«Μια προοπτική, μη τυχαιοποιημένη, φάσης I/ II , μελέτη ασφάλειας και αποτελεσματικότητας της ενδοβρογχικής έγχυσης αυτόλογων απομονοθέντων από το λιπώδη ιστό βλαστοκυττάρων σε ασθενείς με Ιδιοπαθή Πνευμονική Ίνωση»

HTS – 26-11-2011

A. Tzouvelekis[1], P.Ntolios[1], A. Oikonomou[2], G. Koliakos[3], Vassilis Paspaliaris[4], M.Froudarakis[1], D. Bouros[1]

- 1 Department of Pneumonology, Medical School, Democritus University of Thrace and University Hospital of Alexandroupolis, Greece
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- 3Adistem Ltd. Wanchai, Hong Kong
- 4 Department of Biochemistry, Medical School, Aristotles University of Thessaloniki, Greece

Important fibroproliferative diseases of humans

- **The United States government estimates that 45% of deaths in the United States can be attributed to fibrotic disorders.**
- **Major-organ fibrosis**
  - Interstitial lung disease (ILD)
  - Liver cirrhosis
  - Kidney disease
  - Heart disease
  - Diseases of the eye

Nat Rev Immunol. 2004; 4(8): 583–594

Introduction

IPF Pathogenesis

Treatment
What's Coming Down The IPF Pike?

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 MAY 12, 2011 VOL. 364 NO. 19

Evidence for Human Lung Stem Cells

Jan Kajstura, Ph.D., Marcello Rota, Ph.D., Sean R. Hall, Ph.D., Toru Hosoda, M.D., Ph.D., Domenico D'Amario, M.D., Fumihiro Sanada, M.D., Hanqiao Zheng, M.D., Barbara Ogórek, Ph.D., Carlos Rondon-Clavo, M.D., João Ferreira-Martins, M.D., Alex Matsuda, M.D., Christian Arranto, M.D., Polina Goichberg, Ph.D., Giovanna Giordano, M.D., Kathleen J. Haley, M.D., Silvana Bardelli, Ph.D., Hussein Rayatzadeh, M.D., Xiaoli Liu, M.D., Ph.D., Federico Quaini, M.D., Ronglih Liao, Ph.D., Annarosa Leri, M.D., Mark A. Perrella, M.D., Joseph Loscalzo, M.D., Ph.D., and Piero Anversa, M.D.

The NEW ENGLAND JOURNAL of MEDICINE

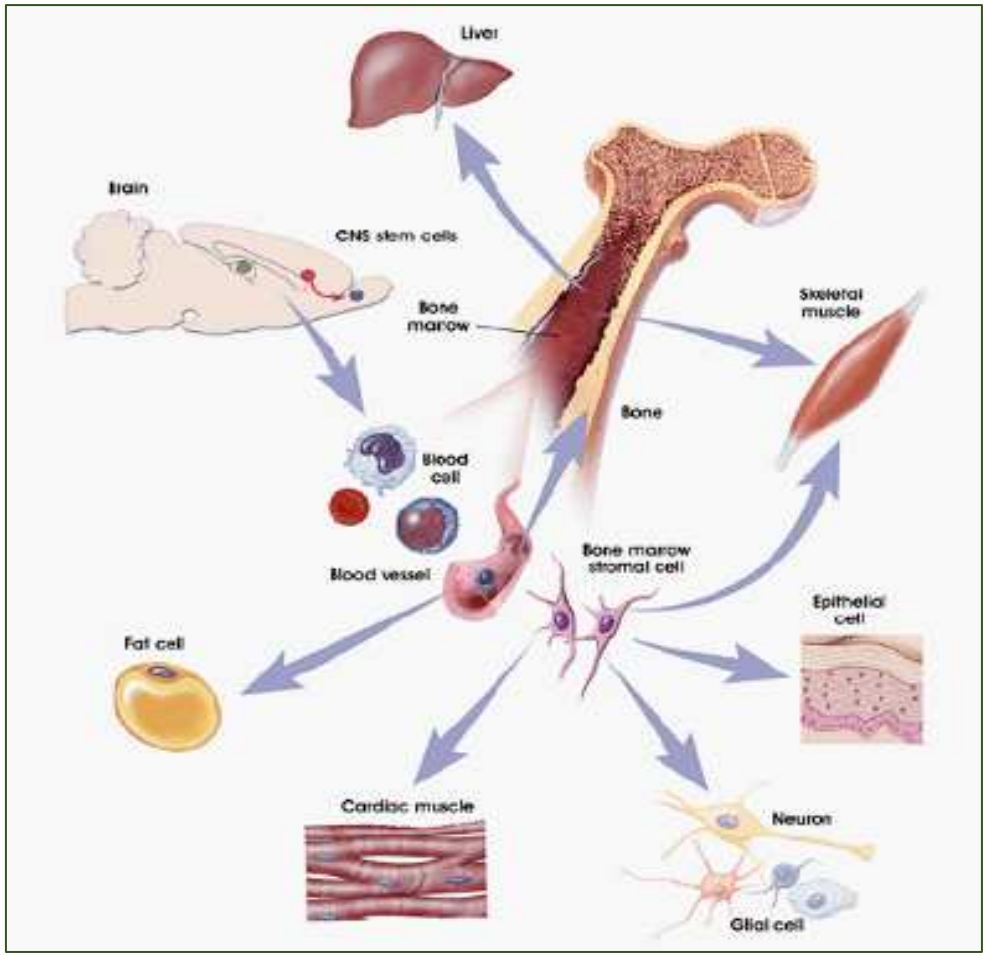
EDITORIALS

Toward Lung Regeneration

Harold A. Chapman, M.D.

What is a stem cell?

- Self-renewal: cell division in undifferentiated state
- Unlimited potency: differentiation into diverse cell types



Stem Cell Sources

Embryonic stem cell

- IVF embryo

Adult stem cell*

- Bone Marrow
- Adipose tissue-SVF
- Peripheral blood
- Blood vessel
- Endogenous progenitors

Why fat?

- ADSCs lie in abundancy (1000 fold more)
- Easily accessible (compared to BM)
- Ethically Uncontested source of MSCs
- No need for manipulation/expansion
- Reduced possibility for carcinogenesis

Tzouvelekis et al.Curr Opin Pulm Med 2011. 17:368-373
Weiss et al. Proc Am Thorac Soc 2008.5:637-667

Phenotypes arising from ADSCs

Mechanisms of action

Casteilla L *et al*. Adipose stroma cells and cell therapy

World J Stem Cells 2011 April 26; 3(4): 25-33
ISSN 1948-0210 (online)

Table 1 Differentiated phenotype given rise from adipose-derived stromal cell and interactive effects of these cells with immune and cancer cells

Phenotype given rise in *in vitro* system	Ref.
Classic mesenchymal phenotype (adipocyte, osteoblast, chondrocyte)	[1]
Hematopoietic supporting cells	[25]
Other phenotypes	Vascular cells (Smooth-muscle cells, Endothelial)[16]
	Neurones[1]
	Cardiomyocyte and skeletal cells in the required presence of 5 azacytidine[16]
Modulation of inflammation and immune suppressive functions	Rheumatoid arthritis[31]
	GVH[30]
	Autoimmune encephalomyelitis[32]
Anti-cancer effect	Tumor progression inhibition[34]
Pro-cancer effects	Tumor progression growth[35,37]

Are stem cells potent enough to be differentiated into AECs??

