Late-Breaking Abstracts I

1. Virotherapy for Diffuse Intrinsic Pontine Glioma: Results from a Phase I Clinical Trial with DNX-2401

Marc Garcia-Moure[1], Jaime Gallego Perez-Larraya[1], Ana Patiño-Garcia[1], Sara Labiano[1], Jasper van der Lugt[2], Jessica Dobbs[3], Joan Robbins[4], Ricardo Diez-Valle[5], Fred F. Lang[6], Candelaria Gomez-Manzano[7], Juan Fueyo[7], Sonia Tejada-Solis[5], **Marta M. Alonso**[1]
[1]Pediatrics, University Hospital of Navarra, Pamplona, Spain,[2]Pediatrics, Princess Maxima, Utrech, Netherlands,[3]DNATrix, SanDiego, CA,[4]DNatrix, SanDiego, CA,[5]Neurosurgery, Fundación Jimenez Díaz, Madrid, Spain,[6]Neurosurgery, MD Anderson Cancer Center, Houston, TX,[7]NeuroOncology, MD Anderson Cancer Center, Houston, TX

Pediatric high-risk brain tumors remain the leading cause of cancer-related death in children. For the last 30 years, numerous treatment approaches for the most aggressive types of pediatric brain tumors have failed to improve survival, leaving the 5-year survival rate at approximately 0%. Diffuse intrinsic pontine glioma (DIPG) is the most aggressive pediatric brain tumor. Median overall survival (OS) with radiation therapy (RT) is approximately 10-11 months and survival at 2 years is <10%. Thus, it is clear that new therapeutic strategies are needed to more effectively treat these tumors. Oncolytic viruses designed to selectively replicate in and destroy tumor cells represent a promising therapeutic strategy that could improve the outcome of this malignancy. A Phase 1, single-center study was conducted to evaluate the oncolytic adenovirus, DNX-2401 (tasadenoturev), followed by RT in patients with DIPG. Newly-diagnosed patients 1-18 years old received a tumor biopsy followed by intratumoral injection of DNX-2401 via cannula and conventional RT 1-3 weeks later. Subjects were enrolled (n=12) from December 2017 to January 2020 and had a median age of 9 (range 3-18) and Lansky/Karnofsky performance scores of 90-100 (n=4; 33%) or 70-80 (n=8; 67%). Genetic assessment was completed for 11 subjects (92%) and histone H3 mutations were identified in 10 subjects, including H3.3 (n=8), H3.2 (n=1), and H3.1 (n=1); 1 subject was H3 wildtype (n=1). p53 mutations were identified in 5 of 11 subjects. DNX-2401 was administered in a dose-escalation manner (1e10 vp (n=4) or 5e10 vp (n=8)), followed by RT (11 of 12; 92%). No dose-limiting toxicities were observed and the treatment regimen was well-tolerated. The most commonly reported AEs (> 5 subjects), regardless of study drug relationship, include asthenia, headache, vomiting, pyrexia, and neurological deterioration. Three SAEs were reported, including grade 3 abdominal pain, grade 3 lymphopenia, and grade 3 clinical deterioration. Tumor reductions were reported for 9 subjects (75%), including 2 confirmed (17%) and 2 unconfirmed (17%) partial responses per RAPNO criteria. As of the data cutoff, median OS is 17.8 months, and OS-24 is 25%, with follow-up ongoing for 3 subjects (33.5, 31.4, 19.6 months). Median OS for subjects with an H3.3 mutation (n=8) is 21.2 months. The immune cell composition of the biopsies was assessed using multiplexed quantitative immunofluorescence. T cells were barely detectable in these tumors, while macrophages were abundant. We detected increased clonal T cell diversity following treatment with the virus in peripheral blood lymphocytes when paired pre- and post-treatment samples from the trial were compared Additionally, we measure pre- and post-treatment neutralizing antibodies and their relationship with survival. DNX-2401 followed by RT can be safely administered to pediatric patients with newly diagnosed DIPG. These encouraging data support the translation of oncolytic viruses for high-risk pediatric brian tumors.

2. Successful Prenatal Delivery of a Therapeutic Antisense Oligonucleotide for Treatment of Angelman Syndrome in Mice

Maria T. Clarke[1], Lea Lentz[1], Laura Remesal Gomez[1], Georgia Kirn[1], Jasmine Wu[1], Antonia Varthaliti[1], Danielle Tan[2], Stephan J. Sanders[1], Scott Dindot[3], Saul Villeda[1], Tippi C. MacKenzie[1]
[1]UCSF, San Francisco, CA,[2]BioMarin, Marin, CA,[3]Texas A&M, College Station, TX

