

Class I-restricted T-cell responses to a polymorphic peptide in a gene therapy clinical trial for α -1-antitrypsin deficiency

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Adeno-associated virus (AAV)-mediated gene therapy is currently being pursued as a treatment for the monogenic disorder α -1-antitrypsin (AAT) deficiency. Results from phase I and II studies have shown relatively stable and dose-dependent increases in transgene-derived wild-type AAT after local intramuscular vector administration. In this report we describe the appearance of transgene-specific T-cell responses in two subjects that were part of the phase II trial. The patient with the more robust T-cell response, which was associated with a reduction in transgene expression, was characterized more thoroughly in this study. We learned that the AAT-specific T cells in this patient were cytolytic in phenotype, mapped to a peptide in the endogenous mutant AAT protein that contained a common polymorphism not incorporated into the transgene, and were restricted by a rare HLA class I C alleles present only in this patient. These human studies illustrate the genetic influence of the endogenous gene and HLA haplotype on the outcome of gene therapy.

polymorphism | α -1-antitrypsin | gene therapy | adeno-associated virus | immune response

Recombinant adeno-associated virus (AAV)-mediated gene therapy holds great promise for the treatment of multiple diseases (1–8). Preclinical mouse studies have demonstrated sustained correction with a relative paucity of transgene-specific immunity. Even when present, a potent cytotoxic T-cell (CTL) response was, by itself, insufficient to eliminate transgene expression without an accompanying inflammatory response (9, 10). However, successful transgene expression in small animal models does not always translate to nonhuman primate models and humans. For example, nonhuman primates injected i.v. with an AAV vector expressing GFP elicited a transgene-specific T-cell response that extinguished transgene expression and caused transient transaminitis, which was not seen in mice injected with a similar vector (11). In a hemophilia B trial, an increase in hepatic transaminases with a concomitant decrease in transgene expression was observed in four patients systemically administered with an AAV vector (12, 13). These and other observations have led to the hypothesis that capsid and transgene immunity may compromise the outcomes of AAV gene therapy clinical trials.

AAV gene therapy is currently being pursued as a treatment for the monogenic disorder α -1-antitrypsin (AAT) deficiency. Results from phase I and II studies have shown a dose-dependent increase in wild-type AAT (m-AAT) after local i.m. administration of AAV1 vectors (14–18). In these studies, AAV capsid-specific CD4⁺ and CD8⁺ T cells with a cytolytic phenotype were detected in blood following AAV administration. Interestingly, m-AAT transgene expression was stable in most patients and was associated with a regulatory T-cell response characterized by increased CD4⁺ CD25⁺FOXP3⁺ cells at the site of vector administration (18). However, T-cell responses to the AAT transgene were observed in

a more limited number of patients who participated in the phase II clinical trial (17).

Here, we describe and characterize the AAT-specific immune responses found in two trial subjects. Our findings demonstrate the previously underappreciated role of coding sequence polymorphisms and HLA genotypes that in one patient triggered a class I-restricted immune response to the AAT transgene following gene transfer.

Results

The T-Cell Response to AAT Is Multifunctional and Is Mediated by CD8⁺ T Cells. The phase II clinical trial consisted of nine subjects stratified into three dose cohorts, 6e11, 1.9e12, and 6e12 viral genomes/kg, who were administered AAV1-AAT i.m. Analysis of AAV1 capsid and AAT transgene immunity by IFN- γ enzyme-linked immunospot (ELISPOT) assays indicated that all nine subjects developed AAV1 capsid-specific T cells (17), but only two subjects (305 and 401) developed AAT transgene-specific T cells (Fig. 1*A* and *B*). None of the subjects had detectable AAV1- or AAT-specific T cells in blood before vector administration,

Significance

The use of adeno-associated virus (AAV)-mediated gene therapy to treat monogenic disorders is currently being tested in phase I, II, and III clinical trials. An immune response to the newly introduced gene product remains a potential problem because of the patient's lack of central and peripheral tolerance to foreign epitopes. In this study, we show the induction of a T-cell response to the therapeutic product in a α -1-antitrypsin (AAT)-deficient subject receiving AAV1-AAT treatment. The response was directed to an AAT epitope upstream of the mutation and was associated with a polymorphism present in the subject but not in the wild-type AAT therapeutic product. Our study highlights the importance of considering polymorphisms in the targeted population when designing a transgene.