Derivation of Lung Epithelium from Human Cord Blood–derived Mesenchymal Stem Cells

Viranuj Sueblinvong[1], Roberto Loi[2], Philip L. Eisenhauer[1], Ira M. Bernstein[3], Benjamin T. Suratt[1], Jeffrey L. Spees[1], and Daniel J. Weiss[1]

[1]Department of Medicine, University of Vermont College of Medicine, Burlington, Vermont; [2]Oncology and Molecular Pathology Unit, Department of Toxicology, University of Cagliari, Cagliari, Italy; and [3]Department of Obstetrics and Gynecology, University of Vermont College of Medicine, Burlington, Vermont

AMERICAN JOURNAL OF RESPIRATORY AND CRITICAL CARE MEDICINE VOL 177 2008

STEM CELLS®

STEM CELL GENETICS AND GENOMICS

Alteration of Marrow Cell Gene Expression, Protein Production, and Engraftment into Lung by Lung-Derived Microvesicles: A Novel Mechanism for Phenotype Modulation

STEM CELLS 2007;25:2245–2256 www.StemCells.com

Published in final edited form as:
Nat Genet. 2008 July ; 40(7): 862–870. doi:10.1038/ng.157.

A Gata6-Wnt pathway required for epithelial stem cell development and airway regeneration

Yuzhen Zhang[1], **Ashley M. Goss**[2], **Ethan David Cohen**[1], **Rachel Kadzik**[2], **John J. Lepore**[1], **Karthika Muthukumaraswamy**[1], **Jifu Yang**[1], **Francesco J. DeMayo**[5], **Jeffrey A. Whitsett**[6], **Michael S. Parmacek**[1,3,4], and **Edward E. Morrisey**[1,2,3,4,*]

Can they attenuate experimental lung fibrosis?

The American Journal of Pathology, Vol. 175, No. 1, July 2009

DOI: 10.2353/ajpath.2009.080629

Stem Cells, Tissue Engineering and Hematopoietic Elements

Human Umbilical Cord Mesenchymal Stem Cells Reduce Fibrosis of Bleomycin-Induced Lung Injury

Adipose Stem Cell Treatment in Mice Attenuates Lung and Systemic Injury Induced by Cigarette Smoking

Kelly S. Schweitzer[1,2], Brian H. Johnstone[3,4], Jana Garrison[1,2], Natalia I. Rush[1,2], Scott Cooper[5], Dmitry O. Traktuev[3,4], Dongni Feng[3,4], Jeremy J. Adamowicz[1,2], Mary Van Demark[1,2], Amanda J. Fisher[2,6], Krzysztof Kamocki[2,7], Mary Beth Brown[1,2], Robert G. Presson, Jr.[2,6], Hal E. Broxmeyer[4,5], Keith L. March[3,4,8*], and Irina Petrache[1,2,4,8*]

AMERICAN JOURNAL OF RESPIRATORY AND CRITICAL CARE MEDICINE VOL 183 2011

Are they safe??

Journal of the American College of Cardiology
© 2009 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 54, No. 24, 2009
ISSN 0735-1097/09/$36.00
doi:10.1016/j.jacc.2009.06.055

CLINICAL RESEARCH — Clinical Trials

A Randomized, Double-Blind, Placebo-Controlled, Dose-Escalation Study of Intravenous Adult Human Mesenchymal Stem Cells (Prochymal) After Acute Myocardial Infarction

53 PATIENTS

Joshua M. Hare, MD,* Jay H. Traverse, MD,† Timothy D. Henry, MD,† Nabil Dib, MD,‡
Robert K. Strumpf, MD,‡ Steven P. Schulman, MD,§ Gary Gerstenblith, MD,§
Anthony N. DeMaria, MD,‖ Ali E. Denktas, MD,¶ Roger S. Gammon, MD,#
James B. Hermiller, JR, MD,** Mark A. Reisman, MD,†† Gary L. Schaer, MD,‡‡
Warren Sherman, MD§§

Miami, Florida; Minneapolis, Minnesota; Phoenix, Arizona; Baltimore, Maryland; San Diego, California; Houston and Austin, Texas; Indianapolis, Indiana; Seattle, Washington; Chicago, Illinois; and New York, New York

Objectives Our aim was to investigate the safety and efficacy of intravenous allogeneic human mesenchymal stem cells (hMSCs) in patients with myocardial infarction (MI).

Background Bone marrow-derived hMSCs may ameliorate consequences of MI, and have the advantages of preparation ease, allogeneic use due to immunoprivilege, capacity to home to injured tissue, and extensive pre-clinical support.

Methods We performed a double-blind, placebo-controlled, dose-ranging (0.5, 1.6, and 5 million cells/kg) safety trial of intravenous allogeneic hMSCs (Prochymal, Osiris Therapeutics, Inc., Baltimore, Maryland) in reperfused MI patients (n = 53). The primary end point was incidence of treatment-emergent adverse events within 6 months. Ejection fraction and left ventricular volumes determined by echocardiography and magnetic resonance imaging were exploratory efficacy end points.

Results Adverse event rates were similar between the hMSC-treated (5.3 per patient) and placebo-treated (7.0 per patient) groups, and renal, hepatic, and hematologic laboratory indexes were not different. Ambulatory electrocardiogram monitoring demonstrated reduced ventricular tachycardia episodes (p = 0.025), and pulmonary function testing demonstrated improved forced expiratory volume in 1 s (p = 0.003) in the hMSC-treated patients. Global symptom score in all patients (p = 0.027) and ejection fraction in the important subset of anterior MI patients were both significantly better in hMSCs versus placebo subjects. In the cardiac magnetic resonance imaging substudy, hMSC treatment, but not placebo, increased left ventricular ejection fraction and led to reverse remodeling.

Conc

Intravenous allogeneic hMSCs are safe in patients after acute MI.

Improved FEV1

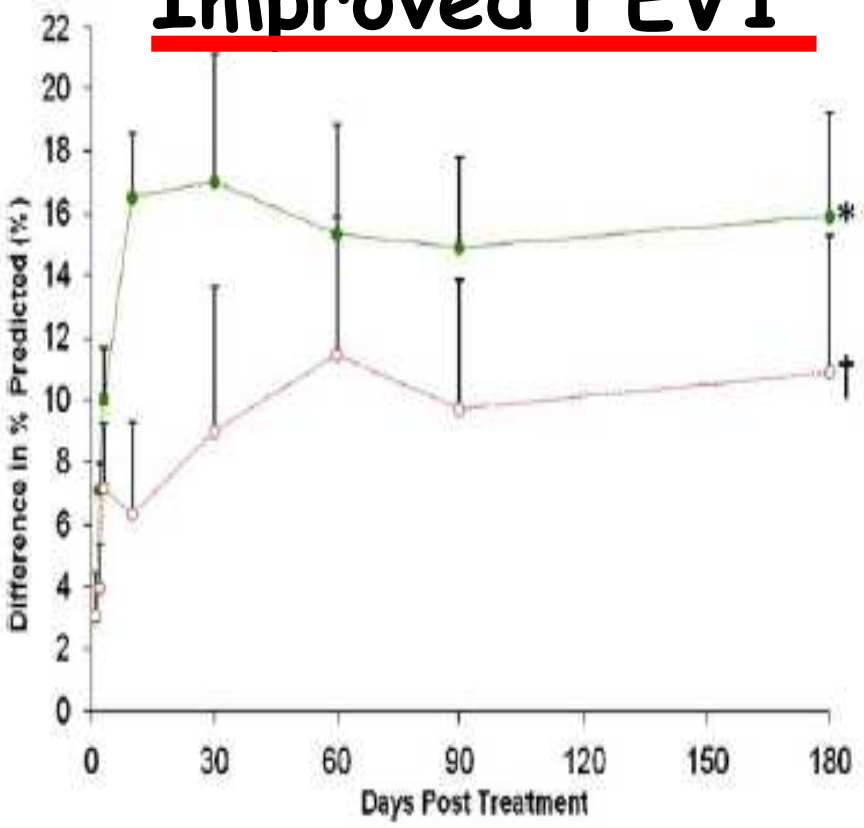


Figure 6 Difference From Baseline in FEV1 % Predicted

Human mesenchymal stem cells = **green line** (n = 31); placebo = **red line** (n = 18). Error bars represent standard error of the mean. *p = 0.003 by repeated measure analysis of variance; †p = 0.01 versus placebo. FEV1 = forced expiratory volume in 1 s.