Introduction: Angelman Syndrome (AS) is a rare early onset neurodevelopmental disorder for which there is currently no cure. AS is caused by the absence of a functional maternally-inherited allele of the imprinted gene *UBE3A*. In neurons, the paternal allele is silenced by a long non-coding RNA, *UBE3A* antisense transcript (*UBE3A-AS*). Degradation of the *UBE3A-AS* transcript by an ASO can activate the intact paternal *UBE3A* allele, leading to genetic rescue of AS. We hypothesized that prenatal delivery of the ASO would result in activation of the paternal *UBE3A* allele in a reporter model, as well as phenotypic improvement in an AS mouse model. *Methods:* We bred wild-type (WT) females to *Ube3a-YFP* males and injected fetuses with a therapeutic ASO via two routes: intra-cerebroventricular (ICV, 14.5) or intra-amniotic (IA, E13.5). Pups were analyzed at P14 and P35 using qPCR to measure *Ube3a-AS* mRNA, miRNA scope for ASO distribution, and *Ube3a-YFP* IF to detect paternal allele expression. After confirming successful delivery, experiments were repeated in AS mice, breeding $AS^{-/-}$ females to WT males such that progeny would be maternal *Ube3a* deficient but have an intact silenced paternal allele. Behavioral analyses were performed on P35 mice alongside analysis of *Ube3a-AS* mRNA and UBE3A protein. *Results*: Both ICV and IA injections were well tolerated, with IA injection allowing a higher dose compared to ICV (Fig. 1A). ICV injection led to decreased *Ube3a-AS* mRNA levels in the cortex (Fig. 1B) and widespread ASO distribution in the brain by miRNA scope and *Ube3a-YFP* IF, particularly in cortical layers III and VI (Fig. 1C). Strikingly, IA injection also resulted in widespread ASO distribution with activation of paternal *Ube3a* (Fig. 1C). Available behavioral data in ICV injected AS mice suggest improvement of cognitive and motor abilities on behavioral tests such as marble burying (Fig. 2A) and accelerating rotarod (Fig. 2B-C). Conclusion: Prenatal injection of ASO into the brain results in reactivation of paternal *Ube3a* in cortical neurons, with improvement of the AS phenotype. Similarly widespread distribution in neurons after amniotic fluid injection supports the potential of a minimally invasive route for prenatal therapy with implications for numerous neurodevelopmental disorders.

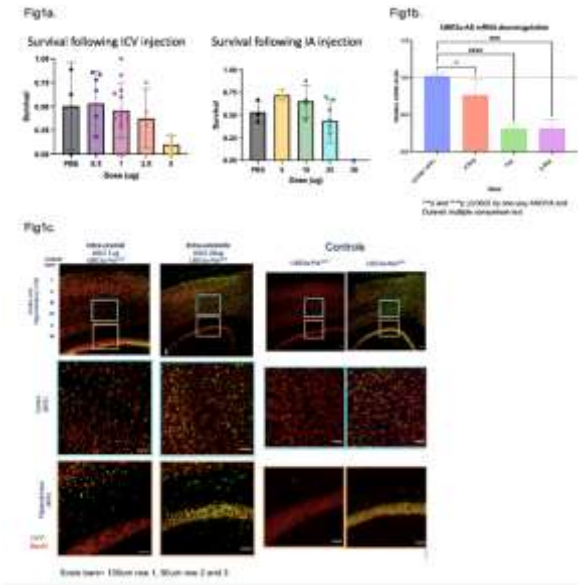


Figure 1: 1a. Survival of in utero injection, each dot=one pregnancy. 1b. *Ube3a-AS* mRNA levels after ICV delivery. 1c. Paternal *Ube3a-YFP* reactivation after ICV delivery (column 1), IA delivery (column 2), or PBS injection (column 3). Maternal YFP (column 4) positive control. Brains stained with neuronal marker NeuN (red) and GFP (green) to detect the endogenous YFP expression.

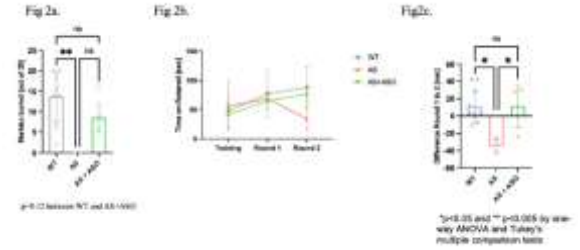
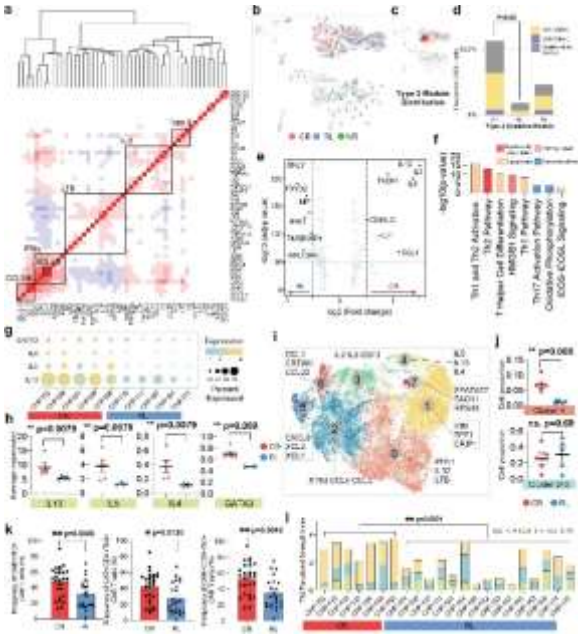


Figure 2: Behavioral testing in wild type (WT) and Angelman syndrome (AS) mice 6 weeks after in utero injection of ASO. 2a. Marble burying, quantified as number of marbles buried out of 20. 2b. Time spent on accelerating rotarod. 2c. Change in time on rotarod between round 1 and round 2.

