PW 26:
Gene therapy
of muscular dystrophy

PW26-320	**UPGRADING U7SNRNA TO COMPLETE EFFICIENT RESCUE OF DYSTROPHIN BY EXON-SKIPPING IN DMD PATIENTS** GOYENVALLE A[1], BABBS A[1], GARCIA L[2], DAVIES KE[1] (1) Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UNITED-KINGDOM. (2) INSERM, S UMR 787, Groupe myologie, Paris, FRANCE.
To contact the author:: aurelie.goyenvalle@dpag.ox.ac.uk.	Most cases of Duchenne muscular dystrophy (DMD) are caused by mutations that disrupt the dystrophin mRNA reading frame. In many cases, skipping of a single exon could supposedly restore the reading frame, giving rise to a shorter but still functional quasi-dystrophin protein. It has previously been proposed to use small nuclear RNAs, especially U7snRNA, to shuttle antisense sequences designed to mask key elements involved in the splicing of targeted exons. Our present project focuses on the upgrading of U7snRNA to complete rescue of dystrophin by exon-skipping in DMD patients. We indeed developed bifunctional U7snRNAs carrying a complementary sequence to the targeted exon and a free tail harbouring canonical binding sites for the heterogeneous nuclear ribonucleoproteins A1/A2 (hnRNP) that are powerful splicing repressors. The presence of this generic strong silencer tail could indeed circumvent the always tricky and time-consuming specific optimization required for each new exon-target. We first focused on the exon 51 of the dystrophin gene and therefore designed new tailed U7snRNA constructs. Each construct has been inserted into lentiviral vectors for *in vitro* analysis on myoblasts from DMD patients. After transduction of these cells with lentiviral vectors encoding the tailed U7-ex51, we confirmed the skipping of the exon 51 by nested RT-PCR and dystrophin restoration by Western blot. By Comparison with their controls with a mutated tail, we could show that the skipping efficiency of these constructs was due to the tail carrying silencer motifs, therefore confirming its splicing repressor action, and not the annealing sequence to the exon. These very encouraging results on exon 51 provide evidence that bifunctional U7snRNA can achieve efficient exon-skipping in myoblasts from DMD patients. Since these tailed U7 could be very useful for the development of the exon-skipping strategy for the other dystrophin exons, the confirmation of their efficiency shown in this study offers very promising tools for clinical treatment of DMD.

PW26-321	**THE USE OF IMMORTALISED HUMAN FIBROBLASTS FROM A DMD PATIENT TO TEST EXON SKIPPING IN VIVO** CHAOUCH S[1], GOYENVALLE A[1], MAMCHAOUI K[1], ; BUTLER-BROWNE G[1], DI SANTO J[2], TORRENTE Y[3], MOULY V[1], GARCIA L[1], FURLING D[1] (1) UMR S 787 Groupe Myologie INSERM/UPMC-ParisVI, Institut de Myologie, Paris, FRANCE. (2) Cytokines and Lymphoid Development Unit, Immunology Department, Institut Pasteur, Paris, FRANCE. (3) Fondazione IRCCS Ospedale Maggiore Policlinico of Milan, Department of Neurological Sciences, Dino Ferrari Center, University of Milan, Milan, ITALY.
To contact the author:: chaouch@chups.jussieu.fr.	We are now at a point in time where gene therapy is becoming a reality. However, in order to validate these strategies, it is essential to have in vitro human cell culture models. For the study of neuro-muscular diseases, the use of patient myoblasts is not always possible due to their drastically decreased proliferative capacity induced by the repeated cycles of degeneration and regeneration. Therefore it is neccessary to envisage new *in vitro* models. In the pioneering studies of Weintraub *et al*, it had been shown that the forced expression of the myogenic transcription factor myoD was able to convert fibroblasts into myogenic cells. Based on this, we have developed a universal in vitro model from skin fibroblasts which have been immortalised using hTERT and then converted into myoblasts by a lentivirus containing an inducible myoD contruct. We have then used this model to validate a strategy for exon skipping using fibroblasts isolated from a DMD patient. These fibroblasts were immortalized and then transduced using an inducible myoD construct. We first confirmed the expression of myoD in vitro and the potential of these cells to form differentiated myotubes. These cells were then transfected with an U7 construct that promote exon skipping in the mutated dystrophin transcript. In order to test if these cells could reconstitue muscle fibres *in vivo* expressing human dystrophin, they were injected into cryodamaged TA muscles of immunodeficient RAG-/- gammaC-/- C5-/- mice. Muscle were analysed after 27 days of regeneration and fibres expressing human dystrophin were observed. Therefore this cellular model provides us with an alternative model system to test different therapeutic strategies for various neuromuscular diseases when patient myoblasts are not available.

