplantation in Thailand. Religious perspectives and political events leading to regulation of stem cell research are also presented and discussed, with special attention directed toward the use of embryonic stem cells for therapeutic cloning. The methods for gathering data were qualitative technique using focus group interview in stakeholders. The results showed both pro and con opinions for autologous stem cell renal transplantation. In conclusion Thai medical scientists, practicing clinicians and bioethicists have to inform both pro and con information to the society continuously and let it formulate the final solution by itself.



**STEM CELLS: WHAT FUTURE FOR THERAPY?  
scientific aspects and bioethical problems**

**AUTHORS' INDEX**

Baylis F	page 61, 71
Brevini TAL	page 47
Bosch Barrera J	page 60
Carrasco I	page 56
Caso C	page 63
Condic ML	page 45
De Luca M	page 42
Di Pietro ML	page 18
Doerflinger RM	page 21
Faggioni M	page 57
Ferreira AT	page 64
Forraz N	page 38
Gérman Zurriarán R	page 65
Habib NA	page 41
Hess DC	page 25, 52
Huarte J	page 66
Huriet C	page 13
Kukura JW	page 67
Lima C	page 27
Lucena E	page 68
Mackay –Sim A	page 29
Mancuso S	page 34
Markeljevic J	page 70
Mauceri J	page 15
McGuckin CP	page 32
Mélançon MJ	page 61, 71
Neri D	page 20
Parolini O	page 39
Perrella G	page 72
Pisu S	page 73
Planinc-Peraica A	page 74
Prentice DA	page 10
Quaini F	page 54
Sherley JL	page 43
Silburn P	page 12
Silva F	page 19
Sipr K	page 75
Sotomayor G	page 37
Strauer BE	page 23
Suwancharas T	page 76
Takahashi K	page 49



**STEM CELLS: WHAT FUTURE FOR THERAPY?  
scientific aspects and bioethical problems**

Vescovi A  
Vout B  
Yamanaka

page 11  
page 16  
page 49



**STEM CELLS: WHAT FUTURE FOR THERAPY?**  
scientific aspects and bioethical problems