3. Single-cell Antigen-specific Activation Profile of CAR-T Infusion Product Identifies Th2 Deficiency in CD19-Positive Relapsed ALL Patients

Zhiliang Bai
Yale University, New Haven, CT

The remissions in a significant fraction of Chimeric antigen receptor-modified (CAR) T treated subjects are short-lived and 30-60% of patients relapse within one year. Although antigen loss could explain a majority of CD19-negative relapse, the mechanisms of CD19-positive relapse remain elusive. We hypothesized that the functional capacity of CAR T cells in the infusion product could be an essential factor determining long-term therapeutic response. Herein we present 101,326 single cell transcriptomic landscape from the CAR T infusion products of 12 pediatric ALL patients upon CAR antigen-specific stimulation. Ten responders were subdivided into those who had a very durable complete remission (>54 months, CR) and those who had a CD19-positive relapse during the trial (RL, median relapse-free remission duration = 9.6 months). Two patients did not show an objective response to the therapy (NR). We performed an unsupervised analysis to identify modules of co-expressed cytokines (Fig. 1a). In the landscape of responsive states, CAR+ cells from CR, RL, and NR patients were localized in the representation (Fig. 1b), suggesting a distinct cytokine module expression profile for these cells. Notably, the Th2 module was found to be enriched in a region containing mostly cells from CR patients (Fig. 1c), implying that Th2 function might be indispensable for maintaining a long-term remission in CAR T therapy. A quantitative comparison identified a significant depletion of CAR+ cells expressing the Th2 module in RL compared to CR patients (Fig. 1d), whereas other functional modules remained comparable (data not shown). We performed differential expression analyses between CR and RL and found the up-regulation of genes associated with Th2 helper related cytokine production, such as IL4, IL5 and IL13, in CR patients (Fig. 1e). Other immune pathways like Th1 cytokine production, T helper differentiation, and ICOS-ICOSL signaling were comparable between the two groups (Fig. 1f). The assessment of IL13, IL5, IL4 and GATA3 expressions in each of the patient showed uniform enrichment of the four genes in CR compared with RL subjects (Fig. 1g,h). Clustering analysis identified 8 transcriptionally distinct subpopulations, and CAR T cells in cluster 4 mainly functioned as Th2 helpers (Fig. 1i). The cell proportion of cluster 4 was significantly elevated in CR patients and no fraction difference was observed for the combination of clusters 2+3 (Fig. 1j), suggesting that the lack of Th2 function rather than Th1 response could induce CD19-positive relapse. We performed an independent functional validation of these findings in two cohorts comprising 49 patients by means of intracellular flow cytometry and a multiplexed secretomic assay. The combined proportions of IL-4+, IL-5+, and IL-13+ CAR T cells (Th2+) were significantly higher in CR group, with consistent discrimination observed in CD4+ or CD8+ subpopulation (Fig. 1k). In the multiplexed secretomic data set, we used the functional strength index (FSI) to describe the specific functionality profile of CAR T cells, which was defined as the frequency of cells secreting a cytokine multiplied by the average signal intensity of the cytokine. We found a significantly higher Th2 FSI in CR patients as compared with RL patients (Fig. 1l). In aggregate, these findings suggest a potential strategy to improve the therapeutic outcome by re-engineering the CAR T product with augmented type-2 signaling or by boosting Th2 functions post infusion to maintain long term remission.



4. MOSAIC Enables In Situ Saturation Mutagenesis of Genes and Optimization of CRISPR Prime Editor Activities in Human Cells

Jonathan Y. Hsu[1,2,3,4], Kin Chung Lam[2,3,4], **Justine Y. Shih**[2,3,4], Luca Pinello[2,3,4], Keith Joung[2,3,4]

[1]Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA,[2]Molecular Pathology Unit, Massachusetts General Hospital, Charlestown, MA,[3]Center for Cancer Research and Center for Computational and Integrative Biology, Massachusetts General Hospital, Charlestown, MA,[4]Department of Pathology, Harvard Medical School, Boston, MA

CRISPR prime editing enables tremendous versatility and precision for creating a broad range of genetic edits in human cells. Here we describe the development of the Multiplexing Of Site-specific Alterations for In situ Characterization (MOSAIC) method, a non-viral PCR-based strategy that enables the rapid installation of thousands of defined prime editing-mediated genetic edits in pooled fashion. Using MOSAIC, we were able to rapidly and easily perform pooled in situ saturation mutagenesis screens of the BCR-ABL1 gene translocation and the IRF1 untranslated region (UTR), re-confirming known imatinib drug-resistance coding sequence mutations in BCR-ABL1 and non-coding regulatory elements in the IRF1 UTR. Furthermore, we also leveraged MOSAIC to enable high-throughput, pooled screening of hundreds of prime editing guide RNA (pegRNA) designs for any given targeted modification of interest. Using MOSAIC, we screened more than 18,000 pegRNA designs and identified optimized pegRNAs for 89 different genomic target modifications. The results of these screens reveal the lack of simple rules for pegRNA design and demonstrate the requirement to perform experimental optimization, a process that is now greatly simplified and can be practiced by any scientist using MOSAIC. In sum, MOSAIC provides an important and novel technology that should facilitate the deployment of CRISPR prime editing for pooled screens and optimize pegRNAs for a wide variety of different research and therapeutic applications.

5. Genome Editing of Human Hematopoietic Stem Cells to Induce Fetal Hemoglobin for Autologous Cellular Therapy of Sickle Cell Disease

Varun Katta[1], Kiera O'Keefe[1], Racheal Wood[1], Cicera R. Lazzarotto[1], Thiyagaraj Mayuranathan[1], Jonathan Yen[1], GaHyun Lee[1], Yichao Li[1], Naoya Uchida[2], Shondra M. Pruett-Miller[3], John Tisdale[2], Akshay Sharma[4], Mitchell J. Weiss[1], Shengdar Q. Tsai[1]

[1]Hematology, St Jude Children's Research Hospital, Memphis, TN,[2]Molecular and Clinical Hematology Branch, National Heart, Lung and Blood Institute, Bethesda, MD,[3]Cell and Molecular Biology, St Jude Children's Research Hospital, Memphis, TN,[4]Bone Marrow Transplantation and Cellular Therapy, St Jude Children's Research Hospital, Memphis, TN

Sickle cell disease (SCD) is a severe genetic blood disorder that affects millions of individuals worldwide. Individuals that co-inherit homozygous sickle mutations in *HBB* and natural genetic variants that cause hereditary persistence of fetal hemoglobin are typically asymptomatic. Thus, genome editing strategies to re-activate developmentally silenced fetal hemoglobin (HbF, $\alpha 2\gamma 2$) represent promising clinical approaches to replace defective sickle hemoglobin (HbS, $\alpha 2\beta^S 2$). Recent reports from an early-stage clinical trial targeting BCL11A erythroid specific enhancer to induce fetal hemoglobin have been encouraging.