PW26-322	**SYSTEMIC DELIVERY OF ANTISENSE OLIGONUCLEOTIDES RESTORES DYSTROPHIN EXPRESSION AND FUNCTIONALITY IN THE MDX MOUSE** VAN PUTTEN M[1], DE WINTER C[1], HEEMSKERK H[1], DE KIMPE S[2], VAN DEUTEKOM J[2], VAN OMMEN G-J[1], AARTSMA-RUS A[1] (1) Leiden University Medical Center, Leiden, THE NETHERLANDS. (2) Prosensa BV, Leiden, THE NETHERLANDS.
To contact the author:: m.van_putten@lumc.nl.	Duchenne Muscular Dystrophy (DMD) is a chromosome X-inherited disorder, characterised by the absence of the protein dystrophin which plays a role in the protection of muscle fibers against damage. Patients display progressive degeneration of skeletal muscles leading to functional impairment and premature death. One of the most promising therapeutic approaches for DMD involves the restoration of the disrupted reading frame through exon skipping with antisense oligonucleotides (AON). In cultured cells from patients and in the *mdx* mouse model AON treatment resulted in the generation of dystrophin proteins similar to those seen in Becker Muscular Dystrophy patients, who generally display a milder phenotype than Duchenne patients. Recently, we showed local restoration of dystrophin expression after intramuscular injection of an exon 51 specific 2'-O-methyl phosphorothioate AON in four Duchenne patients in a first-in-man clinical trial. For future clinical studies on full body treatment we are currently optimizing systemic AON delivery. We here compared different doses (25 mg/kg to 100 mg/kg) and treatment periods (4 to 8 weeks) with murine exon 23 specific AONs in *mdx* mice applying subcutaneous administration. The exon skip levels varied between specific muscle groups from 8% in the diaphragm to 26% in the extensor muscles of the lower forelimb and up to 2% in the heart, after twice weekly repeated injections with a 100 mg/kg AON dose during 8 weeks treatment. Dystrophin expression was observed in all samples analysed. This was accompanied by trends of decreased CK and improved rota-rod running times of treated mice compared to saline injected control mice. Markers for kidney and liver function remained unaffected, indicating that repeated AON treatment was well tolerated even when performed over longer time periods. Our results show that systemic injections of 2'-O-methyl phosphorothioate AON lead to restoration of dystrophin and an improved functionality without apparent toxicity.

PW26-323	**IN VIVO DELIVERY OF NAKED AND LIPID-COMPLEXED ANTISENSE OLIGOS IN MDX MICE: EFFECTS ON SKELETAL AND CARDIAC MUSCLE** PIGOZZO S[1], REPELE A[1], REGGIANI C[2], AUSONI S[3], ZAGLIA T[3], BARONI MD[1], AMBEGIA E[4], MCLACHLAN I[4], VITIELLO L[1] (1) Department of Biology, University of Padova, Padova, ITALY. (2) Department of Anatomy and Physiology, University of Padova, Padova, ITALY. (3) Department of Biomedical Sciences, University of Padova, Padova, ITALY. (4) Protiva Biotherapeutics Inc., Burnaby, CANADA.
To contact the author:: libero.vitiello@unipd.it.	Antisense-mediated exon skipping holds great potential for the treatment of DMD. In mdx mice, functional recovery of skeletal muscle has been reported upon systemic delivery of “naked” oligonucleotides or viral vectors encoding for antisense snRNAs. However, only one study achieved dystrophin restoration in cardiac muscle (using an adeno-associated vector). Here we report the in vivo delivery of morpholino oligos in aged mdx mice, both in skeletal muscle, via intra-arterial injection, and in cardiac muscle, via intramuscular injection. Intra-arterial delivery yielded levels of dystrophin restoration comparable to those reported in the literature with the intra-venous approach, but with smaller amounts of oligonucleotides. Intra-cardiac injections, on the other hand, showed that the level and duration of the skipping effect found in cardiac muscle were greatly decreased compared to skeletal muscle. This latter finding provides the first direct evidence that antisense-mediated dystrophin restoration in cardiac muscle might suffers from limitations that do not exist in skeletal muscle. All data published so far have indicated that systemic delivery via the vasculature requires large amount of naked oligos to achieve therapeutically significant results. Here we also report that the use of lipid carriers has the potential to greatly improve the delivery efficiency; in particular, we found that the use of lipid-encapsulated oligo RNA allowed to detect dystrophin re-expression with a single dose of ~40 µg of oligos per adult mdx mouse. Importantly, dystrophin restoration could be seen not only in skeletal and but also (albeit to a smaller extent) in cardiac muscle.

