Autologous Myoblast Transplantation for Oculopharyngeal Muscular Dystrophy: a Phase I/IIa Clinical Study

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Oculopharyngeal muscular dystrophy (OPMD) is a late-onset autosomal dominant genetic disease mainly characterized by ptosis and dysphagia. We conducted a phase I/IIa clinical study (ClinicalTrials.gov NCT00773227) using autologous myoblast transplantation following myotomy in adult OPMD patients. This study included 12 patients with clinical diagnosis of OPMD, indication for cricopharyngeal myotomy, and confirmed genetic diagnosis. The feasibility and safety end points of both autologous myoblast transplantation and the surgical procedure were assessed by videoendoscopy in addition to physical examinations. Potential therapeutic benefit was also assessed through videoendoscopy and videofluoroscopy of swallowing, quality of life score, dysphagia grade, and a drink test. Patients were injected with a median of 178 million myoblasts following myotomy. Short and long-term (2 years) safety and tolerability were observed in all the patients, with no adverse effects. There was an improvement in the quality of life score for all 12 patients, and no functional degradation in swallowing was observed for 10 patients. A cell dose-dependant improvement in swallowing was even observed in this study. This trial supports the hypothesis that a local injection of autologous myoblasts in the pharyngeal muscles is a safe and efficient procedure for OPMD patients.

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INTRODUCTION

Oculopharyngeal muscular dystrophy (OPMD) is an inherited slow progressing and late-onset autosomal dominant genetic disorder. The mutation that causes OPMD is an abnormal expansion of a (GCG)n trinucleotide repeat in the coding region of the poly(A) binding protein nuclear 1 (PABPN1) gene, located on chromosome 14 (14q11.2-q13). This leads to an expanded polyalanine tract at the N-terminal of the PABPN1 protein.[1] This degenerative muscle disease is mainly characterized by the selective involvement of the pharyngeal muscles, resulting in swallowing disorders, and of the levator palpabrae superioris muscles, resulting in ptosis. Swallowing difficulties are determinant in the prognosis of the disease, and create potentially life-threatening aspiration pneumonia[2–4] and denutrition. Degenerative dystrophy and progressive onset of fibrosis in the pharyngeal muscles cause difficulties in propulsing the food bolus in the pharynx, which together with a decreased relaxation of the cricopharyngeal muscle (the main muscle of the upper esophageal sphincter (UES), located between the pharynx and the esophagus), results in a delay in the transfer of the bolus through the UES, a phenomenon known as dysphagia. The most common treatment for this dysphagia in OPMD patients is a myotomy of the cricopharyngeal muscle (*i.e.* UES myotomy), consisting in an extramucosal section of the cricopharyngeal muscle.[5,6] However, despite the high rate of immediate improvement in swallowing with relief of dysphagia following myotomy, subsequently a very high proportion of the patients present a secondary progressive reoccurrence of dysphagia.[7] Although not totally satisfactory, there are currently no other therapeutic options for this disease. Since only a few small muscles are initially targeted by the disease, OPMD has been identified as a typical school case for autologous cell transplantation therapy. A previous study using transplantation of muscle progenitors between monozygotic twin girl carriers of Duchenne Muscular Dystrophy has demonstrated the feasibility of this approach,[8] suggesting that myoblasts would be good candidates for autologous cell therapy. Moreover, in the case

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of OPMD where only a few well-defined muscles are initially targeted, autologous myoblasts can be isolated and expanded from muscles clinically spared by the pathological process. In a preliminary study, we have shown that cells isolated from the nonaffected muscles of OPMD patients are able to proliferate and differentiate normally, whereas cell cultures isolated from affected OPMD muscles (cricopharyngeal muscle) are characterized by a reduced myogenicity.[9] These results suggested that a therapeutic strategy based on autologous grafting of myogenic progenitors (referred to as myoblasts) isolated from clinically unaffected muscles of OPMD patients, and implanted into the dystrophic pharyngeal muscles, could be used to reinforce the motricity and restore contractility to the affected pharyngeal muscles, and thus improve the life threatening dysfunction of the pharynx for OPMD patients.

