



RoFAR

Foundation for Anemia Research

Cell and gene therapy with erythropoietin-secreting marrow stromal cells for renal failure

Nicoletta Eliopoulos

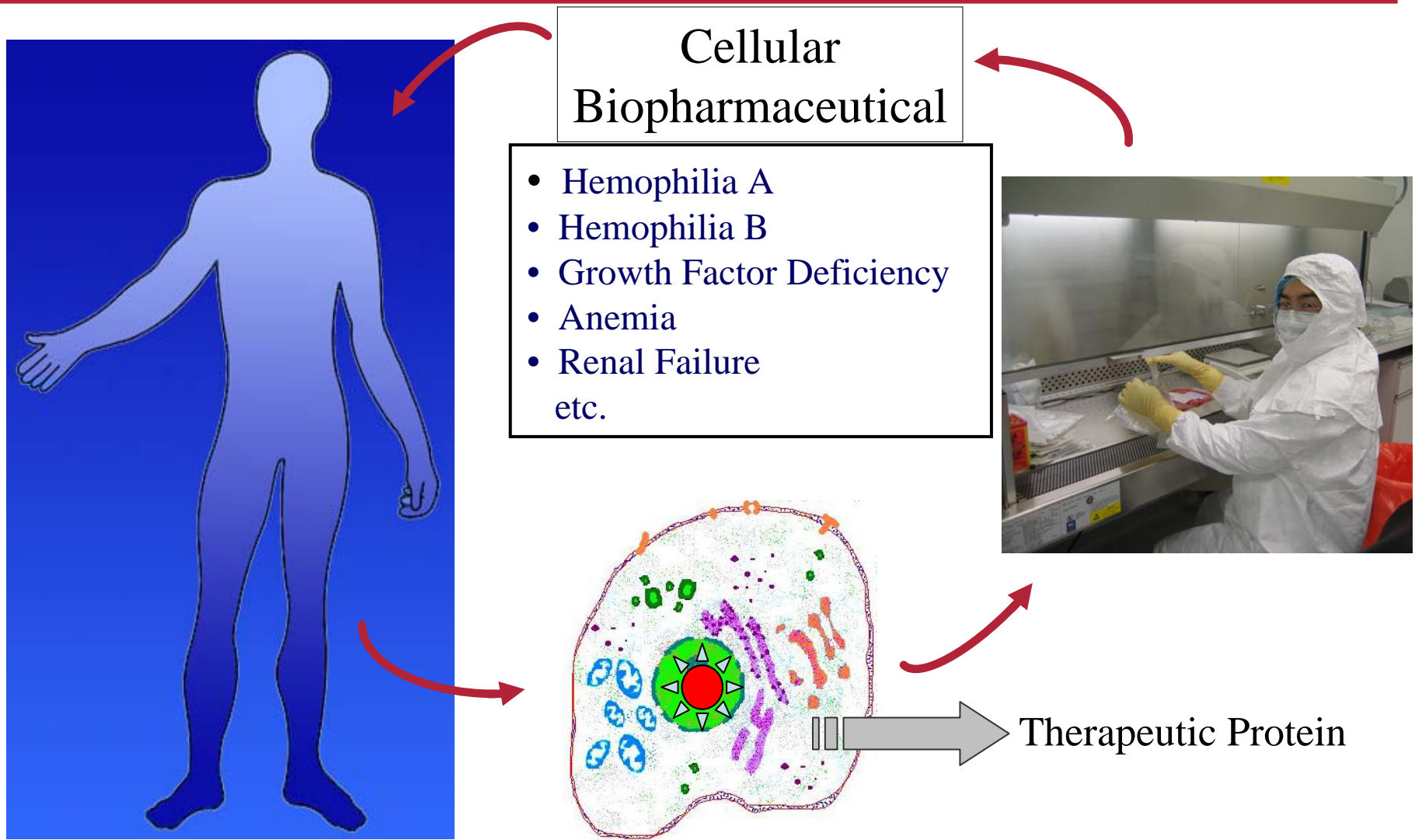
Lady Davis Institute for Medical Research
Montreal, Quebec, Canada



World Congress of
Nephrology

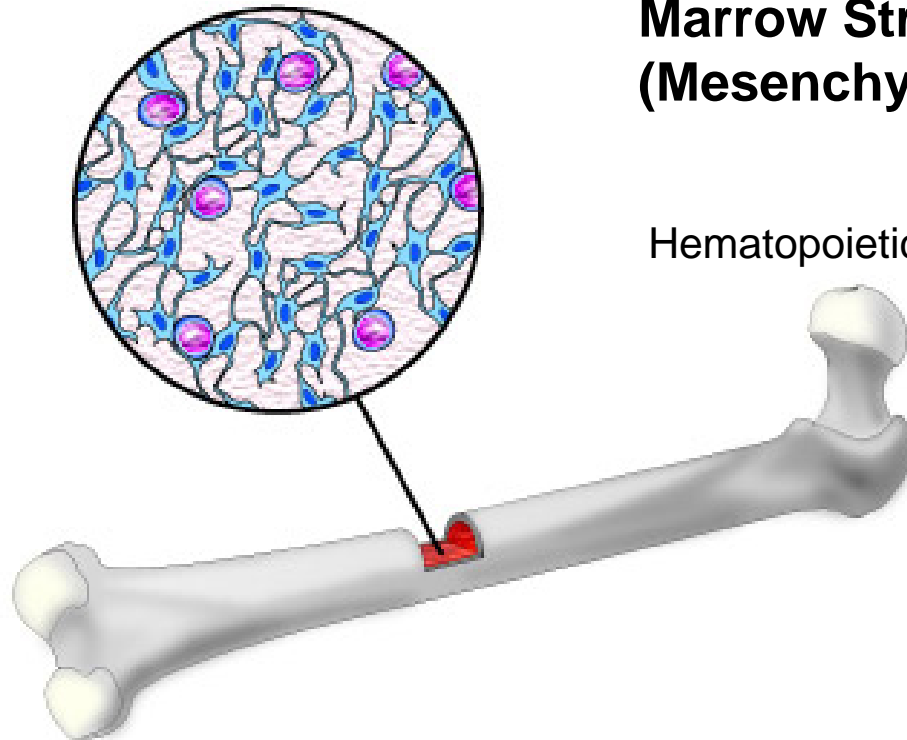
Rio de Janeiro
24 April 2007

Cell and Gene Therapy at a Glance



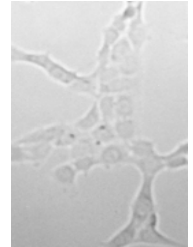


Can Genetically-Engineered Bone Marrow Stromal Cells Serve as a Cellular Platform for Delivery of Therapeutic Proteins?



**Marrow Stromal Cell (MSC)
(Mesenchymal Stem Cell)**

Hematopoietic Stem Cell

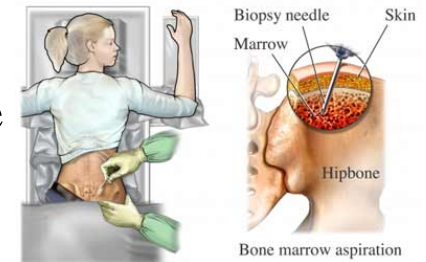


(www.kumc.edu/stemcell/mature.html)

Bone Marrow Stromal Cells as the Ideal “Cellular Vehicle”



- Readily harvested from patients by a simple outpatient procedure



(<http://services.epnet.com/GetImage.aspx/getImage.aspx?ImageID=5018>)



(http://www.bdbiosciences.com/image_library/flask_family.jpg)

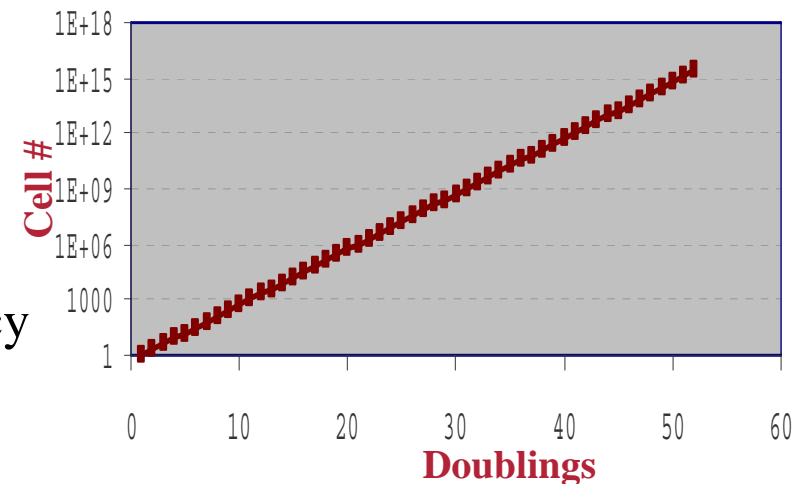
Bone marrow aspirate placed in tissue culture:

Free-floating Hematopoietic Cells

Adherent Fibroblast-like Cells: Marrow Stromal Cells (MSCs)

