

**PW 28:
Cell therapies –
Myogenic precursor cells**

PW28-348	<p><u>ROLE OF NECDIN IN SKELETAL MUSCLE REGENERATION AND IN THE DIFFERENTIATION OF MESOANGIOBLAST STEM CELLS</u> PESSINA P¹, FRANÇOIS S¹, AZZONI E², COSSU G², BRUNELLI S² (1) DIMS-University Milano Bicocca, Milano, ITALY. (2) DIBIT HSR, Milano, ITALY.</p>
To contact the author:: brunelli.silvia@hsr.it.	<p>Muscular dystrophies are heterogeneous diseases characterized by a primary wasting of skeletal muscle. Replacement of diseased muscles with new healthy and functional muscle fibers has been for a long time a major therapeutic strategy for muscular dystrophies. In particular the mesoangioblasts have been show to contribute to muscle repair in dystrophic mice and dogs when injected intra-arterially.</p> <p>Despite of the identification of mesoangioblasts as potential source of skeletal muscle, the molecular mechanisms regulating their growth and differentiation into skeletal muscle remained unexplored. Detailed studies on the molecular pathways regulating their response to specific cues are needed to manipulate their fate, since it would be of great interest to be able to improve their ability to differentiate and fuse to the fiber, as well as to proliferate and resist to cell death and as such act as pool of resident stem cells.</p> <p>Necdin is a member of the MAGE protein family, a large family of genes initially isolated from melanomas. Ndn^{-/-} animals show a defect in muscle regeneration. On the other hand Necdin appears to improve muscle regeneration in gain of function transgenic mice by promoting satellite cells survival and differentiation: it would therefore be of great interest if this could be exploited in mesoangioblasts. Preliminary experiments show that transient overexpression of Necdin increases the differentiation ability of mesoangioblasts in vitro.</p> <p>We are isolating mesoangioblasts from the dorsal aorta of MlcNec2 and Ndn^{-/-} embryos at. In parallel with studies carried on on satellite cells, we are investigating mesoangioblasts proliferation, migration, response to apoptotic stimuli and myogenic differentiation potential in vitro and in vivo in comparison with wt cell.. We will also evaluate their migration potential.</p>

PW28-349	<p><u>IN VIVO MYOGENIC POTENTIAL OF HUMAN AC133 MUSCLE-DERIVED STEM CELLS</u></p> <p>NEGRONI E¹, RIEDERER I¹, WOLFF A¹, DI SANTO J², TORRENTE Y³, MOULY V¹, BUTLER-BROWNE GS¹</p> <p>(1) UMRS787 – Groupe Myologie; Inserm / UPMC-ParisVI; Institut de Myologie, Paris, FRANCE. (2) Institut Pasteur – Département d' Immunologie, Unité des Cytokines et Développement Lymphoïde, Paris, FRANCE. (3) Stem Cell Laboratory – Department of Neurological Sciences, Fondazione IRCCS Ospedale Maggiore Policlinico, Centro Dino Ferrari, University of Milan, Milan, ITALY.</p>
To contact the author:: enegroni@ext.jussieu.fr.	<p>After birth, adult skeletal muscle growth and repair are mediated by a population of cells, normally mitotically quiescent and located under the basal lamina of the myofibers, called satellite cells. In response to injury, resident satellite cells become activated and proliferate, differentiate and fuse to form new muscle fibers. We have investigated the myogenic potential of human muscle-derived cells based on the expression of two stem cells markers, CD34 and AC133, as compared to bona fide satellite cells.</p> <p>The efficiency of these cell populations to participate to muscle regeneration and contribute to replenish the satellite cell pool is evaluated in an <i>ex vivo</i> model, the RAG^{-/-} gammaC^{-/-} C5^{-/-} immunodeficient mouse in which degeneration of the Tibialis Anterior (TA) is induced by cryoinjury. Human cells were then injected into the regenerating TA, each animal receiving satellite cells in one leg and stem cells in the other. 1 month post-injection, human nuclei are visualised using an human-specific antibody directed against lamin A/C, while fibres containing human proteins are identified with an antibody directed against human spectrin.</p> <p>Our results demonstrate that human muscle-derived AC133+ cells showed a better regenerative capacity than human myoblasts derived from satellite cells. The number of fibres expressing human proteins, the number of human nuclei and the number of human cells in a satellite cell position are all increased in TA injected with AC133+ cells as compared to those injected with human myoblasts. In addition AC133+/CD34+ cells exhibited a better longitudinal dispersion in the host muscle when compared to human myoblasts.</p> <p>We propose that human muscle-derived AC133+ cells could be a potentially attractive candidate for cellular therapy, provided that sufficient numbers of cells could be available either at isolation or more likely after their amplification <i>in vitro</i>.</p>