An autologous cell therapy phase I/IIa clinical study was thus set up to assess feasibility and toxicity, based on the grafting of autologous myoblasts isolated from unaffected quadriceps (Q) or sternocleidomastoid (SCM) muscles into the pharyngeal muscles of patients suffering from OPMD following a cricopharyngeal myotomy. Due to its pathophysiological specificity, that is, limited target and potential source of autologous myoblasts, OPMD represents an ideal pathological situation to establish a proof of principle for autologous cell therapy in muscular dystrophies.

RESULTS

Twelve OPMD patients (50% women) with ptosis, dysphagia, and confirmed genetic diagnosis, were included in the study and their characteristics are presented in **Table 1**. None of them had been treated previously by myotomy. Patients gave their written consent for participation in the study. All patients were followed for at least 2 years after grafting (**Supplementary Figure S1**). Ten patients accepted to be monitored for a longer period of time.

Table 1 Subject characteristics: age, sex, genetic diagnosis, global evaluation of the myopathy (with a muscular testing), and follow-up are indicated as the mean (± SD) or the number out of 12 (%)

Characteristics	Subjects
Mean age, year (SD)	62.3 (8.3)
Sex, n (%)	
Female	6 (50%)
Male	6 (50%)
Genetic diagnosis, n (%)	
Heterozygous, GCG_6/GCG_8 (Ala12)	2 (16.6%)
Heterozygous, GCG_6/GCG_9 (Ala13)	6 (50%)
Heterozygous, GCG_6/GCG_{10} (Ala14)	2 (16.6%)
Heterozygous, GCG_6/GCG_{11} (Ala15)	2 (16.6%)
Global evaluation of the myopathy	
Before	
Ptosis	12 (100%)
Lower limb weakness	6/12 (50%)
Upper limb weakness	4/12 (33%)
At 2 years	
Ptosis	12 (100%)
Lower limb weakness	9/12 (75%)
Upper limb weakness	7/12 (58%)
Follow-up, year (SD)	3.5 (1.4)

Muscles selection

Biopsies of both Q and SCM muscles were performed in eight patients. OPMD patients in the late phase of the disease may present some dystrophic features in quadriceps muscles, which may not be apparent on clinical examination, while the SCM is always spared and available through the same neck incision as for cricopharyngeal myotomy. In four patients, the SCM was the unique biopsy performed, since they exhibited proximal limb weakness. Telomere length (TRF), myogenicity, and proliferation kinetics were determined on the cells isolated from the biopsies (**Table 2**). The choice of the selected muscle for final amplification, based on this first evaluation, was SCM for seven patients and Q for the five other patients. The second biopsy of the selected muscles was performed 3 weeks after the initial biopsies for final amplification before grafting. A cervical hematoma occurred in one patient following the initial biopsy of the SCM, requiring hemostasis under general anesthesia. There were no other complications observed related to muscle biopsies during the 2 years of the protocol.

Cricopharyngeal myotomy and injections of autologous myoblasts

The mean percentages of cell viability and myoblast purity were 95 and 86%, respectively. The differentiation potential of the myoblasts was confirmed *in vitro* (**Supplementary Figure S2**). A median of 178 million autologous myoblasts was obtained after 21 days. Cells were injected into the constrictor muscles of the pharynx, associated with surgical cricopharyngeal myotomy: a total of 11 to 16 injections were performed in each patient. All injections in the same patient were equivalent in terms of volume (200 µl per injection) and cell number (4.4 ml injected for all patients with variable concentration of cells). Patients received an antibiotherapy for the first 7 days after grafting and omeprazole for 1 month. Patients were able to eat soft food the day after surgery, and they returned home after 4–5 days (mean 4.2 days). There were no adverse events related to the extramucosal cricopharyngeal myotomy, with no accidental incision of the mucosa. In one patient with a previous left thyroid lobectomy, there was a unilateral transient recurrent laryngeal nerve paralysis despite the fact that the recurrent laryngeal nerve was visualized and preserved during the surgery. Complete recovery of laryngeal motion was observed after 6 months.

Pharyngeal propulsion

Patients were examined before surgery using videoendoscopic swallowing study (VESS) and videofluoroscopy of swallowing (VFS) to determine the status of their dysphagia, and all patients exhibited a dysfunction of the pharyngeal propulsion (decreased in 10, severely impaired in 2, **Figure 1a**, **b**), with salivary and diet pooling in the hypopharynx. After 2 years, examination of pharyngeal propulsion with VESS and VFS was globally unchanged (**Figure 1a**,**b**).

