Myoblast transplant therapy (MTT) can be envisioned as a clinical alternative in the treatment of several myopathies. One of the problems that remain to be solved is the efficiency of MTT due to low proliferation and limited migration of transplanted cells. We have used a model of xenotransplantation in which human myoblasts were transplanted intramuscularly into immunodeficient Rag2−/−;C−/− mice, in order to investigate the kinetics of proliferation and differentiation of the transplanted cells. In standard conditions, most of the injected myoblasts had already differentiated by day 5 post-engraftment. This early differentiation correlated with reduction in proliferation and limited migration of the donor cells within the regenerating muscle. These results suggest that the precocious differentiation, already detected at 3 days post-injection, is a limiting factor for both the migration from the injection site and the participation of the donor cells to muscle regeneration. When we stimulated in vivo proliferation of human myoblasts, transplanting them in a serum-containing medium, we observed 5 days post-transplantation a delay of myogenic differentiation and an increase in cell numbers, which colonized a much larger area within the recipient’s muscle. Importantly, these myoblasts maintained their ability to differentiate, since we found higher numbers of myofibers seen one month post-engraftment, as compared to controls. Our data now place this rapid differentiation of the transplanted human myoblasts as a new problem to be taken into account in future cell therapy trials for muscular dystrophies. Conceptually, these data reveal that in experimental myoblast transplantation therapy, any intervention upon the donor cells and/or the recipient’s microenvironment aimed at enhancing myogenic precursor engraftment should be done before day 3 post-engraftment and could result in different injection protocols being adopted.

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P4.35
In vivo stem cell tracking using scintigraphy in the GRMD dog
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A major challenge in cell therapy for muscle diseases is to specifically target transplanted cells to the muscle. To address this issue, it is necessary to develop non-invasive cell tracking tools at the preclinical stage. The aim of this study is to develop a method of in vivo cell tracking, in the GRMD dog model of Duchenne Muscular Dystrophy (DMD). Three healthy and five GRMD dogs received an injection of 40.106 111In-labelled mesoangio-blasts (MABs) in one femoral artery. Scintigraphic acquisitions were performed during injection, and 1, 3, 24, 48 h and 7 days post-injection. Three other healthy dogs were injected with 111In-labelled leukocytes, myoblasts, and 111In alone, respectively, to assess the specific migratory behaviour of MABs. In healthy and GRMD dogs, the early biodistribution of MABs was linked to the two first capillary filters they encountered: the injected leg, and the lung. However, a greater amount of cells stayed into the leg in GRMD (mean 50.4%; SD 10.9%) than in healthy dogs (34.7% SD 5.2%), suggesting an affinity of MABs for the dystrophic muscle. The radioactivity values in the injected leg remain constant while the strong radiouclide observed in the lung tend to cease in favour of a progressive increase in the liver and kidneys. These observations suggest cells recirculation, or cell death and 111In release. The three control dogs injected with 111In, myoblasts or leukocytes showed different radioactivity distribution patterns, attesting to the method ability to detect specific behaviours. Even if questions remain regarding the distinction of free and cell-linked radioactivity and the superimposition of anatomical structures within the injected leg, this study represents the first in vivo cell-tracking method translated into the GRMD model. Its ability to quantify the amount of cells migrating into the injected leg paves the way for in vivo evaluation.

One interesting alternative regarding muscular dystrophies treatment is stem cell therapy. However, the stem cell to be transplanted has to be allo or xenogenic. This imposes a major limitation driven by the immune system: transplant rejection. Our goal is to set an experimental protocol that allows myoblast transplantation in vivo and that is simple enough to be translated into the clinic. We chose oral antigen administration in combination or not with Lactococcus lactis, a probiotic known to block intestinal inflammation therefore favoring tolerance rather than immunity. None of the substances are toxic, and could be easily tested in humans after the pre-clinical studies. The experimental model consisted of C57BL/6 mice which received for 5 days, by gavage, an extract of human myoblast cell lines (CHQ or LHCN-M2), accompanied or not of L. lactis (continuous ingestion –5 days). After 4 days mice were immunized with CFA without or with 60 μg of a protein extract from the cell line to be transplanted. One week after immunization all groups were boosted with myoblast extract.

