A Case for Immunosuppression for Myoblast Transplantation in Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is caused by an X-linked genetic defect that results in the absence of the structural protein dystrophin. It is characterized by repeated myofiber necrosis leading to progressive destruction and replacement of the skeletal muscle parenchyma with fibrotic and fat tissues. Patients develop progressive muscle weakness by age 4, wheelchair confinement by age 10, and death from respiratory complications or secondary heart disease by the early 20s. Cell-based therapy is a potential solution toward restoring dystrophin expression in skeletal muscles and restoring lost muscle parenchyma. This therapy exploits the ability of wild-type muscle precursor cells to fuse with damaged DMD myofibers, thereby introducing nuclei that express the normal dystrophin gene in the muscle syncytia. Currently such wild-type cells must come from an allogeneic donor, thus requiring the use of immunosuppression, as for any allotransplantation.

The first cells to be studied for experimental cell-based therapy of myopathies such as DMD were adult myoblasts, mononucleated muscle precursor cells derived from satellite cells, which are the stem cells of skeletal muscle.[1,2] Satellite cells are easily isolated from muscle samples by standard cell-culture procedures and can be expanded *in vitro* to obtain large numbers of myoblasts. The first clinical trials of normal myoblast allotransplantation, performed in DMD patients in the 1990s, demonstrated significantly increased dystrophin expression in cell-grafted vs. placebo-injected sites.[3–6] However, only one study showed unequivocally that the dystrophin in the cell-grafted site was derived from donor cells.[6] Thus, the conclusion at the time was that cell-based therapy based on the protocols used at that time was ineffective and new animal studies were needed.

These continuing animal studies have identified two important factors that support further clinical tests of myoblast transplantation. The first factor is immunosuppression. A comparison of immunosuppressant drugs in mice revealed that superior myoblast transplantation was obtained when using the calcineurin inhibitor tacrolimus.[7] Similar results were obtained in the monkey model,[8] which is crucial for translational transplantation research. It was also observed in the latter model that myoblasts fuse predominantly with the myofibers surrounding the injection trajectories.[9–11] Thus, the second factor to consider in future clinical studies of myoblast transplantation is the method of cell implantation: closely spaced injections throughout the muscle are required so as to deliver the cells homogeneously to the tissue.[11]

We conducted a phase IA clinical trial of normal myoblast allotransplantation to test whether these two factors identified in animal studies could produce more consistent results than those of previous clinical trials. Normal allogeneic myoblasts obtained from either parent were transplanted within 1 cm^3 of muscle in nine DMD patients (8–17 years old) who were immunosuppressed with tacrolimus. Muscle biopsies performed 1 month after transplantation revealed dystrophin expression in the cell-grafted sites of eight of nine patients, reaching 26% of the myofibers in the best case.[12,13] Because the patients who participated in this clinical trial had identified dystrophin mutations, it was demonstrated by reverse transcriptase–polymerase chain reaction that the dystrophin messenger RNA was wild type and thus originated from the donor. Moreover, monoclonal antibodies reacting with epitopes encoded by exons deleted in the patients confirmed that the dystrophin-positive myofibers in the cell-grafted site expressed wild-type donor-derived dystrophin. Thus, the trial clearly demonstrated that myoblast transplantation could restore the expression of normal dystrophin in a limited number of myofibers, depending mostly on the inter-injection distance and on adequate immunosuppression. The logical continuation of these results would be to monitor any functional improvement following myoblast transplantation, which would require that a whole muscle be transplanted with normal myoblasts and followed up for a longer period of time.

Evidence that such a protocol could be applied in whole muscles and for longer periods came from a special circumstance: coincident with the end of the phase IA clinical trial, our team had the opportunity to transplant allogeneic normal myoblasts as "compassionate treatment" into an older (26-year-old) DMD patient.[14] In this particular case, normal myoblasts were transplanted both to a small region of a gastrocnemius and throughout some entire muscles, including those of the left thenar eminence. The patient was immunosuppressed with tacrolimus for 18 months. In the cell-grafted site of the gastrocnemius, 27.5 and 34.5% of the myofibers expressed wild-type donor–derived dystrophin 1 month and 18 months after transplantation, respectively.