UES function

UES function was observed in all patients using VESS and VFS (**Figure 2**). Preoperatively, the UES function was disturbed in all patients (**Figure 2a,b**). At 2 years, the UES function was considered to be normal in six patients using VESS (**Figure 2a**) and in two patients using VFS (**Figure 2b**).

Salassa grade, McHorney score

Preoperatively, concerning the grade of Salassa (grade 0 to grade 5, where 0 is considered to be normal and 5 is considered as an extremely severe swallowing disorders), nine patients were at grade 2, whereas three patients were at grade 3 (**Figure 3a**). The mean McHorney score was 34.75 (ranging from 15 to 58, **Figure 3b**). Two years after surgery and grafting, the Salassa score was found to be improved: nine patients exhibited grade 0 or 1 and three patients exhibited grade 2 (**Figure 3a**). The mean McHorney score 2 years after combined surgery and cell grafting was 18.6 (ranging from 4 to 45, **Figure 3b**).

Time of swallowing 80 ml of water ("drink test")

The drink test represents a global functional evaluation of swallowing. Preoperatively, the mean time taken to swallow 80 ml of water in the 12 patients was 23.7 seconds. Two years after surgery and cell transplantation, the mean time taken to swallow 80 ml of water was reduced to 10.2 seconds (**Figure 4**; $P < 0.01$).

In order to evaluate if there was a correlation between the number of cells injected and the improvement in the swallowing time, we compared the improvement in swallowing of patients injected with less than 178 million cells (median of injected cells) with patients injected with more than 178 million cells. Preoperatively, the time of swallowing for these two groups was comparable (data not shown). During the 2-year follow-up, we observed a greater improvement in patients injected with more than 178 million cells than in patients injected with less than 178 million (**Figure 5**; $P < 0.01$). The same comparison made on the two quality of life questionnaires reinforce the observation that we have a cell dose improvement in this study (**Supplementary Figure S3**).

Table 2 Criteria of selection: proliferative kinetics (i.e. the number of divisions achieved per day), and length of telomeres (TRF) were the criteria used to select the appropriate muscle (sternocleidomastoid, SCM or quadriceps, Q), for amplification prior to grafting

	N division/day	TRF	Selected muscle	Myogenicity			
#	SCM	Q	SCM	Q	Selected muscle	Myogenicity	N cells injected × 10^6 (nb site)
1	0.38	0.66	9.2	10.4	Q	78%	262 (14)
2	0.27	0.54	9.3	5.8	SCM	70%	164 (12)
3	0.25	0.24	10.0	9.4	Q	97%	44 (13)
4	0.38	0.38	10.4	10.6	SCM	98%	380 (14)
5	0.34	0.34	8.8	8.8	Q	96%	90 (14)
6	0.42	0.20	10.3	9.9	SCM	93%	380 (16)
7	0.34		8.8		SCM	89%	460 (11)
8	0.71		10.7		SCM	58%	350 (13)
9	0.57	0.33	7.2	8.9	Q	95%	192 (16)
10	0.52		9.2		SCM	85%	97 (13)
11	0.39	0.51	9.2	9.5	Q	85%	125 (12)
12	0,41		ND		SCM	50%	140 (12)

SCM, sternocleidomastoid.

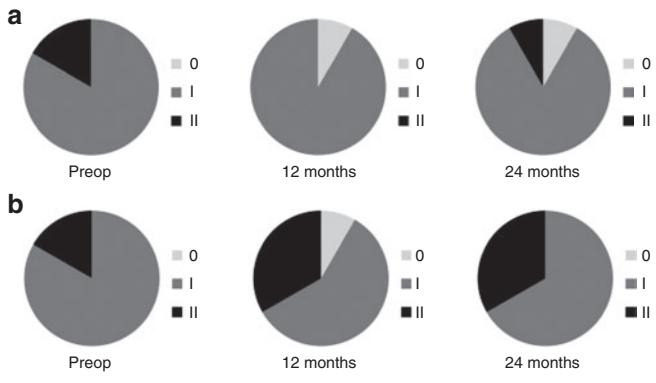

Figure 1 Pharyngeal propulsion preoperatively, and at 12 and 24 months observed by (a) videoendoscopic swallowing study and by (b) videofluoroscopy of swallowing. Parameters were scored as normal (0), decreased (I), or severely impaired with no peristaltic waves (II).