PW28-350	<p>MYOGENIC AND ADIPOGENIC POTENTIAL OF HUMAN SKELETAL MUSCLE-DERIVED CD34-SORTED CELLS</p> <p>PISANI D.F¹, DECHESNE CA¹, DESNUELLE C², BELMONTE N², DELPLACE S², COCHET O¹, BAGNIS C³, DI SANTO J⁴, KURZENNE JY⁵, DANI C¹, SACCONI S²</p> <p>(1) UMR 6543 CNR/UNSA, Nice, FRANCE. (2) UFR Médecine, Nice, FRANCE. (3) Etablissement Français du Sang, Marseille, FRANCE. (4) Institut Pasteur, Paris, FRANCE. (5) Hôpital de l'Archet, Nice, FRANCE.</p>
To contact the author:: dani@unice.fr.	<p>Myoblast transplantation in clinical trials is based on intramuscular injection of a population of muscle-derived cells. Up to date, homogeneity of this population throughout culture has been evaluated using the CD56 marker. According to our data, the CD56 population contains stem cells able to give rise to adipocytes <i>in vitro</i>. Differentiation of stem cells after transplantation into muscles is driven towards the myogenic lineage. However, adipocyte accumulation is observed in human dystrophic muscular diseases and likely, stem cells transplanted in a muscle environment permissive to fat development may be committed towards adipogenesis at the expense of myogenesis. Therefore, for muscular cell therapy, it is critical to identify a cell population with a high myogenic and a low adipogenic potential from the mixture of cells to be transplanted.</p> <p>The stem cell marker CD34 allowed us to sort two distinct populations from human pediatric and adult muscle biopsies. <i>In vitro</i>, the CD34+ cells were myogenic and adipogenic whereas the CD34- cells were only myogenic. Both cell populations have muscle regeneration potential after transplantation in cryo-injured muscle of immunodeficient Rag2^{-/-} γc^{-/-} mice.</p> <p>To our knowledge, there is no convenient mouse model fully mimicking human muscular dystrophies, i.e. fat infiltration in regenerative muscles. However, we have observed a higher fat development in cryo-injured <i>tibialis anterior</i> muscle of Rag2/gc^{-/-} mice when using clodronate-containing liposomes. Therefore, experiments are carrying out to determine the fate of CD34 cell populations after their muscle transplantation in this new mouse model having a micro-environment permissive for differentiation of transplanted cells into adipocytes. In conclusion, the muscle CD34 negative cell population could represent a new alternative cell population in cell therapy of muscular dystrophy.</p>

PW28-351	<p>MYOGENIC POTENTIAL OF GENETICALLY-COMMITTED HUMAN MULTIPOTENT ADIPOSE-DERIVED STEM CELLS</p> <p>GOUDENEGE S¹, PISANI DF¹, DI SANTO JP², DANI C¹, DECHESNE CA¹ (1) CNRS UMR 6543, Institute of Signaling Developmental Biology and Cancer, Nice, FRANCE. (2) Inserm U668, Pasteur Institute, Paris, FRANCE.</p>
To contact the author:: dechesne@unice.fr.	<p>We have previously shown that human multipotent adipose-derived stem (hMADS) cells have a myogenic potential. They contribute to skeletal muscle regeneration after transplantation into <i>mdx</i> mouse muscle although only a very small proportion of cells shows <i>in vitro</i> myogenic commitment under myogenic culture conditions. In addition, co-culture experiments led us to propose that hMADS cells participate in myotube formation via cell fusion and that only a sub-population has the capacity to fuse with myoblasts. The goal of our work is to optimize hMADS cells myogenic potential to evaluate their interest in cell therapy, since adipose tissue is easily available. One option is to experimentally commit hMADS cells to myogenic differentiation. Here we present the effect of a myogenic pre-commitment of hMADS cells that were genetically modified by MyoD-forced expression. Myogenic potential of wild-type hMADS and MyoD-hMADS cells was assessed <i>in vitro</i> and <i>in vivo</i>. <i>In vitro</i> amplified hMADS cells isolated from a fat pad of the prepubic area of a 4-month old boy were transduced with a lentivirus vector expressing human MyoD. Transduced hMADS cells formed numerous characteristic multinucleated myotubes and expressed muscle markers at RNA and protein levels when maintained for 1-2 weeks in myogenic differentiation medium. Thus, forced expression of MyoD dramatically increased the <i>in vitro</i> myogenic potential of hMADS cells. Then, we investigated the capacity of MyoD-hMADS cells to participate to muscle regeneration. Cells were injected into cryo-injured regenerating <i>Tibialis anterior</i> muscles of RAG2 (-/-) αc (-/-) mice and expression of human muscle specific markers such as dystrophin, α-sarcoglycan and spectrin α chain was analyzed by immunohistochemistry and RT-PCR. Contribution of wild-type hMADS and MyoD-hMADS cells to muscle regeneration was clearly observed in this mouse model. Efficiency quantitative comparisons between wild-type hMADS and MyoD-hMADS cells will be presented and discussed.</p>

