

New strategy and possibility for using ADRCs for Treating Scleroderma

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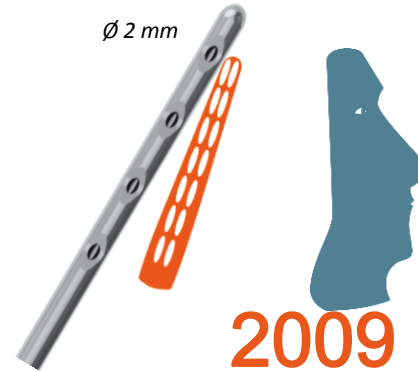
FROM FAT TO STEM CELLS

Treatment for FACE and HANDS in SCLERODERMA



1996

SYDNEY COLEMAN
LIPOSTRUCTURE ®



2009

MICROFAT GRAFTING
FACE SCLERODERMA PATIENTS



P. NGUYEN



2012

SVF INJECTION
HANDS SCLERODERMA DISABILITY

ATMP

Advanced Therapy Medicinal Products

FACE ASSESSMENT
IN PATIENTS
WITH SCLERODERMA

Facial autologous fat grafting

- **Indications**

- **Reconstructive surgery**

- Traumatic sequelae
 - Burns
 - Radiation therapy,
 - Iatrogenic lipodystrophy
 - Facial hemiatrophy ...

- **Aesthetic surgery**

- Filling and reduction of wrinkles
 - Restoring facial contours and face volumes

- **In systemic sclerosis (SSc)**

- **One case in systemic sclerosis**

« Deep phenol peeling and fat injection: treatment option for perioral wrinkles in a scleroderma patient. »

Ramon Y, Fodor A, Ullmann Y. *Dermatol Surg*. **2005** Jul;31:777-9.

- **« En coup de sabre » scleroderma**

« Frontallinear scleroderma: long-term result in volumetric restoration of the fronto-orbital area by structural fat grafting. »

Consorti G, Tieghi and L.C. Clauser. *J. Craniofac Surg*, **2012**. 23(3): p.e 263-5

- **Peri oral fat injection. Average mouth opening**

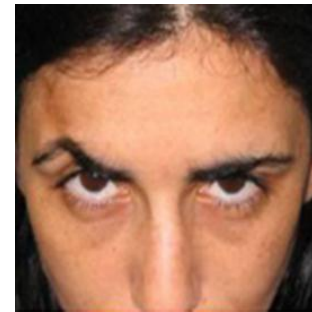
« Autologous fat grafting in the treatment of fibrotic perioral changes in patients with systemic sclerosis. »

Del Papa N, Caviggioli F, Sambataro D, Zaccara E, Vinci V, Di Luca G, Parafioriti A, Armiraglio E, Maglione W, Polosa R, Klinger F, Klinger M. *Cell Transplant*. **2015**;24(1):63-72.

- **Facial treatment on Systemic Sclerosis Patients**

« Efficacy of Autologous Microfat Graft on Facial Handicap in Systemic Sclerosis Patients. »

Nolwenn Sautereau, Aurelie Dumas, Romain Truillet, Elisabeth Jouve, Jeremy Magalon, Julie Veran, Dominique Casanova, Yves Frances, Guy Magalon, Brigitte Granel. *PRS Global Open* • **2016**



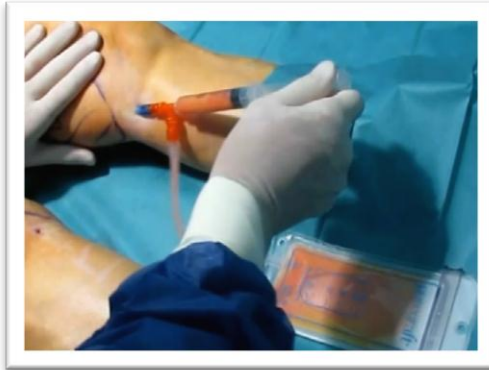
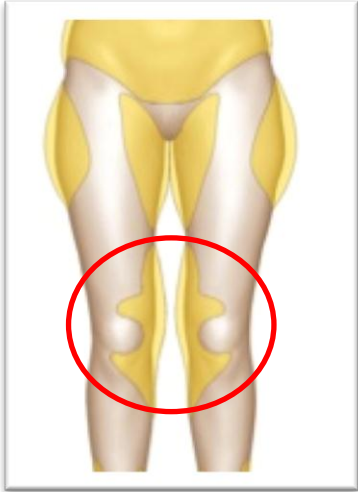
M0



M24

Surgical procedure

1. Fat harvesting : 10-30 min



about 50ml

3. Fat delivery : 5-10 min



15 to 25ml

2. Fat purification: 15 min

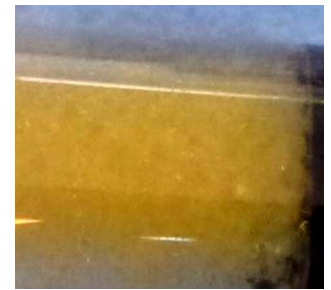
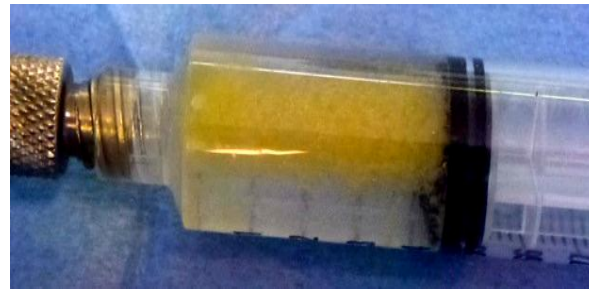
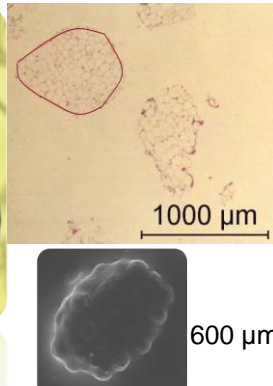
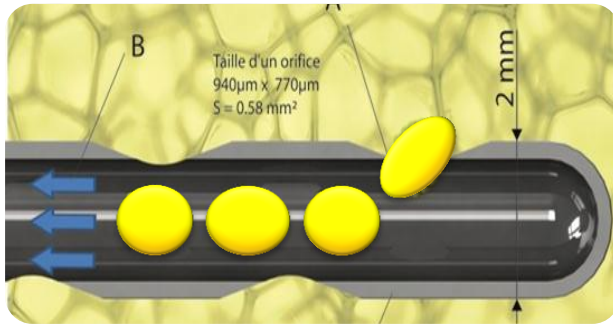