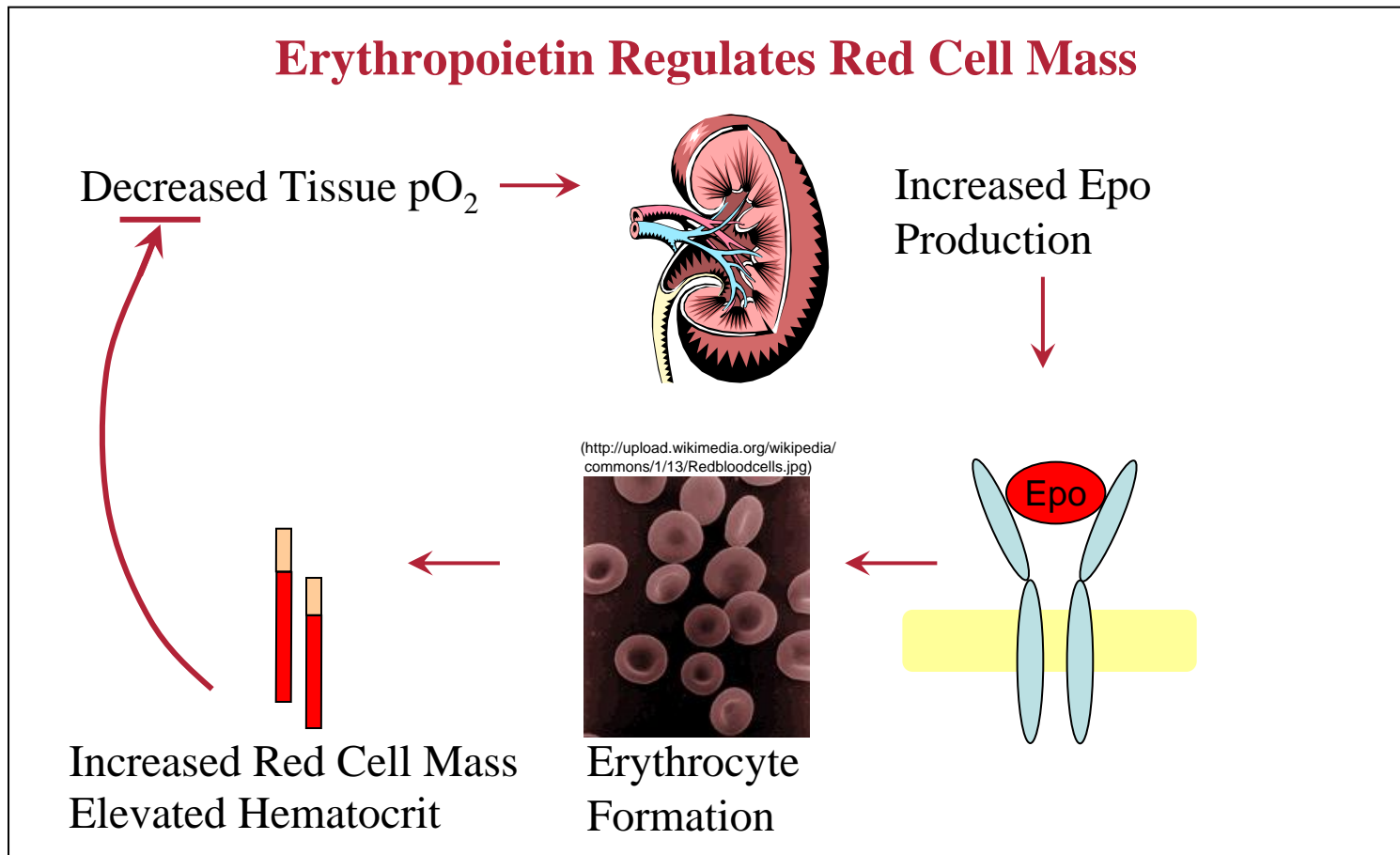


- Abundant in humans of all age groups
- Number expandable *in vitro* for >52 doublings
- Genetically-engineered with reasonable efficiency

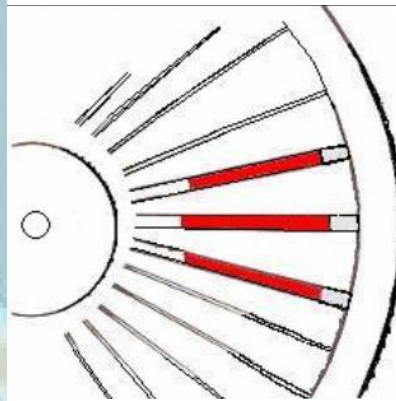
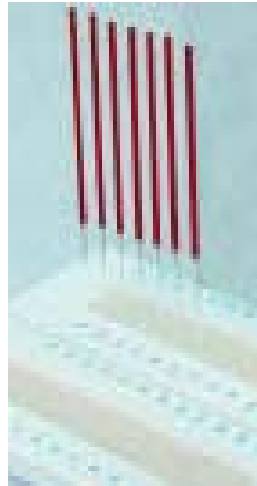


Erythropoietin Delivery by Genetically-Engineered MSCs

Use of Erythropoietin (Epo) for gene modification of MSCs for a Proof-of-Principle, with the expectation that MSCs will behave similarly for the delivery of any recombinant plasma protein.



Hematocrit Determination

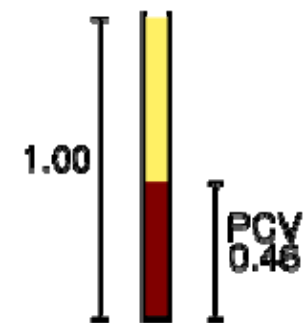
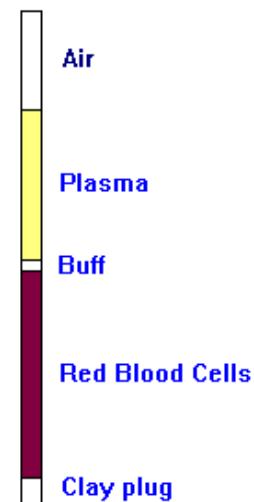


