“Secretions of Mesenchymal Stem Cells: The Next Generation in Orthomolecular Medicine. ”

Neil H. Riordan, P.A., Ph.D

Conflicts

* Stem Cell Institute, Panama, Director, Shareholder
* Medistem Panama, Panama, Director, Shareholder
* Riordan-McKenna Institute, Southlake, TX, Director, Shareholder
* Amniotic Therapies, Inc. Dallas, TX, Director, Shareholder
* Biologic Therapies, Inc. Ocala, FL, Director, Shareholder
* Aidan Products, Chandler, AZ, Director, Shareholder

OM Definition

* Orthomolecular medicine, as conceptualized by double-Nobel laureate Linus Pauling, aims to restore the optimum environment of the body by correcting imbalances or deficiencies based on individual biochemistry, using substances natural to the body such as vitamins, minerals, amino acids, trace elements and fatty acids.
* http://www.orthomed.org/

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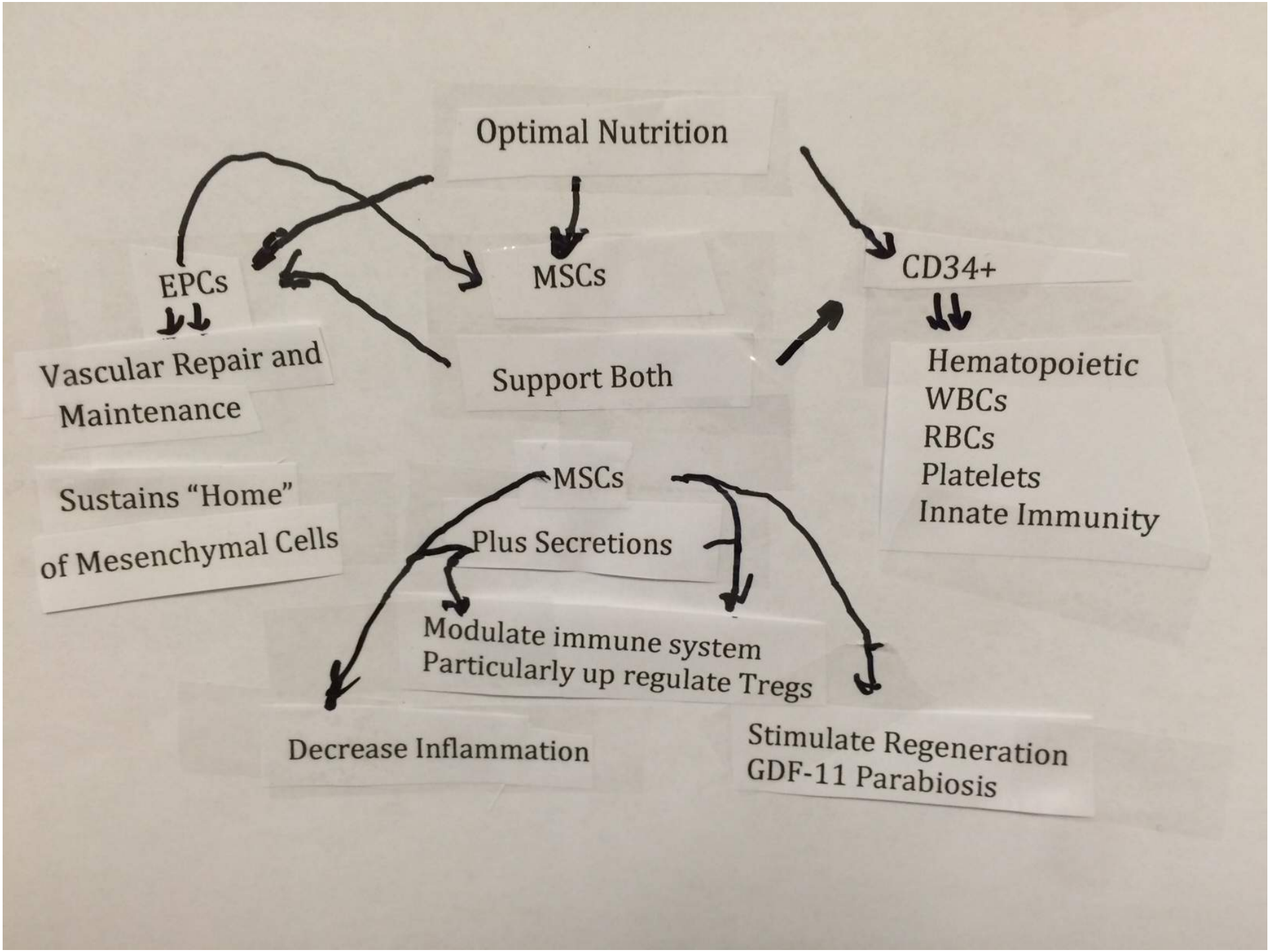
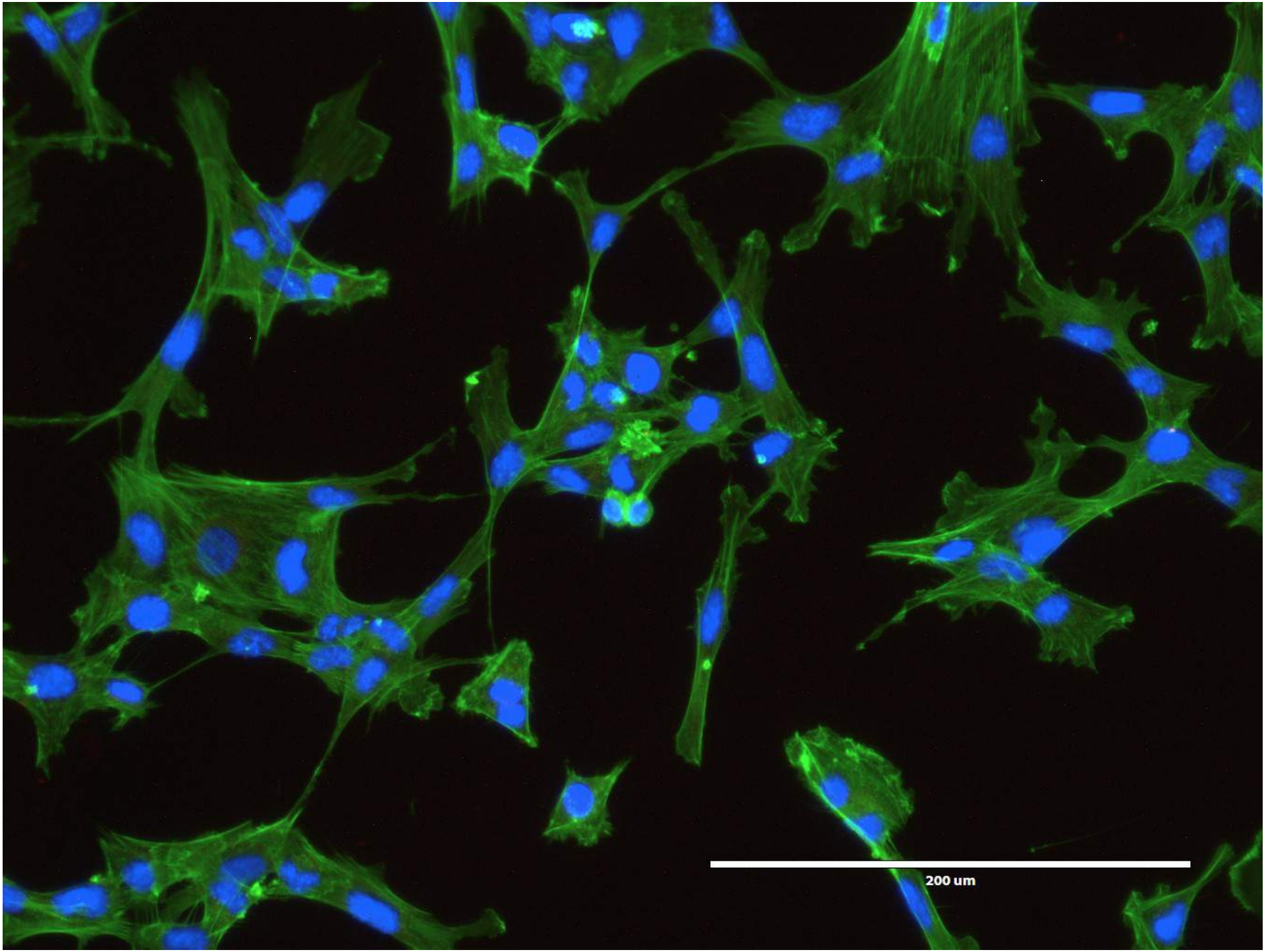
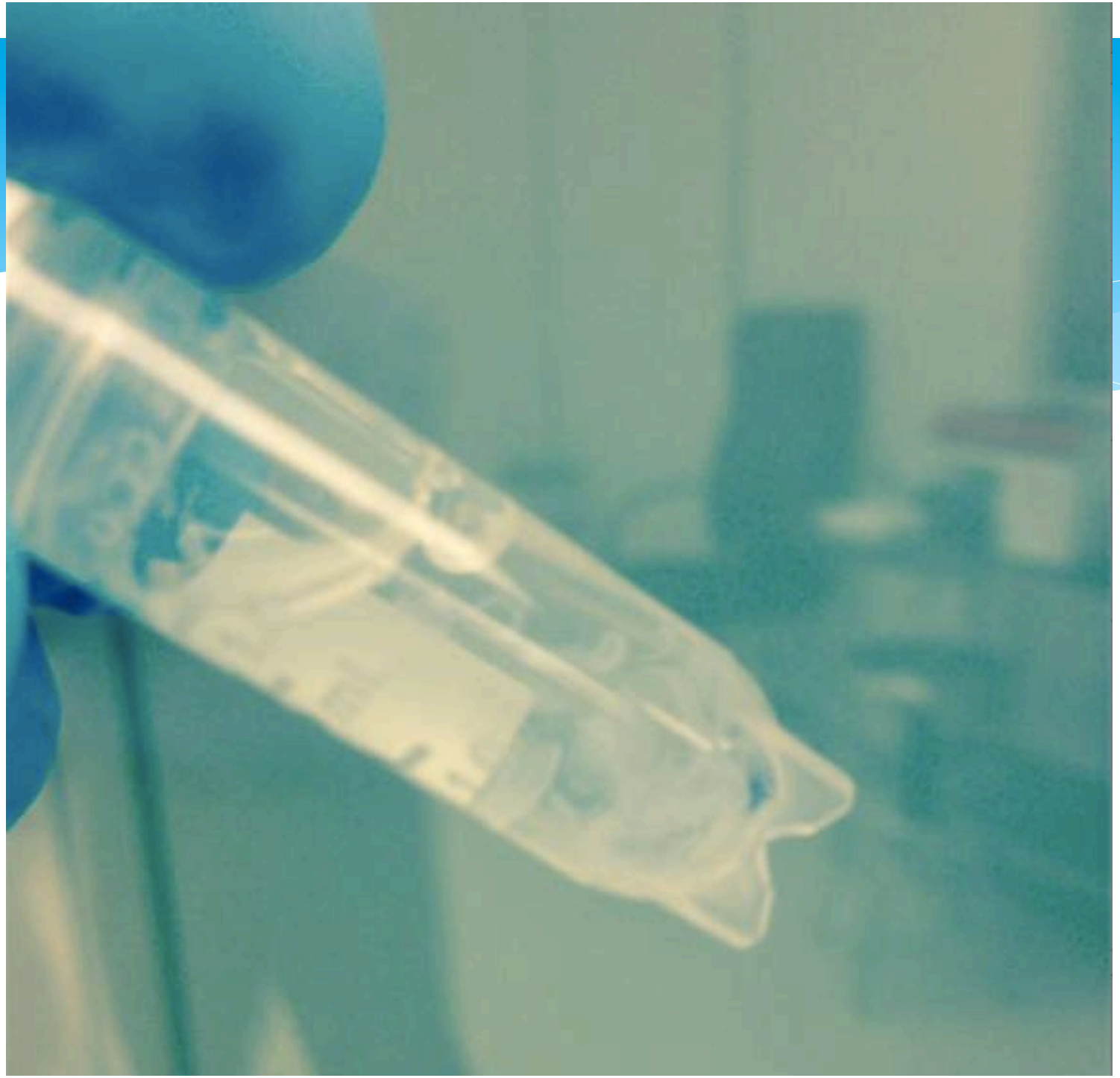
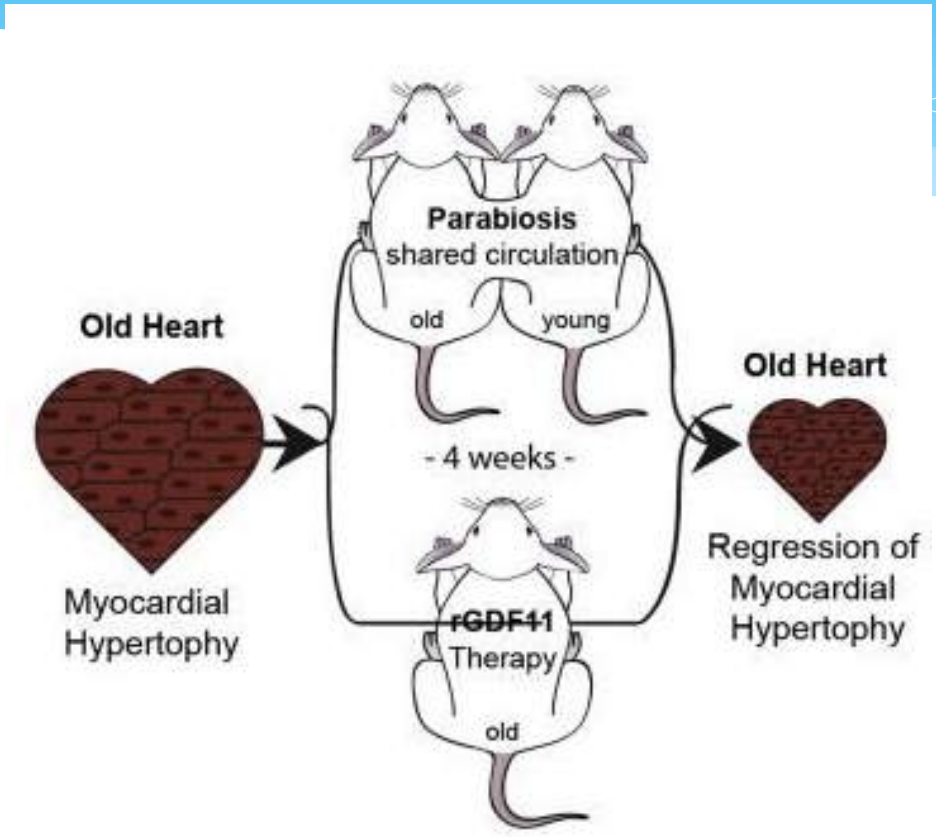