MICRO INJECTION

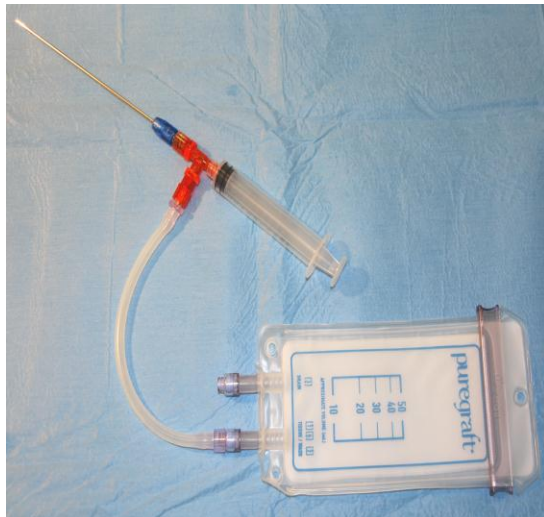
HARVESTING CANNULA – CLOSED SYSTEM

$\varnothing=2\text{mm}$ – 14 Gauge – 130mm

$S=0.58\text{ mm}^2$



PURIFICATION

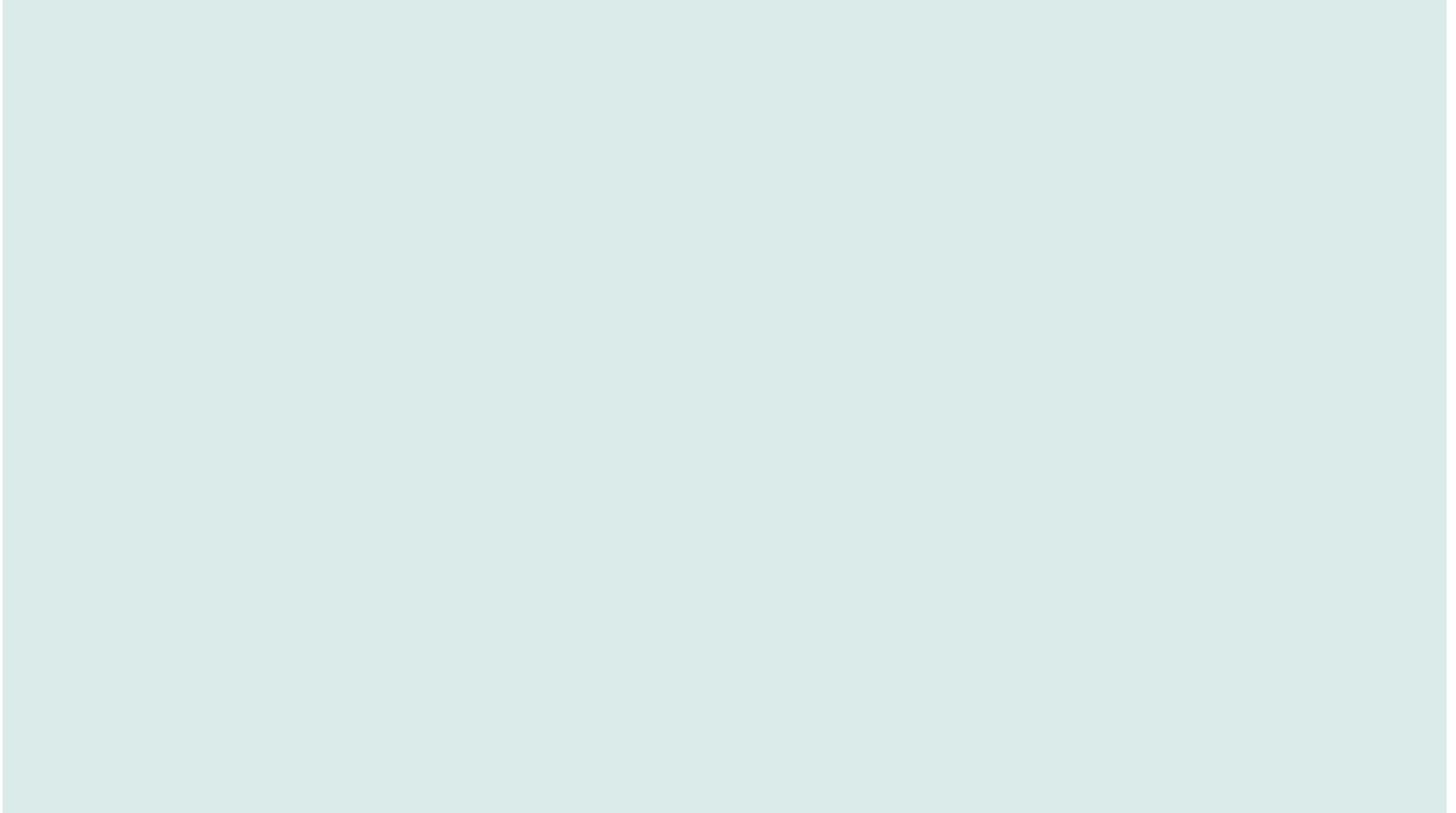


INJECTION CANNULA



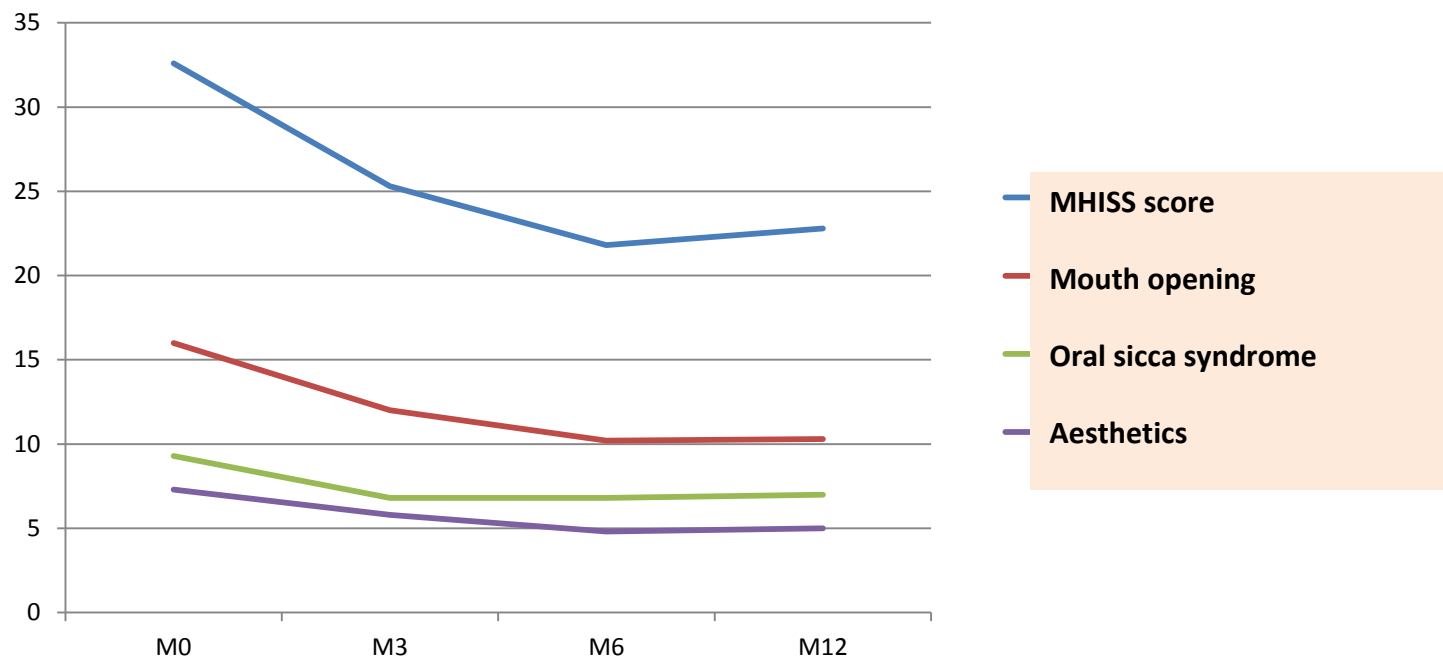
$\varnothing=0.8\text{mm}$ – 21 Gauge – 4mm

Treatment of Scleroderma injuries of the face using a mixture of Fat and PRP



MHISS score

MHISS score (/48)	Inclusion (M0)	6 months (M6)	Variation M0-M6	12 months (M12)	Variation M0-M12
Mean (±SD)	32,6 (± 6,3)	21,8 (± 8,9)	-10,7 (± 5,1) p<0,0001	22,8 (± 8,9)	-9,8 (± 6) p=0,0002