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Conflict of interest statement: J.M.W. is an advisor to REGENXBIO, Dimension Therapeutics, and Solid Gene Therapy and is a founder of, holds equity in, and has a sponsored research agreement with REGENXBIO and Dimension Therapeutics; in addition, he is a consultant to several biopharmaceutical companies and is an inventor on patents licensed to various biopharmaceutical companies. T.R.F. is a founder of Applied Genetic Technologies Corporation, is scientist advisor to and has equity in Dimension Therapeutics, and is a scientific advisor to Editas Medicine.

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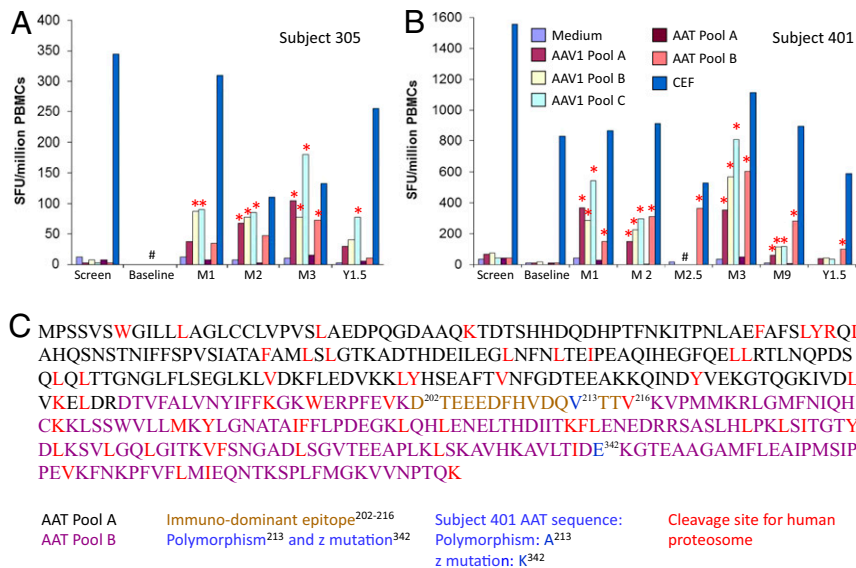


Fig. 1. Time course of immune response and epitope location. The time course of the T-cell response to AAV1 capsid and AAT transgene in PBMCs from subjects 305 (A) and 401 (B) was analyzed in duplicate by IFN- γ ELISPOT assay using peptide libraries specific for the full-length AAV1 capsid (pools A and B) and the AAT transgene (pools A and B). The CEF peptide pool was used at each time point as a positive stimulation control. M, month; SFU, spot-forming units; Y, year. Asterisks indicate a positive response; the hashtag indicates that no sample analysis was available. (C) Full amino acid sequence of the AAT transgene indicating peptides included in pool A (black) and B (purple), the location of immunodominant epitopes (brown), the location of polymorphisms and z-AAT mutations (blue), and C-terminal cleavage sites for human proteasome (red). Superscript numbers indicate the amino acid position of the mature AAT protein.

suggesting that these were primary responses. Nonetheless, we cannot rule out the presence of AAV1- or AAT-specific memory T cells in lymphoid organs before vector administration and the induction of an anamnestic response. Of the two subjects with anti-AAT immunity, subject 305 had a delayed T-cell response to AAT peptide pool B 3 mo after vector administration. However, the low magnitude of the response precluded further analysis of this subject.