PW26-324	**NON-VIRAL VECTORS BASED ON AMPHIPHILIC POLYMERS: PARAMETERS INFLUENCING THEIR IN VIVO EFFICIENCY** ROQUES C[1], BOUCHEMAL K[2], SALMON A[1], FATTAL E[2], FROMES Y[1] (1) Institut de Myologie - INSERM U582, Paris, FRANCE. (2) UMR CNRS 8612 - Université Paris 11, Châtenay-Malabry, FRANCE.
To contact the author:: c.roques@institut-myologie.org.	Gene transfer is an interesting therapeutic approach for inherited muscular dystrophies with no curative treatment currently available. Nevertheless, DNA does not freely cross the membranes as it is a hydrophilic, negatively charge macromolecule, rendering thus formulation of nucleic acids a major concern. Synthetic vectors appear as a promising approach and particularly polymer based formulations. Among those, Polyethyleneimine (PEI), a polymer exhibiting a high density of positive charges and amphiphilic Tetronic 304 and Pluronic L64, displaying few or no charges, seem of particular interest to transfer DNA. Our work has focused on determining the influence of several formulation parameters on the organization of polymer/DNA systems. We have studied the correlation between these modifications and the toxicity and efficiency of the systems *in vivo*. Complexation of PEI with DNA is leading to small nanoparticles displaying relatively strong interactions. After *in vivo* administration, PEI/DNA complexes exhibited a high toxicity towards skeletal muscle. Amphiphilic polymers associated to DNA are generating more complex systems displaying weaker interactions. *In vivo*, no lesions were detected with amphiphilic polymers based formulations. Moreover, these formulations allowed significant improvement of gene transfer to the skeletal muscle with reference to naked DNA, even at low DNA doses. Our experiments highlighted the role of the medium and the temperature to optimize *in vivo* efficiency of Pluronic L64 vectors. Moreover, these findings could be correlated to modifications of the supramolecular organization of these systems depending on the conditions utilized. Our studies have emphasized the interest of amphiphilic polymers displaying few or no charges to transfer DNA in the skeletal muscle. The supramolecular organization of Pluronic L64 based formulations, as well as the interactions between polymer and DNA, is strongly dependent on the temperature and the medium used. These modifications have a direct impact on the *in vivo* efficiency of such vectors.

PW26-325

<u>CLINICAL DEVELOPMENT OF A GENE THERAPY PRODUCT FOR THE TREATMENT OF LIMB GIRDLE MUSCULAR DYSTROPHY TYPE 2C</u>

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In late November 2006, GENETHON initiated its first gene therapy clinical trial in gamma-sarcoglycanopathy (LGMD2C), a rare autosomal recessive muscular disorder caused by mutations in the gamma-sarcoglycan gene. Patients commonly present with proximal and progressive muscular weakness before the age of 10 and loose ambulance by age 12 on average. Cardiomyopathy and respiratory insufficiency may develop during the course of the disease, leading to poor prognosis and premature death.

Earlier during the clinical development, GENETHON had obtained an Orphan Drug Designation by EMEA. Approval from the French Agencies was obtained in November 2006 to initiate the phase I/IIa trial, which is being held at Pitié–Salpêtrière Hospital in Paris. The investigational product, a serotype 1 adeno-associated virus (AAV1) vector harboring the human gamma-sarcoglycan gene, is administered by a single intramuscular injection into the carpi radialis muscle.

The primary objective of this trial is to evaluate the clinical safety of local intramuscular injection of the gene therapy product. Secondary objectives are to monitor local and systemic immune responses, assess histological modifications and gene transfer into injected muscles. 9 patients, aged above 15, will be enrolled sequentially in the study, assigned to 3 cohorts with a single dose-escalation and followed-up for 6 months. Evaluation will address clinical, histological, biological, immunological and functional parameters as well as MRI.

As of today, four patients have been treated. The first cohort has been completed and the first patient of the second cohort has been treated. Encouraging data will be presented on the status of the product activity, immunologic response and patient compliance to the treatment.