To identify which target when edited in the γ-globin gene promoters would elicit the most robust HbF induction, we compared editing of BCL11A (-115) and ZBTB7A (-198) binding motifs. We electroporated Cas9-3xNLS ribonucleoprotein complexes (RNPs) into human primary $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) and attained high editing rates ranging from 76.2% to 85.7%. 17 weeks after transplanting edited $CD34^+$ cells into immunodeficient *NBSGW* mice, we observed consistent and high indel mutations frequencies of 63.5% to 92.7% in all hematopoietic lineages with no apparent impairment of multilineage differentiation. We observed associated higher levels of HbF induction in erythroid progeny of $CD34^+$ cells edited at the -115 γ-globin promoter target (31.8%) than those edited at the -198 ZBTB7A binding site (13%-18%).

To further test our -115 γ-globin promoter editing approach in SCD patient cells, we edited plerixafor-mobilized $CD34^+$ HSPCs from one healthy donor and three individuals with SCD. 17 weeks after transplanting edited cells into *NBSGW* mice, we observed consistently high rates of editing (75.1%) in all hematopoietic lineages, and substantial levels of HbF (27.6%) in erythroid progeny compared to controls (<2.9%). Single cell western showed expression of gamma globin in a majority of edited erythroblasts (58%) compared to unedited controls (<6%). Moreover, RBCs derived from edited $CD34^+$ cells from SCD patients exhibited significant three-fold reduction in hypoxia-induced sickling.

We characterized potential genotoxicities associated with our -115 γ-globin promoter editing approach. With CHANGE-seq discovery and rhAMP-seq validation, we did not detect off-target mutations above background at 194 identified sites. PacBio-Hifi long-range sequencing revealed that the only high-frequency large deletion observed (4.9 kb) results from simultaneous double strand beaks in HBG1 and HBG2 promoters. By digital droplet PCR, we detected an average of 23.9% of this 4.9 kb deletion in engrafted cells at 17 weeks that has no apparent impact on erythropoiesis and HbF induction.

To support plans for a future clinical trial, we have advanced our efforts to manufacture of GMP-grade Cas9 and optimize editing at clinical-scale using the Maxcyte electroporation system. In summary, our preclinical results suggest that disruption of the -115 BCL11A repressor binding site in the γ-globin gene promoters can

induce therapeutically effective levels of HbF and is a promising approach for genomic SCD therapy.

6. A Collaborative Analysis by Clinical Trial Sponsors and Academic Experts of Anti-transgene SAEs in Studies of Gene Therapy for DMD

Carsten G. Bonnemann[1], Beth A. Belluscio[2], Serge Braun[3], Carl Morris[4], Teji Singh[5], Francesco Muntoni[6]
[1]Neuromuscular and Neurogenetic Disorders of Childhood Section, NINDS, NIH, Bethesda, MD,[2]Pfizer, New York, NY,[3]Genethon, Evry-Courcouronnes, France,[4]Solid Biosciences, Cambridge, MA,[5]Sarepta Therapeutics, Cambridge, MA,[6]Great Ormond Street Institute of Child Health, University College London, London, United Kingdom

Currently four sponsors have ongoing clinical trials to evaluate the safety and/or efficacy of investigational gene therapies for the treatment of Duchenne muscular dystrophy (DMD). All approaches use an adeno-associated virus, albeit of different serotypes, to deliver various versions of a shortened dystrophin transgene driven by different promotors. Recently, serious adverse events (SAEs) characterized by muscle weakness with variable cardiac involvement occurred in five patients, across three trials, with a strikingly similar clinical presentation and time course. Following the events, all four sponsors chose to collaborate and share relevant clinical and laboratory data and further convened an international panel of experts to analyze the SAEs, minimize their recurrence, and assess potential therapeutic and preventative strategies.

The SAEs observed exhibited generally consistent clinical presentations including extremity and bulbar muscle weakness, occurring approximately 3-7 weeks following investigational gene therapy infusion. Other findings were noted in some individual patients including severe respiratory muscle compromise and increased cardiac troponin-I levels. Following various immunosuppressive and supportive therapies, muscle strength improved, and cardiac enzyme levels normalized over 6-8 weeks after onset.

Given that similar events were observed across multiple investigational gene therapy products with different capsids, promoters, and transgene sequences, they are most likely to be a specific transgene/genotype-related 'class effect.' The hypothetical mechanism is thought to involve a T-cell mediated immune response to the expressed transgene protein in a cross-reactive immunological material (CRIM)-negative setting, determined by the patient's genotype. Supportive evidence includes: 1) SAEs only occurred in patients with genomic deletions including N-terminal epitopes which are present in the transgene protein, 2) when positive ELISpot (T-cell) tests were recorded in patients with SAEs, they were reactive specifically to the corresponding N-terminal peptide pool, and 3) preliminary epitope mapping of anti-dystrophin antibodies from one patient suggests a prominent signal at the transgene Hinge1 segment within the N-terminus of dystrophin.