Aesthetics : perioral radial folds improvement

59 years old, diffuse cutaneous form



M0



M6



M12

67 years old, limited cutaneous form



M0



M6



M12

Aesthetics : mouth opening

55 years old
Limited cutaneous form



M0



M6: +6mm



M12 : +9mm

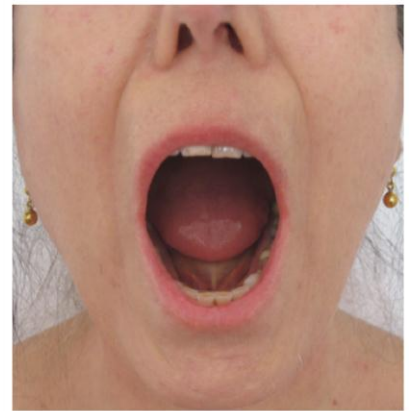
65 years old
Diffuse cutaneous form



M0



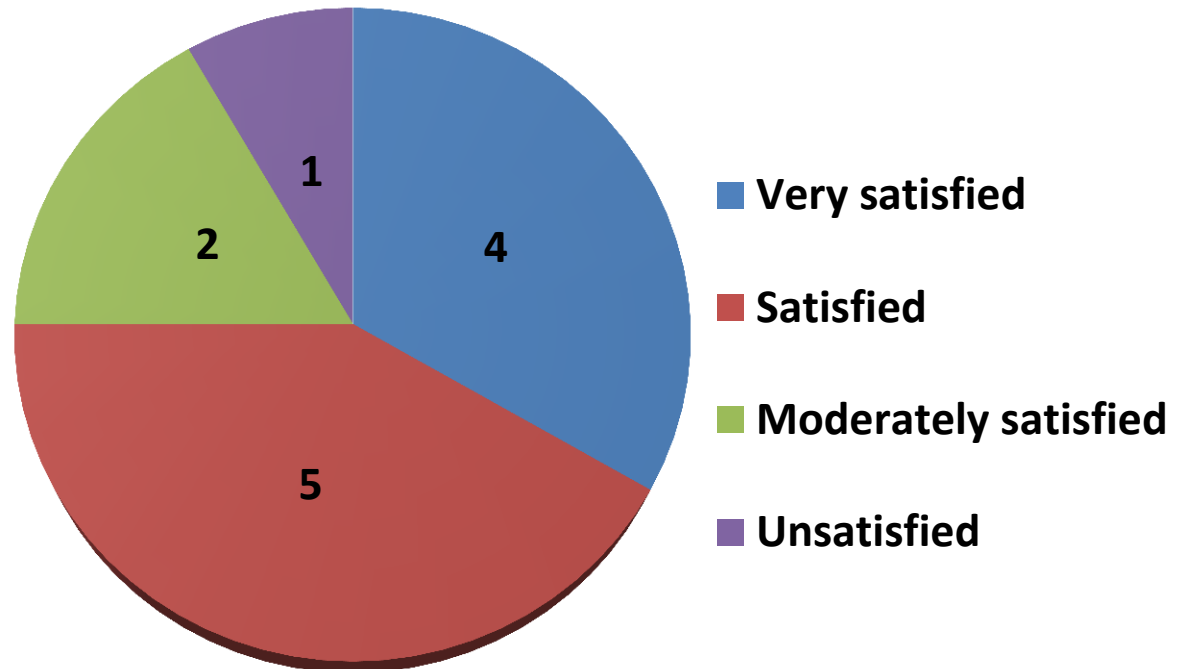
M6: +10mm



M12 : +11mm

Patient's satisfaction

Same satisfaction at 6 and 12 months

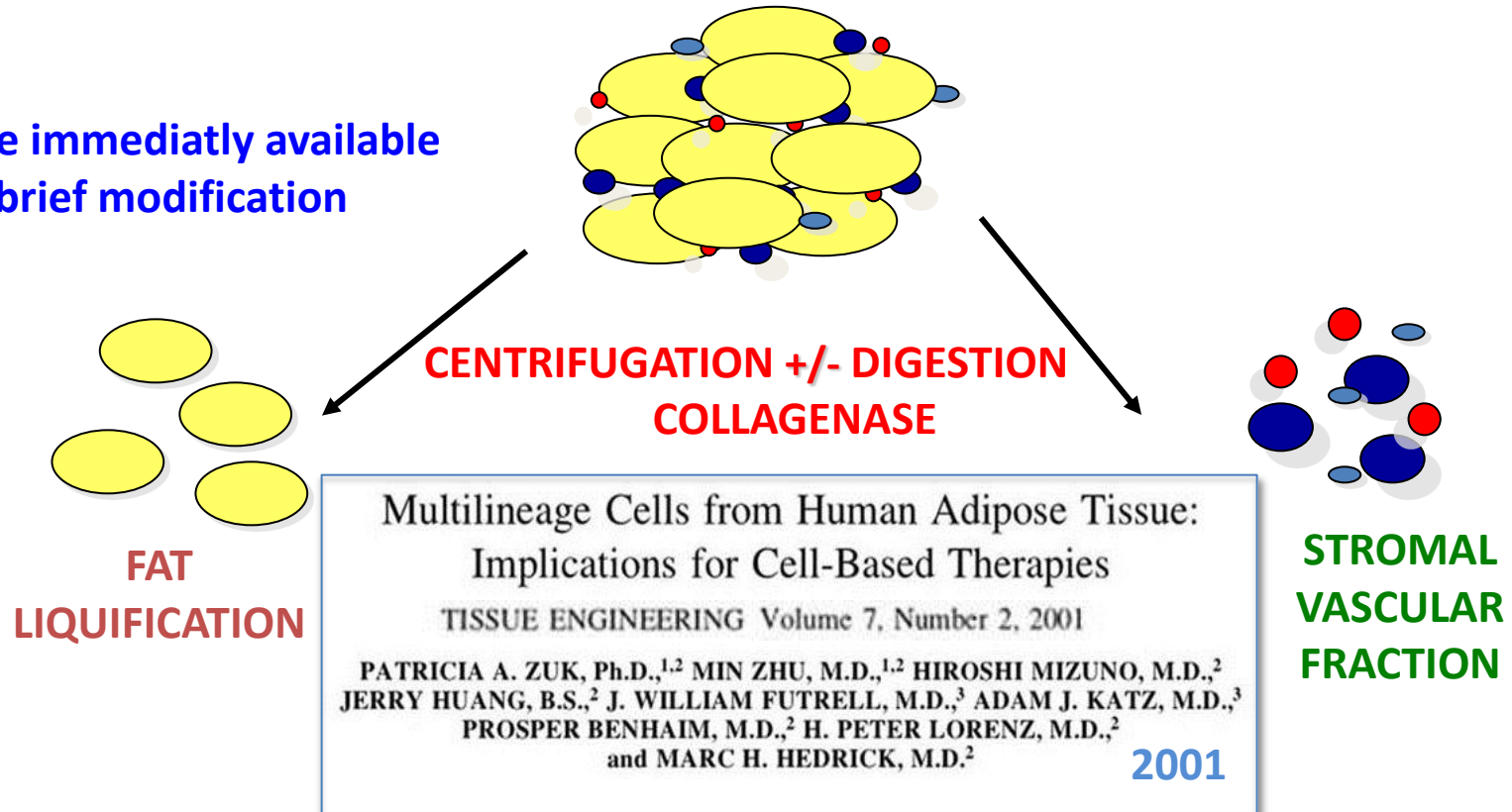


HANDS ASSESSMENT
IN PATIENTS
WITH SCLERODERMA

STROMAL VASCULAR FRACTION

SVF

Cells are immediately available
after a brief modification



Adipose-Derived Stem Cells in Tissue Regeneration: A Review

Patricia Zuk

Regenerative Bioengineering and Repair Laboratory and Division of Plastic and Reconstructive Surgery, Department of Surgery, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA

ISRN Stem Cells, Volume 2013 (2013), Article ID 713959, 35 pages
<http://dx.doi.org/10.1155/2013/713959>

2013

September 2011...

L'autorisation de mise en œuvre de la recherche biomédicale :

« Evaluation de l'effet de la réinjection sous-cutanée de la fraction vasculaire stromale autologue d'origine adipeuse (Système Celution®) au niveau des mains chez les patients atteints de sclérodémie systémique »

est octroyée à :

L'AP HM
Direction de la Recherche
80 rue Brochier
13354 MARSEILLE Cedex 5



Saint-Denis, le **19 OCT. 2012**

Ann Rheum Dis doi:10.1136/annrheumdis-2014-205681

Safety, tolerability and potential efficacy of injection of autologous adipose-derived Stromal Vascular Fraction in the fingers of patients with systemic sclerosis: An open-label phase I trial.