Optimal Nutrition
EPCs
MSCs
CD34+
Vascular Repair and Maintenance
Support Both
Hematopoietic
WBCs
RBCs
Platelets
Innate Immunity
Sustains "Home"
of Mesenchymal Cells
MSCs
Plus Secretions
Modulate immune system
Particularly up regulate Tregs
Decrease Inflammation
Stimulate Regeneration
GDF-11 Parabiosis

Stem Cell Institute Panama

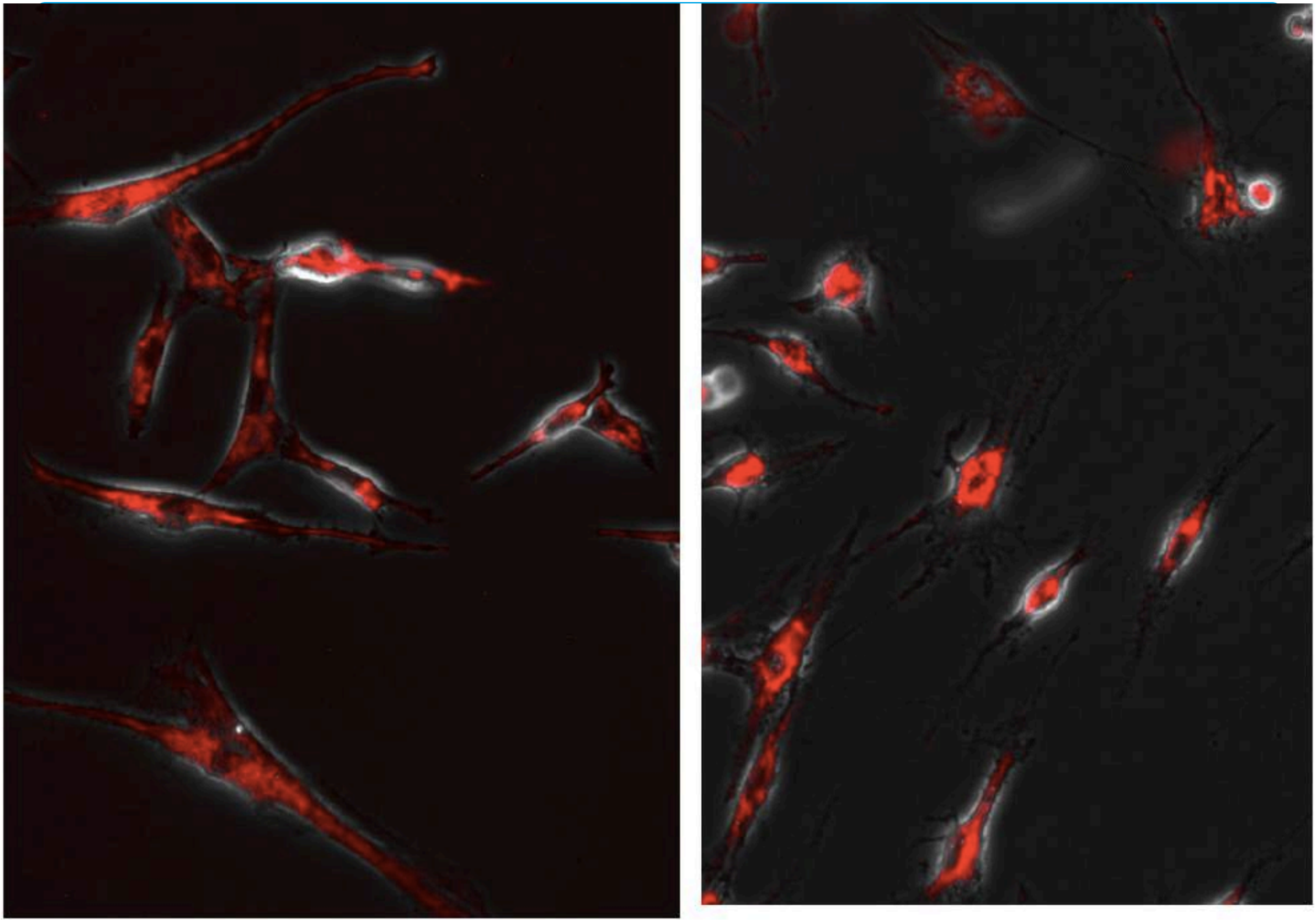

200 um



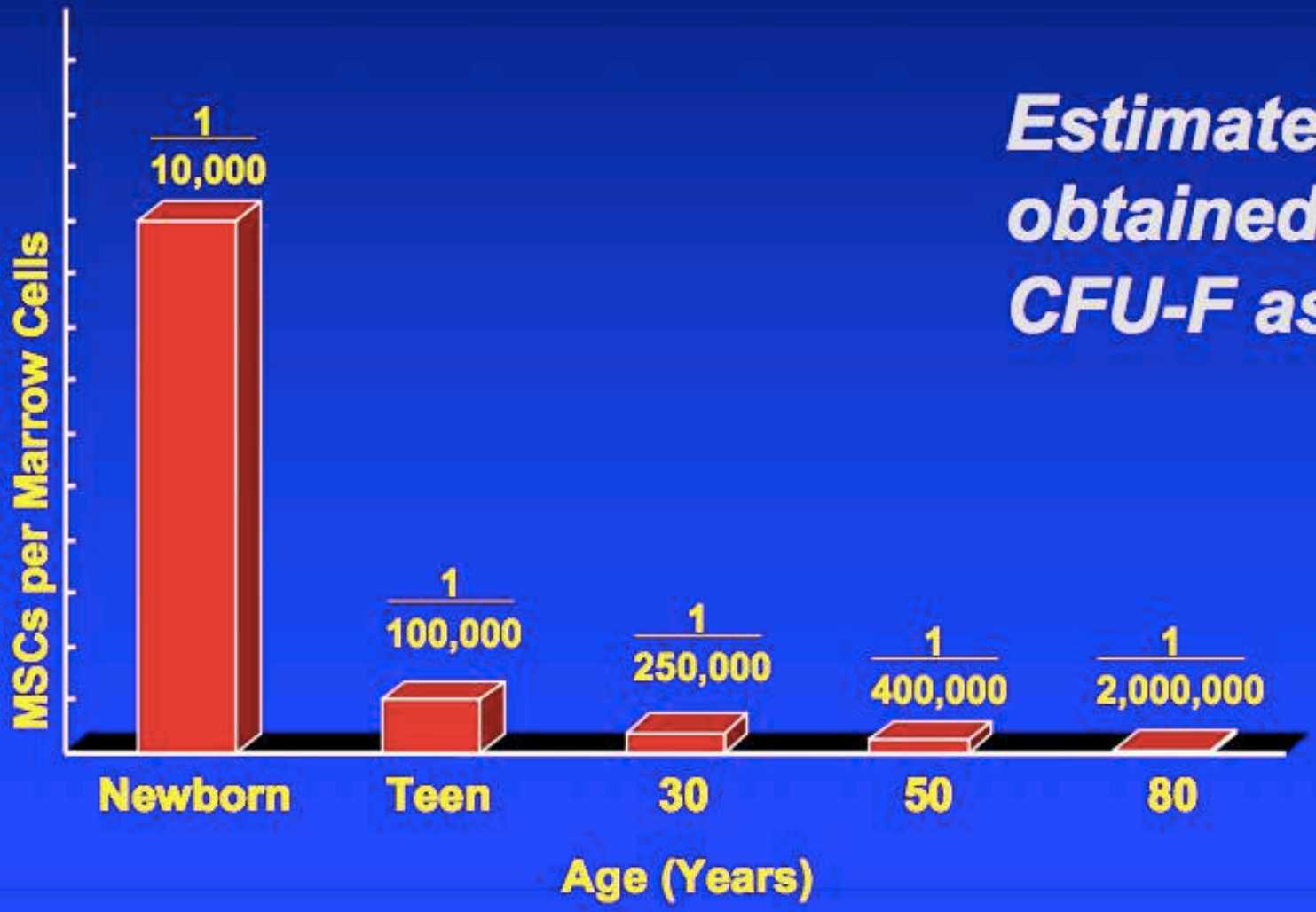
Parabiosis



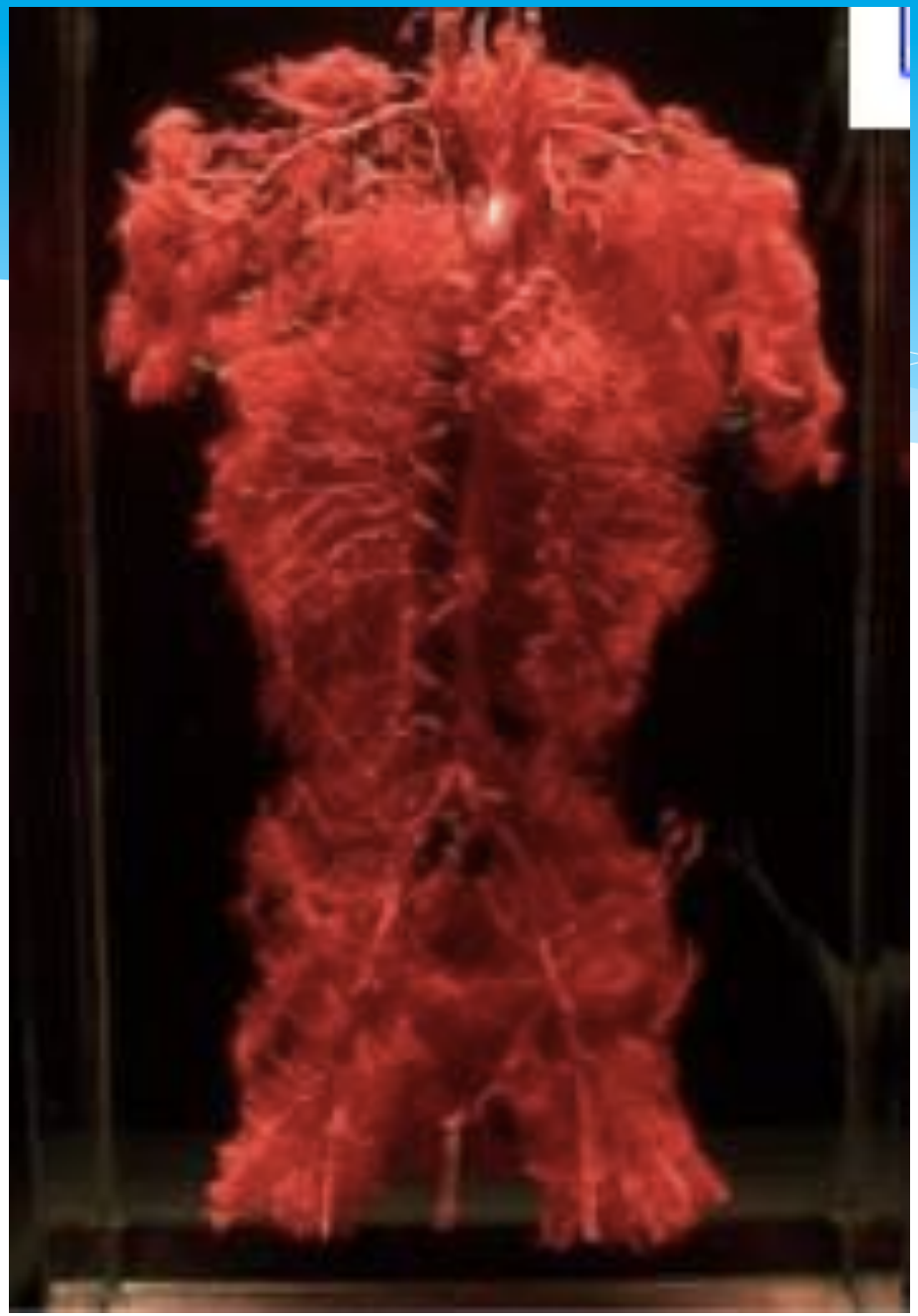
Hetrochronic parabiosis rejuvenates heart muscles

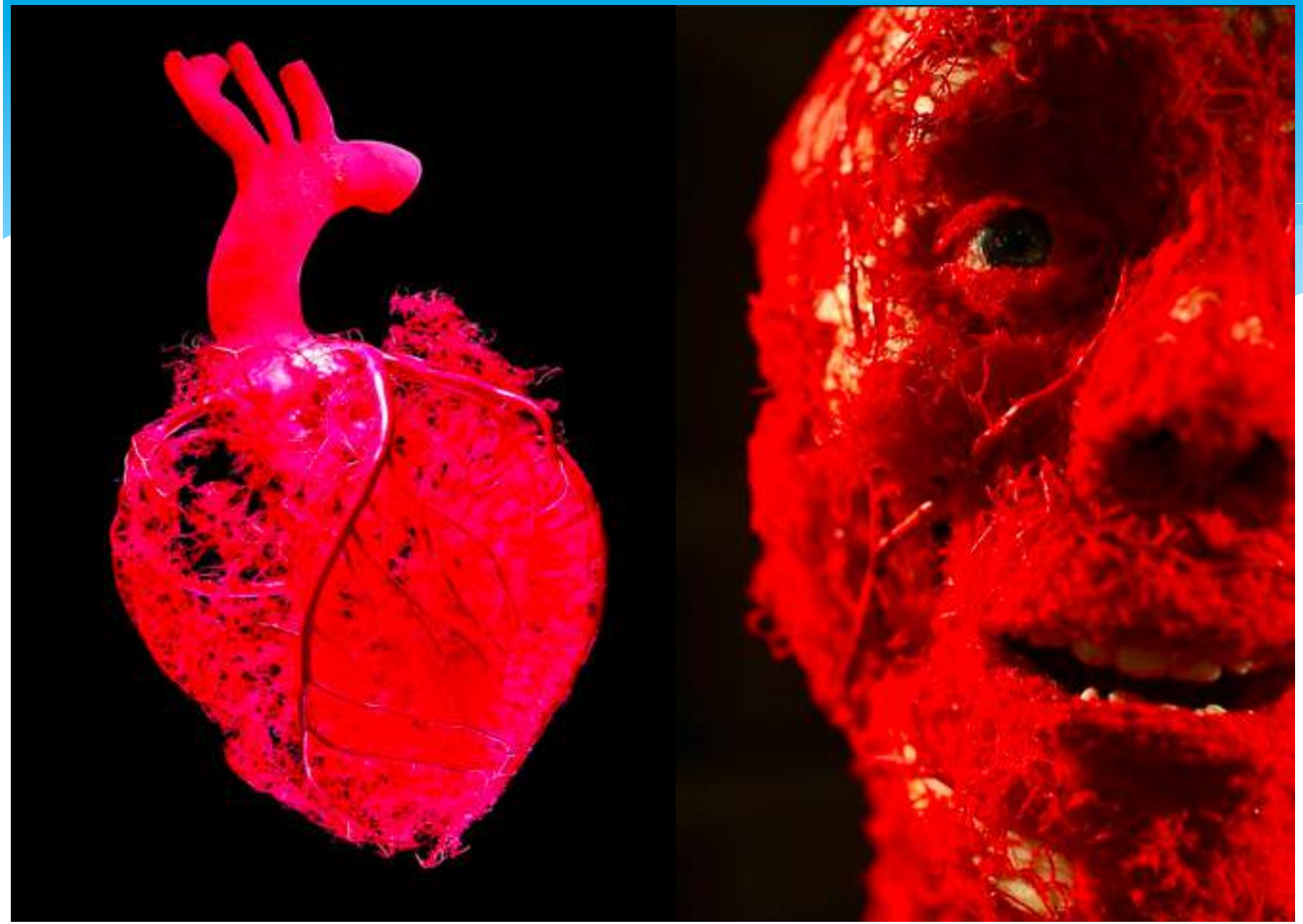


Human MSCs Decline With Age:

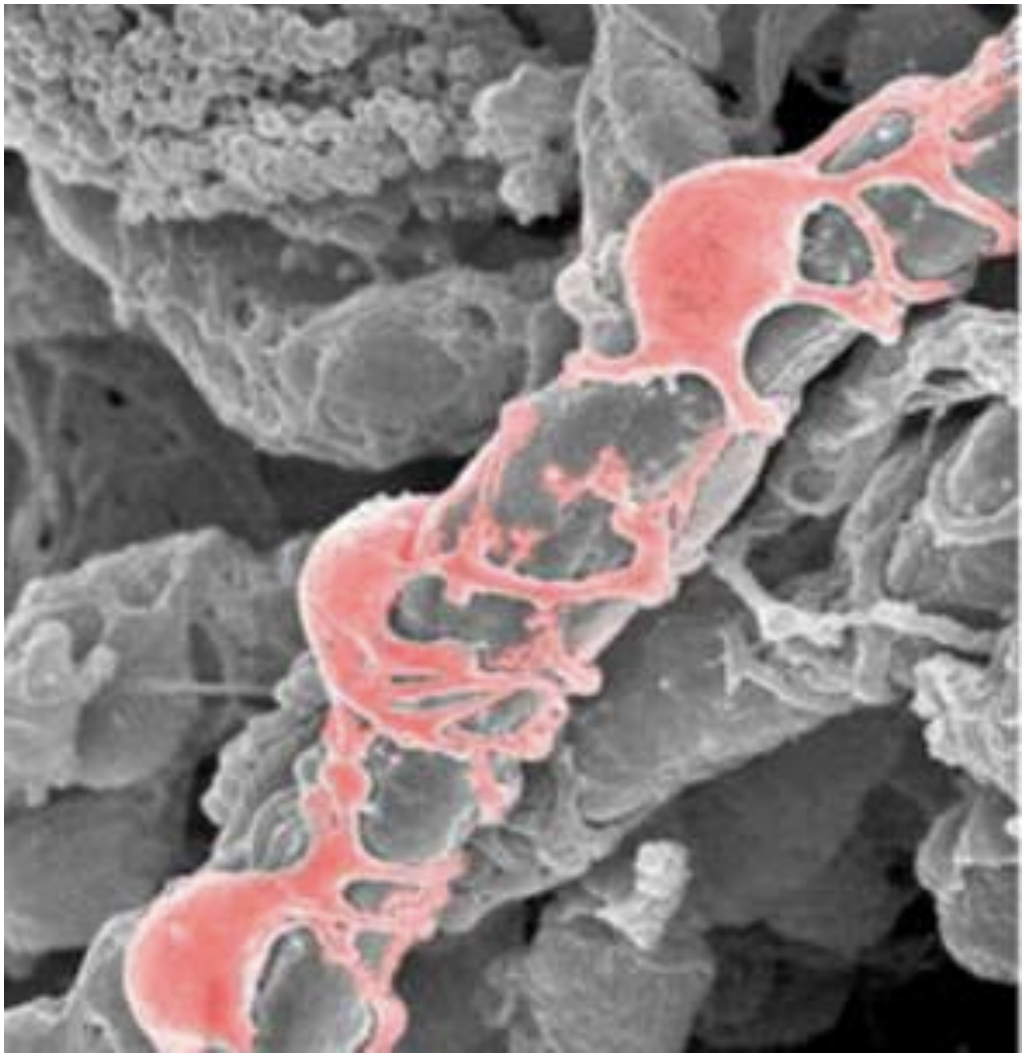


Estimates obtained by CFU-F assay.