The unique and timely formation of an open, collaborative working group including four sponsors of the ongoing studies and multiple academic experts was instrumental in being able to quickly identify an anti-transgene mechanism and the associated risk factors for observed SAEs. A plan for further investigation is underway to comprehensively define the immune mechanism and associated risk factors. This collaborative approach and its conclusions may have implications to mitigate risks in gene therapy development programs beyond DMD.

7. Long-term Follow-up of Subjects With Diabetes 2 Type Treatment with *ex vivo* Gene Therapy

Svitlana Gramatiuk[1], Julia Ivanova[2]
[1]Ukraine Association of Biobank, Kharkiv, Ukraine,[2]Kharkiv National Medical University, Kharkiv, Ukraine

Metabolic syndrome and finally diabetes 2 type (DT2) as a result of progressive obesity, insulin resistance, abnormal cholesterol or triglyceride levels are newfound problems in the current endocrinology. As reported by the International Diabetes Federation, in the entire world 382 million of adults (8.3%) are living with diabetes; the number is estimated to increase to 592 million in the next 20 years. Human of peripheral blood mesenchymal stem (MSCs) represent promising stem cell therapy for the treatment of type 2 diabetes mellitus (DT2), but the results of autologous auto-MSC administration in DT2 patients are contradictory. The purpose of this study was to test the hypothesis that autologous MSC administration in DT2 patient is safe and that the efficacy of the treatment is dependent on the quality of the autologous MSC population and administration routes and is to focus on mitochondrial dysfunction. Materials and methods Mesenchymal stem cell separated of peripheral blood from diabetes 2 type patients was collected in the context of a clinical protocol authorized by the local Ethics Committee of Ukraine Association of Biobank (Ukraine), with a license from the Ministry of Health of Ukraine 04/10/2018 №1813 and 27/03/2019 №1231 by the national competent authority for biobank cord blood, cell and, tissue therapy. The study population (n = 96) was represented by diabetic patients from SI «ZIGUS NAMSU» in Kharkiv, Ukraine, and healthy volunteers. DT2 patients were enrolled, randomly assigned (1treated patients, and the efficacy was evaluated based on the absolute changes in the hemoglobin A1c, fasting blood glucose, and C-poated in 30 DT2 patients. Patients were divided into two groups: group I consisted of patients with diabetes 2 type (DT2), group II - patients with DT2 complicated course of NASH (DT2 + NASH). The control group consisted of 25 conditionally healthy persons (men and women) of the same age. Conclusion: DT2 duration directly altered the proliferation rate of auto-MSCs, abrogated the glycolysis and mitochondria respiration of MSCs, and induced the accumulation of mitochondria DNA mutation. In the modern scientific space, various directions have been proposed in the diagnosis of metabolic syndrome and the treatment of D2T.

Late-Breaking Abstracts II

8. Results of One Year Follow-Up After Treatment With Fordadistrogene Movaparvovec (PF-06939926) for Duchenne Muscular Dystrophy (DMD) in A Phase 1b, Open-label Study

Russell J. Butterfield[1], Perry B. Shieh[2], Florence Yong[3], Michael Binks[3], Tara G. McDonnell[3], Kelly A. Ryan[3], Marielle Delnomdedieu[3], Beth A. Belluscio[3], Srividya Neelakantan[3], Daniel

I. Levy[3], Pamela F. Schwartz[3], Edward C. Smith[4]
[1]University of Utah School of Medicine, Salt Lake City, UT,[2]University of California at Los Angeles (UCLA), Los Angeles, CA,[3]Pfizer Inc, New York, NY,[4]Duke University School of Medicine, Durham, NC

Background: Fordadistrogene movaparvovec (PF-06939926) is an adeno-associated virus serotype-9 (AAV9) gene-replacement construct containing a truncated dystrophin transgene (mini-dystrophin), which aims to restore functional protein to cardiac and skeletal muscle. We present 1 year data from ambulatory participants in a phase 1b, multicenter, single-arm, open-label trial (NCT03362502). **Methods:** Ambulatory boys with a genetic diagnosis of DMD and receiving a stable, daily glucocorticoid regimen were eligible. Fordadistrogene movaparvovec was administered as a single intravenous infusion, at low- or high-dose. Mini-dystrophin expression and distribution in biceps biopsies were analyzed by liquid chromatography-mass spectrometry (LCMS) and immunofluorescence (IF), respectively. Functional endpoints included change from baseline in the North Star Ambulatory Assessment (NSAA) and other measures of motor and respiratory function. **Results:** Nineteen ambulatory boys received fordadistrogene movaparvovec (n=3 low-dose; n=16 high-dose). Median age at gene therapy infusion was 8.8 yrs (range: 6.2-13.0 yrs); median baseline NSAA total score was 27 (range 17-32). Three treatment-related serious adverse events occurred (dehydration, acute kidney injury, thrombocytopenia) as previously reported; all resolved within 15 days. For participants in the high-dose group, mean dystrophin/mini-dystrophin levels were 22% and 40% of normal at 2 and 12 months, respectively (measured via LCMS). Dystrophin-positive fibers were 39% and 62% at 2 and 12 months, respectively (measured via IF). A consistent trend towards improved function was seen at 1-year following treatment with fordadistrogene movaparvovec compared with the decline observed in the external control cohort (placebo trial participants of similar age, weight, baseline function, stable steroid use) (**Table**). **Conclusion:** Preliminary results indicate that fordadistrogene movaparvovec has an acceptable safety profile in this population, provides for substantial expression of mini-dystrophin that increases (on average) between 2 and 12 months, and has the potential to benefit ambulatory DMD patients across a range of functions.