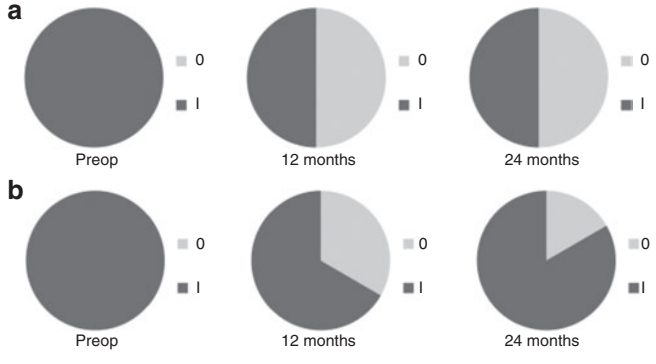

Figure 2 Upper esophageal sphincter (UES) function preoperatively, and at 12 and 24 months observed by (a) videoendoscopic swallowing study and by (b) videofluoroscopy of swallowing. UES function was evaluated by the quality of the UES opening and closure and on the salivary or diet pooling in the hypopharynx, results scored as normal (0) or abnormal (I).

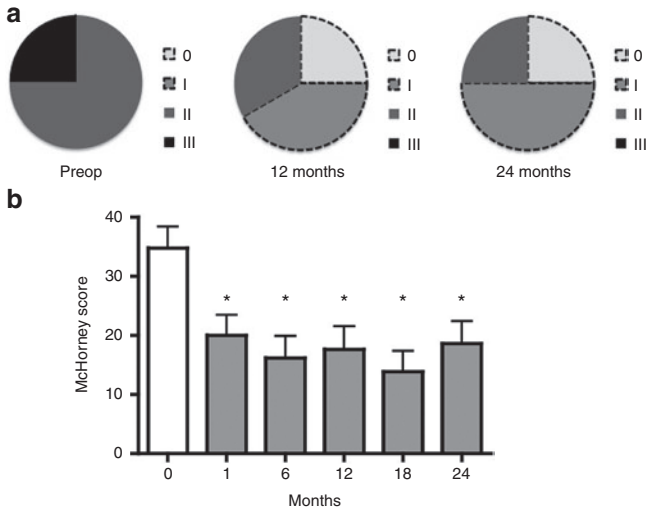

Figure 3 Score of Salassa (a) ranging from grade 0 (normal) to grade V (extremely severed swallowing disorders) and questionnaire of McHorney (b) to evaluate symptoms and quality of life preoperatively or at different time point following the graft. Dotted lines indicate a normal deglutition. ANOVA and Dunnett's post-test (*P < 0.05 compared with preinclusion score).

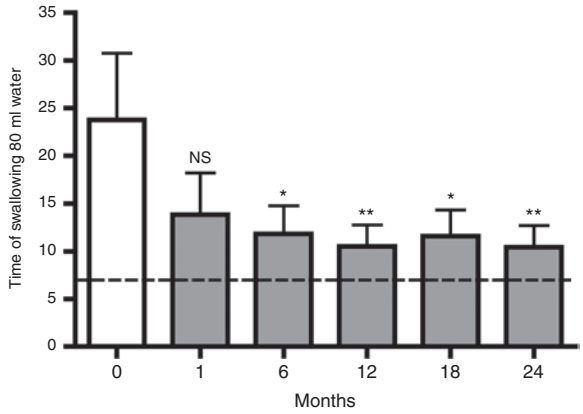

Figure 4 Quantitative evaluation of the global swallowing using the time in seconds to swallow a glass of 80 ml of water. Dotted lines indicate the averaged standard value in the normal population (7s). ANOVA and Dunnett's post-test (NS non significant; *P < 0.05; **P < 0.01 compared with preinclusion score).

Neuromuscular examination

Ptosis was present in all patients. In addition, seven patients also exhibited a lower limb weakness (**Table 1**). After 2 years, whereas five patients exhibited an increase of the limb weakness during the follow-up, no parallel deterioration was observed in pharyngeal function.