MSCs found on every small blood vessel



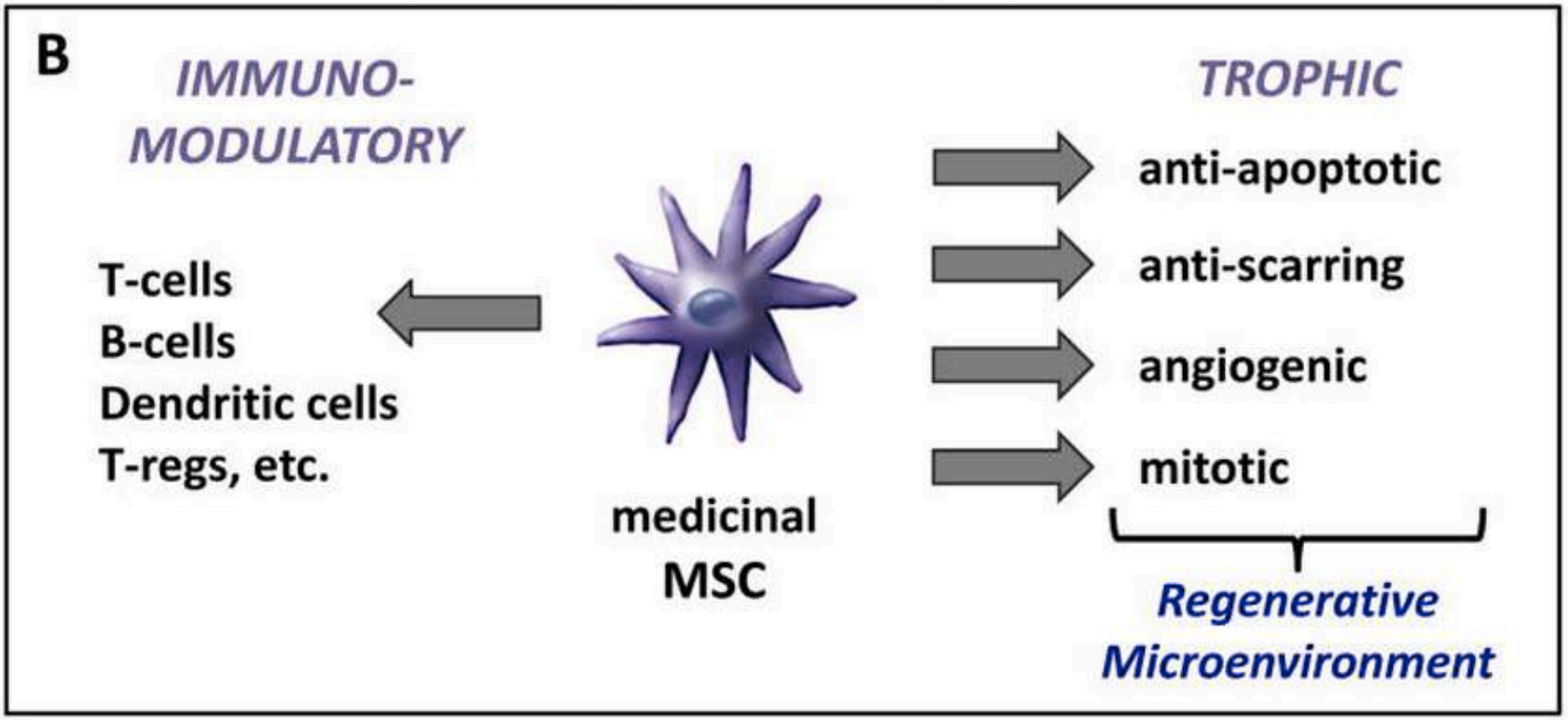
Bruno Peault via Arnold Caplan

The MSC: An Injury Drugstore

Cell Stem Cell. 2011 July 8; 9(1): 11–15.
doi: 10.1016/j.stem.2011.06.008

THE MSC: AN INJURY DRUGSTORE

ARNOLD I. CAPLAN, Ph.D. and DIEGO CORREA, M.D., Ph.D.



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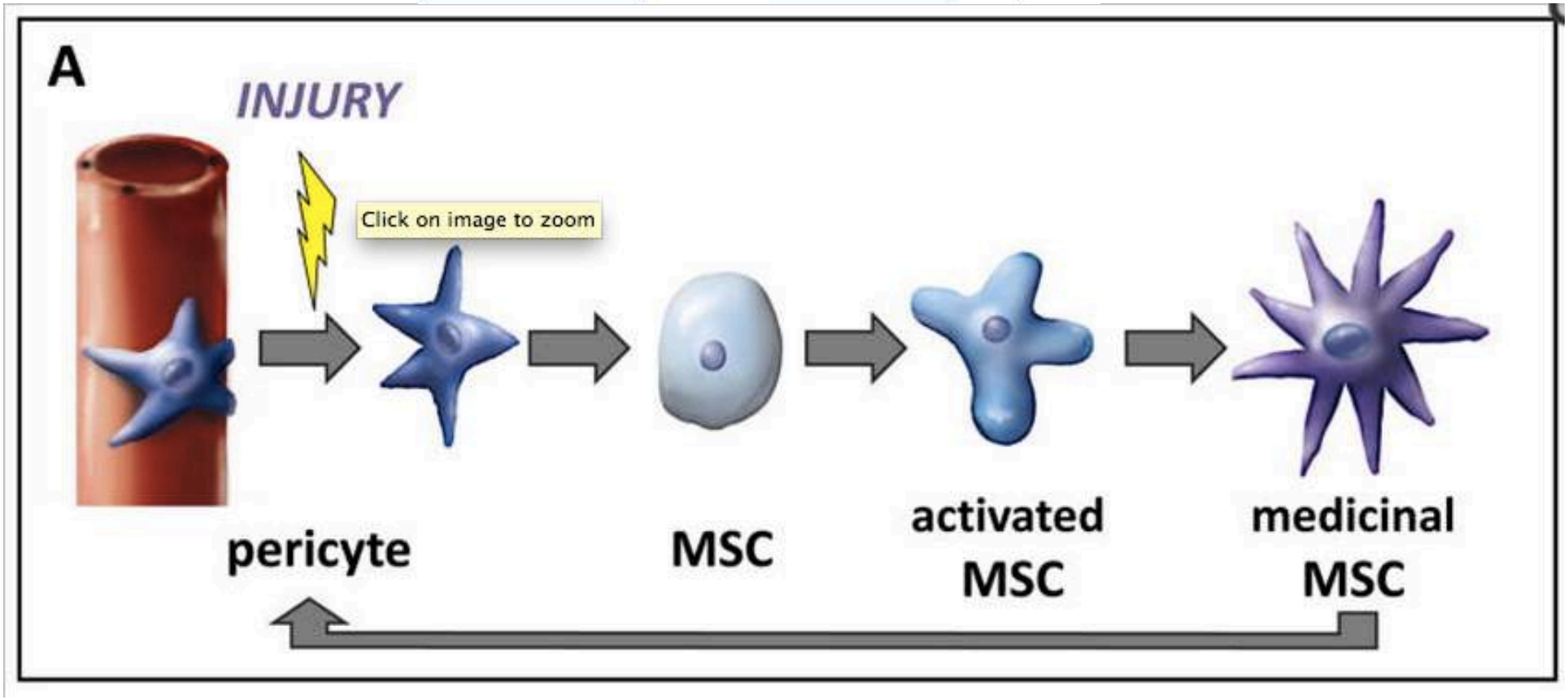


Secretome

IGFBP-4	OPG	G-CSF	OPN	Activin A	ICAM-2	LIMPII	GM-CSF
Follistatin	TIMP-2	MMP-3	ALCAM	PDGF-AA	Trappin-2	Galectin-7	DR6
GROa	TGF-b2	VCAM-1	CD14	GRO	EGF R	PDGF Rb	6Ckine
NSE	PAI-I	VEGF-C	RANTES	ANG	IL-13 R1	L-Selectin	IGF-I SR
DKK-1	sgp130	B2M	MCP-3	NCAM-1	MICB	hCGb	SCF
ANG-1	GCP-2	IL-11	MMP-10	GDF-15	IL-13 R2	HVEM	Dtk
IGFBP-3	TIMP-1	TNF RI	LIF	FGF-7	MIP-1a	Shh N	IL-2 Ra
IGFBP-6	MMP-1	LAP	Endoglin	BDNF	TRAIL R3	Leptin	IL-1b
Nidogen-1	MMP-2	Ferritin	DAN	CXCL16	VEGF	MIP-3a	EpCAM
VEGF R1	ENA-78	bFGF	TPO	IL-8	Catheprin S	VEGF R2	TNF RII
uPAR	MIF	HGF	TIMP-4	MCP-1	IL-17B	CD30	IL-1a
ANGPTL4	ICAM-1	IL-6	GDNF	Procalcitonin	IGFBP-2	IL-17	AgRP

* IGFBP-4 protein consistently inhibits several cancer cells in vivo and in vitro
* Follistatin—antagonist to myostatin
* HGF- major role in adult organ regeneration and in wound healing, markedly accelerates remyelination in multiple sclerosis

Panama

* First Country in western hemisphere to pass an adult stem cell regulatory law
* Multiple clinical trials under IRB recognized by the NIH—National Bioethics Committee

Medistem Panama Laboratory

* BSL-2 (Biosafety level-2) Laboratory
* cGTP, cGMP compliant
* ISO-9001-2008
* Licensed by the Ministry of Health of Panama
* Located in the City of Knowledge, Building 221
* Since 2007

ISO 9001

IGC

EMPRESA CERTIFICADA

LABORATORY
MEDISTEM PANAMA

ReCap

* Two categories of stem cells: CD34+ (Bone marrow and umbilical cord blood) and Mesenchymal Stem Cells (MSCs) found in most tissues of the body
* MSCs
  * Modulate the immune system
  * Calm Inflammation
  * Enhance Repair
  * Stimulate local stem cells