[Brigitte Granel](#), [Aurélie Dumas](#), [Elisabeth Jouve](#), [Jean-Robert Harlé](#), [Pierre-Sébastien Nguyen](#), [Christian Chabannon](#), [Nathalie Colavolpe](#), [Jean-Charles Reynier](#), [Romain Truillet](#), [Stéphanie Mallet](#), [Antoine Baiada](#), [Dominique Casanova](#), [Laurent Giraudo](#), [Laurent Arnaud](#), [Julie Veran](#), [Florence Sabatier](#), [Guy Magalon](#).

... July 2014

A service of the U.S. National Institutes of Health

ClinicalTrials.gov

Clinical trial SCLERADEC

« Assessment of the Subcutaneous Reinjection of Human Autologous Adipose-derived Stromal Vascular Fraction (Celution® System) in the Hands of Patients Suffering From Systemic Sclerosis »

Systemic Sclerosis

The Cell Therapy

Phase I – II

- Screening for safety
- Establishing the testing protocol

APHM multidisciplinary team

Daumas A, Serratrice J, Swiader L, Weiller PJ, Rossi P, Frances Y, Granel B.	Internal Medicine
Samson D.	Anaesthetic and Reanimation Department
Baïada A.	Physiotherapy
Petit P, Colavolpe N.	Medical Imaging
Mallet S.	Dermatology
Nguyen P, Serri J, Eraud J, Hautier A, Magalon G.	Plastic Surgery
Sabatier F, Veran J, Giraudo L, Arnaud L, Aboudou H, Roussey A, Dignat-George F.	Laboratory of Cell Therapy and Clinical Investigation Laboratory of Hematology and Vascular Biology
Jouve E, Charles E, Reynier JCh.	CPCET - Therapeutic Evaluation Center

Hands: the visible and obvious signs of the disease



HOW TO ASSESS THESE HANDS ?

INTEREST OF AUTOLOGOUS ADIPOSE TISSUE ON THE RAYNAUD'S PHENOMENON

« Fat Grafting to the Hand in Patients with Raynaud Phenomenon: A Novel Therapeutic Modality »

Jonathan Bank, M.D., Sam M. Fuller, M.D., Ginard I. Henry, M.D.
Lawrence S. Zachary, M.D.

Plastic and Reconstructive Surgery • May 2014, vol 133, number 5

Conclusions: Preliminary results of fat grafting to the hands of patients with Raynaud phenomenon revealed improved symptomatology with evidence suggestive of measurably increased perfusion in some cases. Fat grafting may benefit the management of this patient population. (*Plast. Reconstr. Surg.* 133: 1109, 2014.)

INTEREST OF AUTOLOGOUS ADIPOSE TISSUE IN TREATMENT OF ISCHEMIC DIGITAL ULCERS (DU) IN SYSTEMIC SCLEROSIS

« Regional implantation of autologous adipose tissue-derived cells induces a prompt healing of long-lasting indolent digital ulcers in patients with Systemic Sclerosis »

Nicoletta Del Papa; Gabriele Di Luca; Domenico Sambataro; Eleonora Zaccara; Wanda Maglione; Armando Gabrielli; Paolo Fraticelli; Gianluca Moroncini; Lorenzo Beretta; Alessandro Santaniello; Gianluca Sambataro; Roberto Ferraresi; Claudio Vitali

Cell Transplantation, 2014

« Fifteen patients with SSc having a long-lasting DU in only one fingertip, unresponsive to intensive systemic and local treatment, were enrolled in the study. The grafting procedure consisted in the injection, at the basis of the corresponding finger, of 0.5-1 ml of autologous ATDCs fraction. »

INTEREST OF AUTOLOGOUS ADIPOSE TISSUE DERIVED STROMAL VASCULAR FRACTION (ADSVF) IN THE TREATMENT OF HANDS IN SYSTEMIC SCLEROSIS PATIENTS

« Safety, tolerability and potential efficacy of injection of autologous adipose-derived stromal vascular fraction in the fingers of patients with systemic sclerosis: an open-label phase I trial. »

Granel B, Daumas A, Jouve E, Harlé JR, Nguyen PS, Chabannon C, Colavolpe N, Reynier JC, Truillet R, Mallet S, Baïada A, Casanova D, Giraudo L, Arnaud L, Veran J, Sabatier F, Magalon G.

Ann Rheum Dis. **2014** Aug 11

This study outlines the safety of the autologous SVF cells injection in the hands of patients with SSc. Preliminary assessments at 6 months suggest potential efficacy needing confirmation in a randomised placebo-controlled trial on a larger population.

	Bank et al, 2014 USA	Del Papa et al, 2014 ITALIE	Granel et al, 2014 FRANCE
STUDY CHARACTERISTICS			
Indication	Raynaud phenomenon	SSc	SSc
Number of patients	13	15	12
Number of hands/ DUs treated	21 hands	15 Digital ulcers	24 hands
INJECTED PRODUCT			
Harvested volume of fat (ml)	Not precised	Not precised	174 ± 46
Preparation	Decantation - 5 hands Lipivage® -16 hands	Centrifugation Coleman Procedure	Enzymatic digestion (Stromal Vascular Fraction) Cytory's Celution®
Adipocytes	YES	YES	NO
Number of SVF cells	Not precised	Not precised	YES 3.76 millions per finger
Volume (cc)	30 cc per hand	0.5 -1 cc per finger affected	1 cc per finger

12 Patients – 24 HANDS

CLINICAL EXAMINATION

- **Cochin** hand functional disability scale - **CHFS**
- Scleroderma Health Assessment Questionnaire - **SSc – HAQ**
- Hand mobility in scleroderma - **Hamis Test**
- **Rodnan** skin score and **Rodnan** score focused on hand
- Evaluation of **Raynaud's** syndrom
- Visual Analogic pain Scale for the hand - **VAS**

PARACLINICAL EXAMINATION

- X-ray
- Doppler : Ulnar and radial arteries
- Laser Doppler tissue imaging
- Capillaroscopy