Normal Hematocrit Range

42-50% in men

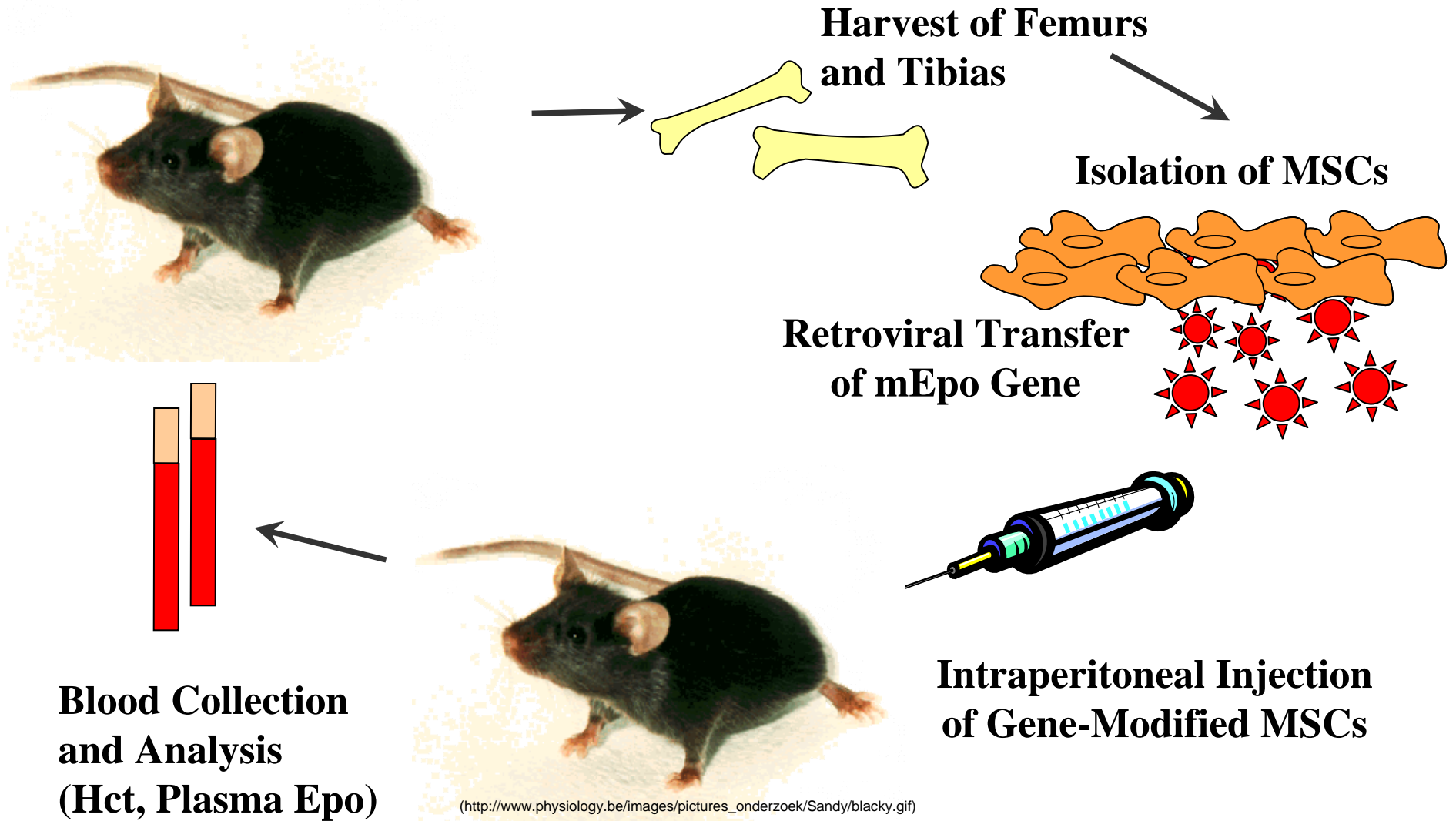
39-48% in women

50-56% in C57Bl/6 mice

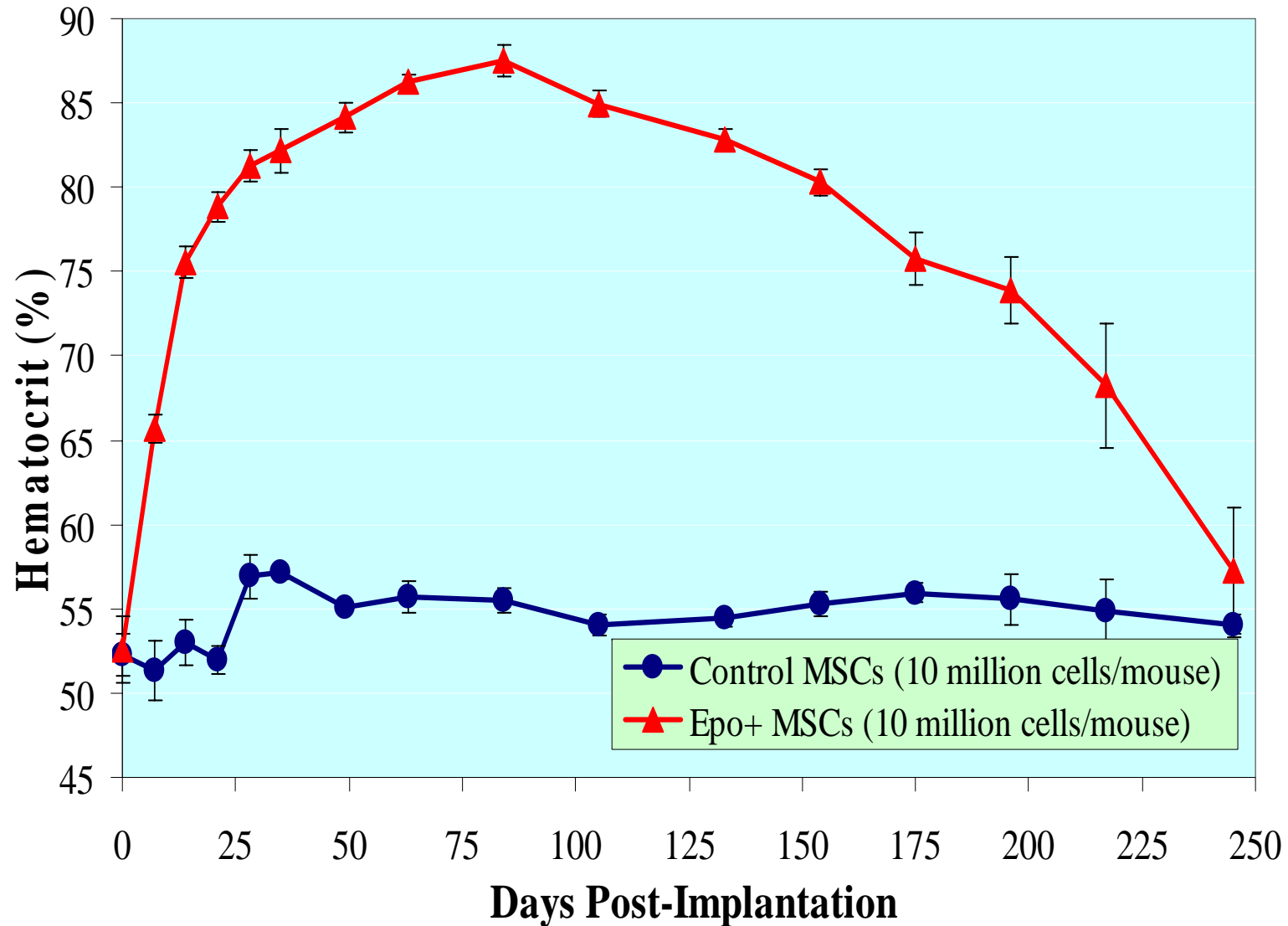


(http://www.medaille.edu/vmacer/120_lab_rodent3_saphenous2.jpg; <http://faculty.southwest.tn.edu/williams/hemato1.gif>; http://static.flickr.com/48/189921011_b0ab89880b_o.jpg; <http://en.wikipedia.org/wiki/Hematocrit>; http://faculty.washington.edu/kepeter/119/images/hematocrit_tube_trio.jpg)

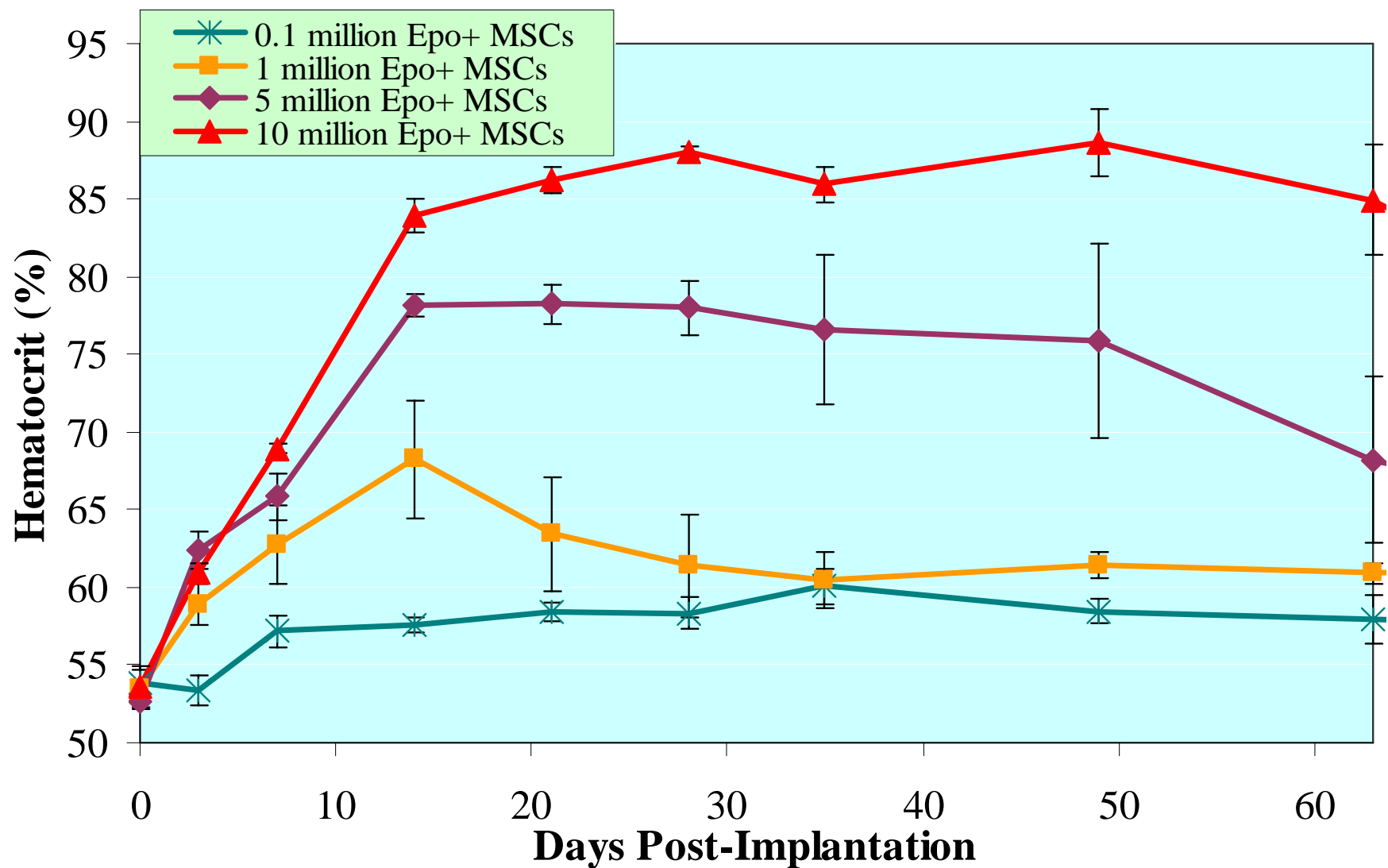
Experimental Outline



Increased Hematocrit in Mice with Intraperitoneal Epo⁺ MSCs



Dose-Dependent Increased Hematocrit in Mice with Intraperitoneal Epo+ MSCs



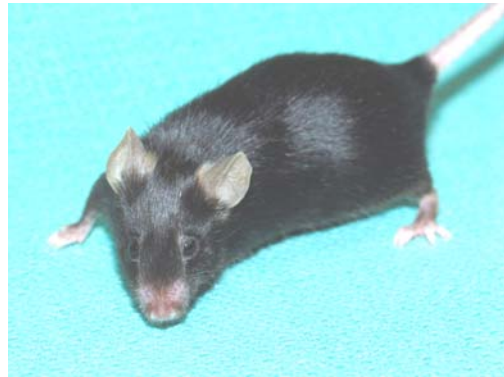
**Can a Human-Biocompatible Substance
Serve to Embed Genetically-Engineered MSCs
and Lead to a Pharmacological Effect Following
Subcutaneous Implantation?**