In contrast, subject 401, who is homozygous for the z-AAT mutation (Glu342Lys), had a sustained and persistent T-cell response to peptide pool B of AAT. The T-cell response increased soon after vector administration, peaked at 3 mo (Fig. 1B), and was detected for more than 1 y. Transgene expression was lower in this subject than in the other two subjects who received the same dose of vector (17), as were vector genomes from muscle biopsies (18). AAT expression before vector administration was 20–30 nM, and the levels were similar to those observed in other subjects within the same cohort (17). Deep sequencing of peripheral blood mononuclear cells (PBMCs) isolated before and 9 mo after gene therapy revealed an expansion of the T-cell repertoire, although the antigen specificity of the expanded T-cell subsets could not be identified by this analysis (SI Text and Fig. S1, and Table S1). Epitope mapping with overlapping peptide subpools at 2.5 and 3 mo identified a positive ELISPOT response against peptide DTEEEDFHVDQVTTV (17). This epitope, hereafter called “peptide 46,” was located at position 202–216 of the mature protein and was flanked by proteasome cleavage sites identified by *in silico* analysis using the NetChop server (Fig. 1C) (19). The epitope is distant from the z-AAT mutation, which is located at position 342 of the AAT protein.

We investigated whether the immune activation of subject 401 to wild-type AAT could be the result of a previously identified Val213Ala polymorphism found in a minority of wild-type AAT alleles and in most z-AAT alleles (20). DNA sequencing revealed that subject 401, who was homozygous for z-AAT, was also homozygous for the Val213Ala polymorphism that resides in the portion of the AAT protein that spans the immunodominant peptide 46 from subject 401 (Fig. 1C and Fig. S2). The existence of the polymorphism in the immunodominant peptide suggests but does not prove that it played a role.

Next, we evaluated the robustness of the AAT immune response in subject 401 by measuring T-cell polyfunctionality (i.e., the ability to secrete or activate multiple immune modulators). Activated CD8⁺ T cells secreted multiple cytokines/chemokines, e.g., TNF, IFN- γ , IL-2, and macrophage inflammatory protein 1 α (MIP-1 α), and the degranulation marker CD107a, which is a surrogate for cytotoxic activity (Fig. 2A). T-cell responses to peptide 46 were dominated primarily by IFN- γ - and TNF-secreting CD8⁺ T cells, and more than half of the activated CD8⁺ T cells were polyfunctional, coexpressing at least three of the five evaluated factors (Fig. 2B, pie charts). Further phenotypic characterization of peptide 46-stimulated CD8⁺ T cells showed an abundance of cells with the effector T-cell (Teff) phenotype (CD27⁻CD45RO⁻) (Fig. 2B, density plots). The skewing of the immune response to a Teff phenotype suggests ongoing antigen exposure, as is consistent with the low but persistent m-AAT expression in blood and in muscle biopsies (17, 18).

The activation profiles of CD4⁺ T cells were more distinct following stimulation with the AAT peptide library, peptide pool B, or peptide 46. CD4⁺ T-cell response to the AAT peptide library was dominated by the coexpression of IFN- γ and IL-2 in more than half of the CD4⁺ T cells (Fig. 2C). In contrast, the response to pool B was significantly lower and was dominated by cells coexpressing IL-2 and TNF. In both instances, the CD4⁺ T-cell response was mediated by central memory (Tcm) (CD27⁺CD45RO⁺) and effector memory (Tem) (CD27⁻CD45RO⁺) T cells (Fig. 2D). CD4⁺ T-cell responses were absent in cells stimulated with peptide 46. Overall, these data indicate that the response to AAT immunodominant epitope peptide 46 is dominated by effector CD8⁺ T cells with a cytotoxic profile.

We also detected a T-cell response to AAV capsid in subject 401, and we compared this response with two other trial subjects (306 and 302) who did not develop AAT immune responses. These studies indicated that capsid T-cell responses are multifunctional and diverse (SI Text and Fig. S3).