DISCUSSION

Swallowing disorders in OPMD patients are usually encountered in the fifth to sixth decade of life and their evolution can be more or less severe resulting in a progressive degradation in the quality of life of the patients. Extramucosal cricopharyngeal (or UES) myotomy is the most common and effective treatment for OPMD dysphagia, providing immediate improvement in the majority of cases.[4–6,10] This surgical intervention consists of sectioning UES

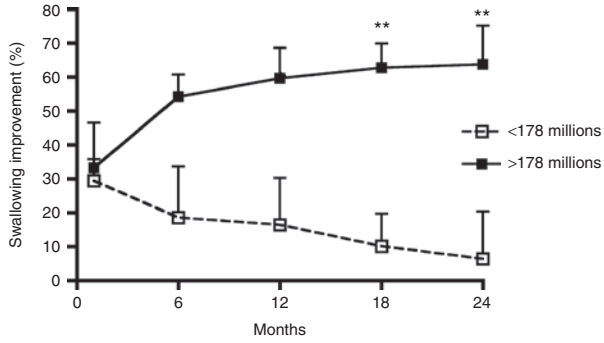

Figure 5 Correlation between the number of cells injected and the improvement of the time of swallowing. The percentage of swallowing improvement is determined between the time of swallowing preoperatively and each time point. 178 millions cells represent the median of the number of cells injected in this trial. Each group contains six patients, with no statistical difference in swallowing preoperatively. Mann-Whitney test (**P < 0.01).

muscles, improving swallowing by releasing the contraction of the fibrotic muscles, and facilitating the passage of food from the pharynx to the esophagus, despite the decrease in the force of pharyngeal contraction due to the myopathy. Although it has been shown to provide satisfactory immediate results in a majority of patients, many of them suffer a secondary deterioration of their deglutition after the myotomy since this surgical intervention does not prevent the progressive degradation of the pharyngeal muscles located above the UES muscles.[7] This progressive loss of contractility will increase aspiration and difficulties in swallowing, with risks of aspiration pneumonia and severe weight loss, which are the most common causes of mortality in OPMD patients.[2–4] In a series of 39 patients,[7] 12 out of the 35 patients (34%), although improved in the short term by the cricopharyngeal myotomy, exhibited a recurrence of dysphagia between 10 and 81 months (mean 39 months) after surgery. Moreover, this surgical procedure is not a curative treatment for the myopathy.

In a preliminary study, the major abnormality, which was observed in cultures of muscle progenitor cells isolated from OPMD patients, was a rapid decrease in the myogenicity of cells isolated from the cricopharyngeal muscle in contrast to other muscles.[9] The behavior of progenitor cells isolated from unaffected limb muscles or SCM was in fact identical to that of progenitors isolated from age-matched controls. Based on these results and the fact that the target muscles are small in OPMD, a therapeutic strategy based on grafting autologous myoblasts isolated from unaffected Q or SCM muscles and implanted into the dystrophic pharyngeal muscles was proposed. This could restore contractility of the affected pharyngeal muscles, limiting the life threatening dysfunction of the pharynx and improve quality of life in these patients. Our objective is to offer a possibility of avoiding the secondary degradation of pharyngeal function by enhancing skeletal muscle regeneration following the incorporation of new muscle progenitor cells.[11] The feasibility of this cell therapy approach for pharyngeal muscles was first demonstrated in a preclinical study of toxicity and safety of this procedure in dogs,[9] resulting in a validation of the surgical procedure and a demonstration of its safety. A follow-up at 1 month following autologous cell transplantation

revealed the presence of the implanted myoblasts and their differentiation: labeled cells were observed integrated in the pharyngeal muscles.[9] The proof of concept to use autologous myoblast transplantation as a therapeutic strategy for limited targets has been well described in previous studies for heart infarction[12] and for discrete muscular sites in Duchenne muscular dystrophy.[8]

In our study, two small biopsies were obtained from two different unaffected muscles, the Q and SCM from the same patient. One of the aims in this phase I/IIa study was to identify for future trials which muscle would be the best source to isolate and amplify progenitor cells. Interestingly, we observed that the different parameters studied – proliferative capacity, myogenicity, TRF, and differentiation **(Supplementary Figure S2)** – were very similar for both the Q and SCM. Due to the simple surgery required for the SCM biopsy by the same neck incision as for the cricopharyngeal myotomy and the fact that lower limb muscles were often affected in the older OPMD patients, it has been decided that this will be the muscle of choice for future clinical trials using autologous myoblast transplantation.