PW28-352	<p>ARE SATELLITE CELLS AN HOMOGENOUS POPULATION? ADIPOGENIC POTENTIAL IS LINKED TO THEIR PROLIFERATIVE CAPACITY.</p> <p>ROSSI CA¹, DITADI A¹, MALERBA A¹, FRANZIN C², SANNA M², POZZOBON M¹, VETTOR R², DE COPPI P¹</p> <p>(1) Pediatric Oncohematology, Stem Cell Transplantation Unit, Department of Pediatrics, University of Padova, Padova, ITALY. (2) Endocrine-metabolic laboratory, Department of Medical and Surgical Sciences, University of Padova, Padova, ITALY.</p>
To contact the author:: carloalberto.rossi@unipd.it.	<p>Cell therapy represents a valid tool for tissue replacement, in particular in the contest of muscle dystrophies or structural defects. Satellite cells (SCs) have been frequently used as source of cells for skeletal muscle replacement, because they represent <i>in vivo</i> the pool of myogenic precursors. SCs are located between the basal lamina and the plasma membrane of skeletal myofibers. They offer the possibility of <i>in vitro</i> expansion and autologous transplantation. According to recent studies, they seem to be divided into two subpopulations, one of committed muscle precursors and one of cells with more stem-like properties. This distinction correlates to both a diversity in markers expression and differentiation potential.</p> <p>In this study, for the first time, two subpopulations of SCs obtained from rat <i>flexor digitorum brevis</i> muscle through single fiber selection and disgregation, were distinguished based on both different proliferative and differentiative capacities. Quantitative analyses showed that there is an almost fixed proportion of SCs that possess a great proliferative potential, and that spontaneously give rise to adipocytes in culture. Immunofluorescence and PCR analyses showed that while initially SCs are homogenously positive for early myogenic markers such as Pax7 and Myf5, pluripotent clones lose in culture myogenic markers and form lipid droplets in cytoplasm, becoming adipocytes.</p> <p>These observations could be relevant in muscle regeneration therapies. Selection of satellite cells by their proliferative ability could have implication in their <i>in vivo</i> regeneration potential.</p>

PW28-353	<p><u>RESTORING CELL-BASAL LAMINA INTERACTION TO RESCUE TISSUE DEGENERATION IN CONGENITAL MUSCULAR DYSTROPHY</u> PORRELLO E¹, CAPOTONDO A², TRIOLO D¹, SAMPAOLESI M³, BRUNELLI S³, COMI G¹, RUEGG M⁴, COSSU G³, BIFFI A², QUATTRINI A¹, PREVITALI S¹ (1) Dept. of Neurology, S. Raffaele Scientific Institute, Milan, ITALY. (2) TIGET, S. Raffaele Scientific Institute, Milan, ITALY. (3) SCRI, S. Raffaele Scientific Institute, Milan, ITALY. (4) Biozentrum, University of Basel, Basel, SWITZERLAND.</p>
To contact the author:: previtali.stefano@hsr.it.	<p>Congenital Muscular Dystrophy (CMD) is characterized by progressive wasting muscular dystrophy and dysmyelinating neuropathy with variable involvement of the central nervous system, which may lead to severe disability in early childhood. The most frequent form is due to mutations of the LAMA2 gene encoding the laminin alpha2 chain, which forms merosin the predominant laminin isoform of muscle and nerve basement membrane. Although much is known about clinical aspects and genetic causes of CMD, and about the pathological mechanisms that lead to muscle and nerve degeneration, no useful therapy to arrest neuromuscular degeneration and to promote tissue repair is available to date. As a proof of principle the overexpression of laminin2 or mini-agrin, a cross-linker molecule that allows reconnection of the basement membrane to the resident cells, showed amelioration of CMD in animal models. However, at present direct viral transduction of exogenous proteins into human tissues is not feasible. Cell therapy may instead constitute a promising tool to speed translation into clinical practice. Mesoangioblasts have shown promising results in terms of amelioration of muscular dystrophy phenotype and reconstitution of missing proteins in pre-clinical experiments. We infected mesoangioblasts with lentivirus vectors carrying a mouse mini-agrin gene. Mesoangioblasts can synthesize and deliver mini-agrin in vitro and in vivo. We injected the engineered mesoangioblasts in the vein tail of CMD model, dy2J/dy2J mice. Mesoangioblasts carrying the mini-agrin gene were able to fuse into myotubes of dy2J mice, many of these myotubes expressed the mini-agrin protein, and these mice displayed amelioration of muscle histology and clinical phenotype.</p>