The Importance of HLA Restriction on the Presentation of the Immunodominant AAT Epitope. The observation that only one trial subject developed a robust T-cell response, although all are

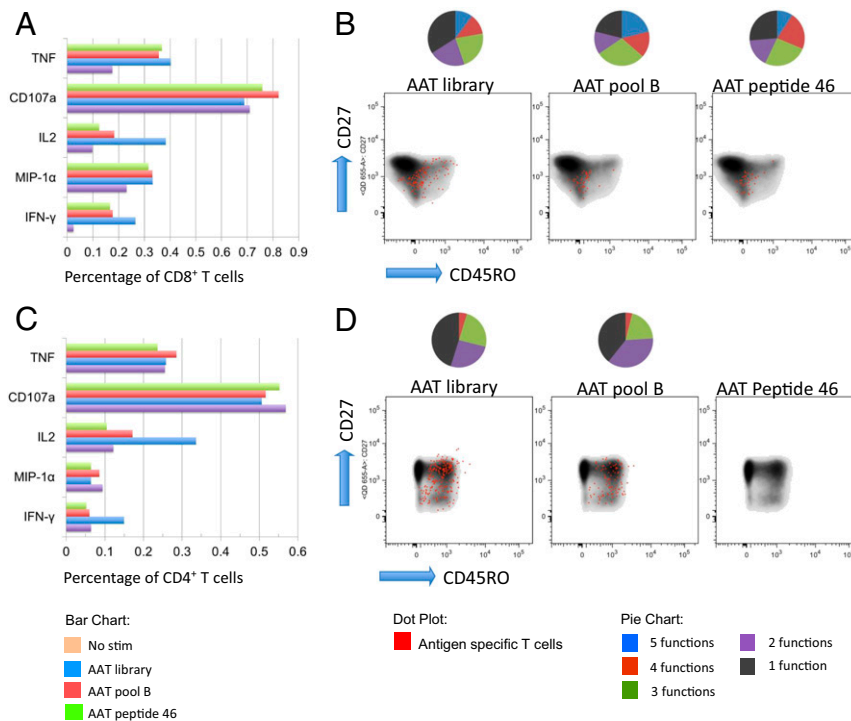


Fig. 2. Functional and phenotypic analysis of the AAT transgene-specific T-cell response in PBMCs from subject 401. (A and C) Functional profile of cytokine (IFN- γ , TNF, and IL-2), chemokine (MIP-1 α), and surface expression of a degranulation factor (CD107a) by CD8⁺ (A) and CD4⁺ (C) T cells from PBMCs obtained at the 2.5-mo time point following stimulation with AAT peptide library, AAT pool B, or AAT immunodominant peptide 46 DTEEDFHVDQVTTV. (B and D) Memory phenotype characterization of AAT-specific CD8⁺ (B) and CD4⁺ (D) T cells. Naive cells were defined as CD27⁺CD45RO⁻, Tcm cells were defined as CD27⁺CD45RO⁺, Tem cells were defined as CD27⁻CD45RO⁺, and Teff cells were defined as CD27⁻CD45RO⁻. Pie charts represent the total AAT-specific T-cell response. Each slice indicates the proportion of cells that produce one to five cytokines as indicated by different colors.

expected to carry the Val213Ala polymorphism (20), suggests a possible HLA allele restriction. To identify the allele(s) most likely responsible for presenting the immunodominant epitope, we HLA-typed all trial subjects and compared the haplotype of subject 401 with that of the subjects who did not activate T cells (Table 1). We were looking for a rare HLA allele that segregated with the development of AAT-specific T cells. In this analysis, any HLA allele that was found in a subject who did not mount an AAT-specific T-cell response would be precluded from being responsible for presenting the immunodominant epitope in subject 401. Of the HLA alleles present in subject 401 (HLA-A*02/03, HLA-B*35/44, and HLA-C*04/05), HLA-C*04/05 uniquely segregated with the T-cell response in subject 401 (Table 1). Furthermore, we performed a computational HLA class I affinity prediction for 8- to 12-mer peptides spanning the polymorphic site for patient 401's HLA genotype. This analysis revealed a single 8-mer peptide (HVDQVTTV) predicted to bind with high affinity to HLA-C*05:01; however, no peptides were predicted to bind with high affinity to HLA-A or -B alleles (Tables S2 and S3). This result suggests that the polymorphic site generates a high-affinity neoepitope in patients carrying the HLA-C*05:01 allele.