One of the most intriguing observations of this study was the improvement which we observed in both swallowing and quality of life questionnaires in the patients who had been injected with more than 178 million cells. Consequently, in future clinical trials, we will ensure that the protocol will allow to amplify the myoblasts so that each patient will be injected with at least 178 million cells even though this may delay the scheduled date for the graft.

Our primary aim was to improve both swallowing and contractility of the dystrophic pharyngeal muscles, a cricopharyngeal myotomy was therefore carried out at the same time as myoblast transplantation to provide an immediate clinical benefit to the patients, this surgical procedure being the only, although transitory, referential treatment for OPMD dysphagia.[4,6,7,13,14] This may mask any change in the immediate result on dysphagia, but since OPMD is a rare disease, it was not possible to include a group of patients with myotomy alone. However, since the evolution of the disease is different from one patient to another, each patient is in his own control. It is very encouraging to note that we did not observe any secondary degradation 2 years postoperation in any of these 12 patients. A long-term follow-up has been carried out on 10 patients. One patient died from sudden aspiration pneumonia 40 months after transplantation. In the other nine patients who accepted to continue the follow-up for a mean duration of 58.6 months, eight remained stable whereas we observed a degradation of swallowing in one patient. We conclude that these results are very promising, with a potential individual benefit for the patients, since a large proportion of patients in the protocol did not present any further degradation of their pharyngeal function, in contrast to the patients with cricopharyngeal myotomy alone.[7]

The results of this study have successfully shown that autologous myoblast transplantation is safe. The transplantation was well tolerated with no adverse side effects. We also found, using the global functional evaluation, that swallowing was improved (time of swallowing and questionnaires), although our evaluation did not allow us to observe any improvement of pharyngeal propulsion, using the qualitative tools available (VESS, VFS). In order to evaluate pharyngeal propulsion, other more sensitive functional tools (*e.g.* magnetic resonance imaging), will have to be developed for future clinical trials.

In light of these results, the perspectives of this protocol are to continue to follow the patients of the present study, and to improve both the procedure and the methods of evaluation for future clinical studies. A new study has been initiated in which patients with or without previous cricopharyngeal myotomy will be included. It should be noted that the autologous cells grafted in this study still carry the mutation, although the late onset of the disease argue for a delayed effect of this mutation. In order to ensure a long-term effect of cell therapy, a modification of the autologous cells should be considered using a gene therapy approach to repair the mutation. Therefore, a logical step toward an efficient cell therapy trial for OPMD should aim at delivering autologous muscle cells in which only a normal PABPN1 protein would be expressed; however, since the mutation consists of a short expansion of a repeat, a simple elimination of the mutated allele seems difficult to obtain. Finally, other stem cells with a myogenic potential could be considered for future cell autologous cell therapy trials, such as mesoangioblasts or CD133-expressing cells.[15,16] Since these cell types are currently being tested in preclinical or clinical assays, the results of this assay will soon confirm if they can be safely used in clinical context.

MATERIALS AND METHODS

Study design. This study was a noncomparative, nonrandomized pilot phase I/IIa clinical study (French Clinical Research National Program, PO20908 – AOM02100 – 2003, referenced NCT00773227 on clinicaltrial.gov, sponsored by AP-HP). It was approved by the local ethical committee "Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale" (CCPPRB, Paris-Cochin) and by the "Agence Française de Sécurité Sanitaire des Produits de Santé" (AFSSAPS). Twelve patients were prospectively enrolled in the clinical trial and fully completed the protocol (flow diagram in **Supplementary Figure S1**). The period of inclusion lasted for 4 years. All patients were operated and followed in the same center (Tenon Hospital, Paris), although they had been initially referred from six different clinical centers.

Patient inclusion. Written informed consent from the patients was required prior to their participation. All subjects had to be at least 18 years old and less than 75 years old. They all had a clinical diagnosis and a confirmed genetic diagnosis of OPMD and all patients exhibited disabling dysphagia with a positive indication for cricopharyngeal myotomy.

Patients with previous cricopharyngeal myotomy were excluded from this protocol, as were patients with HIV, active hepatitis B and C, active tuberculosis or tuberculosis which had been treated during the past 5 years; patients with lupus polyarthritis, rhumatoid polyarthritis, sarcoidosis, collagenose diseases, previous cancer, previous cervical radiotherapy; or patients with renal or liver failure, pregnancy, or breast-feeding women. In addition, patients in whom a follow-up of 24 months was not possible for any reason, as well as patients with no social protection were excluded.