Improved LVEF

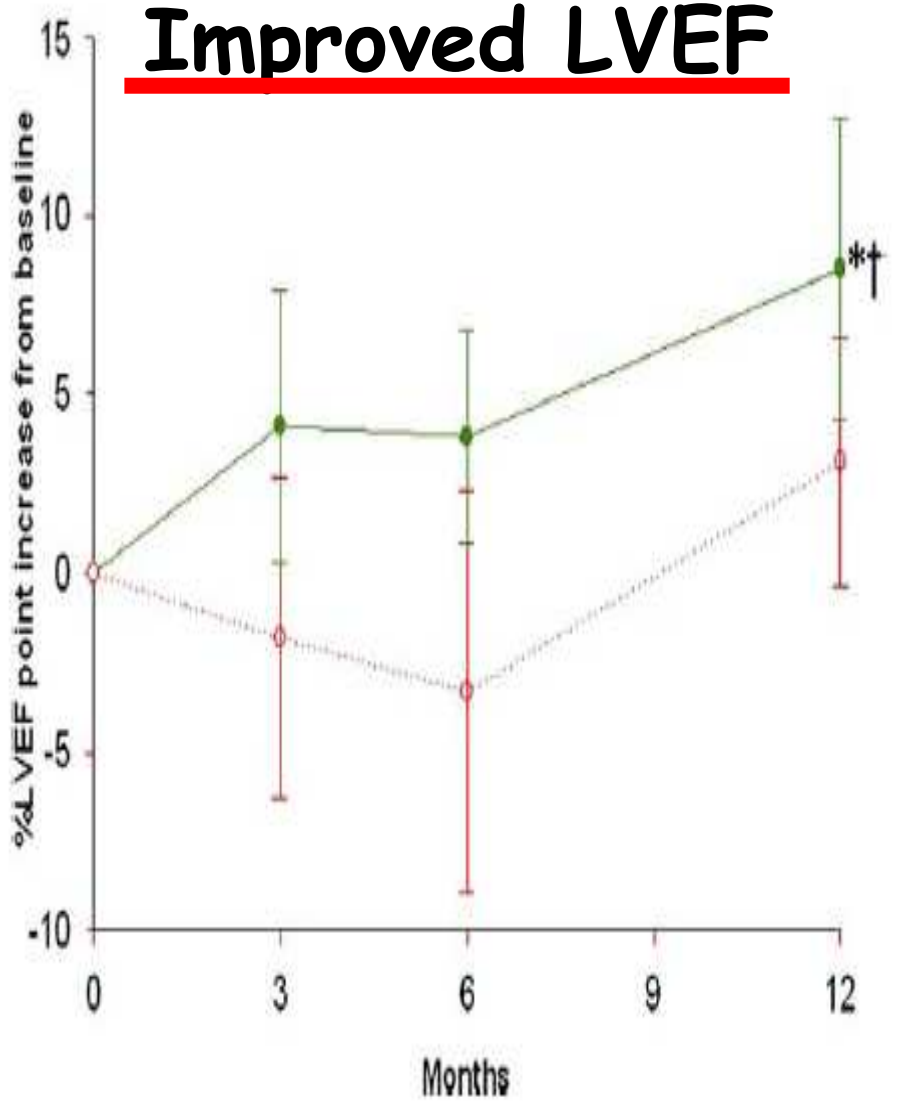


Figure 4 Impact of hMSC Treatment on LVEF Evaluated by Cardiac MRI

Aim - Hypothesis

- Endobronchial autologous infusion of ADSCs is safe, well tolerated and effective as a therapeutic modality in IPF patients

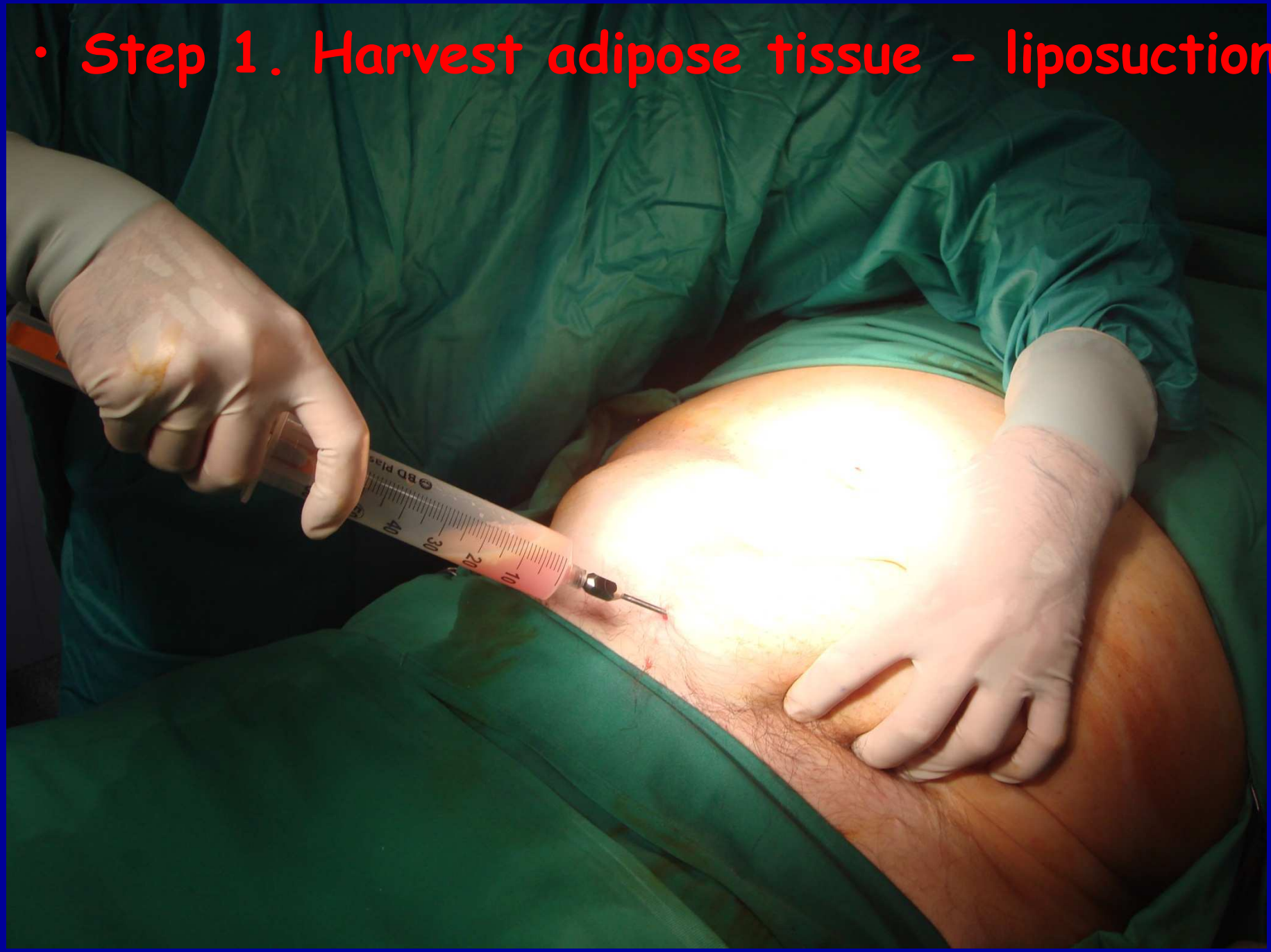
- ADSCs may exert their beneficial role through paracrine anti-inflammatory, immunomodulatory activity and reparative capacity through differentiation into alveolar and endothelial progenitor cells