To test HLA restriction of the dominant AAT epitope, we devised a strategy using an ex vivo antigen-presentation model. Individual HLA alleles were transfected into K562 cells, a human myelogenous cell line lacking endogenous HLA expression, and these cells subsequently were used to activate subject 401's AAT-specific T cells (Fig. 3). Next, transiently transfected cells were immune-stained to monitor cell-surface class I expression. As expected, HLA staining was absent in K562 cells transfected with a sham construct, whereas strong cell-surface staining was observed in more than 50% of K562 cells transiently transfected with HLA-A*02, HLA-C*04, or HLA-C*05 (Fig. 3). Next, peptide-loaded transfected

cells were used as antigen-presenting cells (APCs) by coculturing with PBMCs from subject 401. HLA-C*05 K562 cells activated subject 401's AAT-specific T cells threefold over unloaded targets (Fig. 3, Upper Right). Activation was lower when target cells were transfected with HLA-A*02. Although subject 401 carried two unique HLA-C alleles, C*04-transfected cells did not activate T cells and were similar to control mock-transfected cells.

We observed a similar T-cell response when we used our ex vivo approach for antigen presentation (Fig. 3, Upper Right) or when we used professional APCs (Fig. 3, Lower Left); as expected, the magnitude of the response was lower with nonprofessional APCs.

Table 1. HLA haplotyping of the subjects enrolled in the clinical trial

Subject	HLA class I		
	A	B	C
301	02, 29	18, 44	07, 16
302	01, 31	18, 57	06, 07
303	11, 30	13, 13	06, 06
304	03, 29	44, 51	15, 16
305	02, 02	35, 50	06, 15
401	02, 03	35, 44	04, 05
306	01, 02	07, 07	07, 07
307	02, 03	08, 45	06, 07
308	NA	NA	NA

NA, sample not available for HLA typing. Numbers indicate the two alleles for A, B, and C loci.

We conclude that the apparent reduction in transgene expression that developed in subject 401 in this trial was the result of a class I-restricted T-cell response to a peptide in AAT. This T-cell response and its consequence were limited to a single patient who happened to have a rare HLA-C allele that efficiently presented a peptide from the transgene-derived protein. We suspect that a polymorphism in the immunodominant peptide of both z-AAT alleles of subject 401 resulted in a lack of central tolerance that, combined with vector-induced inflammation, resulted in a robust T-cell response to vector-transduced cells. This study shows that HLA genotype and polymorphism play a substantial role in gene therapy and should be considered in the investigation of transgene expression in clinical trials. We speculate that class I restriction may influence the substantial patient-to-patient variability that has been seen in some of the AAV gene therapy hemophilia trials.

Methods

Clinical Trial. This study was conducted under the Food and Drug Administration's Investigational New Drug program with approval from the institutional review boards and biosafety committees of the University of Massachusetts Medical School and Cincinnati Children's Hospital. All procedures and protocols were in accordance with the tenets of the declaration of Helsinki. This study is registered with ClinicalTrials.gov (NCT01054339). All subjects provided written informed consent.

IFN- γ ELISPOT Assay. We analyzed IFN- γ released in PBMCs using previously described methods (1). PBMCs were stimulated in duplicate with medium alone, AAV1, or AAT peptide libraries. The CEF peptide pool [comprising 32 MHC class I-restricted T-cell epitopes (8–12 amino acids in length) that contain sequences derived from the human cytomegalovirus, Epstein-Barr virus, and influenza virus] (Mabtech) served as a positive control, as previously described (25). Positive ELISPOT counts were defined as >55 spot-forming units per million lymphocytes, i.e., values three times over background (25).

Peptide Libraries. We synthesized a peptide library tailored for the full lengths of the AAT and the AAV1 capsid as 15-mers with 10-aa overlap (Mimotopes). The AAT peptide library was divided into two pools, A and B, and the AAV virion peptide 1 (VP1) peptide library was divided into three pools, A, B, and C, as previously described (14).

