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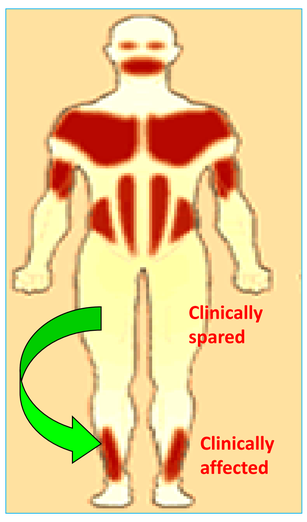
Introduction

Facioscapulohumeral muscular dystrophy type 1 (FSHD1) is one of the most frequent adult myopathies (1/20.000), with selective involvement of specific groups of muscles : facial, scapular fixator, anterior foreleg muscles, abdominal and humeral muscles.
Vastus lateralis (VL) is usually spared clinically until late stages of the disease, and myoblasts grown from VL have similar behaviour in vivo and in vitro than myoblasts from control patients

-> Proposal:
Transplantation of autologous myoblast from spared muscle (VL) into an affected muscle as the Tibialis anterior (TA) muscle could locally improve the muscle's regenerative capacities.



Purpose of the study

Primary endpoints: Safety, feasability
- Feasability of cell preparation
- Safety of intramuscular injections of cells
- Clinical and biological tolerance of cell transplantation

Secondary endpoints : Follow-up of muscle strength and resistance to fatigue **over 2 years**
- Mechanical testing of strength and resistance to fatigue
- Surface electromyography, MRI and FDG fixation by PET-Scan

Specific inclusion criteria

- Men and women aged 18-65.
- Clinical manifestations of DMFSH confirmed by molecular diagnosis (D4Z4 repeats).
- Lack of clinical deficit in at least one VL muscle (assessed by score at knee extension, MRC = 5), and absence of adipo-fibrotic invasion (assessed by MRI).
- Motor deficiency of at least one anterior leg (assessed by score at foot dorsiflexion, MRC < 4) and fatty infiltration in at least one TA muscle (assessed by MRI).

-> **Three groups of at least three patients selected in a sequential fashion**.

Clinical parameters

Informations collected repeatedly from early (D0) to late phases (1mo).
Clinical monitoring: overall wellness, heart rate and pressure, fever, cutaneous status, pain, redness, edema…
Biological monitoring: blood formulation, sedimentation, inflammation (CRP), CPK, myoglobinemia, myoglobinuria, creatininemia, ions, calcemia, phosphoremia, transaminases, transferases.

MMT testing and Electromyography

Global manual testing performed at time of inclusion, then at 15 d, 21 d, 1, 2, 3, 4, 5, 6, 12 months. Measurement of dorsal and plantar flexion of ankles, extension and flexion of knees, abduction and antepulsion of shoulders, extension and flexion of arms.
Maximal voluntary contraction (MVC) of ankle dorsiflexor muscles were measured with an ankle dynamometer (isometric strength).
Fatiguing exercise consisted in a maximal isometric dorsiflexion lasting 30 s. A fatigue index (FI) was determined.
Muscle activity of TA muscle was recorded by bipolar sEMG electrodes (10 mm diameter, 20 mm inter-electrode distance).
Common peroneal nerve stimulation was induced with a constant-current stimulator to evoke the electrophysiological and associated mechanical responses. **The peak-to-peak amplitudes** of the three M_{max} responses of the TA muscle and the **maximal amplitude** of the three mechanical twitches (Pt) elicited by single stimuli at rest were averaged. The **root mean square (RMS)** of the sEMG recordings of the TA muscle was analyzed over a 500ms period around the peak force.

NMR imagery

At inclusion, absence of fatty infiltration in one VL (donor muscle) and the presence of abnormal fatty infiltration in one TA (recipient muscle) are documented **without Gadolinium.**
G**raded semi-quantitative analysis** expressing the **ratio fat signal / skeletal muscle** signal obtained by NMR imaging using **Gadolinium contrast agent and T1 ponderation** done before, 1, 3 and 6 months after implantation to evaluate the inflammatory reaction and the evolution of the volume of fatty infiltration (at middle and lower parts of the thigh and of the leg).