As the clinical trial is halfway, a next phase IIb clinical trial is being planned, in which systemic administration will be performed. This implies important preclinical and clinical points to consider, as well as a series of preclinical studies to achieve.

PW26-327	**CORRECTION OF MUSCLE FUNCTION IN MYOTUBULAR MYOPATHY BY AAV-MEDIATED MTM1 REPLACEMENT** BUJ BELLO A[1], FOUGEROUSSE F[2], JAMET T[1], DURAND M[2], KRETZ C[1], DANOS O[2], DOUAR AM[2], MONTUS M[2], DENÈFLE P[2], MANDEL JL[1] (1) IGBMC, INSERM U596, CNRS UMR7104, ULP, Collège de France, Illkirch, FRANCE. (2) Généthon, Evry, FRANCE.
To contact the author:: abb@igbmc.u-strasbg.fr.	Myotubular myopathy (XLMTM) is a severe congenital muscular disease due to mutations in the myotubularin gene (*MTM1*) and characterized by the presence of small non-regenerative myofibres with frequent occurence of internalized nuclei. No specific treatment exists to date. Recombinant adeno-associated virus (rAAV) vectors appear as one of the most promising tools for gene therapy of muscular disorders. We have constructed rAAV vectors expressing myotubularin under either a ubiquitous (CMV) or a muscle-specific (desmin) promoter in order test their therapeutic potential in a faithful XLMTM mouse model. We show that a single intramuscular injection of either of these vectors in symptomatic *Mtm1*-deficient mice rescues the pathological phenotype. Myotubularin replacement corrects nuclei and mitochondria positioning in myofibres and leads to a strong increase in muscle volume. Importantly, the contractile force of mutant muscle becomes comparable to that of wild-type animals 4 weeks after rAAV transduction. This study provides a proof of principle that viral-mediated *Mtm1* gene delivery may be an effective therapeutic approach for patients with myotubular myopathy.

PW26-328

NO NEUROMUSCULAR DISEASE CLINICAL TRIAL WITHOUT GMP PHARMACEUTICAL CLINICAL LOT PRODUCTION.

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One way to design drugs for neuromuscular diseases goes through gene therapy products from Biodrugs list. Regarding the regulatory legislation, gene therapy products are considered as pharmaceutical products and their manufacturing requires compliance with Good Manufacturing Practices (GMP).

Manufacturing processes complexity in addition with genetically modified organisms manipulation entail for biodrugs production a pharmaceutical structure as well as a know-how that Genethon has implemented more than three years ago.
This structure named ETGC has obtained a GMP certification. It has been inspected by Afssaps and has received the authorisation to produce gene therapy vectors for phase I and II clinical trials. We are able to produce AAV, HIV and MLV vectors up to 50 litres scale. Our production capacity is 6 lots per year and per production suite.

From the 2200 m2 facility surface, approximately 800m2 are dedicated to clean rooms where clinical lots are produced. The other rooms are used for raw materials and media preparation, storage, quality control laboratory, quality-assurance and administration areas.
Facility qualification including materials and clean rooms must be done and annually validated, according to Q10 ICH guidelines.

Production processes development including upstream and downstream process, technological transfers to pharmaceutical GMP norms, aseptic filling validation, in process and final products controls are as many necessary steps that must be validated. .

Today Genethon has the expertise to produce, control and aseptic fill gene therapy products including AAV, HIV and MLV vectors.
We have produced different AAV vectors clinical lots which are already, or about to be, used in neuromuscular disease clinical trials (for example gamma-sarcoglycanopathy phase 1 trial or polymiositis disease).

Our capacity today is sufficient to meet the local intramuscular phase 1 clinical trial approach, but as we already have assessed, the systemic treatment for neuromuscular disease will require huge amounts of GMP products. We already have started, in collaboration with the bioprocess development department of Genethon, the industrial vectors production scale, for GMP vector production up to 300 litres.