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The contralateral gastrocnemius was dystrophin-negative. In addition, a significant increase in strength was observed in the left thumb of the patient following myoblast transplantation into his left thenar muscles, remaining significantly (50%) higher than the pretransplantation values even 14 months after transplantation.

On the basis of these encouraging results, permission was sought to conduct a phase IB clinical trial. Because there was reticence by the relevant institutional human ethics committee to allow multiple close injections in a large important muscle such as the biceps brachii, the extensor carpi radialis longus was selected. This medium-sized muscle of the forearm allowed measurement of wrist extension strength but was not predicted to result in a significant reduction in the global functionality of the forearm in the unlikely case of adverse events. Myoblasts were to be transplanted throughout one randomly selected extensor carpi radialis longus, whereas the contralateral muscle would be injected with saline as a control. The duration of follow-up was to be only 6 months. Because of the perceived risks associated with the planned immunosuppression, the human ethics committee restricted the participation to DMD patients older than 18 years. Moreover, because of the risks of infection/reinfection due to immunosuppression, Health Canada also stipulated that participants be seronegative for cytomegalovirus (CMV) and not on a respirator.

However, these restrictions raised several practical problems, as well as an ethical dilemma. First, there are very few DMD patients over the age of 18 who are healthy enough to meet both the respiratory restrictions and general health considerations required for such a trial. In addition, 70% of adults in Canada are seropositive for CMV. Thus, extremely few patients would qualify for such a study. Second, most adult DMD patients have severely restricted movement in their forearm muscles, limiting meaningful strength assessment after myoblast transplantation. Third, clinical results suggest that muscles that are severely degenerated and replaced with fat and conjunctive tissue are not good targets for myoblast transplantation because it is difficult to form new functional myofibers in this environment. Thus, the restrictions for performing a clinical trial of myoblast transplantation in Canada represent a catch-22 situation. Efficacy of the procedure must be demonstrated to offset the perceived risk and allow trials that include younger patients, but older patients fulfilling the criteria are very rare and may not respond adequately, limiting the potential to demonstrate efficacy of the treatment. Thus, the current guidelines essentially preclude assessment of myoblast transplantation in Canada.

We believe that myoblast transplantation remains a plausible mode of therapy in myology and that the early data warrant further study to confirm and improve the initial clinical results. One limitation of myoblast transplantation is that it may target only individual muscles, thereby limiting the potential benefit to patients. However, in clinical practice, the difficulty of healing an individual completely does not preclude the implementation of all possible measures to alleviate patient suffering and to produce some real benefit. Preserved or increased strength of only the respiratory and limb muscles would mean major improvements in morbidity and mortality for DMD patients. Although alternative potential therapies have been proposed for DMD, it seems unlikely that they will be 100% effective or without side effects. Should an alternative therapy prove partially successful, myoblast transplantation could be an effective second-line or adjunctive treatment. Moreover, solutions to the technical challenges of intramuscular cell transplantation are likely to be identified with further study. In addition, future studies may involve the transplantation of autologous cells that have been genetically corrected, obviating the need for immunosuppression. However, one of the difficulties with using autologous cells is that the patient's own satellite cells are close to senescence as early as 6 years of age. Thus, an important question is how ethics committees and regulatory agencies will view trials attempting to examine the efficacy of such genetically modified autologous cells. Will they require the trials to be done in patients over 18 years old? Regulatory agencies and human ethics committees in Canada question the risk-to-benefit ratio of the proposed phase Ib myoblast transplantation clinical trial. We contend that, as detailed above, the potential benefits are significant and the technological aspects of the approach are surmountable.