Summary of the protocol. The protocol was organized in several phases detailed below and the monitoring of the protocol was followed by the "Unité de Recherche Clinique Paris Est" (URC Est). A safety committee of five expert members was constituted to examine every 6 months the follow-up of the protocol, the potential adverse effects, and the ethical issues.

Analytic biopsies and selection of the donor muscle. This first step aimed at selecting the muscle from the patient from which myoblasts can be expanded for the graft. This phase included two small biopsies

(about 100–250 mg each), one from the quadriceps (Q) and one from the SCM muscles. Both biopsies were excised under local anesthesia, except in patients with obvious clinical impairment/weakness of proximal limbs in whom only the SCM was biopsied. Biopsies were performed 2 or 3 months prior to the final graft. Each biopsy was divided into two equal parts that were sent to two different laboratories (Cell therapy Unit in Hospital St Louis and Institute of Myology in Hospital Pitié Salpêtrière) in order to compare their respective proliferative capacities. For each passage, the number of divisions was calculated as: $\ln(N/n)/\ln 2$, where N is the number of cells counted and n is the number of cells initially plated. The determination of proliferative kinetics, that is, the number of divisions achieved in a given time, the length of telomeres (TRF) to assess the proliferative capacity by an independent method,[17] and the myogenicity[18–21] were the criteria used to select the appropriate muscle for amplification prior to grafting. The total proliferative capacity, that is, the number of population doublings achieved before reaching senescence, was also determined for each culture. The muscle with the highest myogenicity and with the highest proliferative capacity was selected as the donor muscle.

The second step included a biopsy of approximately 1.7 g taken from the muscle selected in the first step, either SCM or Q. If there was no difference between the two different muscle biopsies, then the SCM muscle was preferentially biopsied, since this muscle is located directly in the trajectory/incision used for surgery (cricopharyngeal and myoblasts grafting). This second biopsy was performed under local anesthesia 3 weeks after the initial biopsies. The biopsy was then sent to the Cell Therapy Unit of Hospital Saint-Louis, where myoblasts were isolated and expanded for 21 days under Good Manufacturing Practice conditions as described previously.[22] The minimal target for expansion of the cells was set at 50×10^6. The total number of injected cells could be different from one patient to another, and was not related to patient's body weight. After 3 weeks of expansion, cells were collected by trypsinization, washed, counted, and suspended in Phosphate Buffer Saline supplemented with albumin 0.1% and transferred into a sterile vial. Criteria for product release included a percentage of myoblasts (identified by flow cytometry of CD56-positive cells) ≥50%, a cellular viability (as determined by 7-AAD) ≥80% and quality control tests such as sterility and the absence of endotoxins. The amplified cells were then shipped back to the transplantation centre.

Surgery. Myoblasts were injected into the constrictor muscles of the pharynx, associated with surgical cricopharyngeal myotomy.[4,6,13,14] Both cricopharyngeal myotomy and autologous myoblast transplantation were carried out during the same surgical procedure under general anesthesia. Following the extramucosal cricopharyngeal myotomy, the inferior constrictor muscles of the pharynx were widely exposed. Scarifications of the pharyngeal muscles with a scalpel were performed prior to cell injection, in order to induce muscle regeneration. Cells were injected into an average of 12 sites in the constrictor muscles of the pharynx over an area of approximately 10 cm^2 above the myotomy using a Hamilton syringe with an angulated needle (Hamilton, Reno, NV). The suture of the dissected tissue was then performed with or without drainage. The use of nasoesophageal tube was not necessary and all the patients resumed soft diet on the day following surgery.

Follow-up and end points. The evaluation of the protocol was performed during a follow-up period of 24 months after injections, at periodic intervals: before the surgical intervention as a control, and at 1, 6, 12, 18, and 24 months after the graft. The criteria of evaluation were based on the quality of pharyngeal propulsion using VESS, VFS, as well as global evaluation of swallowing, based on the results of questionnaires related to symptoms and quality of life, physical examination, and time of swallowing 80 ml of water ("drink test"). In addition, we evaluated the tolerance to muscles biopsies and cell grafting (see next paragraph). Results were analyzed for each patient, so that each patient was his own reference (control was before operation and was compared with values observed at different time points after graft and myotomy). Beyond the initial 2-year period of follow-up, patients were proposed to be monitored for an extended period of time; 10 out of 12 accepted while two are discontinued.