PW26-329	**GENE THERAPY PRODUCTS MANUFACTURING: FRAME AND LIMITATION OF VIRAL CLEANING AND DECONTAMINATION** PAUTREL I[1], CRAPIE C[1], JACQUES D[1], SEQUESTRA N[1], NOGUIEZ-HELLIN P[1] (1) GENETHON-ETGC, EVRY, FRANCE.
To contact the author:: pautrel@genethon.fr.	Généthon's ETGC is working in 5 production rooms - dispatched on two sites - among which 3 are dedicated to viral vectors production for development of therapeutic products for phase I and II clinical trials. These productions are performed in compliance with GMP standards. One of the standards applies to cross contaminations control during manufacturing and recommends production in separate zones, implementation of air locks and air extraction device, protective clothing, use of manufacturing closed system, checking for absence of residues and cleaning as well as decontamination processes known for their efficacy. To prove the efficacy of cleaning or decontamination, attachment 15 of GMP standards recommends validation of disinfectants efficacy on facilities and equipments used at all different production steps. At ETGC, cross contamination control concerns viral vectors, like lentivirus and rAAV vector, produced in GMP zones. Validation of disinfectants virucidal activity must be validated in this context. According to NF72-180 norm, a virucidal activity is defined as follows: "a disinfectant virucidal activity must allow a decrease of at least 10^4 times the number of infectious units". Validation is performed on surface disinfection (liquid-liquid validation) and facilities air disinfection. Validation first step consists in selecting one or several marketed disinfectants with virucidal activity beyond the 4 logs recommended by norm. Among all marketed disinfectants, for liquid-liquid validation, 10 products have been tested and only 2 have a virucidal activity decreasing lentivirus and rAAV vector by 4 logs. For air disinfection, 10 products have been tested and only 2 may be efficient enough to be selected further to validation steps. Disinfection validations have shown a strong resistance of AAV vector to disinfectants (surface and air) usually used in laboratories. One of the most efficient disinfectants still is Javel water at 2,6% on a period of 30 minutes

PW26-330	**GMP GENE THERAPY VECTORS MANUFACTURING** FAUCHILLE S[1], ADIN P[1], BARNAY-TOUTAIN F[1], BURIE C[1], DUFOUR D[1], HALBOUT C[1], LE PROVOST G[1], MALEAU G[1], SANDER B[1], NOGUIEZ-HELLIN P[1] (1) GENETHON-ETGC, EVRY, FRANCE.
To contact the author:: sfauchille@genethon.fr.	Genethon has been provided with GMP manufacturing facilities of about 600 m^2 for the production of viral vectors for human gene therapy trials. All steps are performed on the site: plasmid production, cell banking, cell culture, purification and final filling. Genethon can assume a complete process for AAV, HIV and MLV viral vectors. Cell culture can be performed in flasks (up to 24 CF 10 cell factories) or bioreactors (up to 10 liters volume). The viral vectors are purified by ion exchange or affinity chromatographic steps. The batch size, depending on the process, is from 10^{13} to 10^{14} total physical particles. The process covers 3 to 5 weeks. The production site is operational since 2005. Genethon is producing clinical batches for four inner projects but also for outer customers, depending on the available production slots. Genethon's facilities are also designed for feasibility studies in pilot laboratories before GMP production. The production is performed on two independent sites: one is L2 classified and the other one L3. In 2010, new facilities will be built for Genethon and designed for large scale production and will be equipped with 300 liters bioreactors and 4 independent production suites. It will be located in Evry.

PW26-331

STRATEGY OF CONTROLS FOR GENE THERAPY CLINICAL BATCHES

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Généthon's aim is to find medicine for rare and heavy neuromuscular or immunological diseases. In the '90s, Généthon's adopted the Gene Therapy to find solutions for these diseases.
In this purpose, the E.T.G.C. from Généthon (Cell and Gene Therapy Company) was created in 2005 for the production and control of viral vector batches from AAV, HIV or MLV. These batches are dedicated to Phase 1 for human clinical trials. The AFSSAPS (French regulatory for Health products authorisation) accredited the ETGC to produce these batches following restrictive quality rules.
In consequence, each step of the production is controlled:
Raw material involved into the production process was elected in regards to quality standards (respect to European Pharmacopeia for example), its traceability, and agreement of each subcontractor to a quality charter written by Généthon. Each subcontractor is then audited every two years to verify the Charter regulation engagements. Raw material is also controlled a second time at the E.T.G.C. by operators. These operators are qualified to work according to the GMPs recommendations: Identity testing is then performed on chemical raw materials whereas sterility, endotoxin and viability on culture media.
In process control consisting in Physical and Infectious Particles quantification is done to calculate the yield of the production.
On the final product, at least 15 tests are performed: Identity testing (sequencing, gel migration), purity (SDS-Page, residual or total proteins quantification) and functionality testing are realised in the QC team. Security viral testing (as human adventitious viruses, viral contaminants, replicative viruses, infectious genomes quantification…) are however done by qualified subcontractors.