Autism Spectrum Disorder Rationale

* MSCs modulate immunity and inflammation
* Neurological inflammation found
* Immune dysregulation found—in the gut in many
* Elevated levels of several inflammatory molecules in the blood—MDC and TARC and others

Autism Clinical Study

* 13 subjects, now enrolling 20 more ages 6-16
* Intervention: 4 intravenous infusions of MSCs 1 time per day
* Repeated 4 times (0, 3, 6, and 9 months)
* Primary Endpoint: Safety
* Measurements:
  * CARS (Childhood Autism Rating Scale)
  * ATEC (Autism Treatment
  * Quantitative EEG (qEEG)
  * Inflammation markers in the blood
    * MDC (Macrophage derived chemokine)
    * TARC (Thymic and related chemokine)

Safety

* No serious adverse events
* Pain at site of infusion

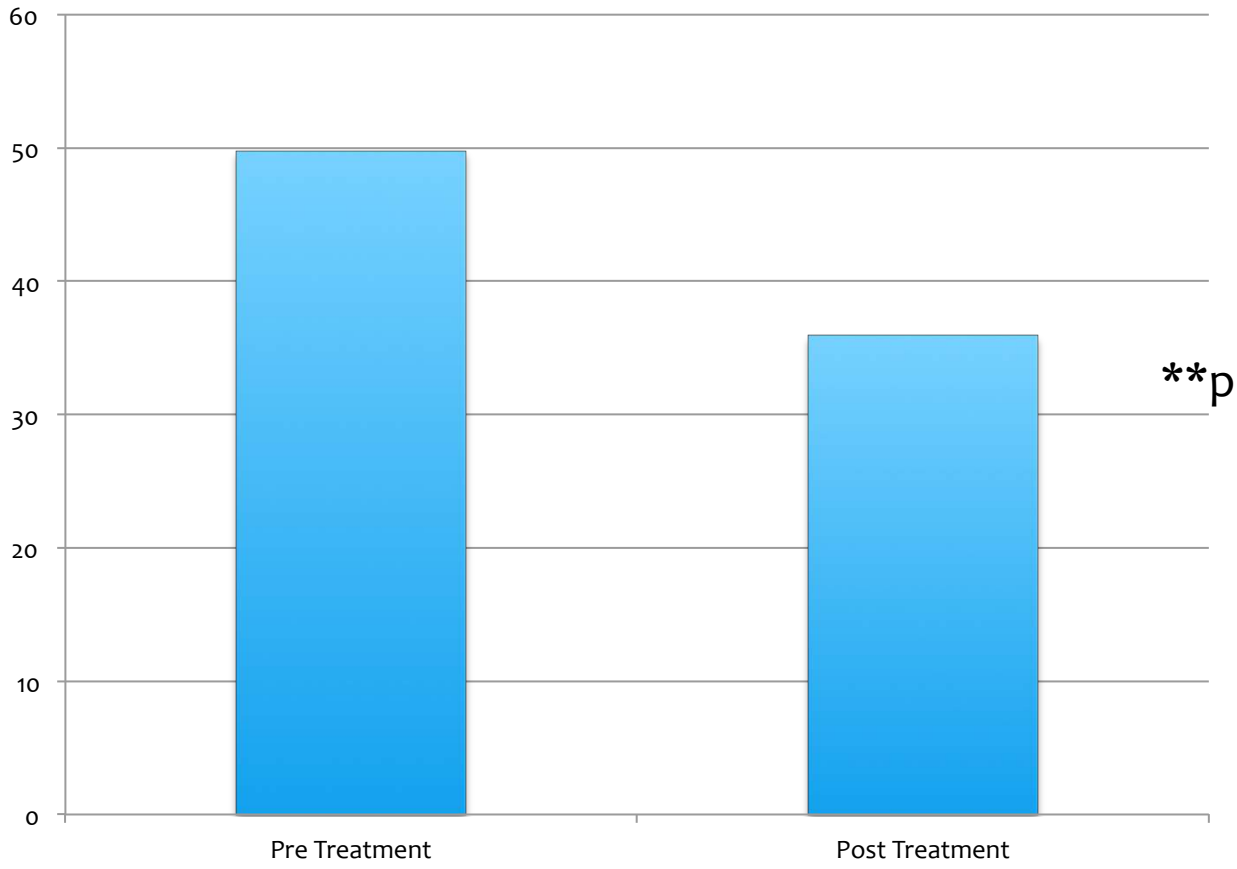
CARS—Childhood Autism Rating Scale--Schopler, Reichier and Renne

* Scores range form 15 to 60 with 30 being the cutoff rate for a diagnosis of mild autism
* Scores 30-37 indicate mild to moderate autism
* Scores between 38 and 60 are characterized as severe autism

CARS—Childhood Autism Rating Scale

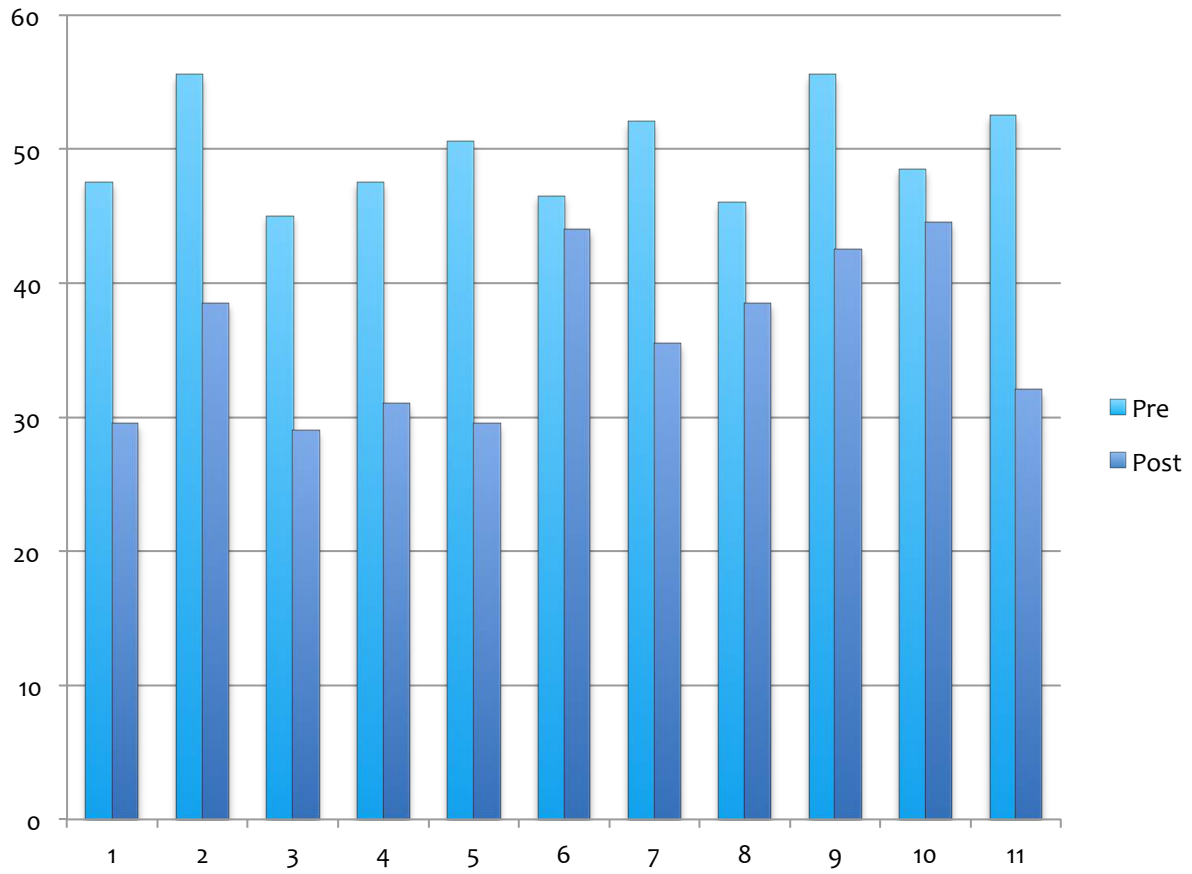
SUBJECT		Baseline	After Treatment	Average	Reduction
SUB001		47.5	29.5	38.5	18
SUB002		55.5	38.5	47	17
SUB003		45	29	37	16
SUB004		47.5	31	39.25	16.5
SUB005		50.5	29.5	40	21
SUB006		46.5	44	45.25	2.5
SUB007		52	35.5	43.75	16.5
SUB008		46	38.5	42.25	7.5
SUB009		55.5	42.5	49	13
SUB010		48.5	44.5	46.5	4
SUB011		52.5	32	42.25	20.5
SUB012		Treatment is ongoing			
SUB013		Treatment is ongoing			