On the next day the draining nodes were harvested and in vitro analyses were undertaken. Proliferation was 60–85% less on the tolerized groups. In terms of cytokine production, T cells from the tolerized mice produced less than 60% of the Y-interferon detected in the experimental group demonstrating a reduction in the Th1 response induced by oral administration of antigen. Phenotypically we observed an increase in the absolute number of B220+ cells in the experimental groups and no alteration in the numbers of CD3+ or regulatory T cells in comparison with the negative control group. We are currently testing if this protocol allows a successful myoblast graft in vivo in a model of cyro-leision using an immunocompetent host.

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P4.34
Induction of tolerance to human myoblasts in mice
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One interesting alternative regarding muscular dystrophies treatment is stem cell therapy. However, the stem cell to be transplanted has to be allo or xenogenic. This imposes a major limitation driven by the immune system: transplant rejection. Our goal is to set an experimental protocol that allows myoblast transplantation in vivo and that is simple enough to be translated into the clinic. We chose oral antigen administration in combination or not with Lactococcus lactis, a probiotic known to block intestinal inflammation therefore favoring tolerance rather than immunity. None of the substances are toxic, and could be easily tested in humans after the pre-clinical studies. The experimental model consisted of C57BL/6 mice which received for 5 days, by gavage, an extract of human myoblast cell lines (CHQ or LHCN-M2), accompanied or not of L. lactis (continuous ingestion –5 days). After 4 days mice were immunized with CFA without or with 60 μg of a protein extract from the cell line to be transplanted. One week after immunization all groups were boosted with myoblast extract.

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P4.33
Rapid differentiation of engrafted human myoblasts into immunodeficient mice correlates with the reduced proliferation and migration
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Myoblast transplant therapy (MTT) can be envisioned as a clinical alternative in the treatment of several myopathies. One of the problems that remain to be solved is the efficiency of MTT due to low proliferation and limited migration of transplanted cells. We have used a model of xenotransplantation in which human myoblasts were transplanted intramuscularly into immunodeficient Rag2−/−;C−/−− mice, in order to investigate the kinetics of proliferation and differentiation of the transplanted cells. In standard conditions, most of the injected myoblasts had already differentiated by day 5 post-engraftment. This early differentiation correlated with reduction in proliferation and limited migration of the donor cells within the regenerating muscle. These results suggest that the precocious differentiation, already detected at 3 days post-injection, is a limiting factor for both the migration from the injection site and the participation of the donor cells to muscle regeneration. When we stimulated in vivo proliferation of human myoblasts, transplanting them in a serum-containing medium, we observed 5 days post-transplantation a delay of myogenic differentiation and an increase in cell numbers, which colonized a much larger area within the recipient’s muscle. Importantly, these myoblasts maintained their ability to differentiate, since we found higher numbers of myofibers seen one month post-engraftment, as compared to controls. Our data now place this rapid differentiation of the transplanted human myoblasts as a new problem to be taken into account in future cell therapy trials for muscular dystrophies. Conceptually, these data reveal that in experimental myoblast transplantation therapy, any intervention upon the donor cells and/or the recipient’s microenvironment aimed at enhancing myogenic precursor engraftment should be done before day 3 post-engraftment and could result in different injection protocols being adopted.

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of methods to improve cell homing to muscles in a large pre-clinical model of DMD.

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P4.36
Development of pluripotent stem cells as vectors for viral gene therapy
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Several approaches to restore functional protein expression in Duchenne Muscular Dystrophy (DMD) patients, which target post-transcriptional processing of the dystrophin gene, are currently in clinical trials. Inevitably these exon skipping and premature stop codon read-through studies only give hope for a fraction of the patients due to the variation in types and locations of mutations in the dystrophin gene. Studies on the in vitro restoration of dystrophin and personalised therapies are hindered by the diminished ex vivo expansive capacity of biopsy-derived cells. Therefore alternatives must be sought for a more effective method for deriving cells bearing patient-specific mutations. Induced Pluripotent Stem Cells (iPSC) can be used for patient-specific cell line derivation and subsequent differentiation into more appropriate lineages for therapeutic testing. In collaboration with Nottingham University we have successfully generated iPSCs from patient fibroblasts and subsequently differentiated them into cardiomyocytes. Adeno-associated viruses (AAV) have successfully been used as a vector for delivering micro-dystrophin to cardiac cells, reliably attenuating phenotype in mouse models. Capsid engineering and serotype affinity have been employed to improve efficiency of organ-specific transduction. Optimization of appropriate vectors for transducing patient iPSC-derived cardiomyocytes with dystrophin constructs is an interdisciplinary approach with a prospect that gives hope to a larger cohort of DMD patients.