Table: Change from baseline (CFB) to 1-year in functional assessments following treatment with fordadistrogene movaparvovec, or for placebo participants in the External Control cohort, derived with similar age, weight, baseline function, and stable steroid use

CFB to 1-year	Fordadistrogene movaparvovec (N=19)*	External Control (N = 66)*	P-value comparison 1-year
NSAA total score			
Median	+1	–4.0	<0.005
Mean ± SD	+0.9 ± 4.0	–4.4 ± 5.3	
6MWD			
Median	–1	–23.0	0.08
Mean ± SD	+7.8 ± 63.2	–20.5 ± 59.7	
4SC velocity (1/s)			
Median	–0.02	–0.06	0.008
Mean ± SD	+0.02 ± 0.1	–0.05 ± 0.1	
%pFVC			
Median	+6.0	–1.4	0.14
Mean ± SD	+6.4 ± 16.1	–3.8 ± 22.7	

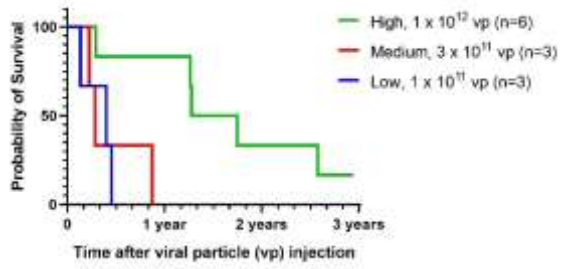
*Final n may vary depending on number of participants with data available at 1-year.
P-value based on Wilcoxon test
4SC vel, 4-stair climb velocity; 6MWD, 6-min walking distance; %pFVC, percent predicted forced vital capacity; NSAA, North Star Ambulatory Assessment.

9. Replication Competent Adenovirus-mediated cytotoxic and Interleukin-12 Gene Therapy in Stage IV Pancreatic Cancer: 36 Month Follow-Up Data from a Phase I Clinical Trial

Shyam Nyati[1], Farzan Siddiqui[1], Kenneth Barton[1], Robert Pompa[2], Svend O. Freytag[1], Gazala Khan[3], Irina Dobrosotskaya[3], Munther Ajlouni[1], Yingshu Zhang[1], Jingfang Cheng[1], Stephen Brown[1], Benjamin Movsas[1], David Kwon[4]
[1]Radiation Oncology, Henry Ford Health System, Detroit, MI,[2]Gastroenterology, Henry Ford Health System, Detroit, MI,[3]Oncology, Henry Ford Health System, Detroit, MI,[4]Henry Ford Pancreatic Cancer Center, Henry Ford Health System, Detroit, MI

Introduction: Pancreatic cancer (PC) is the fourth leading cause of death from cancer in the United States. Metastatic PC (mPC) has a median survival of less than 9 months. The tumor immune microenvironment (TIME) of mPC is marked by an immunosuppressive network which contributes to immunotherapy resistance. There is an urgent clinical need to develop novel therapeutic approaches that improve survival for mPC patients. We conducted a phase I clinical trial in mPC using replication-competent (RC) Ad5-yCD/mutTK$_{SR39}$rep-hIL12 adenovirus in combination with chemotherapy. The primary endpoints of this dose escalation study were maximum tolerated dose (MTD) and dose limiting toxicities (DLTs) at Day 21, secondary endpoint was rates of grade 3 CTCAE and exploratory endpoints were viral distribution, level of immunological cytokines and clinical outcomes. Overall survival was not a study end point. **Method:** This was a single site, nonrandomized, dose-escalation phase 1 trial of a replication competent adenovirus harboring two suicide genes (HSV-TK, yCD) and an IL-12 expression cassette for treatment of metastatic pancreatic cancer

patients (men and women older than 18 yrs). Patients were enrolled between October 2017 and May 2019. Each subject received a single endoscopic ultrasound guided intratumoral injection of the IL-12 adenovirus. The three patient cohorts received either $1X10^{11}$ virus particles, vp (N=3), $3X10^{11}$ vp (N=3) or $1X10^{12}$ vp (N=6). Two days later, subjects were administered 7 days of 5-fluorocytosine (150 mg/kg/day, orally) prodrug therapy. Fourteen days after completion of the 5-FC prodrug therapy course, subjects initiated chemotherapy at the discretion of the treating physician. Toxicity, viral load, immunological cytokines, and immune cell activation was measured up to day 21. Overall survival analysis was conducted on March 9th, 2022. One patient is still alive 35.7 months after adenoviral injection and chemotherapy. **Results and conclusion:** This trial demonstrated that Ad5-yCD/mutTK$_{SR39}$rep-hIL12 adenovirus was safe and well tolerated by mPC patients. Moreover, intratumoral injection of this virus showed viral load in several patients for up to 2 weeks post-injection, led to increased serum cytokines (IL12, IFNγ, and CXCL10), and increased CD4+, CD8+ T-cells indicative of immune system activation. MTD was not reached in the study. Additionally, this non-randomized, phase I trial demonstrated that higher doses ($1X10^{12}$ vp, cohort 3) of the Ad5-yCD/mutTK$_{SR39}$rep-hIL12 gene therapy virus provided a clinically meaningful median OS benefit of 18.4 months (Figure 1) compared with 4.2 months for patients receiving low doses ($1X10^{11}$ and $3X10^{11}$vp, cohorts 1 and 2) of the adenovirus. Log-rank (Mantel-Cox) test P values were 0.01 while Logrank test for trend were 0.009 (Figure 1). To our knowledge this is the first phase-1 trial which indicated median OS ~18 months for mPC patients. Our future plans include conducting a Phase II trial in the same patient population to establish efficacy of this therapeutic approach.