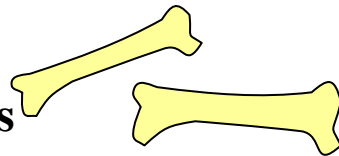
+

Contigen™

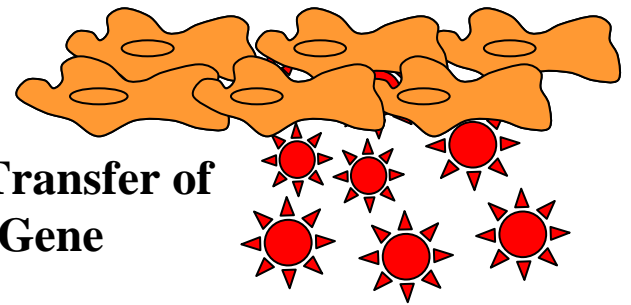
Experimental Outline



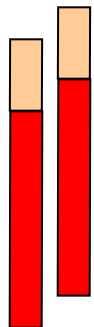
Harvest of
Femurs and Tibias



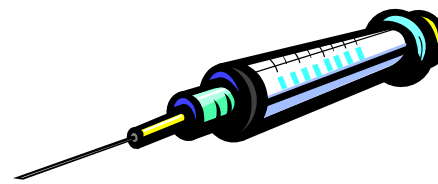
Isolation of MSCs



Retroviral Transfer of
mEpo Gene



Blood Collection



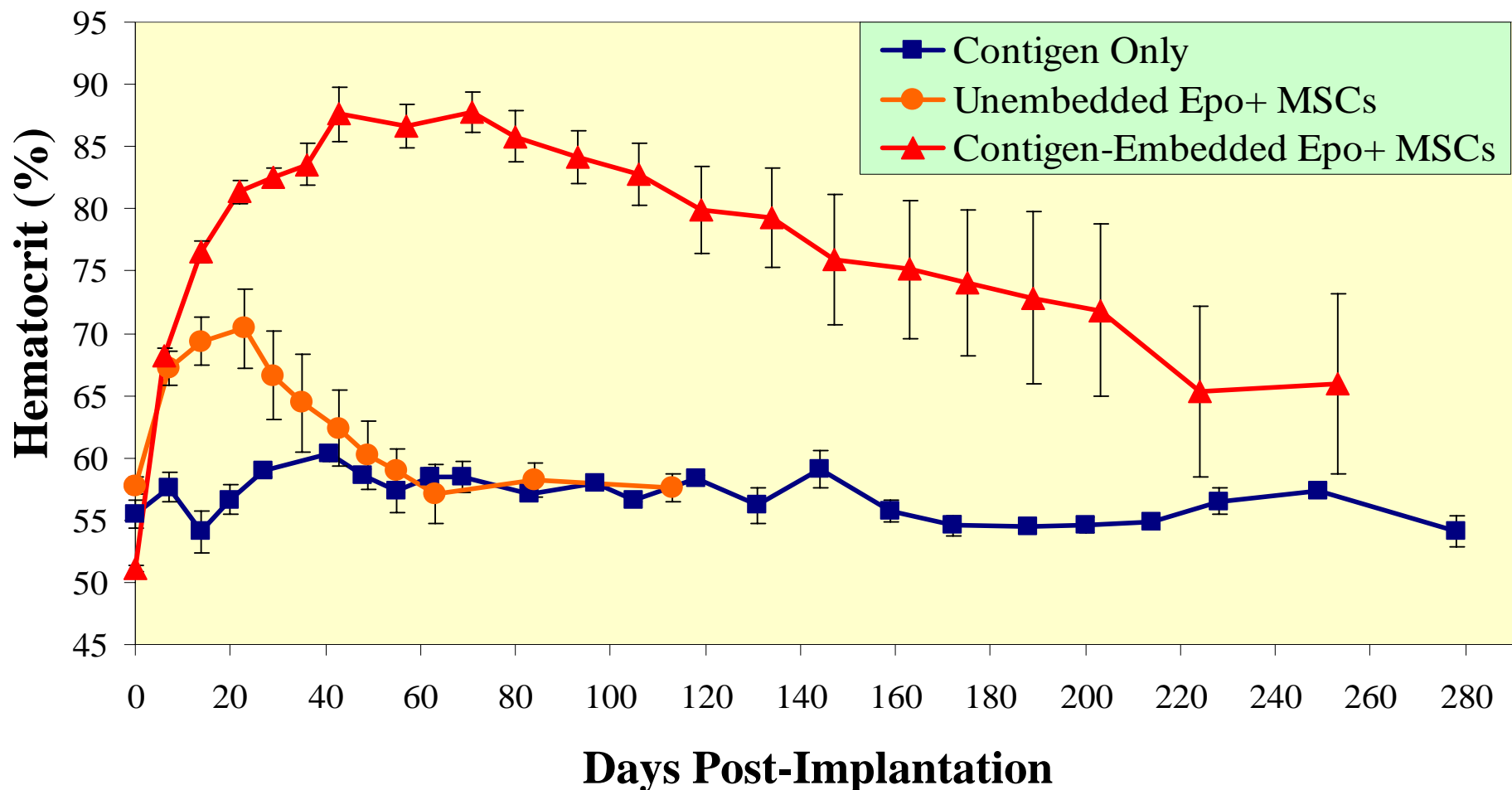
Subcutaneous
Injection
of Contigen™
Embedded MSCs

+

Contigen™



Higher and More Sustained Rise in Hematocrit in Mice with Subcutaneous Implant of Contigen™-Embedded Epo⁺ MSCs



Retrievability Safety Feature of Neo-Organoid Approach

Surgical Implant Retrieval Process

Mouse with Contigen Implant Anesthetized by Isoflurane Inhalation for Surgery



Skin Incision Exposing Contigen Implant



Beginning of Retrieval of Contigen Implant



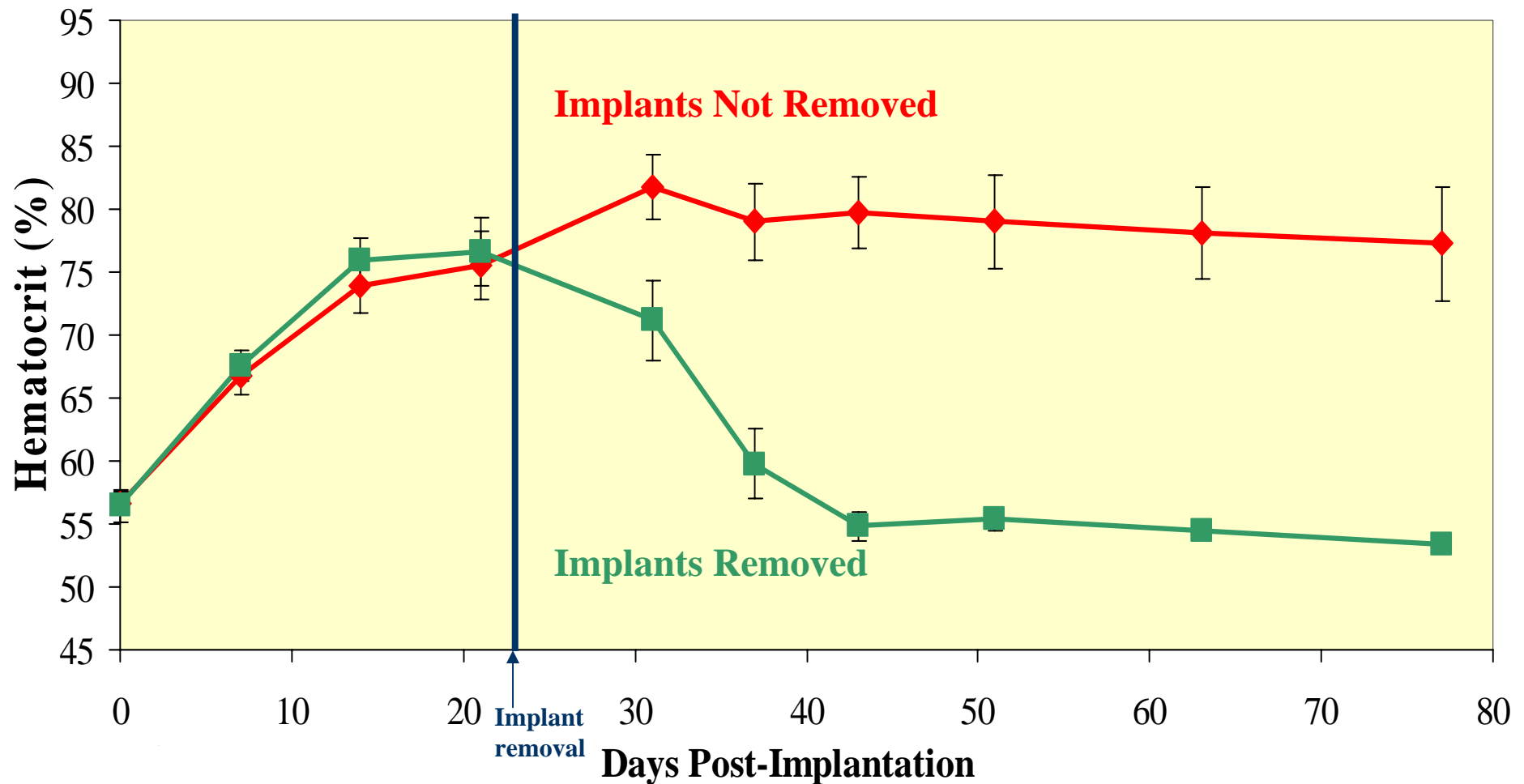
Post-Removal of Contigen Implant

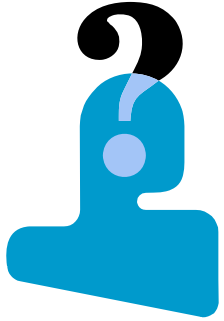


Completion of Metal Clip Placing



Reversal of Effect on Hematocrit Following Removal of Implant of Contigen™-Embedded Epo⁺ MSCs





**Can Epo Gene-Modified
Marrow Stromal Cells
Serve for the Treatment of Anemia
of End-Stage Renal Disease?**

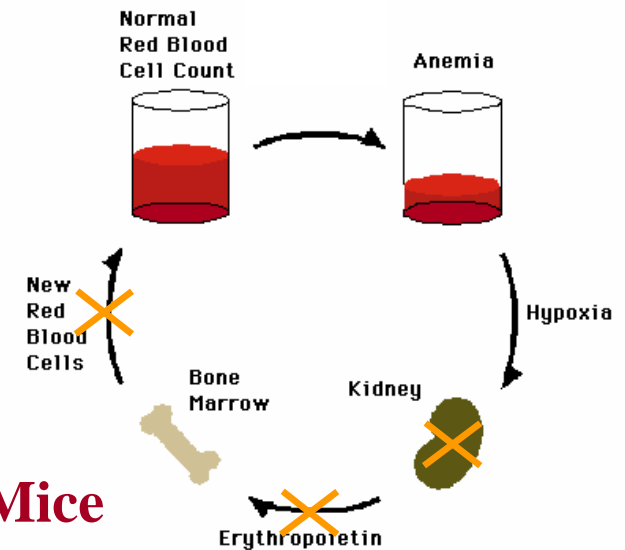


Renal Anemia

Caused by insufficient Epo production by diseased kidneys

Commonly treated with recombinant human Epo (rHuEpo)

Main disadvantages associated with use of rHuEpo



(<http://sickle.bwh.harvard.edu/hctchart.gif>)

Induction of Chronic Renal Failure in C57Bl/6 Mice

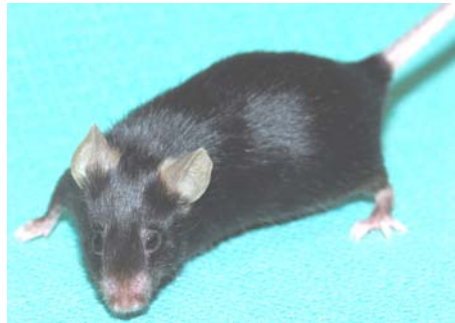
Methodology

- I. thermal injury (electrocoagulation) of surface of right kidney (day 0)
- II. contralateral nephrectomy (day 13-14)