Protocol analysis

Study population	12 phase I 30 phase II
Study Duration	36 mo (12 recr.*2, 12fu)
Number of infusions	3 (one each month) (phase I) 3 (one each month) (phase II)
Inclusion criteria	IPF (ATS/ERS 2011) of moderate disease severity (FVC>50%, DLCO>35%)
Exclusion Criteria	Cancer, End stage CHF, serious CNS abnormalities, HF, RF
Primary endpoints	Safety: Toxicity level (Low, Medium, High)
Secondary endpoints (exploratory)	Improvement in FVC, DLCO (6 and 12 mo after first infusion) Improvement in SGRQ, 6MWD Stabilization HRCT

Methods

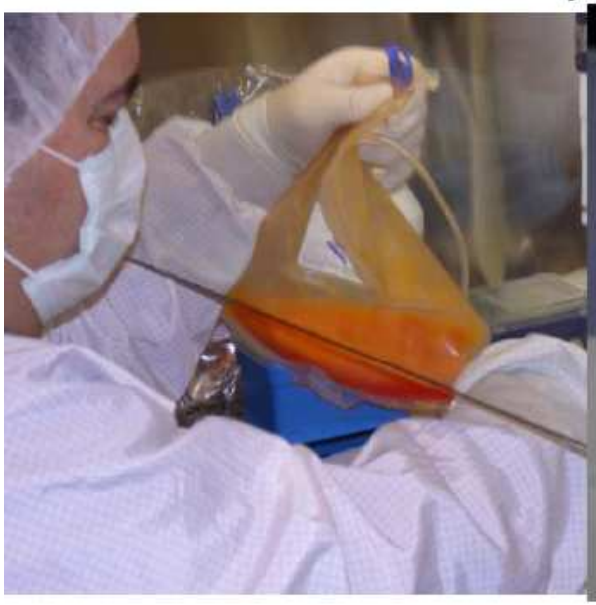
- Step 1. Harvest adipose tissue - liposuction



AdiStem Ltd.

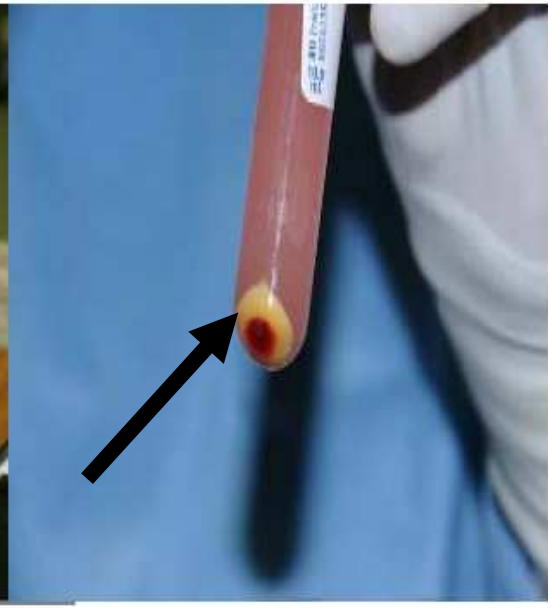
Step 2. Separation

Harvested fat



Centrifuge separates fat cells from MSCs

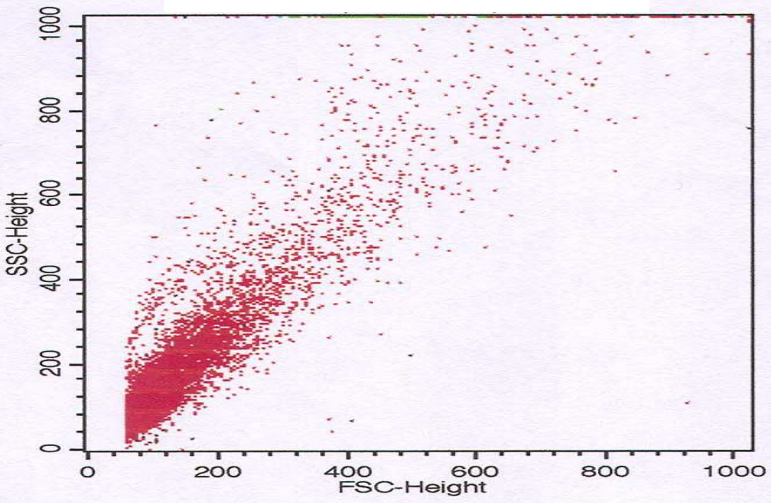
Final Pellet – Stromal Vascular Fraction

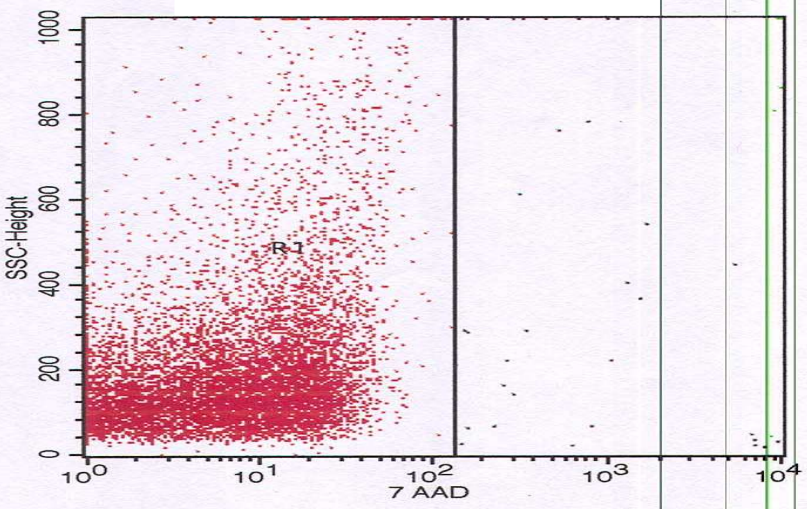


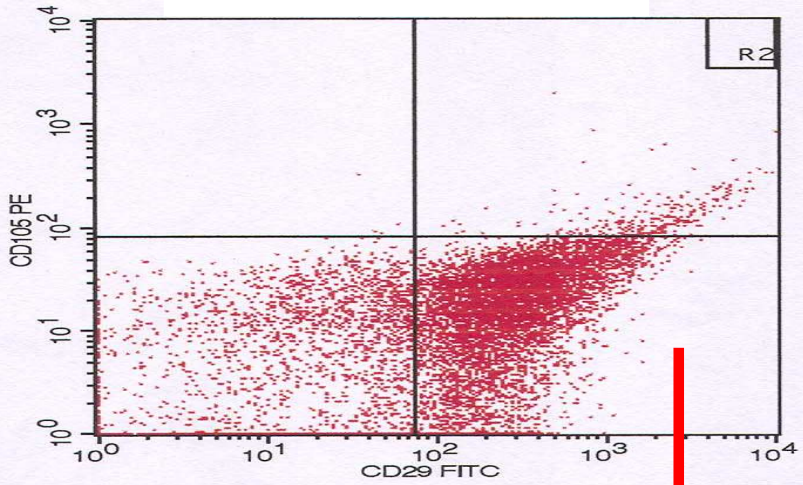
Stromal Vascular Fraction

- Heterogeneous cell population
- 55-70% cells of mesenchymal origin (CD29+, CD105+, CD90+, CD45-, CD34-)
- 20% mature endothelial (CD31+, CD105+) and hematopoietic cells (CD34+)