What really limits the trials, then, is not the potential benefit but perceived risks linked to the immunosuppression with tacrolimus. Here we are surprised to discover such reticence. Calcineurin inhibitors have been used in thousands of patients worldwide for more than 20 years, and their side effects and risks are well known. They are used routinely in organ and pancreatic islet transplantation. The incidence of lymphoma in pediatric renal transplantation patients receiving tacrolimus was 0.3% at 1 year.[15] Moreover, the evidence suggests that it is cumulative immunosuppression, not a specific drug, that promotes the development of lymphoma.[16,17] Renal transplant recipients, including children, receive a combination of immunosuppressive drugs that may include T-cell-depleting monoclonal antibodies that increase the risk of lymphoma. In trials using calcineurin inhibitors as single agents in children, the incidence of lymphoma seems to be much lower. For example, 20 children (averaging 11 years of age) with focal segmental glomerulosclerosis received tacrolimus plus corticosteroids for 12 months, and none developed lymphoma.[18] It is therefore expected that the incidence of lymphoma in DMD patients being treated with tacrolimus as a sole immunosuppressive agent will show a low incidence of malignancy.

Although the incidence of lymphoma in children treated with calcineurin inhibitors alone is lower than that observed in more heavily immunosuppressed renal transplant recipients, the transplant experience provides insight into treatment and prevention. First, of pediatric renal transplant recipients developing a lymphoma, 50% are alive 5 years later.[15] Post-transplant lymphoproliferative disorder, the most common form, is usually linked to Epstein-Barr virus (EBV). A measure to reduce its incidence is serial monitoring of EBV titers, with reduction of immunosuppression and/or treatment with acyclovir or ganciclovir in patients who have a high viral load.[19] Finally, monitoring CMV titers also allows prevention and early treatment of disease with ganciclovir.[20] Given concerns of CMV pneumonitis in DMD patients, who are prone to respiratory infection, it may be prudent to emulate the renal transplant experience and treat all recipients with prophylactic doses of ganciclovir for 6 months. Prophylaxis of

renal transplant patients with ganciclovir or acyclovir during the initial therapy also seems to decrease the incidence of post-transplant lymphoproliferative disorder as a result of its effects on EBV.[21]

Thus, tacrolimus is routinely used in children in transplantation of solid organs, even in situations that are not imminently life threatening, such as kidney failure. Patient survival following kidney transplantation has markedly improved since its inception. Given the relative safety of tacrolimus, it is now being examined for treatment of diseases that are far less life threatening. For example, immunosuppression was used in trials treating children with type 1 diabetes, a disease that can be controlled with insulin administration. Indeed, tacrolimus is currently being used in children for the treatment of various autoimmune diseases,[18, 22, 23] many of which are much less severe than DMD. We argue that mortality and morbidity in teenagers with DMD are clearly worse than in those on dialysis or with diabetes. Of course, one must also weigh the potential benefits of any experimental treatment. In the case of renal transplantation the potential benefits were clear, judging from prior successful results obtained in adult patients. However, attempts to induce remission in new-onset diabetes or childhood nephropathy remain instructive, because this can be tested only in children and adolescents, with hopes of preventing further damage to islets or renal tissue. This strategy is in line with the National Institutes of Health policy, which recognizes the specific need to include children in clinical trials, particularly for diseases of childhood, in which the response may be better than in adult patients. In keeping with the notion that individual participants should benefit, we believe that future DMD trials should insist on continuing immunosuppressive therapy for those who demonstrate improved function. For those in whom improvement is lost or not achieved, immunosuppression can be stopped, limiting potential risks.

In summary, compared with DMD, the risk-to-benefit ratio is significantly higher for islet-cell transplantation in that many such patients have not lost function of vital organs and insulin therapy can sustain diabetics for decades. We have shown that doses of tacrolimus considered safe and effective in many other settings can be used as monotherapy and support myoblast transplantation in DMD. With careful monitoring the risks are very low, especially for the short-term studies being planned.

We believe that a feasibility study is warranted to assess whether the current approach for cellular therapy is safe and effective. Such a trial must include a blinded comparison of myoblast-injected and control muscle in DMD patients young enough to be in sufficiently good health and without irreparable muscle damage. Restricting study to patients over the age of 18 dooms the approach to failure because we cannot expect to recruit a sufficient number of patients and we do not expect myoblast transplantation to produce enough new myofibers in muscle extensively replaced by fat and fibrous tissue. We believe that the available scientific and clinical data indicate that the potential benefits outweigh the risks and that no better treatment currently exists.

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