Consequences of poor remaining kidney function

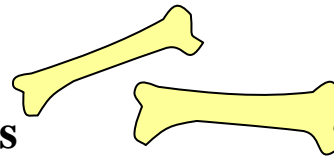
- Renal failure → Elevated blood urea nitrogen levels (BUN)
Anemia → Decreased hematocrit

Experimental Outline



NORMAL MOUSE

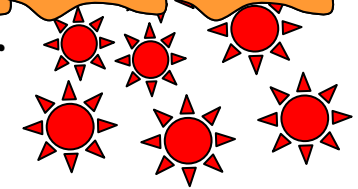
**Harvest of
Femurs and Tibias**



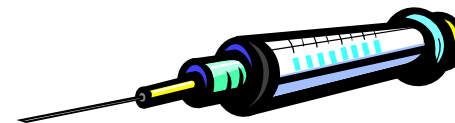
Isolation of MSCs



**Retroviral Transfer
of mEpo Gene**



Contigen™

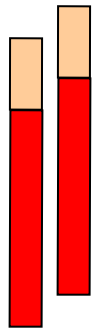


**Subcutaneous Injection of
Contigen™-Embedded MSCs**

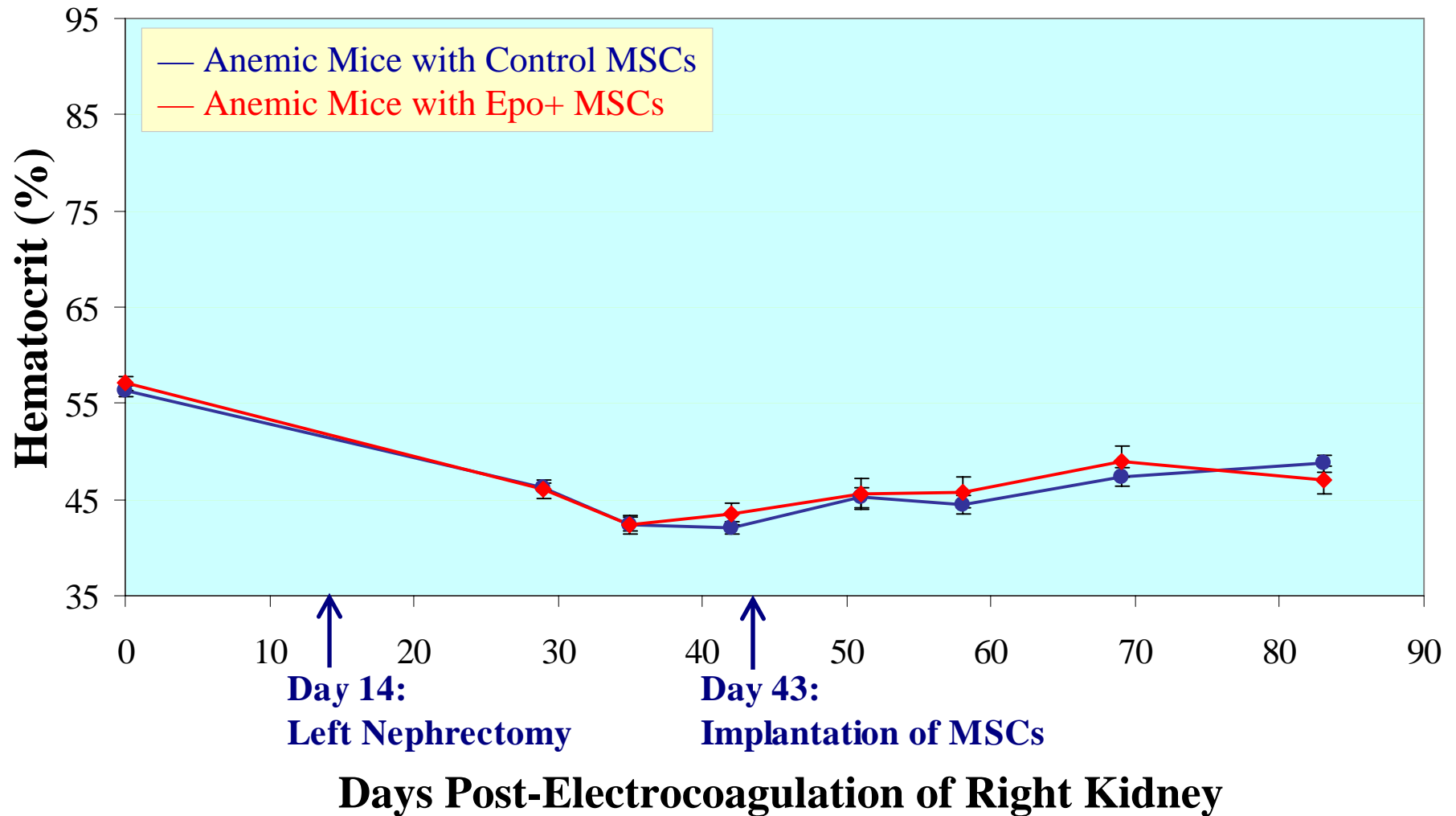