World J Stem Cells 2011 April 26; 3(4): 25-33
ISSN 1948-0210 (online)







Sample ID: katsavaras iperikeime I
Acquisition Date: 01-Mar-10
X Parameter: CD29 FITC (Log)
Y Parameter: CD105 PE (Log)

Quad	Events	% Gated
UL	4	0.04
UR	278	2.68
LL	2480	23.91
LR	7612	73.38

Sample ID: katsavaras iperikeime lipos
Acquisition Date: 01-Mar-10
X Parameter: 7 AAD (Log)
Y Parameter: SSC-Height (Linear)

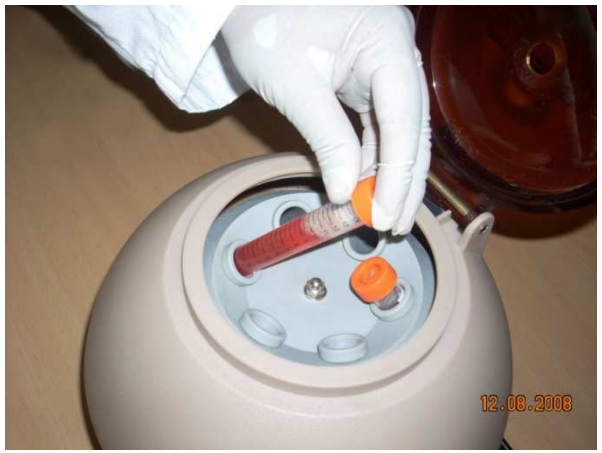
Region	Events	% Gated	Px,Py
R1	10374	91.17	5, 2
R2	972	8.54	3, 4

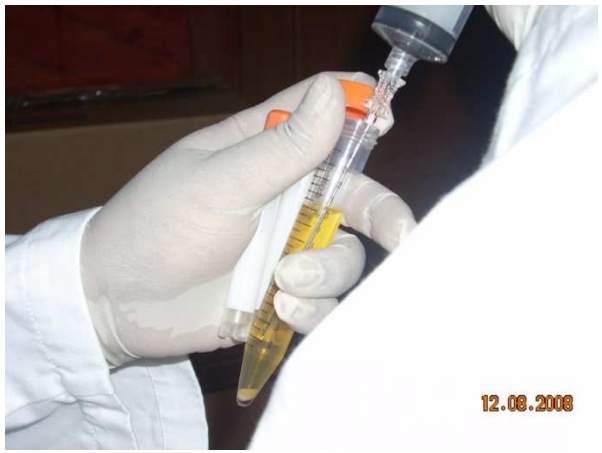
LR=73.38%
CD29+,CD105+

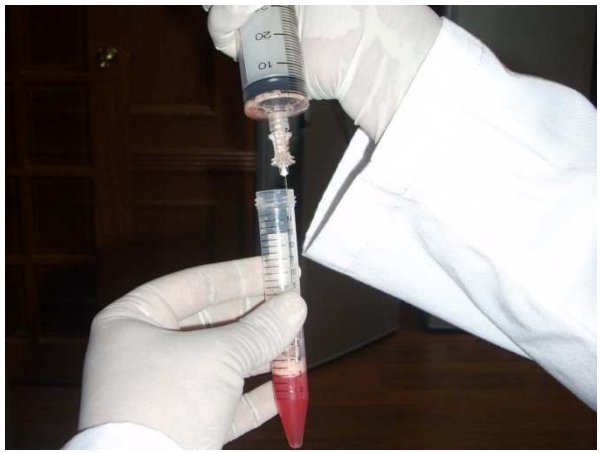
AdiStem Ltd.

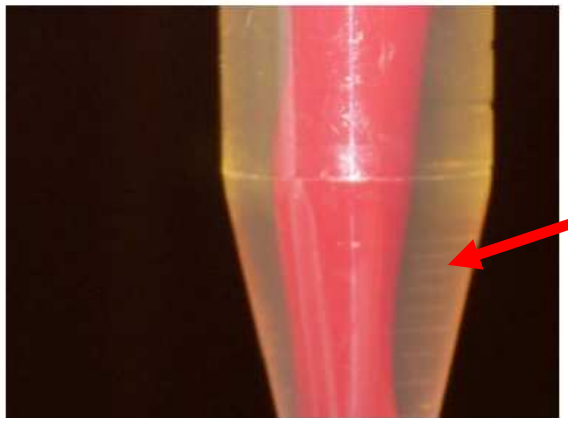
Step 3. Activation

A. Platelet Rich Plasma (PRP)





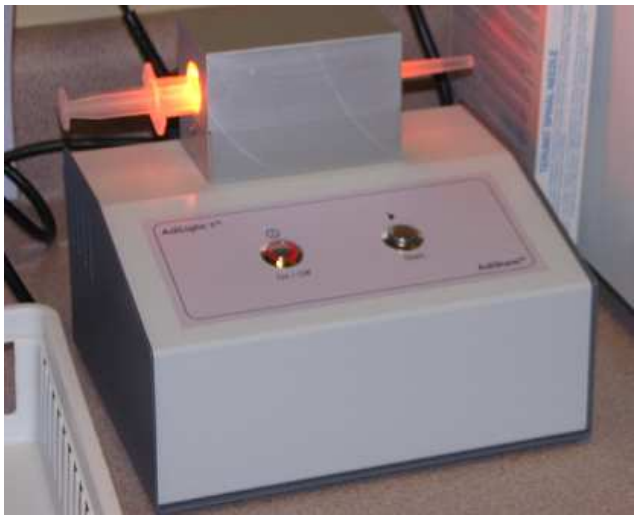




Growth Factors (VEGF, KGF, IGF)

B. Photo-modulation

- Place syringe in Adilight for 20 min
- Enhanced expression of VEGF, IL1-ra, proliferation