PW28-354	<p><u>RESIDENT CD34⁺/Ac133⁺ FETAL MUSCLE-DERIVED CELLS : LOCATION AND MYOGENIC POTENTIAL</u> AUDA-BOUCHER G¹, ROUAUD T¹, ROUGER K², FONTAINE-PÉRUS J¹, CHÉREL Y², GARDAHAUT MF¹ (1) CNRS UMR 6204 Fac Sciences, 44322 Nantes Cedex 3, FRANCE. (2) INRA UMR 703 Ecole Nat Vét, 44307 Nantes Cedex 3, FRANCE.</p>
To contact the author:: Gwenola.Boucher@univ-nantes.fr.	<p>We previously showed that CD34⁺ mouse fetal muscle-derived cells transplanted into <i>mdx</i> dystrophic mice efficiently regenerate skeletal muscle and improve its function. Following several reports on the existence of heterogeneity in CD34⁺ adult muscle-derived cells we attempted to identify the specific subset of fetal CD34⁺ population that possessed the highest myogenic potential. CD34⁺ fetal cells sorted by magnetic bead selection were characterized on the basis of their expression for several markers as Sca1, CD31, Ac133, CD45 using FACS analysis. CD31, Ac133 and Sca1 were expressed at different levels by CD34⁺ cells. In contrast the CD34⁺ cells were negative for CD45 indicating that the sorted CD34⁺ population comprised muscle resident cells. In fetal muscle sections CD34⁺/CD31⁺ cells were found in all vessel endothelium, whereas CD34⁺/Ac133⁺ cells were observed on endothelium of some vessels. Double staining for CD34⁺ and Sca1⁺ recognized cells lodged in connective tissue surrounding muscle fibers and muscle bundles. We then examined the myogenic potency of these three cell subsets (isolated from GFP mice) after transplantation into mice EDL muscle. Our results indicated that CD34⁺/CD31⁺ cells preferentially differentiated into endothelial cells while CD34⁺/Sca1⁺ cells were shown to have both endothelial and adipogenic potentials. If CD34⁺/Ac133⁺ subpopulation had a high tendency to differentiate into myogenic cells, nevertheless it displayed less efficient myogenic activity compared to the original whole CD34⁺ sorted population. These observations suggest that the other subpopulations identified into whole CD34⁺ fetal muscle-derived cell population facilitate myogenic differentiation of CD34⁺/Ac133⁺ fraction. <i>In vitro</i> and <i>in vivo</i> experiments are currently in progress to understand and define the conditions that promote the myogenic capacities of CD34⁺/Ac133⁺ fetal muscle-derived cells.</p>