10. Interim Safety, Biomarker, and Efficacy Data From Imagine-1: A Phase 1/2 Open-label, Multicenter Study to Assess the Safety, Tolerability, and Efficacy of a Single Dose, ICM Administration of PBGM01 in Subjects with Type I (Early Onset) and Type IIa (Late Onset) Infantile GM1 Gangliosidosis (GM1)

David A. Weinstein[1], Caroline A. Hastings[2], Debra-Lynn Day-Salvatore[3], Can Ficicioglu[4], Chester B. Whitley[5], Michal Inbar-Feigenberg[6], Geneviève Bernard[7,8], Roberto Giugliani[9], Julien Baruteau[10], Fatih S. Ezgü[11], Jeanine R. Jarnes[5], Yan G. Ni[1], Pruthvi Nagilla[1], Victoria L. Ballard[1], Thomas F. Haws[1], Michael H. Gelb[12], Mark S. Forman[1]

[1]Passage Bio, Inc., Philadelphia, PA,[2]UCSF Benioff Children's Hospital, Oakland, CA,[3]Saint Peter's University Hospital, New Brunswick, NJ,[4]Children's Hospital of Philadelphia, Philadelphia, PA,[5]University of Minnesota, Minneapolis, MN,[6]The Hospital for Sick Children (SickKids), Toronto, ON, Canada,[7]McGill University, Montreal, QC, Canada,[8]McGill University Health Centre, Montreal, QC, Canada,[9]Federal University of Rio Grande do Sul, Porto Alegre, Brazil,[10]Great Ormond Street Hospital for Children, London, United Kingdom,[11]Gazi University, Gazi, Turkey,[12]University of Washington, Seattle, WA

Introduction: GM1 is a neurodegenerative autosomal recessive disorder resulting from mutations in the human galactosidase beta 1 gene (*GLB1*), which encodes beta-galactosidase (β-gal). Currently, no disease-modifying treatments exist. Methods: A first-in-human, global interventional, multicenter, single-arm, dose-escalation, adaptive design clinical trial of PBGM01 (NCT04713475 [ClinicalTrials.gov]; 2020-001109-22 [EudraCTNumber]), an adeno-associated viral vector serotype Hu68 carrying a DNA sequence encoding the human *GLB1* gene, is underway. A single ICM (intra-cisterna magna) administration of PBGM01 delivers a functional copy of the *GLB1* gene directly to the CSF. Patients (pts) with early onset (EO, Type I, ≥4 to <24 mo of age at enrollment) and late onset (LO, Type IIa, ≥6 to <36 mo of age at enrollment) infantile GM1 have enrolled in a dose escalation phase testing low- (LD) and high dose- (HD) PBGM01. Primary outcomes (OCs) include number of subjects with treatment-related AEs, SAEs, and clinically significant laboratory abnormalities, nerve conduction study changes, and immune response to PBGM01 (CSF & serum). Secondary OCs include longitudinal neurodevelopmental assessments (eg, Vineland-II [V-II] & Bayley-III [B-III]), change in key biomarker (BM) activity (CSF & serum β-gal; CSF GM1 ganglioside), neuroimaging, and QoL assessments. Results: Pts have been enrolled in Cohort 1 (LD, LO), with 2 (HD, LO) and 3 (LD, EO) to follow. Early data have shown PBGM01 was well-tolerated and had a favorable safety profile as no SAEs, complications related to ICM injection, or evidence of DRG toxicity were observed. Initial assessments showed increases in CSF and serum β-gal activity post-treatment in both pts in Cohort 1, above NHS patient values. Improvements were documented (V-II) and directly observed (B-III) in all developmental areas through the completed assessment periods, notable as Pt 2 had a severe developmental delay at baseline. The latest safety, BM, and longitudinal developmental data will be presented; results from vector DNA distribution, immunogenicity studies, and brain volumetric changes as assessed by MRI will also be presented. Conclusion: Interim safety, BM, and developmental data from these initial pts support the effectiveness of PBGM01 as a disease-modifying treatment of infantile GM1.

11. Myelodysplastic Syndromes after Eli-cel Gene Therapy for Cerebral Adrenoleukodystrophy (CALD)

David A. Williams[1], Jacob R. Bledsoe[2], Christine N. Duncan[1], Florian S. Eichler[3], Bartosz Grzywacz[4], Ashish O. Gupta[5], Troy Lund[5], Paul J. Orchard[5], Sarah Slauson[6], Dustin Whitney[6], Jakob T. Sieker[6], Geoffrey Parsons[6]

[1]Dana-Farber & Boston Children's Cancer and Blood Disorders Center, Harvard Medical School, Boston, MA,[2]Dept of Pathology, Boston Children's Hospital, Boston, MA,[3]Massachusetts General Hospital & Harvard Medical School, Boston, MA,[4]Department of Laboratory Medicine and Pathology, University of Minnesota Medical Center, Minneapolis, MN,[5]Division of Blood and Marrow Transplantation, Dept. of Pediatrics, University of Minnesota, Minneapolis, MN,[6]bluebird bio Inc., Cambridge, MA