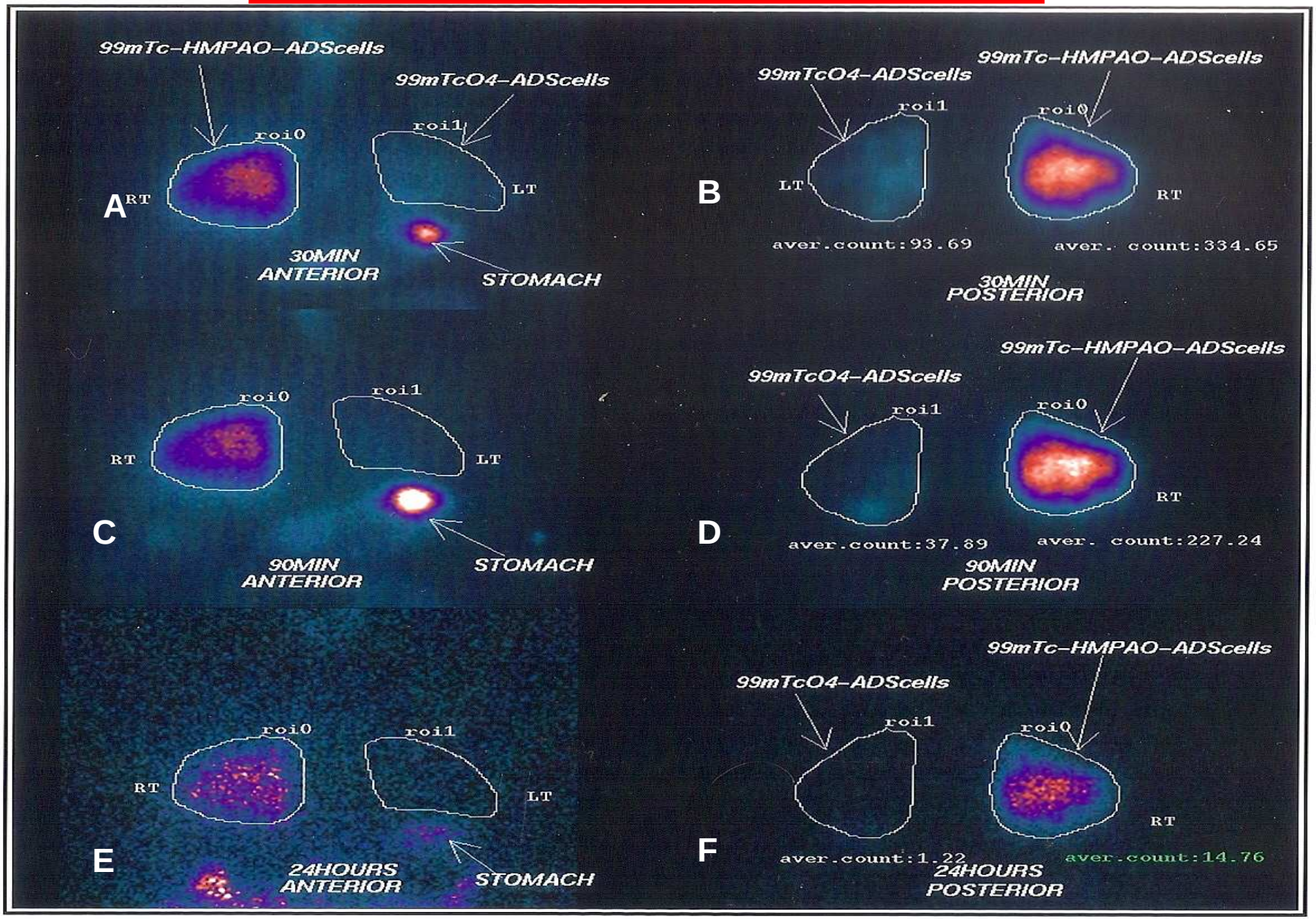
Lin *et al. Journal of Translational Medicine* 2010, **8**:16
http://www.translational-medicine.com/content/8/1/16

REVIEW Open Access

Lasers, stem cells, and COPD

Step 5. Endobronchial infusion of ADSCs in RLL and LLL

Visualization of ADSCs



Tzouvelekis *et al. Journal of Translational Medicine* 2011, **9**:182
http://www.translational-medicine.com/content/9/1/182

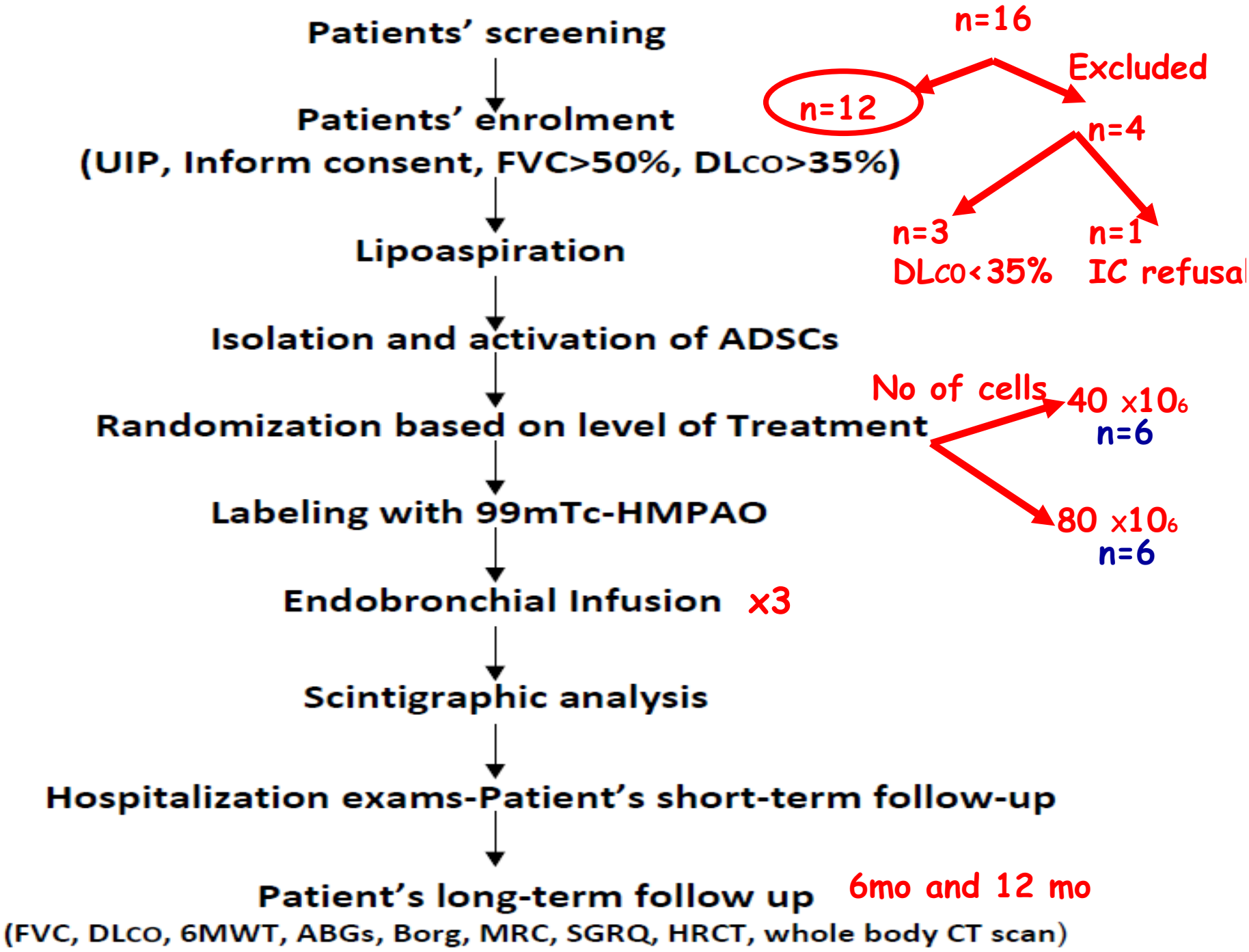
JOURNAL OF TRANSLATIONAL MEDICINE

PROTOCOL Open Access

Stem cell therapy for idiopathic pulmonary fibrosis: a protocol proposal

Argyris Tzouvelekis[1], George Koliakos[2], Paschalis Ntolios[3], Irene Baira[3], Evangelos Bouros[3], Anastasia Oikonomou[4], Athanassios Zissimopoulos[5], George Kolios[3], Despoina Kakagia[6], Vassilis Paspaliaris[7], Ioannis Kotsianidis[8], Marios Froudarakis[1] and Demosthenes Bouros[1*]