PW28-355	<p><u>MESENCHYMAL-LIKE CELL POPULATIONS IN HUMAN SKELETAL MUSCLE.</u> LECOURT S¹, MAROLLEAU JP², FROMIGUÉ O³, VAUCHEZ K⁴, TERNAUX B⁵, LACASSAGNE MN⁵, ROBERT I⁵, PRAUD C¹, ANDRIAMANALJAONA R⁶, BOUMEDIENE K⁶, CHEREAU F⁴, MARIE P³, LARGHÉRO J⁵, FISZMAN M¹, VILQUIN JT¹ (1) Inserm, U582, Institut de Myologie, UPMC Univ Paris 06, UMR S582, IFR14, Paris, FRANCE. (2) CHU Amiens Hôpital Sud, Service Hématologie Clinique, UPJV, Amiens, FRANCE. (3) Inserm U606, Université Paris 7, Hôpital Lariboisière, Paris, FRANCE. (4) Genzyme SA, St-Germain en Laye, FRANCE. (5) Laboratoire de Thérapie Cellulaire, Hôpital Saint Louis, Paris, FRANCE. (6) Laboratoire de Biochimie des Tissus Conjonctifs, Faculté de Médecine, Caen, FRANCE.</p>
To contact the author:: jt.vilquin@institut- myologie.org.	<p><u>Purpose:</u> The comprehension of the human skeletal muscle development, homeostasis and physiopathology, and the set up of new therapeutic tools, mandate the cellular investigation of skeletal muscle compartments. <i>In situ</i>, we assessed the phenotype and localization of muscle cells on biopsy sections. <i>In vitro</i>, we analysed the main cell populations from the onset of cell culture, their potential relationships and phenotypical evolutions, and their differentiation abilities.</p> <p><u>Methods:</u> Immunohistochemistry was performed on human muscle cryostat sections. Primary cell cultures were established upon enzymatic dissociation. Immunophenotypic analyses were performed by flow cytometry and completed by immunocytochemistry. The two main cell populations were separated using immunomagnetic beads, and expanded. Differentiation studies were performed in vitro using specific media.</p> <p><u>Results:</u> Classical mesenchymal (MSC) markers and additional ones (CD 10, 13, 15, 29, 34, 44, 47, 49, 56, 62, 73, 90, 105, 106, 146) were expressed by resident muscle cells, which were classified according to their position relatively to muscle fibers, basement membranes, and intramuscular vessels. CD56+ satellite cells co-expressed rarely the CD90, CD146 or CD34 markers but no other MSC marker. In culture, two main populations were identified, separated, and distinguished by expression of CD56+ or CD15+ antigens, while a third population of CD34+ cells disappeared rapidly. When expanded, CD56+ and CD15+ cells expressed all MSC markers, similar to that harbored by human BM-MSC, while keeping heterogeneity. The CD56+ cells expressed myogenic and chondrogenic abilities and osteogenic markers, while CD15+ presented adipogenic and chondrogenic capacities, and osteogenic markers.</p> <p><u>Conclusions:</u> These data underline the diversity of cells participating to human muscle structures. They suggest that different populations express phenotypical markers typical of myogenic and/or MSC, and present mutually exclusive lineage restrictions.</p> <p>Supported by grants from Genostem and the AFM.</p>

PW28-356	<p><u>MUSCLE-DERIVED STEM CELLS ISOLATED AS NON-ADHERENT POPULATION GIVE RISE TO CARDIAC, SKELETAL MUSCLE AND NEURAL LINEAGES</u> ARSIC N¹, MAMAEVA D¹, LAMB N¹, FERNANDEZ A¹ (1) Institut de Genetique Humaine, CNRS, Montpellier, FRANCE.</p>
To contact the author:: af@acrux.igh.cnrs.fr.	<p>Stem cells with the ability to differentiate in specialized cell types can be extracted from a wide array of adult tissues including skeletal muscle. Here we have characterized a population of stem cells from skeletal muscle that can be reproducibly isolated and grown as a non-adherent, floating population. These cells express the stem cell surface markers Sca-1 and Bcrp-1. Although capable of growing as non-attached spheres for months, when given an appropriate matrix, these cells adhere and give rise to skeletal muscle, neurons and beating cardiac myocytes. Interestingly no similar cell population could be isolated from either bone marrow or cardiac tissue suggesting their specificity to skeletal muscle. When injected into damaged muscle, these muscle-derived floating stem cells are retrieved expressing Pax7, in a sublaminar position characterizing satellite cells and participate in forming new myofibers. These data show that a non adherent-stem cell population can be specifically isolated and expanded from skeletal muscle and this population spontaneously differentiates into muscle, cardiac and neuronal lineages in vitro and contributes significantly to the repair of injured muscle in vivo. These findings support the potential use of a similar muscle derived non-adherent cell population from human muscle in the treatment of neuromuscular disorders.</p> <p>(Key words: muscle; stem cells; multi-lineage differentiation: tissue regeneration)</p>