CARS Pre- and Post n=11



**p<.0001

CARS

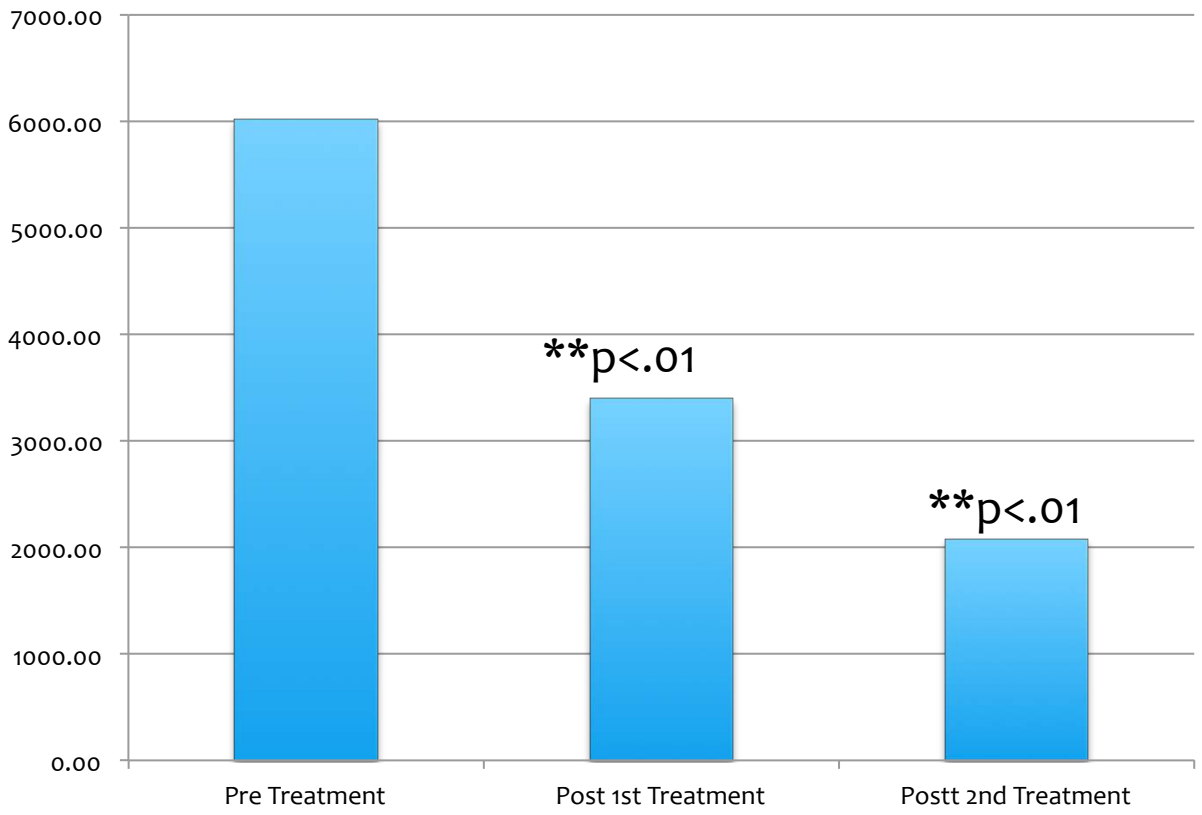


MDC and TARC

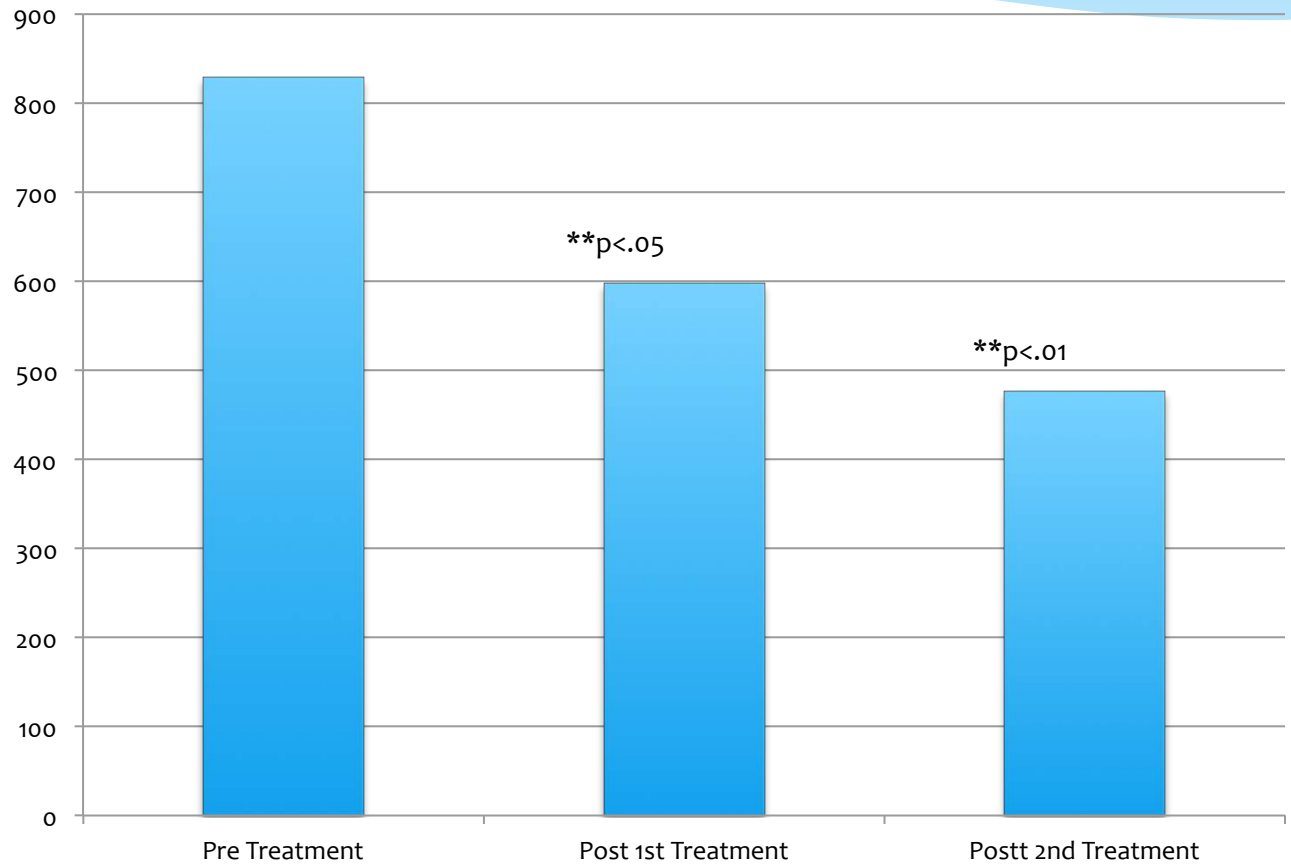
* Study of 56 autistic children and 32 healthy matched children
* Autistic children had significantly higher serum levels of MDC and TARC than healthy controls (P <0.001 and P <0.001, respectively)
* Children with severe autism had significantly higher serum levels of MDC and TARC than patients with mild to moderate autism
* Significant positive correlations between CARS and serum levels of both MDC (P <0.001) and TARC (P <0.001)

Al-Ayadhi LY1, Mostafa GA. Elevated serum levels of macrophage-derived chemokine and thymus and activation-regulated chemokine in autistic children. J Neuroinflammation. 2013 Jun 19;10:72.

MDC (Macrophage derived chemokine) Tx n=13, 11



TARC (Thymus and related chemokine) Tx n=13, 11



China Study

* 37 Subjects: Combination of umbilical MSCs and cord blood MNCs
* Umbilical MSCs plus cord blood MNCs IV an intrathecal
* Cord blood MNCs IV and intrathecal
* Control
* No severe adverse events
* Significant improvements. CARS $p<.05$

Summary Autism

* ASD patients can have neural inflammation, gut immune dysregulation, and increase inflammatory markers.
* MSCs reduce inflammation
* Early study results show reduction in inflammatory markers and clinical improvement via CARS with good safety profile.

Spinal Cord Injury Rationale

* MSCs secrete trophic factors that stimulate resprouting of neural fibers and repair spinal cord in animal models
* CD34+ cells (found in bone marrow and umbilical cord blood) home to and stimulate repair of the spinal cord.
* Multiple Human Clinical Trials using Bone Marrow
* Multiple Animal Trials using MSCs

History

Cytotherapy. 2005;7(4):368-73.

A 37-year-old spinal cord-injured female patient, transplanted of multipotent stem cells from human UC blood, with improved sensory perception and mobility, both functionally and morphologically: a case study.

Kang KS, Kim SW, Oh YH, Yu JW, Kim KY, Park HK, Song CH, Han H.

Laboratory of Stem Cell and Tumor Biology, College of Veterinary Medicine, Seoul National University, Seoul, Korea.

* The patient could move her hips and feel her hip skin on day 15 after transplantation.
* From POD 15 she began to elevate both lower legs about 1 cm, and hip flexor muscle activity gradually improved until POD 41
* 41 days after [stem cell] transplantation" testing "also showed regeneration of the spinal cord at the injured cite" and below it.

History

Case Report

The Journal of TRAUMA® Injury, Infection, and Critical Care

Spinal Cord Injury Treatment With Intrathecal Autologous Bone Marrow Stromal Cell Transplantation: The First Clinical Trial Case Report

Fukuki Saito, MD, Toshio Nakatani, MD, Masaaki Iwase, MD, Yuji Maeda, MD, Akihiko Hirakawa, MD, Yoshinori Murao, MD, Yoshihisa Suzuki, MD, Rie Onodera, MS, Masanori Fukushima, MD, and Chizuka Ide, MD

J Trauma. 2008;64:53–59.

First CD34+ Cells--Rats

Zhongguo Yi Xue Ke Xue Yuan Xue Bao. 2004 Feb;26(1):38-42.

[Transplantation of human umbilical cord stem cells improves neurological function recovery after spinal cord injury in rats].

[Article in Chinese]

Li HJ, Liu HY, Zhao ZM, Lu SH, Yang RC, Zhu HF, Cai YL, Zhang QJ, Han ZC.

State Key Laboratory of Experimental Hematology, Institute of Hematology, CAMS and PUMC, Tianjin 300020, China.

Cell Transplant. 2004;13(2):113-22.

Intraspinal transplantation of CD34+ human umbilical cord blood cells after spinal cord hemisection injury improves functional recovery in adult rats.

Zhao ZM, Li HJ, Liu HY, Lu SH, Yang RC, Zhang QJ, Han ZC.

National Research Center for Stem Cell Engineering & Technology, State Key Laboratory of Experimental Hematology, Institute of Hematology, Chinese Academy of Medical Sciences, Peking Union Medical College, Tianjin, People's Republic of China.

J Neurosurg Spine. 2006 Nov;5(5):424-33.

The use of hemopoietic stem cells derived from human umbilical cord blood to promote restoration of spinal cord tissue and recovery of hindlimb function in adult rats.

Nishio Y, Koda M, Kamada T, Someya Y, Yoshinaga K, Okada S, Harada H, Okawa A, Moriya H, Yamazaki M.

Department of Orthopaedic Surgery, Chiba University Graduate School of dicine, Tougane Chiba Prefecture Hospital, Chiba, Japan.

Whole Bone Marrow

Cell Transplant. 2008;17(12):1277-93.

Administration of autologous bone marrow stem cells into spinal cord injury patients via multiple routes is safe and improves their quality of life: comprehensive case studies.

Geffner LF, Santacruz P, Izurieta M, Flor L, Maldonado B, Auad AH, Montenegro X, Gonzalez R, Silva F.

Hospital Luis Vernaza, JBGYE, Guayaquil, Ecuador.

* Demonstrated improvements in ASIA, Barthel (quality of life), Frankel, and Ashworth scoring
* Eight case studies of SCI (four acute, four chronic) with approximately 2 years of follow-up that were administered bone marrow stem cells (BMSCs) via multiple routes: directly into the spinal cord, directly into the spinal canal, and intravenous
* 52 patients with SCI and have had no tumor formations, no cases of infection or increased pain, and few instances of minor adverse events

* This study demonstrated that intrathecal administration of cultured autologous BMSCs is safe and feasible for treatment of spinal cord injury.

Restor Neurol Neurosci. 2012 Jan 1;30(2):127-36.

Administration of cultured autologous bone marrow stromal cells into cerebrospinal fluid in spinal injury patients: A pilot study.

Saito F, Nakatani T, Iwase M, Maeda Y, Murao Y, Suzuki Y, Fukushima M, Ide C.

Emergency and Critical Care Center, Kansai Medical University, Moriguchi, Osaka, Japan.

Mesenchymal Cells

Cell Mol Neurobiol. 2006 Oct-Nov;26(7-8):1167-80. Epub 2006 Jul 29.

Transplants of human mesenchymal stem cells improve functional recovery after spinal cord injury in the rat.

Cízková D, Rosocha J, Vanický I, Jergová S, Cízek M.