Results


Patients' screening
n=16
Excluded
n=12
Patients' enrolment
(UIP, Inform consent, FVC>50%, DLCO>35%)
n=4
n=3
DLCO<35%
n=1
IC refusal
Lipoaspiration
Isolation and activation of ADSCs
No of cells
40 x10⁶
n=6
Randomization based on level of Treatment
80 x10⁶
n=6
Labeling with 99mTc-HMPAO
Endobronchial Infusion x3
Scintigraphic analysis
Hospitalization exams-Patient's short-term follow-up
Patient's long-term follow up
6mo and 12 mo
(FVC, DLCO, 6MWT, ABGs, Borg, MRC, SGRQ, HRCT, whole body CT scan)

Patients' baseline data

Characteristics	Baseline data
Subjects	12
Male	9
Age (yrs)	63 (55-75)
Smokers	12
Ex-smokers	12
Non-smokers	0
Prior treatment (steroids) received	5
Other treatment received	2
VATS	4
Emphysema (HRCT)	4
sPAP (by echocardiography) mmHg	37.2 $\pm$ 19.2

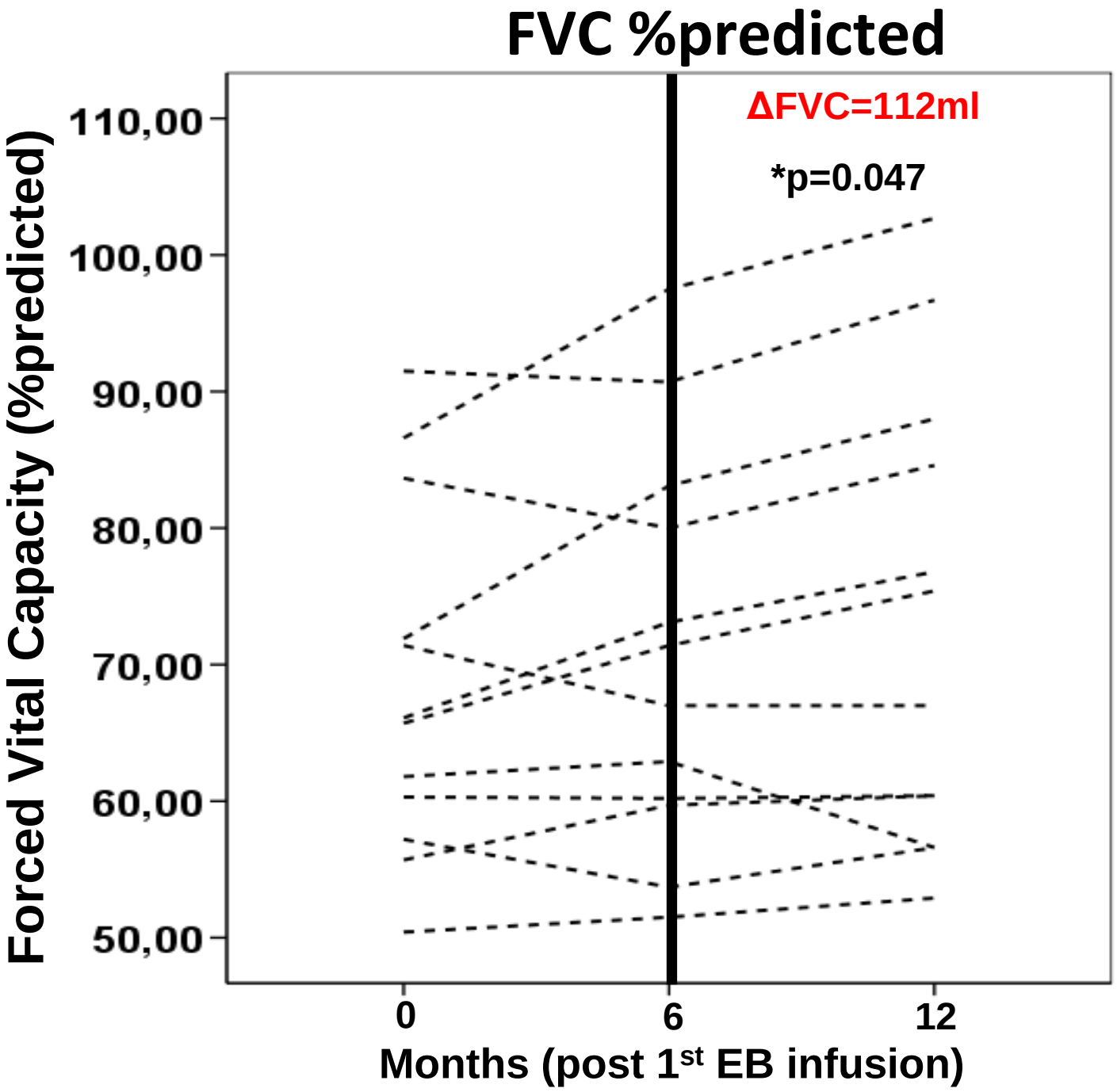
Safety – Primary end-points

Fever (transient – post bronchoscopy)	8/12
Worsening of cough	1/12
Worsening of Dyspnea	1/12
Allergic Reactions	0/12
Infections (fever, ↑ WBCs, ↑ CRP, BAL +culture)	0/12
Liver enzymes, creatinine abnormalities	0/12
Acute Exacerbations/Hospitalizations/deaths	0/12
Ectopic tissue formation (whole body CT scan- 1yr follow-up)	0/12