THE COCHIN SCALE

Answers to the questions: 18

0 = Yes, without difficulty

1 = Yes, with a little difficulty

2 = Yes, with some difficulty

3 = Yes, with much difficulty

4 = Nearly impossible to do

5 = Impossible to do

Maximum score = 90

Osteoarthritis and Cartilage (2001) 9, 570–577
© 2001 OsteoArthritis Research Society
International

**Reliability, validity, and sensitivity to change
of the Cochin hand functional disability scale
in hand osteoarthritis**

*S. Poiraudau, X. Chevalier, T. Conrozier, R.-M.
Flippo, F. Liote, E. Noe, M. Lefevre-Colau, J
Fermanian, M. Revel and R. Rhumato*

THE COCHIN SCALE

In the kitchen

1. Can you hold a bowl?
2. Can you seize a full bottle and raise it?
3. Can you hold a plate full of food?
4. Can you pour liquid from a bottle into a glass?
5. Can you unscrew the lid from a jar opened before?
6. Can you cut meat with a knife?
7. Can you prick things well with a fork?
8. Can you peel fruit?

Dressing

9. Can you button your shirt?
10. Can you open and close a zipper?

Hygiene

11. Can you squeeze a new tube of toothpaste?
12. Can you hold a toothbrush efficiently

At the office

13. Can you write a short sentence with an ordinary pen?
14. Can you write a letter with an ordinary pen?

Other

15. Can you turn a round door knob?
16. Can you cut a piece of paper with scissors?
17. Can you pick up coins from a table top?
18. Can you turn a key in a lock?

CHARACTERISTICS OF PATIENTS AND DISEASE

- **12 females with systemic scleroderma,**
mean age 54.5 years (38-64)
- 7 cutaneous limited (58.33%) and 5 cutaneous diffuse (41.67%)
 - Disease duration : 9.9 years (2-24)
- Raynaud's syndrome : 100%
 - Disease duration : 14.3 years (5-34)
- Digestive symptoms : 75 %
- Respiratory symptoms : 83.3%
- Cardiac symptoms : 8.3 %
- Sicca syndrome : 33.3%

AP-HM



QUALITY CONTROLS

- **STERILITY TESTING**
- **CELL NUMERATION AND VIABILITY**
- **FUNCTIONAL ASSAY**
 - **CFU-F (Colony Forming Unit–Fibroblasts)**
- **FLOW CYTOMETRY ANALYSIS OF CELL POPULATIONS**

Cell Dose Information

Volume of Fat Harvested	174 ± 46 mL
Viable Cell Yield	50.4 ± 24.7 x 10 ⁶
Cell Dose Delivered per Finger	3.7 ± 1.8 x 10 ⁶
% CD45 ⁺ /CD34 ⁻ /CD146 ⁻ cells (leukocytes)	49 ± 18
% CD45 ⁻ /CD34 ^{bright} /CD146 ^{dim} cells (EPC-like)	3.4 ± 2.2
% CD45 ⁻ /CD34 ^{dim} /CD146 ^{bright} cells (endothelial)	6.4 ± 6.8
% CD45 ⁻ /CD34 ^{bright} /CD146 ⁻ /CD90 ⁺ cells (MSC-like)	36 ± 14.5
% CD45 ⁻ /CD34 ^{bright} /CD146 ⁻ /CD90 ⁺ cells (HSC-like)	5.1 ± 2.3
% Fibroblast-like cell Colony-Forming Unit (CFU-F)	3.7 ± 1.9

All samples sterile by gram stain and by Bactec™ testing for both aerobic and anaerobic organisms

AP-HM (Assistance Publique des Hôpitaux de Marseille) **strategy**
based on recommendations of
ANSM (Agence Nationale de la Santé du Médicament)/**EMA** (European Medicines Agency)

2012 – SCLERADEC 1 - clinical trial

Markers combination		Identified Populations
CD90-FITC CD146-PE CD34-ECD	CD45-PC5 DRAQ5 DAPI	1- Leukocytes 2- Endothelial cells 3- Stromal cells

2016 – SCLERADEC 2 - clinical trial

Markers combination		Identified Populations	
Tube 1 : CD90-FITC CD146-PE CD34-ECD CD45-PC5 DAPI	Tube 2 : CD14-FITC CD34-ECD CD45-PC5 CD56-PC7 CD3-AA750 DAPI	1- Pericytes 2- Transitional cells 3- Endothelial cells 4&5- Stromal cells CD90+/-	6- CD34+ macrophages 7- Granulocytes 8- Monocytes 9- NK cells 10- T lymphocytes 11- Others lymphocytes

POST OPERATIVE CONTROLS

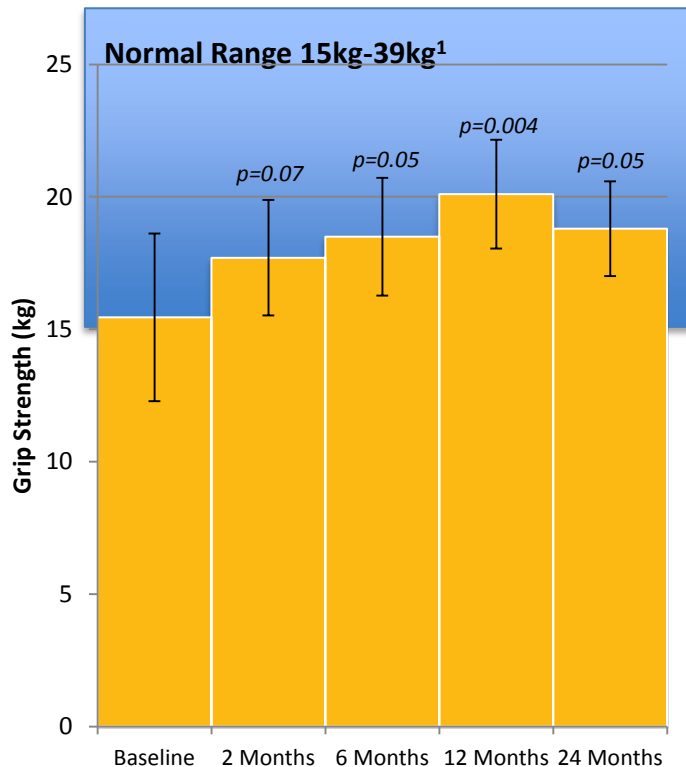
	J1	J7	J21
HANDS			
Sensitive disorders	0	1	1
Hematomas	0	0	0
Infection	0	0	0
Need for amputation	0	0	0
HARVESTING AREA			
Hematomas	1	6	2
Infection	0	0	0