**ANEMIC MOUSE
Post Electrocoagulation
of Right Kidney &
Post Left Nephrectomy**

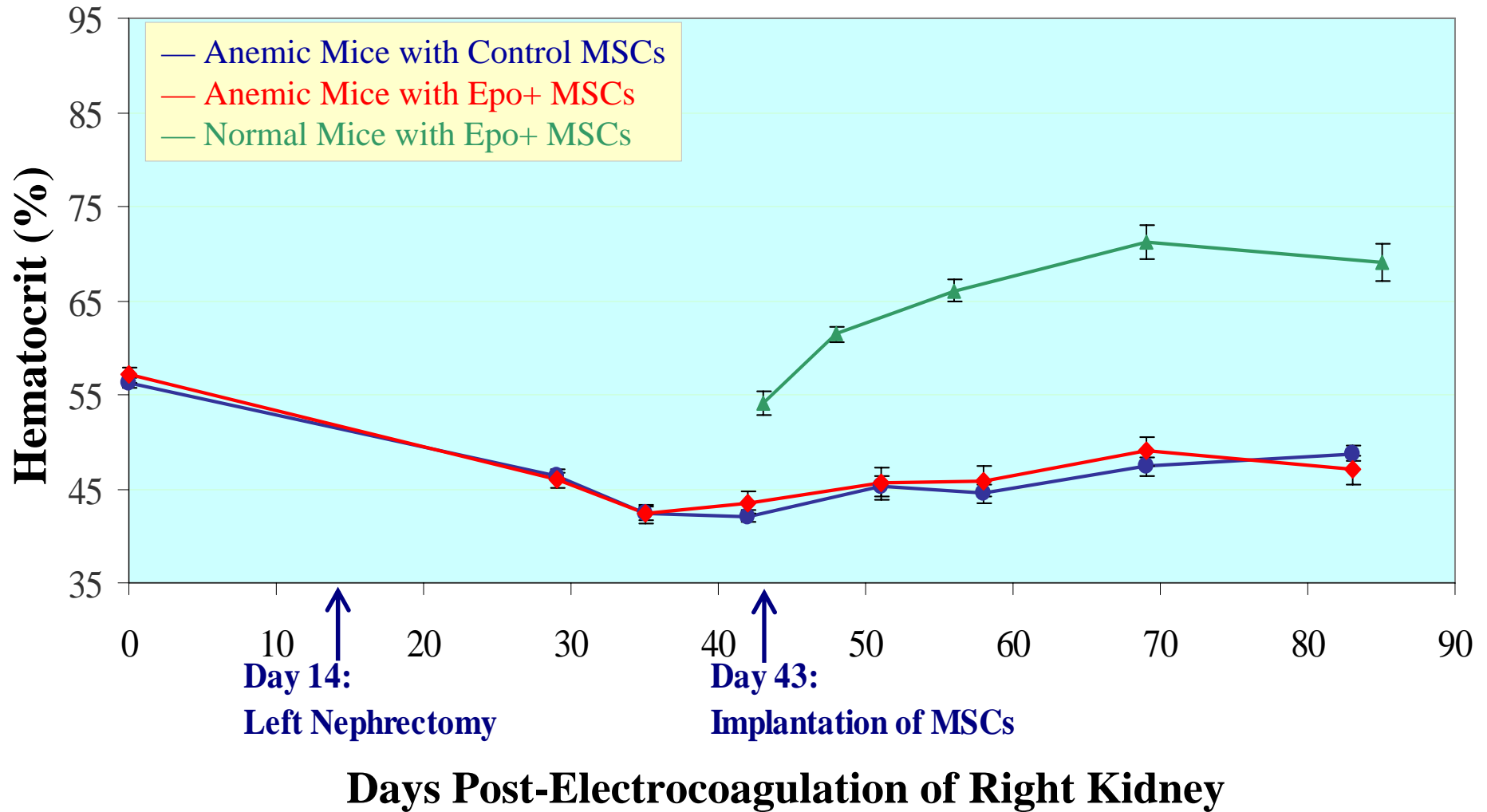
Blood Analysis:
Urea
Hematocrit



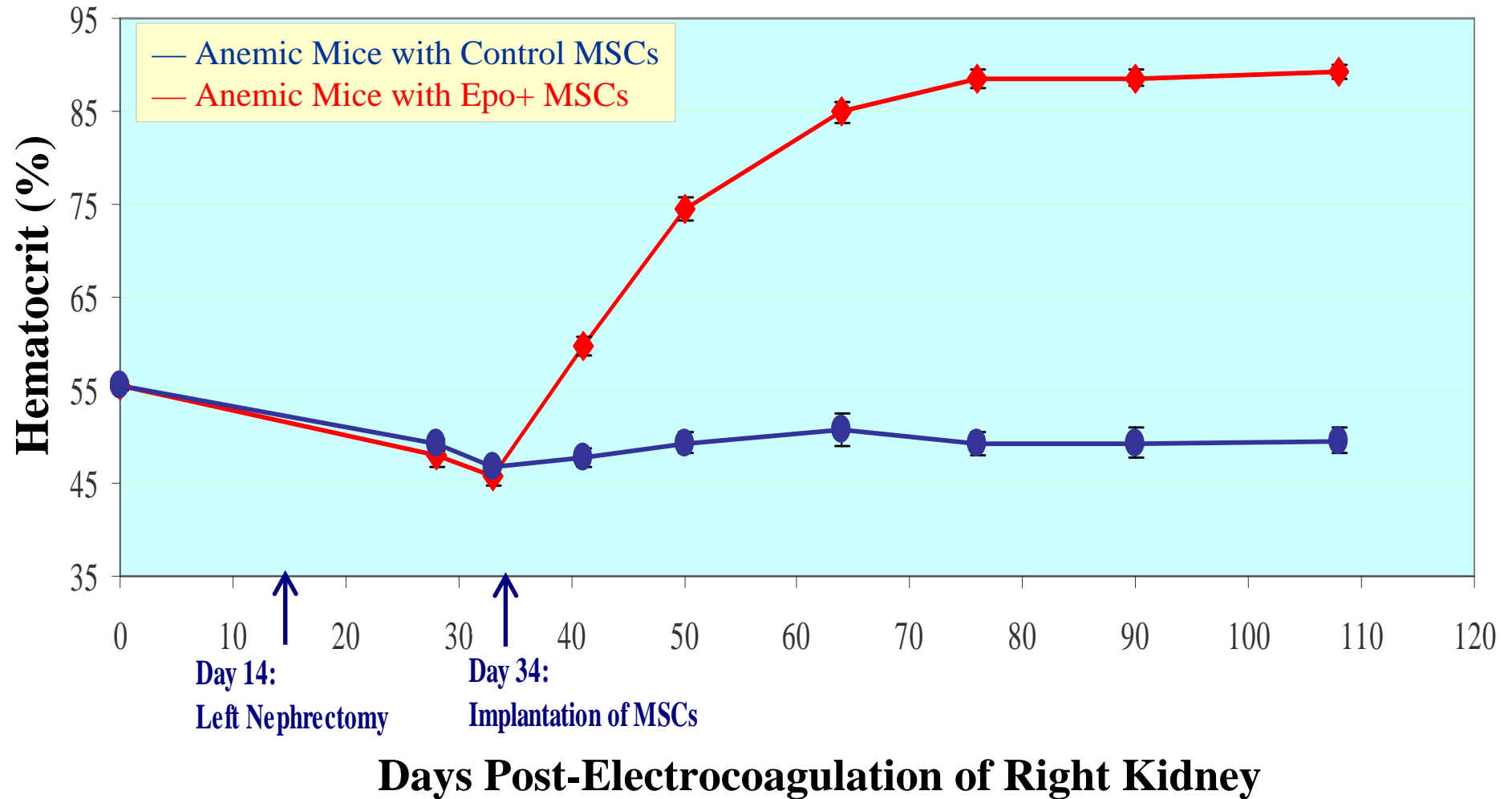
Implant Containing 3 million Epo+ MSCs Shows Epo Refractoriness in Mice with Renal Failure



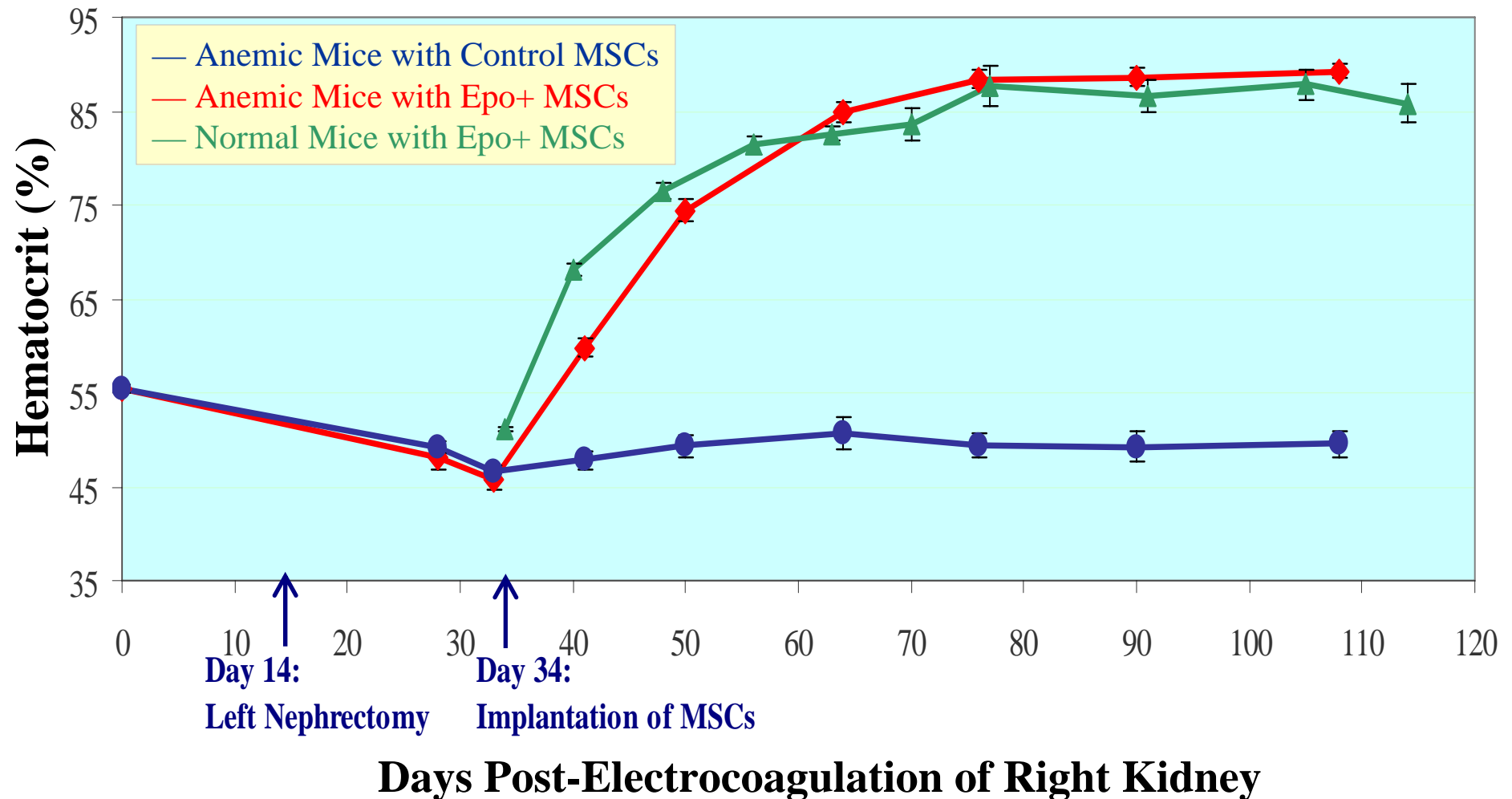
Implant Containing 3 million Epo+ MSCs Shows Epo Refractoriness in Mice with Renal Failure



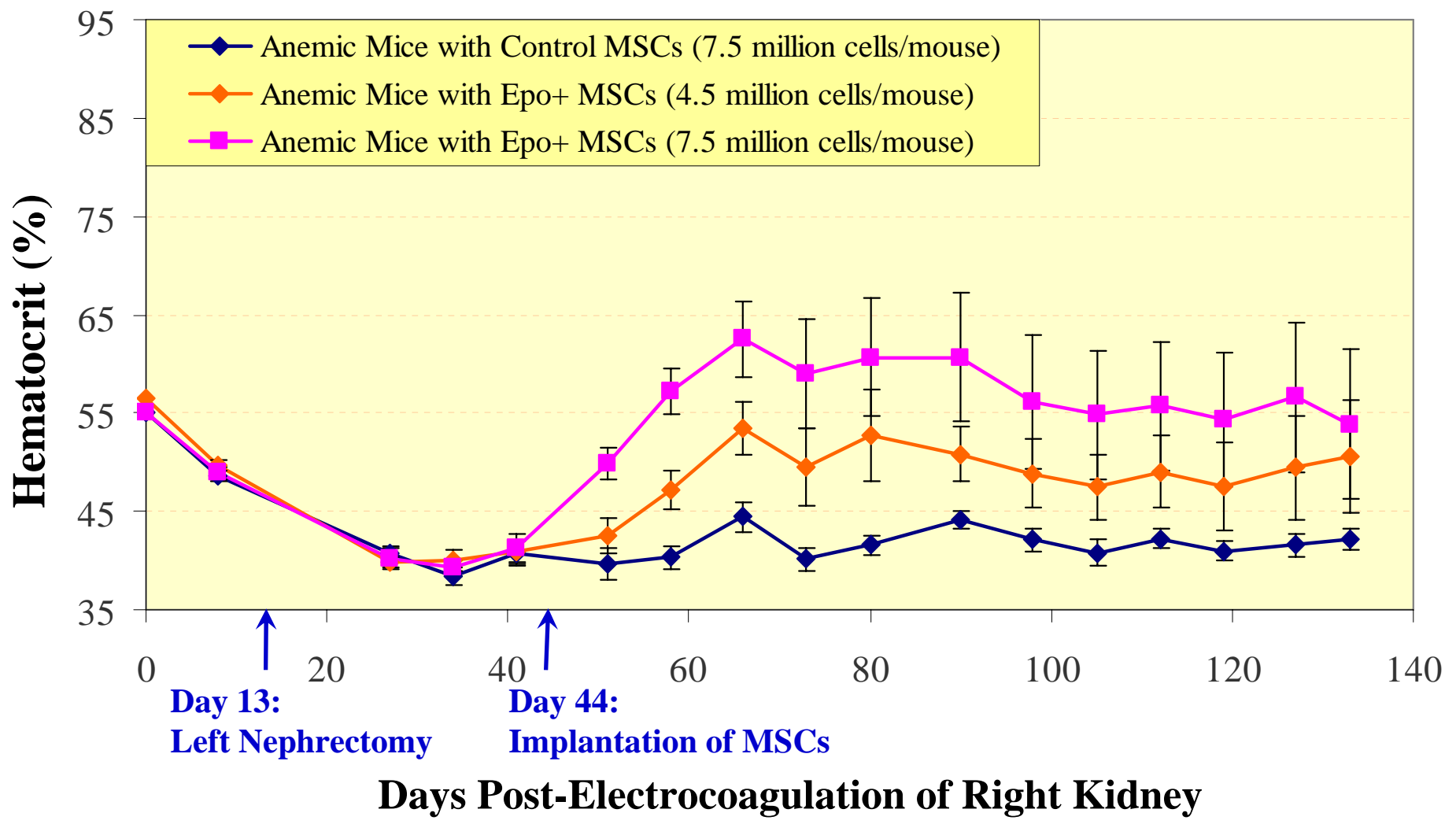
Implant Containing 10 million Epo+ MSCs Leads to Supraphysiological Effect & Need for Dose Adjustment