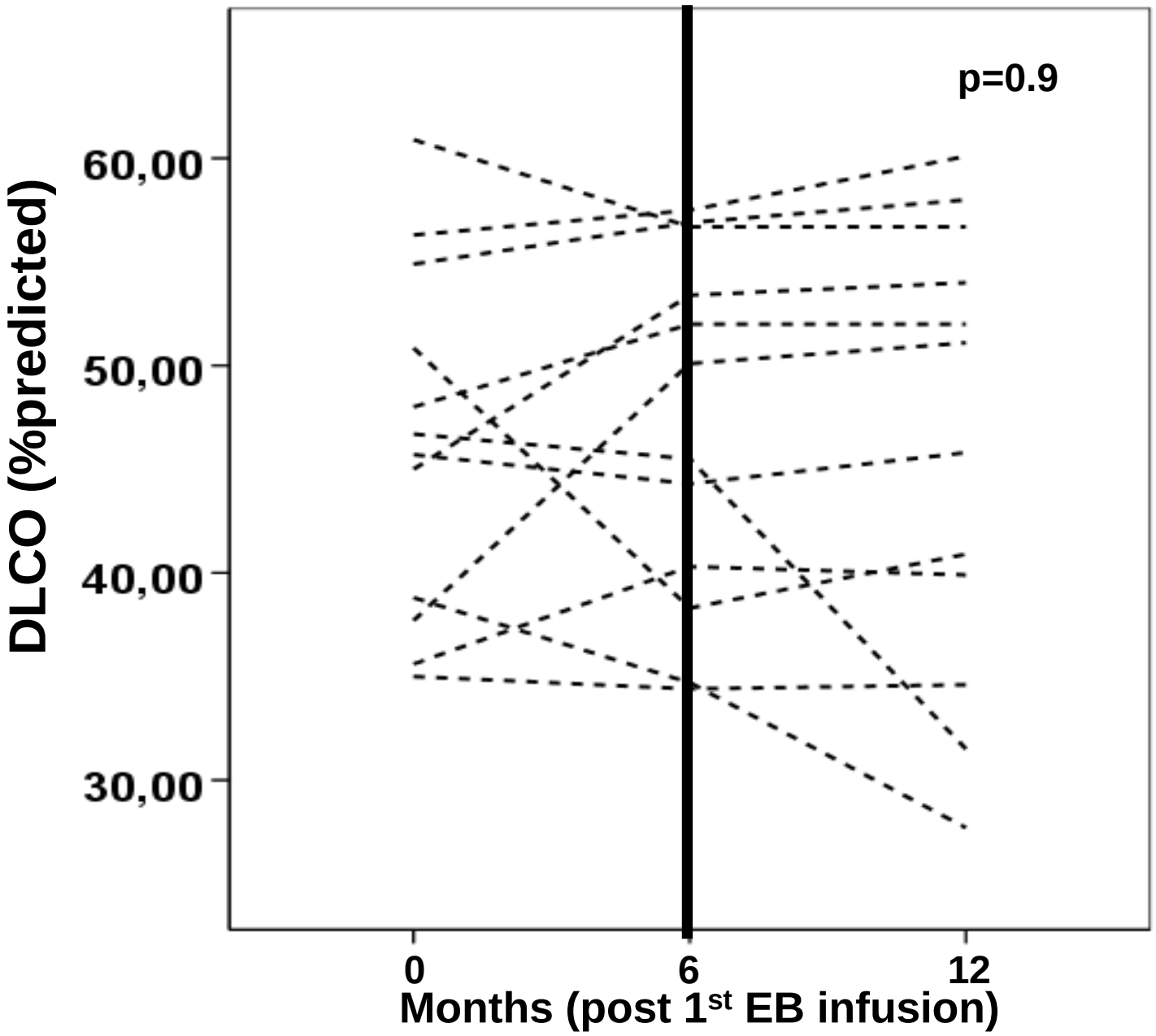
Efficacy – Secondary end-points

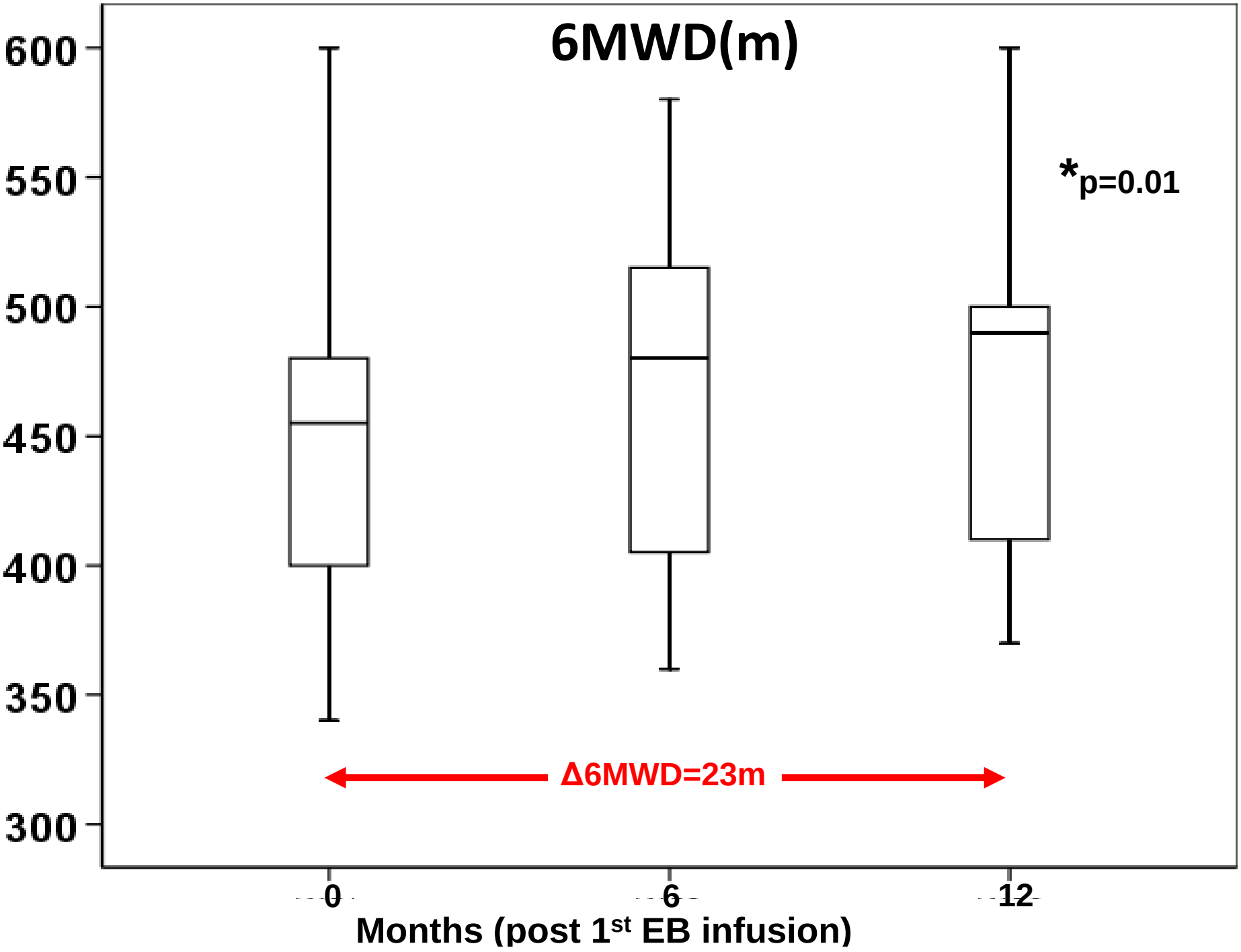
Table 2. FVC, DL_{CO}, 6MWD, SGRQ, and MRC dyspnea scale at baseline and 6 and 12 month post first EB infusion

	Baseline	6 mo	12 mo	p-value [1]	p-value [2]
FVC (%)pred	68,5 ± 12,9	70,9 ± 16,5	73,2 ± 16,8	0.156	0.047
FVC (ml)	2186 ± 514	2251 ± 503	2298 ± 445	ΔFVC_1=65ml	ΔFVC_2=112ml
DL_{CO} (%)pred	46,3 ± 8,4	47 ± 8,6	46,1 ± 10,2	0.7	0.9
6MWD	450 +70	469 +71	473 + 74	0.07	0.01
SGRQ	44,5± 13,2	25± 6,1	26,5± 6,5	<0.001	<0.001
CAT	12,4 ± 4,5	7,9 ± 3,1	7,9± 3,3	0.003	0.002
MRC	2	1,35	1,25	<0.001	<0.001


FVC %predicted
ΔFVC=112ml
*p=0.047
Forced Vital Capacity (%predicted)
110,00
100,00
90,00
80,00
70,00
60,00
50,00
0
6
12
Months (post 1st EB infusion)

DLco %predicted




6MWD(m)
600
550
500
450
400
350
300
*p=0.01
Δ6MWD=23m
0
6
12
Months (post 1st EB infusion)

Limitations

- Limited number of patients
- Non-randomized study
- No placebo – controlled study
- Quality of life parameters – placebo effect??
- Arbitrary randomization based on number of ADSCs infused (however; no differences in both safety and efficacy data btw 2 groups)
- Still don't know the fate of these cells within the fibrotic lung? do they really work? Mechanisms?
- Time of infusion (after lung injury) is critical in animals for stem cell fate….in humans??

Sueblivong et al. Current Opinion Pharmacology 2009, 9:268-273

Conclusions

- EB infusion of ADSCs-SVF is safe, well tolerable, ethically uncontested and costless therapeutic modality in IPF patients
- Marginal improvement in FVC- No differences in DLCO
- Significant improvement in quality of life parameters (even though a potential placebo effect is still important since pt is feeling better)
- Respiratory medicine has significantly lagged behind other specialties regarding cell therapies due to ethical and safety reasons – time to move forward
- Larger multi-center prospective randomized placebo-controlled studies are sorely needed to prove efficacy
- When you inform the patient always separate the hope from the hype