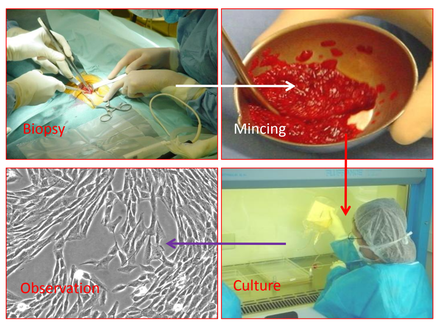
PET-Scan analysis

^{18}F-fluorodéoxyglucose (^{18}FDG) accumulates in some tissues as a function of their metabolic activity, especially in brain, myocardium and skeletal muscle upon exercise.
Measurements have been done at the level of TA upon standardized exercise **before implantation, then 3 and 6 months later** to quantify the volumes of metabolically active muscle tissue.

Acknowledgments

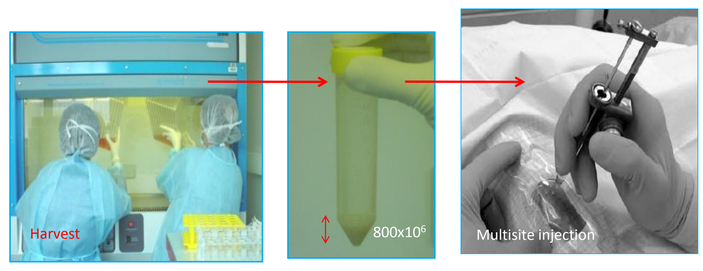
The authors wish to thank the patients and their families for their motivation, together with the several collaborators involved in the set up of this study at multiple levels.

Cell cultures



1-2g of VL muscle harvested under local anaesthesia in Nice Hospital and shipped to the Saint Louis Cell Therapy Laboratory in Paris. Within 24h, biopsies minced, digested, filtered and cells grown in a myogenic proprietary medium. Cells settled and expanded on days 8, 11, 14, 17, 20/21. Phenotypical characterization (CD56 NCAM), viability, microbiological controls, endotoxins assessed according to classical procedures. Methodologies, products and clinical protocols validated by regulatory agencies.

Autologous cell transplantations



800 million cells containing >50% CD56+ cells concentrated in an isotonic saline buffer.
Injection of cells suspended in 10ml, into half of TA muscle (surface: approx. 20 cm^2 ; volume: approx. 40 cm^3).
3 groups of patients : 3 injection modalities with increased densities of needle trajectories per surface unit :
-> Group 1 : 64 sites, interspace 5 mm – Group 2 : 100 sites, interspace 4 mm – Group 3: 189 sites, interspace 3mm.

Description of patients

Patient characteristics

Patient	Age at inclusion	Duration of disease at inclusion	Number of D4Z4 repeats	Global Sumscore at inclusion (/80)	Tibialis MMT score at inclusion	Quadrcieps MMT score at inclusion
FSHD1	54	9	6	62,99	2	5
FSHD2	51	16	7	64,66	3+	5
FSHD3	49	16	6	65,68	2-	5
FSHD4	44	23	7	61,33	1	5
FSHD5	60	10	7	62,65	2+	5
FSHD6	48	32	7	70,00	3-	5
FSHD7	52	15	7	65,33	1+	5
FSHD8	59	2	7	74,33	3-	5
FSHD9	64	15	6	73,33	4+	5
FSHD10	61	20	8	65,69	3-	5
FSHD11	59	8	9	63,33	2	5
Mean +/- SD	55 +/- 6,4	15 +/- 8,1		66,3 +/- 4,3		

Clinical outcomes

Clinical tolerance

Patient	Pain at D8 (Y/N) - Patient evaluation	Pain at D8 (Y/N) - Physician evaluation	Edema at D15 (Y/N) - Physician evaluation	CK > 5000 U/L at D8 (Y/N)	Myoglobinemia > 5000 ng/ml at D0-D8 (Y/N)	Myoglobinuria > 500 ng/L at D0-D8 (Y/N)	Kalemia > 5,5 mmol/L at D0-D8 (Y/N)	Perturbation of inflammatory status (BUN, SS, CRP) at D8 (Y/N)	Renal status (calcemia, creatinemia, phosphoremia, ionogram) at D0 - D8	Liver status (transaminases < 3 x norms) D0-D8 (Y/N)	Final tolerance
FSHD1	Y (< 50 mm)	N	N	N	N	N	N	Y (clinically not significant)	N	Y	Good
FSHD2	ND	ND	N	N	N	N	N	N	N	Y	Good
FSHD3	Y (< 50 mm)	ND	N	N	N	N	N	N	N	Y	Good
FSHD4	N	N	N	N	N	N	N	N	N	Y	Good
FSHD5	N	ND	N	N	N	N	N	N	N	Y	Good
FSHD6	N	ND	N	N	N	N	N	N	N	Y	Good
FSHD7	N	N	N	N	N	N	N	Y (CRP 160,2)	AN	Y	Questionable
FSHD10	N	N	N	N	N	N	N	Y (clinically not significant)	N	Y	Good
FSHD11	N	ND	N	N	N	N	N	N	N	Y	Good

PET-Scan evolution

No significant changes were noted over the follow-up period.