Institute of Neurobiology, Center of Excellence, SAS, Soltésovej 4, Kosice, 04001, Slovakia. cizkova@saske.sk

- Human Cells into rats
- Cells survived without immune suppression
- Intravenous mesenchymal cells migrated to injury
- Cells did not become neurons
- Cells enhanced recovery (significantly)
- Promoted axonal growth and supported re-myelination

Rationale: Yang et al--Taiwan

Transplantation of Human Umbilical Mesenchymal Stem Cells from Wharton's Jelly after Complete Transection of the Rat Spinal Cord

Chang-Ching Yang[1☯], Yang-Hsin Shih[2,3☯], Miau-Hwa Ko[4☯], Shao-Yun Hsu[1], Henrich Cheng[5,6*], Yu-Show Fu[7,8*]

1 Institute of Anatomy and Cell Biology, School of Medicine, National Yang-Ming University, Taipei, Taiwan, Republic of China, **2** Department of Neurosurgery, Neurological Institute, Taipei Veterans General Hospital, Taipei, Taiwan, Republic of China, **3** School of Medicine, Taipei Medical University, Taipei, Taiwan, Republic of China, **4** Department of Anatomy, School of Medicine, China Medical University, Taichung, Taiwan, Republic of China, **5** Neural Regeneration Laboratory, Department of Neurosurgery, Neurological Institute, Taipei Veterans General Hospital, Taiwan, Republic of China, **6** Department of Pharmacology, School of Medicine, National Yang-Ming University, Taipei, Taiwan, Republic of China, **7** Department of Anatomy and Cell Biology, School of Medicine, National Yang-Ming University, Taipei, Taiwan, Republic of China, **8** Department of Education and Research, Taipei City Hospital, Taipei, Taiwan, Republic of China

Taiwan Rat Study

* Spinal Cords Cut
* MSCs placed over the cut in a fibrin gel
* Spinal cords regrew
* MSCs were not part of the new spinal cord

Yeng et al

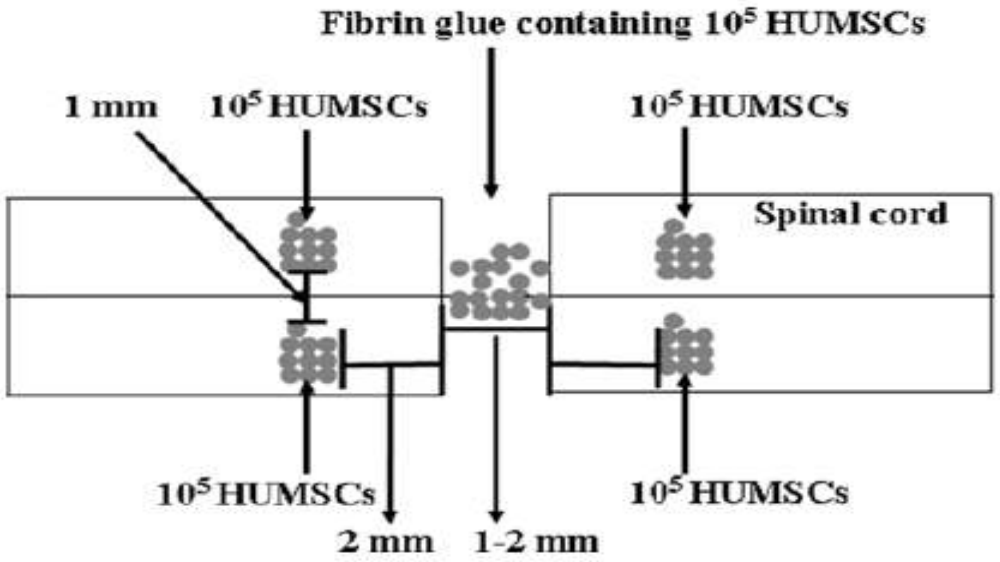
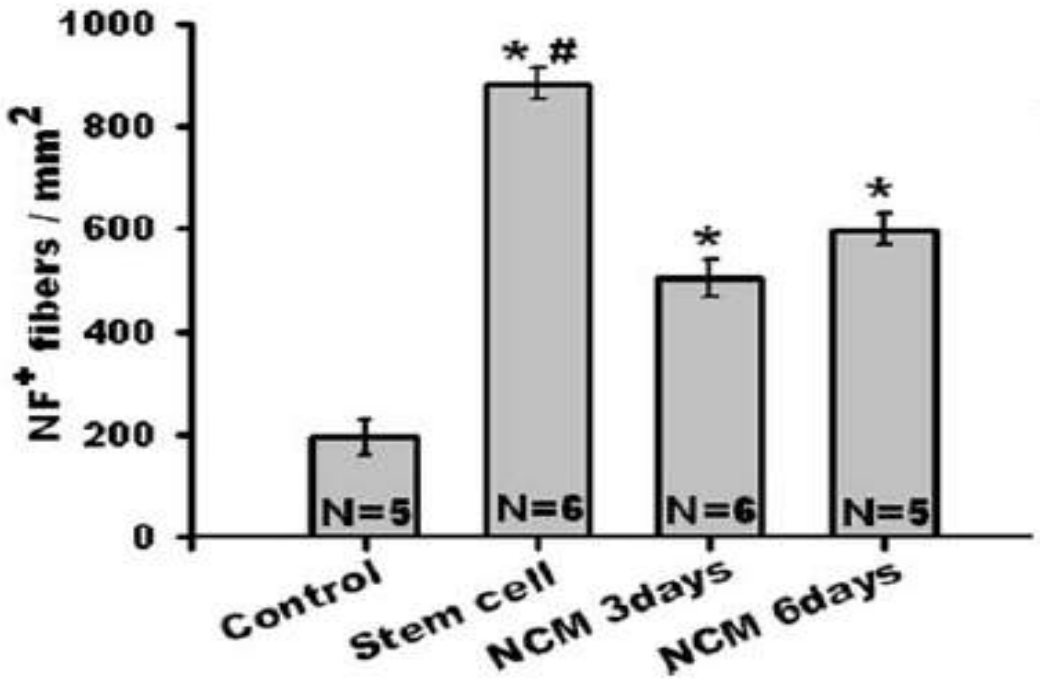


Figure 1. Experimental design for cell grafting after the transection of the spinal cord at the 8th thoracic level.
doi:10.1371/journal.pone.0003336.g001

Yeng et al


1000
800
600
400
200
0
NF+ fibers / mm2
* #
*
*
N=5
N=6
N=6
N=5
Control
Stem cell
NCM 3days
NCM 6days

Take Home

* Cells are not rejected, even trans-species
* Cells do not engraft
* Secretions of cells stimulating the normal response to injury that is lacking in spinal cord
* Reduced the area of the cystic cavity at the site of injury
* Increased the volume of residual white matter
* Promoted the regeneration or sparing of axons in the injured spinal cord
* Cells survived 3 weeks, were gone at 5 weeks
* Cells did not become neuronal tissue
* No evidence of immune reaction to the cells

Best Case

* Juan Carlos Murillo, plane crash
* Commercial pilot, native of Costa Rica
* Received only umbilical cord CD34+ and mesenchymal stem cells using both routes.
* Complete recovery.
* Was evaluated at Miami
* Never see restoration of urinary continence

Published article

Ichim *et al. International Archives of Medicine* 2010, **3**:30
http://www.intarchmed.com/content/3/1/30

INTERNATIONAL
ARCHIVES OF MEDICINE

CASE REPORT **Open Access**

Feasibility of combination allogeneic stem cell therapy for spinal cord injury: a case report

Thomas E Ichim[1], Fabio Solano[2], Fabian Lara[2], Eugenia Paris[3], Federico Ugalde[2], Jorge Paz Rodriguez[2], Boris Minev[4], Vladimir Bogin[1,5], Famela Ramos[1], Erik J Woods[6], Michael P Murphy[7], Amit N Patel[8], Robert J Harman[9], Neil H Riordan[1*]

Study Protocol

* Mesenchymal from cord IV and IT
* Bone marrow cells IT
* 4 weeks in Panama

Safety

* 85 clinical trials being performed using allogeneic, or donor MSCs for a variety of conditions. All have been approved by ethics committees who weight the risk benefit ratio to patients. http://clinicaltrials.gov
* Allogeneic (donor) MSCs from bone marrow have been approved for human use in Canada and New Zealand for the treatment of graft vs. host disease (Osiris). Allogeneic umbilical cord MSCs have been approved for human use in Korea.
* Rheumatoid Arthritis Study
* In our experience>6000 infusions

Umbilical Cord Safety

* Is it natural?
* Every mother has stem cells from every baby she has carried—for up to 30 years—Lancet 2004
* We would not exist as a species if transplantation of cells from one individual to another was lethal
* Once thought that mothers had higher incidence of certain autoimmune diseases—since disproven.

Umbilical Cord Safety

"life span of mothers increased linearly up to 14 children (0.32 years per additional child; p =.004) but decreased with each additional child beyond 14 (p =.0004)."

* J Gerontol A Biol Sci Med Sci. 2006 Feb;61(2):190-5. Does having children extend life span? A genealogical study of parity and longevity in the Amish. McArdle PF, Pollin TI, O'Connell JR, Sorkin JD, Agarwala R, Schäffer AA, Streeten EA, King TM, Shuldiner AR, Mitchell BD.Department of Epidemiology and Preventive Medicine, University of Maryland School of Medicine, Baltimore, MD 21201, USA.

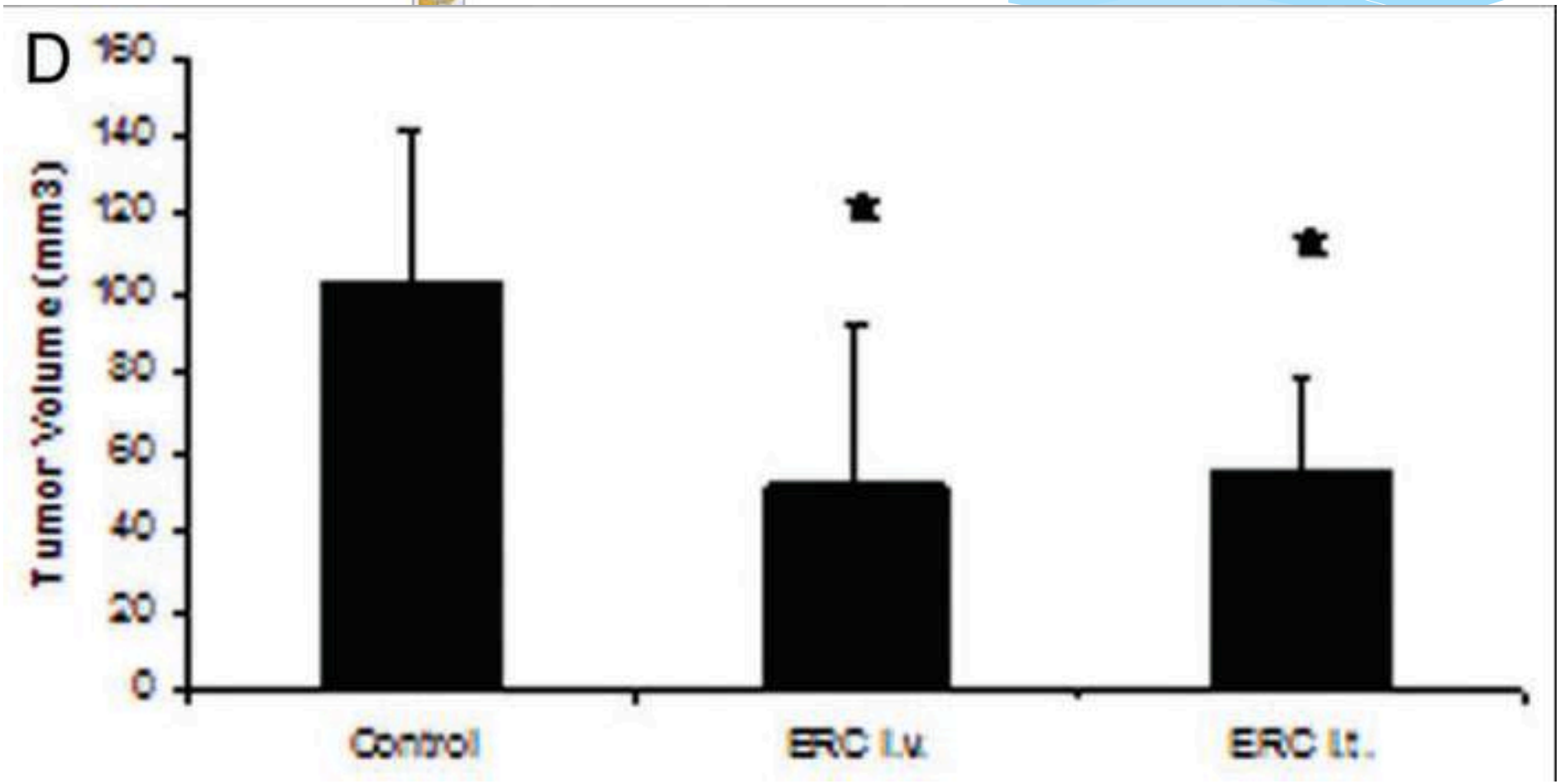
How Many Transplants?