PW26-332	**EFFICIENT IN VIVO DYSFERLIN EXPRESSION BY DUAL AAV VECTORS RECONSTRUCTION.** LOSTAL W[1], BOURG N[1], ROUDAUT C[1], MIYAKE K[2], MCNEIL P[2], BARTOLI M[1], RICHARD I[1] (1) GENETHON, EVRY, FRANCE. (2) Medical College of Georgia, Augusta, USA.
To contact the author:: lostal@genethon.fr.	Dysferlinopathies are recessive muscular disorders caused by defects in dysferlin. Genetic mutations are responsible for two major phenotypes: Limb Girdle Muscular Dystrophy type 2B and Distal Miyoshi Myopathy. These skeletal muscle diseases are characterized by progressive loss of muscle integrity and strength. Recently, dysferlin was demonstrated to be involved in membrane repair process, providing a preliminary understanding of the pathophysiological mechanism in these diseases. Currently, no treatment is available. Considering the recessive nature of dysferlinopathies, a possible therapeutic strategy is gene transfer. To date, the best vector for gene transfer in muscle is Adeno Associated Virus (AAV). However, the human dysferlin cDNA size approximates 7 kb, preventing its direct incorporation into a single AAV vector, since the encapsidation limit is around 4.7 kb. In order to bypass this limitation, we set up a strategy taking advantage of the concatemerization ability of AAV vectors. The dysferlin cDNA was separated into two AAV2/1 vectors, one carrying a muscle specific promoter followed by the 5' half of dysferlin cDNA and a 5' splicing signal and another one carrying a 3' splicing signal, the remaining dysferlin sequence and a polyadenylation signal. We demonstrated the ability of these vectors to produce a human full-length dysferlin mRNA in a cellular model. To test this approach *in vivo,* intramuscular injection of both vectors was performed into dysferlin deficient mice. Dysferlin mRNA was correctly spliced, expressed at a level close to the endogenous one and was stable for an entire year. At the protein level, dysferlin was detected at 237 kDa, the size of the expected full-length protein. The functionality of dysferlin was demonstrated by a membrane repair assay based on 2-photon injury on isolated muscle fibers. Taken together, all these data prove that gene transfer based on AAV concatemerization allows the expression of a full-length functional dysferlin.

PW26-333	**CHARACTERIZATION OF THE IMMUNE RESPONSES TO AAV2/1 VECTORS FOLLOWING INTRAVENOUS INJECTION IN MICE.** SUDRES M[1], GALY A[1] (1) Immunology and Gene Therapy Group - INSERM U790- Genethon, Evry, FRANCE.
To contact the author:: galy@genethon.fr.	Clinical gene therapy studies have demonstrated the immunogenicity of AAV vectors since neutralizing antibodies and cytolytic T-cell responses have been elicited against the capsid, in some patients. For further clinical development, the immunogenicity of AAV vectors should be better understood in preclinical models. We are interested in rAAV2/1 vectors which are promising tools for gene transfer into skeletal muscle. We evaluated the innate and adaptive immune responses following a bolus intravenous (IV) administration in mice. Innate immune responses triggered by AAV vectors are thought to be weak, yet hepatic inflammatory reactions were described in mice following IV administration (Zaiss et al., J. Virol 2002). In our hands, the IV injection of 10^{11} vg endotoxin-free AAV2/1 vector did not trigger a detectable cytokine response. On the contrary, research-grade AAV2/1 preparations induced rapid but transient increases in TNFa, IL6, CCL5 and CXCL10 mRNAs, presumably an effect of the LPS contained in these preparations. Endotoxin-free AAV2/1 vectors triggered strong and neutralizing humoral responses initiated with IgM followed by IgG conversion peaking respectively at 1 and 2.5 weeks. A dose-dependent increase in IgG2a and IgG3 levels was observed with increasing amounts (10^{10}, 10^{11} or 10^{12} vg) of vector whereas IgG2b were high at all doses and little IgG1 was detected. We found no evidence of $CD4^+$ or $CD8^+$ T-cell priming based on proliferation or activation markers. In conclusion, a bolus IV administration of rAAV2/1 vector in mice induces a strong humoral immune response against the capsid but little to no inflammation or cellular immunity. However, antibody isotype profiles suggest an underlying IFNg or TGFb-mediated T cell response. Our current prospects are (i) to further characterize the cellular immune response to the AAV1 capsid using reporter epitopes (ii) to assess the pro-inflammatory potential of AAV1 vector through activation of TLRs or others pathway of inflammasome.