Results of Cell cultures

Cell culture feasibility

Patient	Biopsy: Weight (g)	Biopsy: Initial number of cells (10^6)	Culture duration (days)	Final number of cells (millions)	Final CD56 (%)	Viability (%)	Microbiological controls / Endotoxins	Feasibility
FSHD1	1,7	1,58	22	1238	96,2	93,4	Neg / Neg	Good
FSHD2	1,9	0,95	20	1937	98,2	92,5	Neg / Neg	Good
FSHD3	1,9	0,68	20	993	94,1	95,6	Neg / Neg	Good
FSHD4	1,1	0,71	21	999	92	93,2	Neg / Neg	Good
FSHD5	1,9	0,9	22	1357	71,3	92,5	Neg / Neg	Good
FSHD6	1,4	2,55	21	2037	50,05	91,45	Neg / Neg	Good
FSHD7	1,7	0,85	21	1767	63,6	98,1	Neg / Neg	Good
FSHD8	1,1	0,28	21	1081	25,3 *	97	Neg / Not Done	Not grafted / Not reached
FSHD9	1,2	2,09	21	2000	18,3 *	96,4	Neg / Not Done	Not grafted / Not reached
FSHD10	1,6	1,26	28	804,5	95,94	95,7	Neg / Neg	Good
FSHD11	2	0,38	20	1855	97,63	97,8	Neg / Neg	Good

Inter-individual variabilities may be important. CD56+ cut-off reached 9 times out of 11 within three weeks. In two cases, biopsies looked fibro-adipocytic, purity was not reached. Muscle biopsy were then extemporaneously qualified using histology.

MMT and Electromyography

Mean differential MMT in each methodological series

	Serie 1			Serie 2			Serie 3		
	ΔTreated	ΔUntreated	Global ΔSumscore	ΔTreated	ΔUntreated	Global ΔSumscore	ΔTreated	ΔUntreated	Global ΔSumscore
M1	-19,97%	-6,79%	-17,51%	6,31%	0,00%	-0,94%	23,71%	0,00%	-1,88%
M3	-13,31%	-2,59%	-11,48%	17,31%	0,00%	-1,28%	23,71%	0,00%	-2,24%
M6	-16,62%	-9,38%	-10,16%	17,31%	0,00%	-2,32%	19,79%	0,00%	-1,74%
M9	-22,12%	-9,38%	-8,09%	17,31%	-1,33%	-2,35%	18,21%	-8,27%	0,23%
M12	-15,51%	-9,38%	-7,65%	28,64%	-1,33%	-1,86%	15,44%	-13,37%	-2,07%

The maximal voluntary isometric strength of the dorsiflexor muscles remained constant during the follow-up period. The **fatigue index** of the dorsiflexor muscles of the experimental leg tend to decrease over the 12 months whereas the one from the control leg fluctuated.
The **neuromuscular transmission was preserved** after the cell therapy. The mechanical twitch response of the dorsiflexor muscles of the experimental leg slightly increased over the follow-up period.
Taken together, and considering that no changes were observed on the control leg, these results lead us to conclude that the **cell therapy may have positively affected the experimental leg.**

Discussion and Conclusions

Cell **productions** were **feasible** but the quality of the initial muscle biopsy is important. Cell **administrations** were feasible and **clinically well tolerated by all patients but one**. The control of local cell distribution may be improved by echographic monitoring. Results show **slight increases in twitch response and slight decrease in fatigue** in the 3rd group. The combination of cell type (myoblasts) and of the modality (dense multisite injections) may have positively affected the TA muscle, **BUT**: **No clinically significant gain of function** perceived by FSHD patients, and **no significant changes were noted at MRI and PET-Scan.**The local FSHD1 **degenerated muscle environment** may be detrimental to the stability of the fibers or of the niches, and muscle regeneration may have been inefficient, too transitory, aborted or too unstable.
The slight muscle strength increase may not be clinically significant for FSHD patients, but **may improve the quality of life of patients with more advanced muscle loss** (e.g. DMD patients) in other indications.