Intracellular Cytokine Staining. PBMCs were stimulated with the AAV1 or AAT peptide libraries or with individual immunodominant AAT peptide 46 (DTEEDFHVQVTTV). Reactive T cells were analyzed functionally and

phenotypically using previously described techniques (1). T-cell polyfunctionality was assessed by simultaneous detection of secreted TNF, CD107a, IL-2, MIP-1 α , and IFN- γ . Cellular phenotypes were evaluated by the expression of the surface markers (CD3, CD4, CD8, CD27, and CD45RO). Naive T cells were defined as CD27⁺CD45RO⁻, Tcm cells were defined as CD27⁺CD45RO⁺, Tem cells were defined as CD27⁻CD45RO⁺, and Teff cells were defined as CD27⁻CD45RO⁻.

T-Cell Receptor β Chain Deep-Sequencing Analysis. Deep-sequencing analysis of the T-cell receptor β (TCRB) chain was performed using patient PBMCs to detect all possible rearranged genomic sequences and to determine T-cell receptor variable region b (TCR-Vb) use for each clone (Adaptive Biotechnologies) as described earlier (18).

HLA Haplotypes Analysis. HLA analysis was performed by LabCorp in eight of nine subjects enrolled in the trial. Samples for HLA typing were not available from subject 308.

AAT Polymorphism Analysis. DNA was extracted from eight 10- μ m paraffin-embedded muscle tissue sections using a QIAamp DNA formalin-fixed, paraffin-embedded tissue kit per the manufacturer's instructions (Qiagen). A 545-bp product was amplified and sequenced (Genewiz) using primers (forward: ttggatggtcagtttcagca and reverse: agagggtcaggagggtgaa) flanking exon 3 (26).

Cell Lines and Transfection. K562 cells were a gift from Carl June, University of Pennsylvania, Philadelphia, PA. Cells were transiently transfected using Lipofectamine reagent (Invitrogen). We used indirect immunofluorescence to image transiently transfected K562 cells expressing individual human HLA using an anti-human MHC class I antibody. Images were collected using a Nikon inverted microscope.

Computational HLA Class I Affinity Analysis. To identify peptides predicted to bind MHC class I with high affinity, a sliding window of 8- to 12-mer peptides centered on the amino acid of interest were generated and ranked for binding using the Immune Epitope Database and Analysis Resource (27). Peptides with an IC₅₀ corresponding to a percentile rank of less than one against a standardized database were classified as high affinity.