Tolerance. The functional tolerance of donor muscles to the biopsies and of recipient muscles to the graft were evaluated at 1, 6, 12, 18, and 24 months following the graft. This was based on the physical examination of the pharynx and a cervical palpation in order to follow the evolution of the sites of biopsies and of the scars and to detect any abnormalities, particularly on the site of myoblast implantation. The sites of the muscle biopsies were also examined. An endoscopic examination of the pharynx was performed systematically at 6 and 12 months after the graft to confirm the absence of pharyngeal tumors. Classical blood biochemistry was also performed 1 month before and 6 months after the graft (analysis of the electrolyte composition of the blood, speed of sedimentation, CRP level, liver biochemical markers, serum CPK).

VESS. A VESS was performed at inclusion and at 1, 6, 12, 18, and 24 months following the graft.[23] The VESS was performed using a flexible laryngoscope (on Olympus, ENF type PIII or on Machida, ENT-30 type PIII or on PENTAX) and a video recording, and included a morphological study of the pharynx and a functional assessment of deglutition during dry, thick cream, water, and marshmallow swallowing.[24] The VESS parameters were focused on pharyngeal propulsion/function scored as normal, decreased, or severely impaired with no peristaltic waves (aperistalsis), and on UES function studied indirectly by the presence or absence of salivary or diet pooling in the hypopharynx, above the UES (results scored as normal or abnormal).

VFS. The protocol for the videofluoroscopic study was based on a modification of the barium swallowing procedure.[25–27] This evaluation was performed at inclusion and at 1, 12, and 24 months following the graft. Patients swallowed liquid barium (2–20 cc), and/or paste and/or cookies coated with barium. The images were obtained in lateral and postero-anterior positions. Results were focused on pharyngeal propulsion scored as normal, decreased, or with no peristaltic waves (aperistalsis). UES function was also evaluated by the quality of the UES opening and closure and on the barium pooling in the hypopharynx (results scored as normal or abnormal).

Quality of life assessment based on dysphagia Salassa grade and McHorney score. The symptoms and quality of life, evaluated by patient self-assessment questionnaires using the Salassa grade[28] and the McHorney score,[29] were determined at inclusion and at 1, 6, 12, 18, and 24 months following the graft. Both questionnaires are focused on the subjective difficulties in swallowing. Questions concerned the dysphagia, the diet, problems resulting from aspiration such as pneumonia, as well as the impact of their disease on their quality of life (fatigue, sleep, psychological status, and social aspects). The Salassa scale went from grade 0, normal to grade 5, extremely severe swallowing disorders, and was used in parallel to the score of McHorney (44 items questions), where responses were scored as yes or no (presence or absence of the complaint). The weight of the patients was also registered.

Time of swallowing ("drink test"). A quantitative evaluation of the global swallowing was performed by measuring the time in seconds necessary to swallow a glass of 80 ml of water[30] at 1, 6, 12, 18, and 24 months following the graft.

Neuromuscular examination. A global neuromuscular examination (muscular testing), performed by a neurological expert, was also carried out on an annual basis at inclusion and every year after the graft to follow the general evolution of the myopathy. Attention was also given to ptosis as well as dysfunctions of superior and inferior limb muscles to evaluate the global evolution of disease.

Statistical analysis. All statistical analyses were performed using either the Student t-test when comparing two groups or the ANOVA one-way

analysis of variance followed by the Dunnett's post-test for multiple groups comparison, using GraphPad Prism (version 4.0b; GraphPad Software, San Diego, CA). A difference was considered to be significant at $P < 0.05$ (*), $P < 0.01$ (**) or $P < 0.001$ (***).

Human research. This study was approved by the local ethical committee "Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale" (CCPPRB AOM 02100, Paris-Cochin) and by the "Agence Française de Sécurité Sanitaire des Produits de Santé" (AFSSAPS). Written informed consent from the patients was received prior to their inclusion in the study.

SUPPLEMENTARY MATERIAL

Figure S1. Flow diagram of the OPMD clinical study.
Figure S2. Myogenicity and in vitro differentiation of injected cells.
Figure S3. Correlation between the number of cells injected and the quality of life questionnaires.

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