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POMPE DISEASE: POSTER PRESENTATIONS

P4.37
Towards hematopoietic stem cell gene therapy for the treatment of Pompe disease
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Pompe disease is an autosomal recessive lysosomal storage disease, characterized by progressive muscle weakness and death within the first year of life in its most severe form due to cardiac and respiratory failure. Pompe disease is caused by mutations in the gene encoding acid alpha-glucosidase (GAA) and results in elevated levels of glycogen. Enzyme replacement therapy is the current option for treatment, but is not beneficial to all patients and may result in immune reactions against the recombinant enzyme. Therefore, we have developed an alternative therapy, based on transplantation of gene modified autologous hematopoietic stem cells (HSC). The use of autologous HSC has the intrinsic advantage to induce immune tolerance to the recombinant transgene product and minimizes the risk of transplant related mortality and morbidity. Using this strategy, life-long high levels of (human) alpha-glucosidase were achieved in leukocytes and affected tissues of Pompe mice using mild conditioning. Also glycopeptide storage was reduced significantly in most tissues. We found near normalization of heart morphology and function, as well as a significant improvement in skeletal muscle function. After a robust challenge of the treated animals with the rhGAA protein, an immune response was absent, contrary to a vigorous response in untreated mice. When using a codon-optimized GAA gene, very high levels of GAA activity were achieved in all affected tissues, corresponding to a profound glycopeptide reduction in the examined tibialis, long extensor, quadriceps, gastrocnemius and soleus muscles. Heart morphology and function normalized, and, most importantly, also the skeletal muscle function fully normalized as shown by rotarod and running wheel tests. Initial results also demonstrated a reduction of glycogen in brain tissue. Thus, ex vivo hematopoietic stem cells lentiviral vector mediated gene therapy corrects the Pompe phenotype in the relevant mouse model.

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P4.38
Survival of adult Pompe patients with and without treatment with enzyme replacement therapy
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Pompe disease is a rare hereditary neuromuscular disorder for which since 2006 enzyme replacement therapy (ERT) is available. ERT treatment of adult patients has shown positive effects on respiratory and muscle function outcomes and quality of life. Yet there is paucity of information on the impact of ERT on survival of adult patients. We assessed whether there is differential mortality in adult patients treated with ERT compared to untreated patients. Data were collected in an international observational study conducted between 2002 and 2011. Survival analyses differentiating patients ever treated with ERT and the non-treatment comparison group were performed using the Kaplan–Meier method. Multivariable logistic regression analyses were performed to detect factors associated with mortality. Two-hundred and seventy patients with a median age of 48 years (range 19–79 years) were included in the study. The ERT-group included 195 patients with 15 deaths. The non-treatment comparison group included 75 patients with 26 deaths. Kaplan–Meier analyses showed a greater survival for those patients on ERT compared to patients who were never on ERT (p-value < 0.001) and for patients with lower compared to higher levels of disease severity (p-value = 0.001). For each level of disease severity (based on wheelchair or ventilator dependency) Kaplan–Meier analyses demonstrated greater survival on ERT. In logistic regression analyses controlling for age, gender, and national treatment patterns, both treatment with ERT and disease severity were statistically significantly associated with survival. In our cohort of adult Pompe patients, ERT and disease severity were both associated with survival. Based on the present data, ERT seems to expand the life span of adult patients even when they are severely affected. Longer follow-up is needed to further elucidate the relationship between ERT, disease severity and survival of adults with Pompe disease.

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