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- Maguire AM, et al. (2008) Safety and efficacy of gene transfer for Leber's congenital amaurosis. *N Engl J Med* 358(21):2240–2248.
- Hauswirth WW, et al. (2008) Treatment of leber congenital amaurosis due to RPE65 mutations by ocular subretinal injection of adeno-associated virus gene vector: Short-term results of a phase I trial. *Hum Gene Ther* 19(10):979–990.
- Cideciyan AV, et al. (2008) Human gene therapy for RPE65 isomerase deficiency activates the retinoid cycle of vision but with slow rod kinetics. *Proc Natl Acad Sci USA* 105(39):15112–15117.
- Eberling JL, et al. (2008) Results from a phase I safety trial of hAADC gene therapy for Parkinson disease. *Neurology* 70(21):1980–1983.
- Muramatsu S, et al. (2010) A phase I study of aromatic L-amino acid decarboxylase gene therapy for Parkinson's disease. *Mol Ther* 18(9):1731–1735.
- Nathwani AC, et al. (2011) Adenovirus-associated virus vector-mediated gene transfer in hemophilia B. *N Engl J Med* 365(25):2357–2365.
- LeWitt PA, et al. (2011) AAV2-GAD gene therapy for advanced Parkinson's disease: A double-blind, sham-surgery controlled, randomised trial. *Lancet Neurol* 10(4):309–319.
- Stroes ES, et al. (2008) Intramuscular administration of AAV1-lipoprotein lipase S447X lowers triglycerides in lipoprotein lipase-deficient patients. *Arterioscler Thromb Vasc Biol* 28(12):2303–2304.
- Mays LE, Wilson JM (2011) The complex and evolving story of T cell activation to AAV vector-encoded transgene products. *Mol Ther* 19(1):16–27.
- Faust SM, et al. (2013) CpG-depleted adeno-associated virus vectors evade immune detection. *J Clin Invest* 123(7):2994–3001.
- Gao G, et al. (2009) Adeno-associated virus-mediated gene transfer to nonhuman primate liver can elicit destructive transgene-specific T cell responses. *Hum Gene Ther* 20(9):930–942.
- Nathwani AC, et al. (2014) Long-term safety and efficacy of factor IX gene therapy in hemophilia B. *N Engl J Med* 371(21):1994–2004.
- Manno CS, et al. (2006) Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. *Nat Med* 12(3):342–347.
- Brantly ML, et al. (2009) Sustained transgene expression despite T lymphocyte responses in a clinical trial of rAAV1-AAT gene therapy. *Proc Natl Acad Sci USA* 106(38):16363–16368.
- Brantly ML, et al. (2006) Phase I trial of intramuscular injection of a recombinant adeno-associated virus serotype 2 alpha-antitrypsin (AAT) vector in AAT-deficient adults. *Hum Gene Ther* 17(12):1177–1186.
- Flotte TR, et al. (2004) Phase I trial of intramuscular injection of a recombinant adeno-associated virus alpha 1-antitrypsin (rAAV2-CB-hAAT) gene vector to AAT-deficient adults. *Hum Gene Ther* 15(1):93–128.
- Flotte TR, et al. (2011) Phase 2 clinical trial of a recombinant adeno-associated viral vector expressing α 1-antitrypsin: Interim results. *Hum Gene Ther* 22(10):1239–1247.
- Mueller C, et al. (2013) Human Treg responses allow sustained recombinant adeno-associated virus-mediated transgene expression. *J Clin Invest* 123(12):5310–5318.
- Nielsen M, Lundegaard C, Lund O, Kesmir C (2005) The role of the proteasome in generating cytotoxic T-cell epitopes: Insights obtained from improved predictions of proteasomal cleavage. *Immunogenetics* 57(1-2):33–41.
- Nukiwa T, et al. (1986) Identification of a second mutation in the protein-coding sequence of the Z type alpha 1-antitrypsin gene. *J Biol Chem* 261(34):15989–15994.
- Herson S, et al. (2012) A phase I trial of adeno-associated virus serotype 1- γ -sarcoglycan gene therapy for limb girdle muscular dystrophy type 2C. *Brain* 135(Pt 2):483–492.
- Mendell JR, et al. (2010) Sustained alpha-sarcoglycan gene expression after gene transfer in limb-girdle muscular dystrophy, type 2D. *Ann Neurol* 68(5):629–638.
- Hudrisier D, Mazarguil H, Laval F, Oldstone MB, Gairin JE (1996) Binding of viral antigens to major histocompatibility complex class I H-2Db molecules is controlled by dominant negative elements at peptide non-anchor residues. Implications for peptide selection and presentation. *J Biol Chem* 271(30):17829–17836.
- Mingozzi F, High KA (2013) Immune responses to AAV vectors: Overcoming barriers to successful gene therapy. *Blood* 122(1):23–36.
- Calcedo R, et al. (2013) Self-reactive CFTR T cells in humans: Implications for gene therapy. *Hum Gene Ther Clin Dev* 24(3):108–115.
- Takahashi H, Crystal RG (1990) Alpha 1-antitrypsin Null(isola di procida): An alpha 1-antitrypsin deficiency allele caused by deletion of all alpha 1-antitrypsin coding exons. *Am J Hum Genet* 47(3):403–413.
- Kim Y, et al. (2012) Immune epitope database analysis resource. *Nucleic Acid Res* 40(Web Server issue):W525–530.