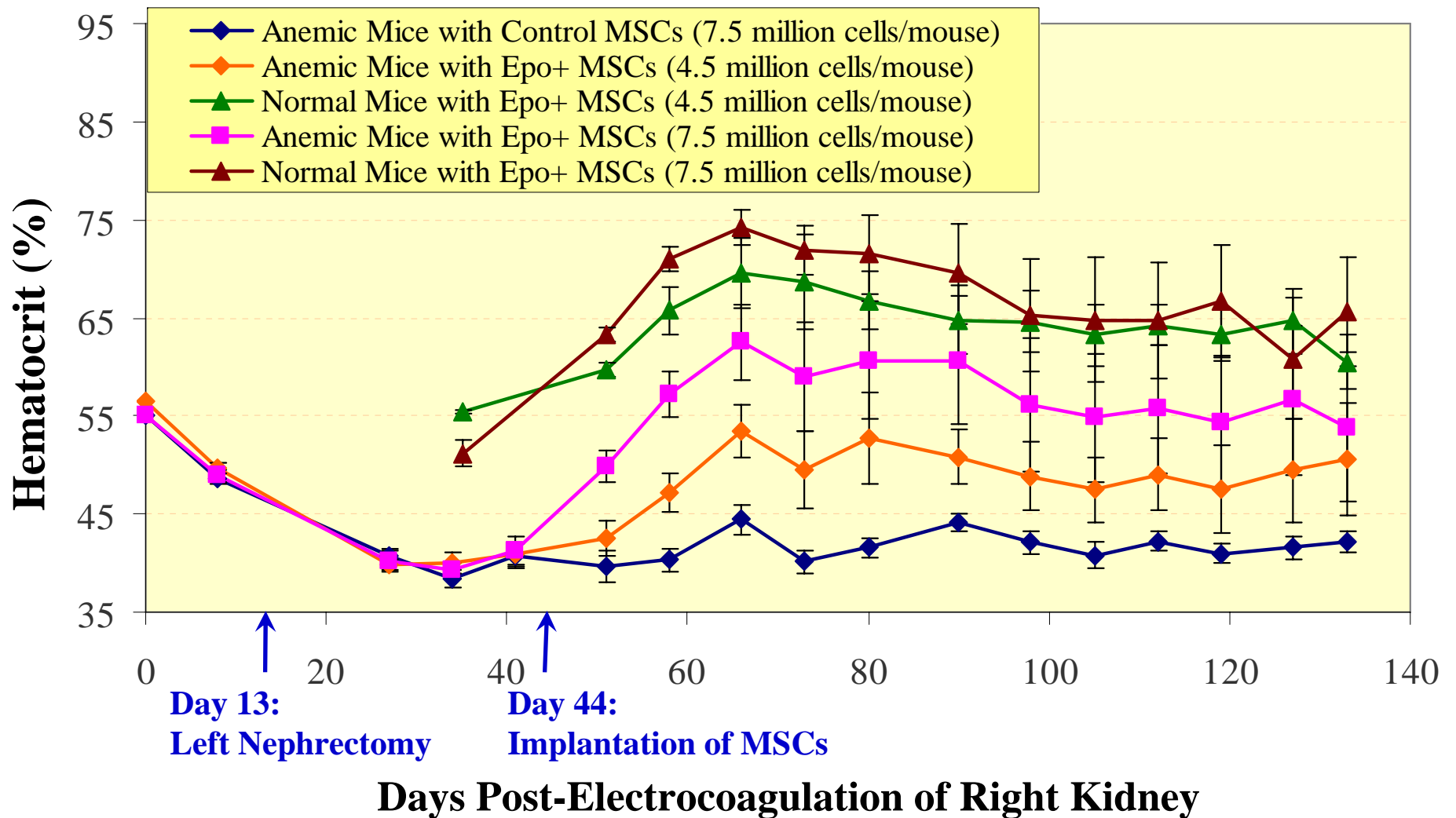
Implant Containing 10 million Epo⁺ MSCs Leads to Supraphysiological Effect & Need for Dose Adjustment



Implants Containing 4.5 and 7.5 million Epo+ MSCs Show Dose-Dependent Increase in Hematocrit in Anemic Mice



Implants Containing 4.5 and 7.5 million Epo⁺ MSCs Show Dose-Dependent Increase in Hematocrit in Anemic & Normal Mice

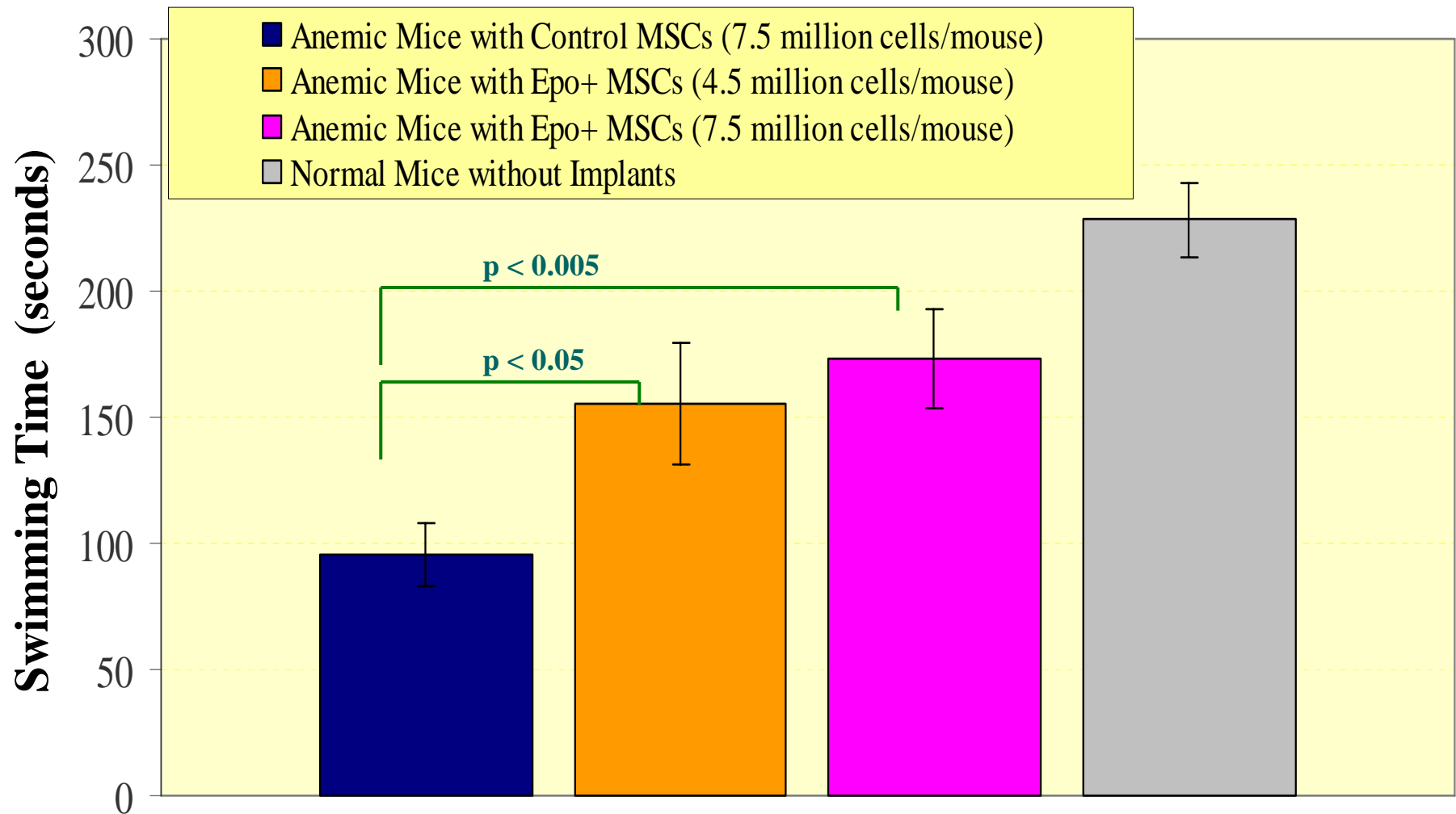


Determination of Exercise Capacity by Swimming Duration



(www.adamstennett.com/home.html)

Increased Swimming Time of Anemic Mice with Implant of Contigen™-Embedded Epo⁺ MSCs Versus Control MSCs



Results at 2-2.5 months post-implantation

Conclusions from Pre-RoFAR Studies

- MSCs can be genetically-engineered and efficiently utilized *in vivo* for the delivery of plasma soluble proteins
- Genetically-engineered MSCs can be embedded in a collagen-based subcutaneous implant and serve as a removable neo-organoid for therapeutic protein delivery
- Contigen-embedded Epo-secreting MSCs lead to increased hematocrit in mice with renal anemia

Publications:

- Eliopoulos N, Al-Khaldi A, Crosato M, Lachapelle K, Galipeau J. A Neovascularized Organoid Derived from Retrovirally-Engineered Bone Marrow Stroma Leads to Prolonged *In Vivo* Systemic Delivery of Erythropoietin in Non-Myeloablated, Immunocompetent Mice. *Gene Therapy*, 10(6): 478-489, 2003.
- Eliopoulos N, Lejeune L, Martineau D, Galipeau J. Human-compatible collagen matrix for prolonged and reversible systemic delivery of erythropoietin in mice from gene-modified marrow stromal cells. *Molecular Therapy*, 10(4):741-748, 2004.
- Eliopoulos, N., Gagnon, R., Francois, M., Galipeau, J. Erythropoietin Delivery by Genetically-Engineered Bone Marrow Stromal Cells for Correction of Anemia in Mice with Chronic Renal Failure. *Journal of the American Society of Nephrology*, 17: 1576-1584, 2006

ROFAR Special Grant 2007-2010

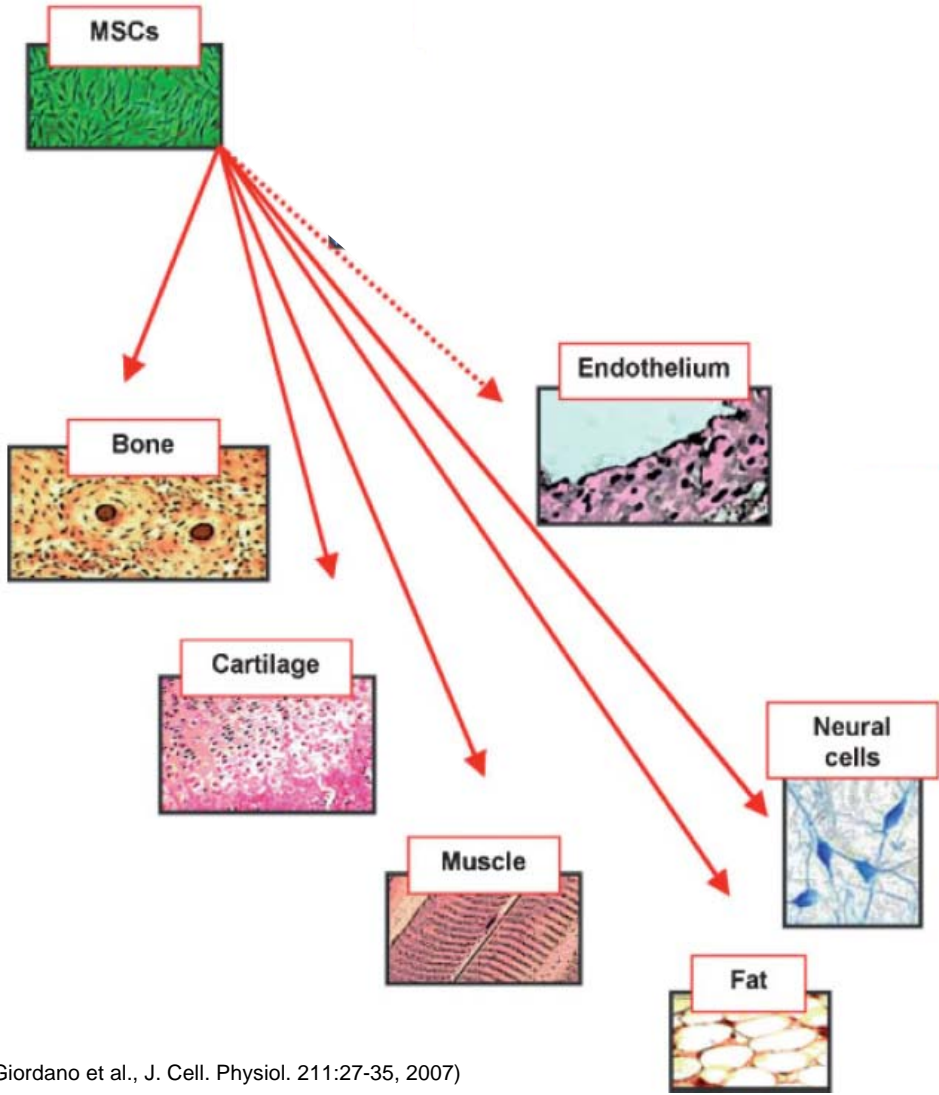
Proposal Title:

Cell and Gene Therapy with Erythropoietin-Secreting Marrow Stem Cells for Kidney Repair

General Question:

**Would MSCs Genetically-Engineered to Secrete Epo
Lead to a Significant Therapeutic Effect
in Acute Renal Failure-Induced Kidney Damage ?**

Plasticity of MSCs

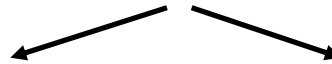


(from Giordano et al., J. Cell. Physiol. 211:27-35, 2007)

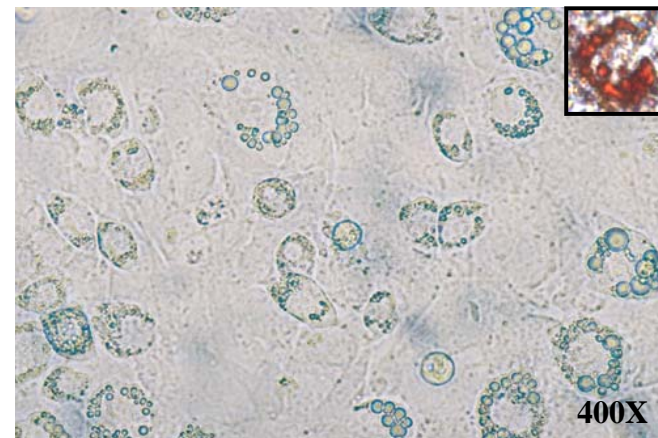
Genetically-Engineered MSCs Retain their Differentiation Capabilities *In Vitro*



Non Differentiated

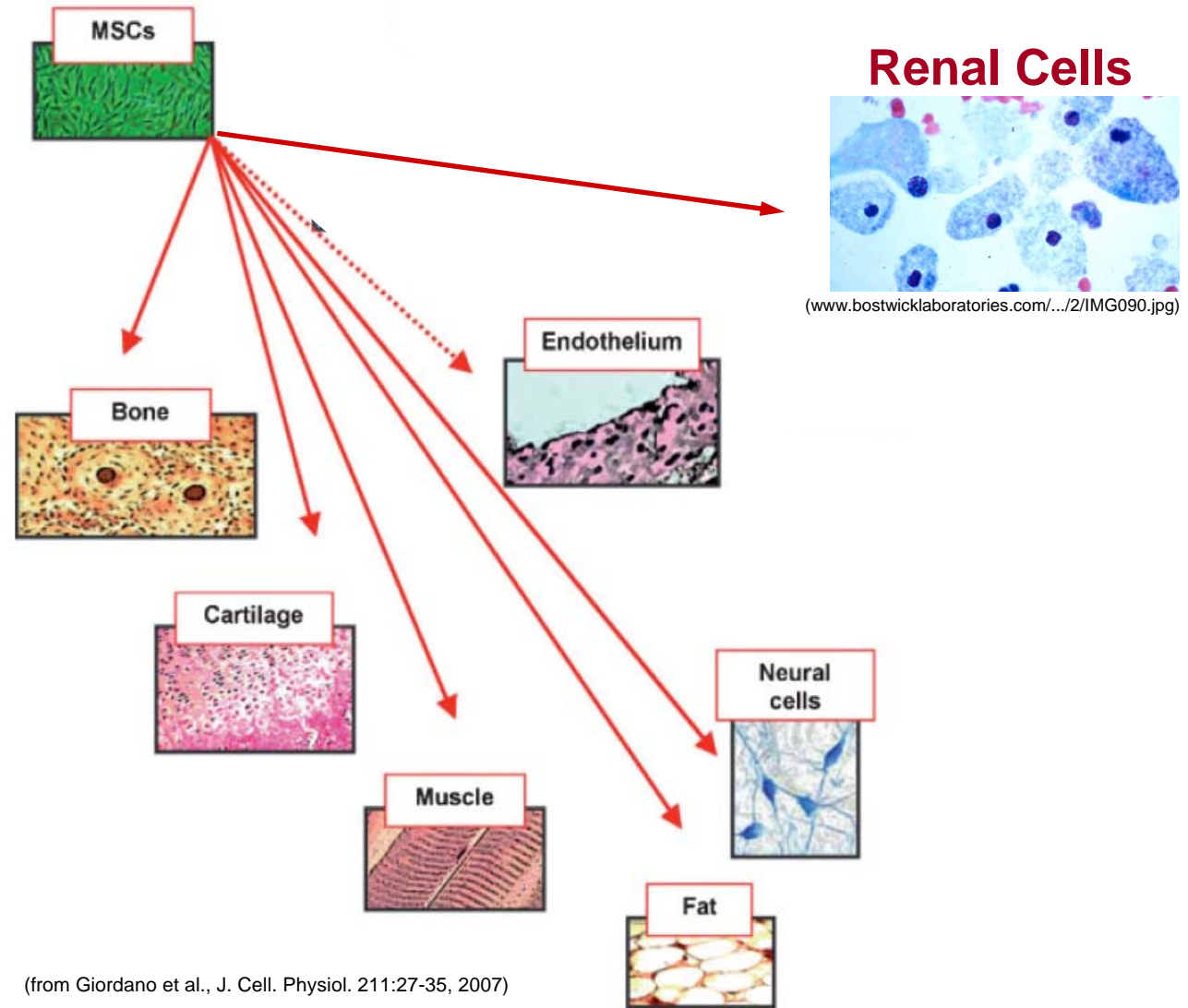


Post Osteogenic Differentiation



Post Adipogenic Differentiation

Plasticity of MSCs Includes Renal Cells



(from Giordano et al., J. Cell. Physiol. 211:27-35, 2007)

Acknowledgments

Jacques Galipeau, M.D., Lady Davis Institute for Medical Research

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Previous funding: Anemia Institute for Research and Education (AIRE)



Current funding: Roche Foundation for Anemia Research (RoFAR)



Special Grant Award