* 1.5 Million corneal transplants have taken place in the past 13 years.
* Each transplant requires limbal cell transplants also for the cornea to "take."
* Limbal cells are mesenchymal stem cells.
* Many of these transplants are done with cells grown, or "expanded" in the laboratory.
* Amniotic Membrane—100 year history of use

Glioma

Inhibition of intracranial glioma growth by endometrial regenerative cells

Xiaodi Han,[1,2] Xiaolong Meng,[1] Zhenglian Yin,[1] Andrea Rogers,[1] Jie Zhong,[1] Paul Rilema,[2] James A. Jackson,[1] Thomas E. Ichim,[3,*] Boris Minev,[4,5] Ewa Carrier,[4] Amit N. Patel,[6] Michael P. Murphy,[7] Wei-Ping Min[8] and Neil H. Riordan[1,3]
Cell Cycle 8:4, 1-5; 15 February 2009]



Ganta et al, K-State

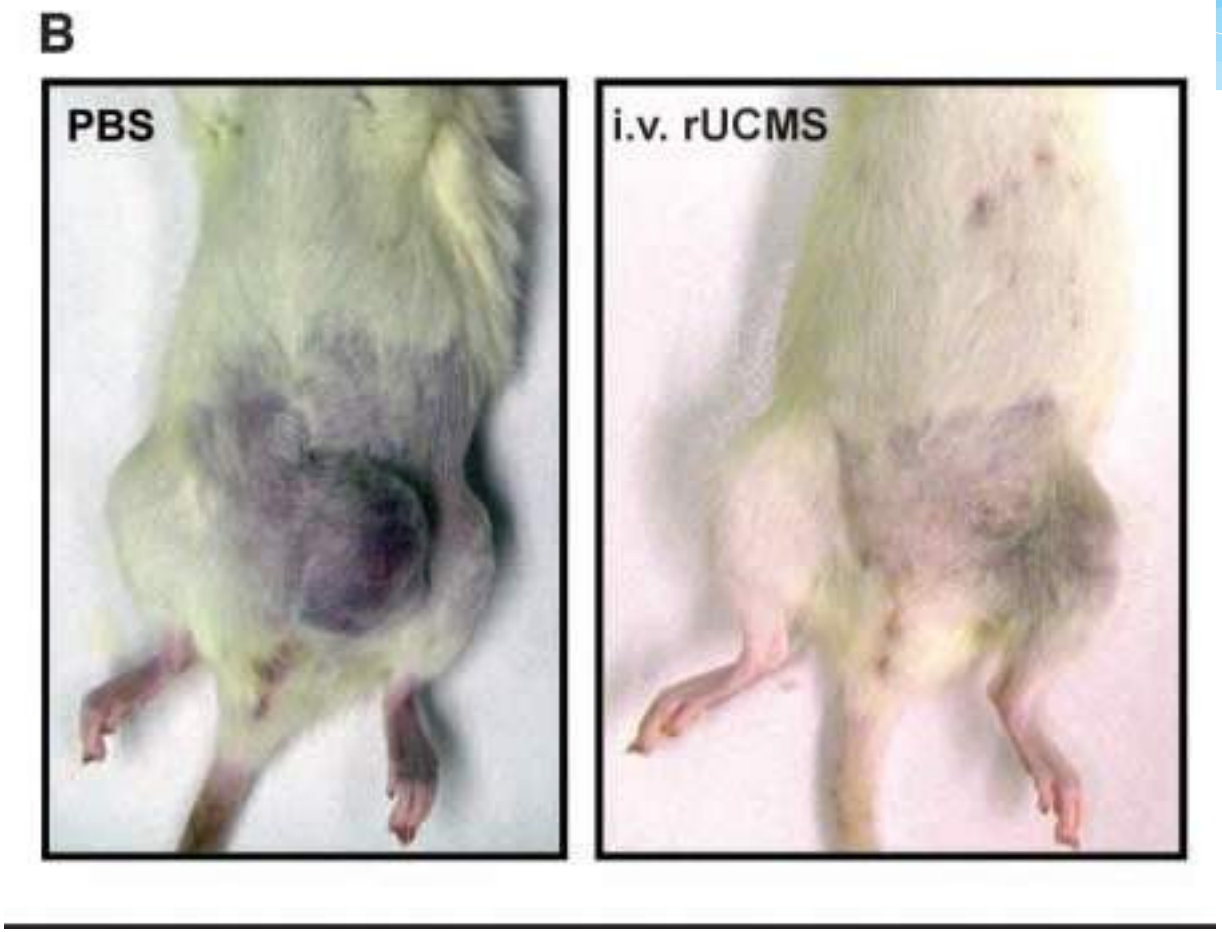
Rat Umbilical Cord Stem Cells Completely Abolish Rat Mammary Carcinomas with No Evidence of Metastasis or Recurrence 100 Days Post–Tumor Cell Inoculation

Chanran Ganta,[1] Doi Chiyo,[1] Rie Ayuzawa,[1] Rajashekar Rachakatla,[1] Marla Pyle,[1] Gordon Andrews,[2] Mark Weiss,[1] Masaaki Tamura,[1] and Deryl Troyer[1]

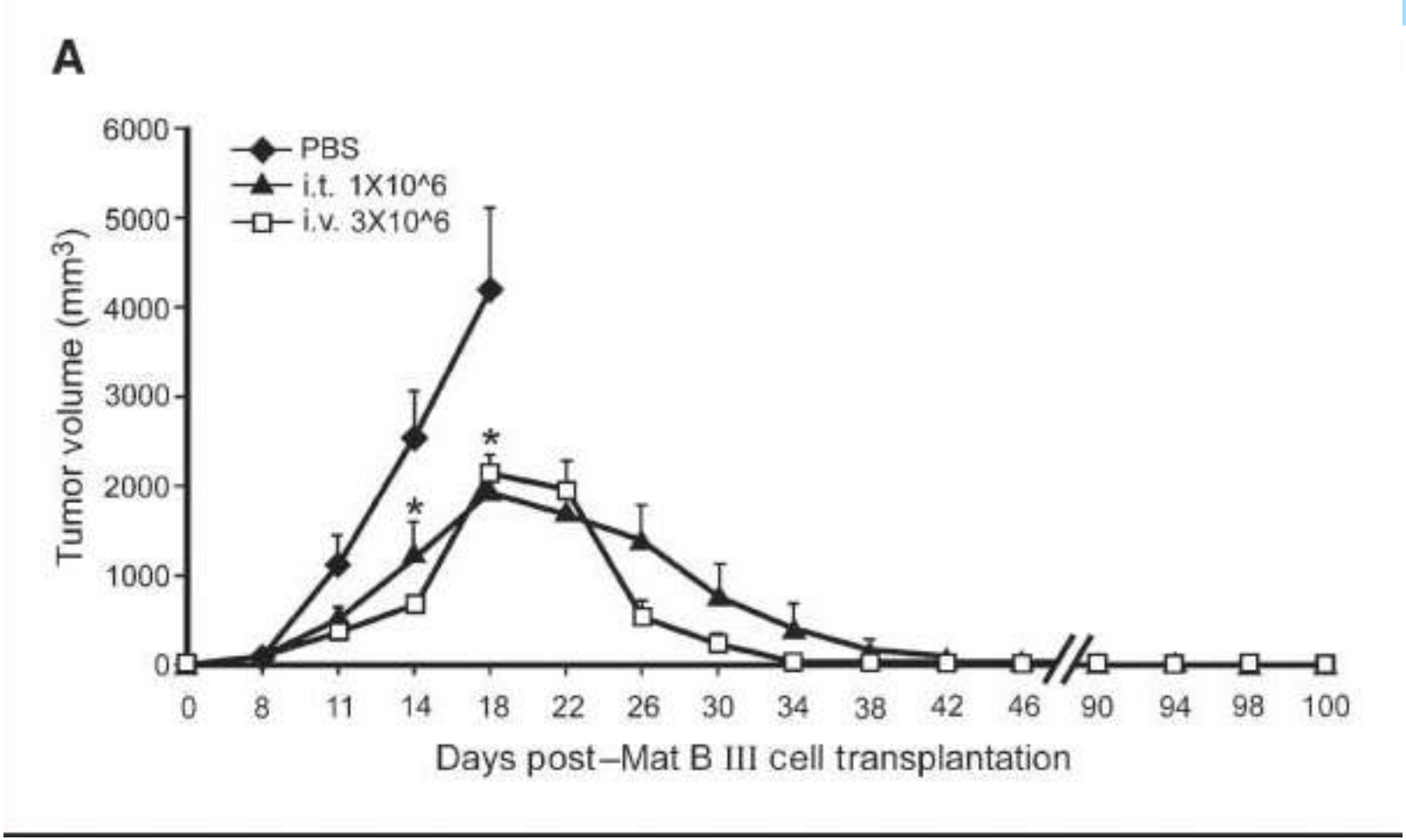
Departments of [1]Anatomy and Physiology and [2]Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas

Cancer Res 2009; 69: (5). March 1, 2009

Ganta et al



Ganta et al


A
PBS
i.t. 1X10^6
i.v. 3X10^6
Tumor volume (mm3)
6000
5000
4000
3000
2000
1000
0
*
*
0
8
11
14
18
22
26
30
34
38
42
46
90
94
98
100
Days post–Mat B III cell transplantation

Summary

* Optimized nutrition supports
* These cells and their secretions are the major repair system of the body.
* Cell secretions may be the second generation of the “right” molecules.