NO SERIOUS ADVERSE EVENT

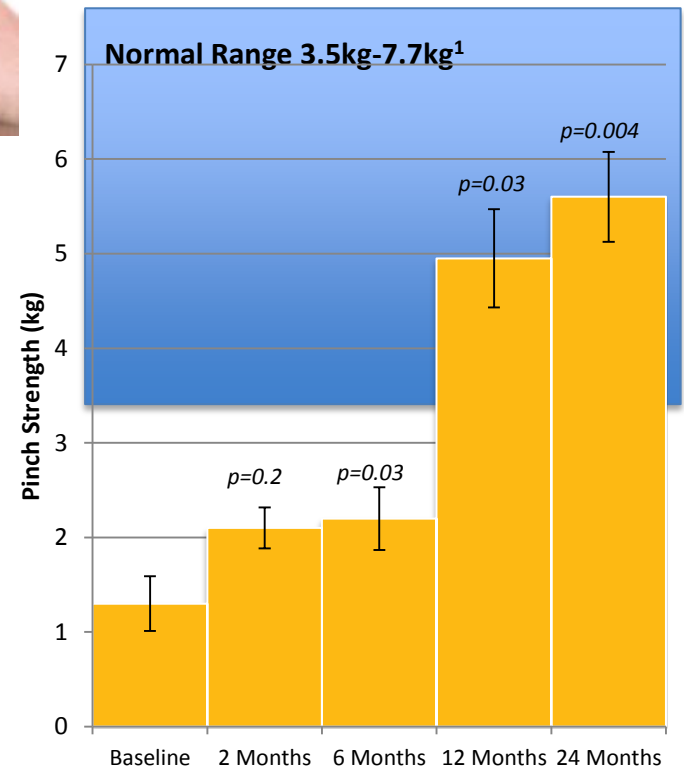
Improvement in Hand Strength

Objective Improvement in Grip and Pinch Strength: Dynamometer

Grip Strength



Pinch Strength

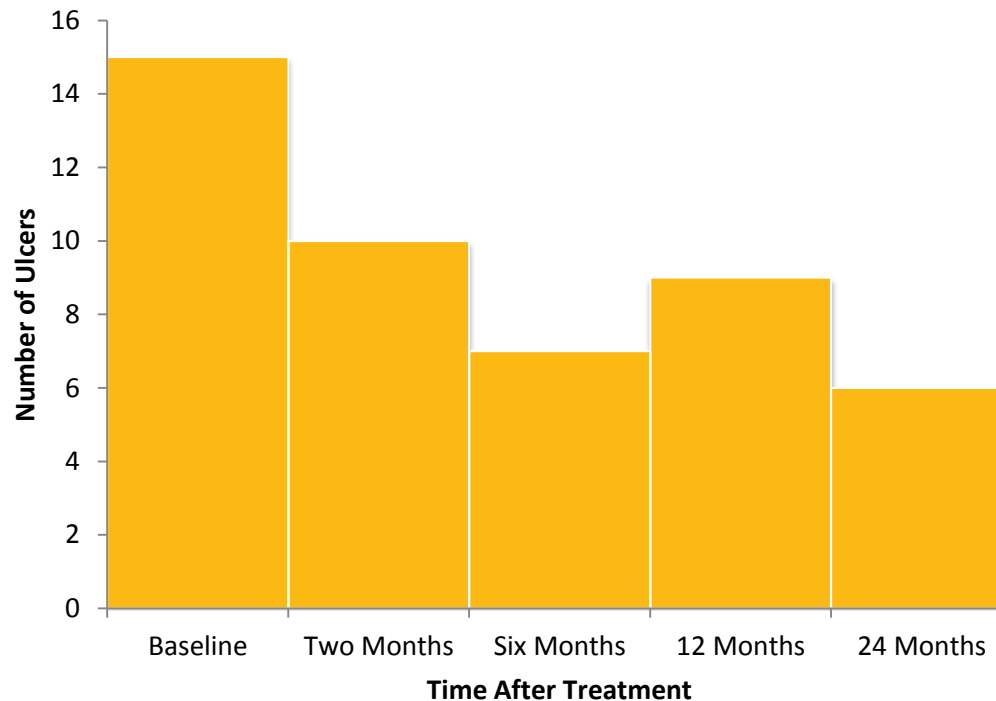


Data presented as mean \pm standard error

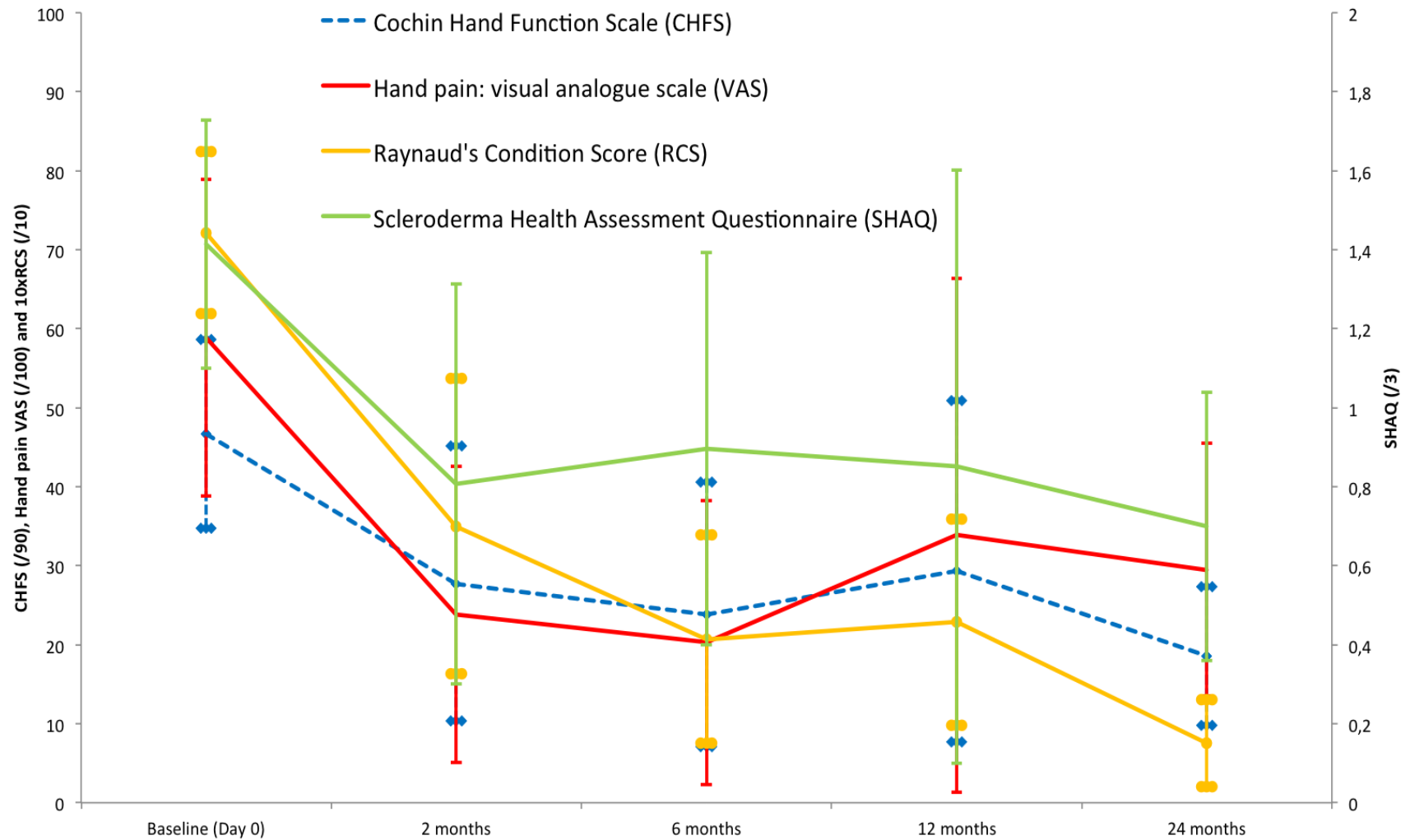
1. Mathiowetz *et al* (1985); Arch Phys Med Rehabil 66:69-72
Granel *et al*. Ann Rheum Dis 2014; Guillaume-Jugnot *et al*. Rheumatology 2015

Digital Ulcers

~60% decrease in total number of digital ulcers at 24 months



24 months follow-up for SSc patients after SVF re-injection



October 2016 - Current Research in Translational Medicine – Brief communication

Long-term follow-up after autologous adipose-derived stromal vascular fraction injection into fingers in systemic sclerosis patients

Autologous adipose-derived stromal vascular fraction in scleroderma

A Daumas, J Magalon, E Jouve, R Truillet, D Casanova, L Giraudo, J Veran, A Benyammine, F Dignat-George, G Magalon, F Sabatier, B Granel

Patient Comments

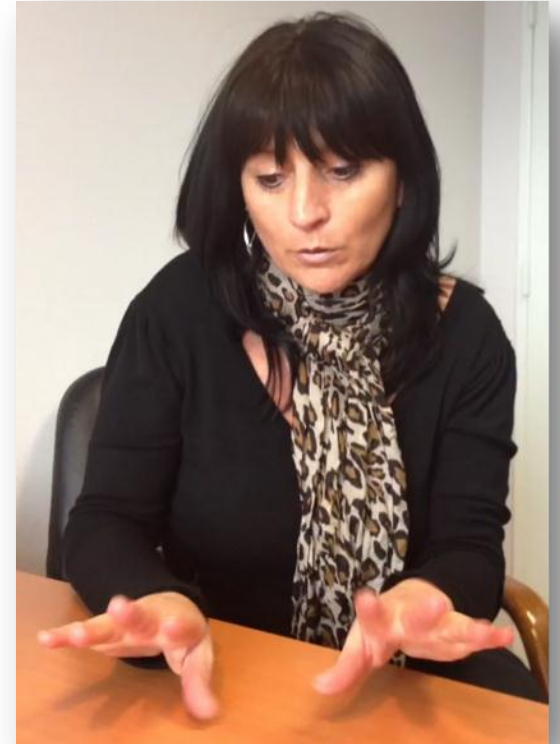
“It felt like ten fingers had been grafted on to me”

“There is no longer any pain at all”

“I’m living again”

“My sense of touch has improved”

“My fingers are pinker”



Conclusion

- **Microfat grafting** has been developed to treat **Scleroderma Faces**.
This is an efficient microinvasive painless procedure with long term results.
- Injection of autologous of **Stromal Vascular Fraction** into **the Hands** can be performed safely.
SVF treatment was associated with an 50% improvement of Cochin hand fonction scale, Raynaud's condition score and global disability at two years. Longer term, larger and controlled studies will be important to confirm wheater this new form of cell therapy can improve the long term prognosis.
- Controlled and comparative studies are needed to confirm the promising results obtained par both autologous **Fat grafting and SVF injection procedure** in open studies
- Next steps : **2 clinical trials, SVF**
 - France: **SCLERADEC II**, randomized, 10 to 70 millions cells, 5
Centers, 40 Cases – *In progress 25 cases*
 - USA: **STAR**, randomized, 40 millions cells,
15 States, 88 Cases – *Completed*
 - European project: **FAST**, randomized, cryopreserved products, 4 countries - *Waiting*