As of March 2021, 55 patients with CALD had been treated with elivaldogene autotemcel gene therapy (eli-cel; Lenti-D lentiviral vector [LVV]-transduced autologous CD34+ cells) in ALD-102 (NCT01896102) and ALD-104 (NCT03852498) studies, with long-term follow-up in LTF-304 (NCT02698579). Lenti-D LVV

was designed to express the *ABCD1* cDNA to enable production of adrenoleukodystrophy protein (ALDP) across different cell lineages and contains the MNDU3 promoter. Non-clinical assessments, including the In Vitro Immortalization (IVIM) assay, did not identify insertional mutagenesis as a quantifiable hazard. In ALD-102, 91% of evaluable patients treated with eli-cel were alive and free of major functional disabilities 24 months (M) post-infusion. To date, the safety/tolerability of eli-cel treatment regimen primarily reflects effects of mobilization/apheresis and conditioning regimens, but 3 cases of myelodysplastic syndrome (MDS) were diagnosed in Jul, Aug and Nov 2021, and one finding suggestive of benign clonal predominance has been identified. Here, we present further details on these cases, including hematologic data received up to 31 January 2022. Two patients from ALD-104 were diagnosed with MDS with single lineage dysplasia without excess blasts at 14 and 26 M post-infusion. Notably, both patients achieved platelet engraftment >100 days after infusion. Insertion site analysis (ISA) revealed clonal predominance at first protocol assessment (M6), with 2 and 4 vector insertions in the predominant clones including, in both cases, a single insertion in the *MECOM* gene. Chromosome analysis, Fluorescence In-Situ Hybridization (FISH) and Rapid Heme Panel (RHP) analysis revealed no typical driver mutation of myeloid neoplasms. MDS cases were considered likely mediated by Lenti-D LVV insertion due to EVI1 dysregulation, specifically mRNA overexpression. Both patients have subsequently undergone allogeneic hematopoietic stem cell transplantation (allo-HSCT). One patient from ALD-102 developed MDS with excess blasts 92 M after eli-cel treatment. ISA detected insertions in multiple genes, including *PRDM16*. There was no evidence of a *MECOM* insertion. RHP showed somatic *KRAS* and *NRAS* mutations; chromosome analysis, hematologic malignancy fusion panel, and FISH did not reveal any abnormal findings. Investigations are ongoing to determine the role of Lenti-D LVV in MDS development. The patient has completed a first cycle of chemotherapy. In the separate patient with benign clonal predominance after treatment in ALD-102, a clone with multiple insertions, including a single *MECOM* insertion with EVI1 dysregulation, expanded and persisted over multiple years (follow up: 77M). Multiple bone marrow evaluations at 6-12 M intervals have revealed no signs of dysplasia or hematologic malignancy and peripheral blood counts remain normal. These cases highlight the need for long-term follow-up and further investigation into the significance of multiple insertions, disease-specific factors, and specific design features of the Lenti-D LVV that could contribute to the development of MDS.

12. AXO-AAV-GM2 Gene Therapy for Infantile- and Juvenile-onset GM2 Gangliosidosis: Preliminary Results from an Ongoing Phase 1/2 Trial

Florian Eichler[1], Terence Flotte[2], Haley Andonian[1], Oguz Cataltepe[2], Rebecca Artinian[2], Albert Misko[1], Benjamin Thorp[3], John Jameson[3], Michael Sheehan[3], Toby Vaughn[3], Donna Valencia[3], Erika De Boever[3]

[1]Massachusetts General Hospital, Center for Rare Neurologic Diseases, Boston, MA,[2]University of Massachusetts Chan Medical School, Worcester, MA,[3]Sio Gene Therapies, Inc, New York, NY

Introduction: GM2 gangliosidosis, known as Tay-Sachs (TSD) and Sandhoff (SD) disease, are rare, recessive lysosomal storage disorders caused by mutations in *HEXA* and *HEXB* genes, respectively, encoding β-hexosaminidase A (HexA) enzyme. HexA enzyme deficiency causes build-up of GM2 ganglioside, leading to diffuse neurodegeneration, progressive symptoms, and early death. No disease-modifying treatments currently exist. **Methods:** This is an ongoing, open-label, dose-ranging, Phase 1/2 trial (NCT04669535) of AXO-AAV-GM2 gene therapy for treatment of GM2 gangliosidosis. AXO-AAV-GM2 uses AAVrh8 vectors encoding the *HEXA* and *HEXB* genes. The study includes subjects with infantile-onset (6-20 months old) and juvenile-onset (2-12 years old) GM2 gangliosidosis, enrolled in 4 cohorts: Cohort 1 ($1.42x10^{14}$ vg), Cohort 2 ($1.95x10^{14}$ vg), Cohort 3 ($2.18x10^{14}$ vg), and Cohort 4 ($3.56x10^{14}$ vg). With each dose level, increasing volumes of vector are infused bilaterally into the thalamus, and by intracisternal magna (ICM)/intrathecal (IT) administration into the CSF. Infusion volumes range from 180-1,250 mcl/thalamus + 4.0-8.4 mL ICM/IT in Cohorts 1 and 4, respectively. CT and MRI are used to confirm bithalamic catheter placement and absence of acute surgery-related thalamic injury. Subjects receive immunosuppression with rituximab, sirolimus, and corticosteroids for up to 6 months. The primary endpoint is safety/tolerability; secondary endpoints include neurodevelopmental and motor function assessments, disease severity, MRI, and biomarkers. **Results:** As of March 2022, 5 subjects have been dosed: Cohort 1 (n=1 infantile-onset SD, followed 13 months); Cohort 2 (n=2 juvenile-onset TSD, n=1 infantile-onset TSD, followed 3-9 months); and Cohort 3 (n=1 juvenile-onset SD, with no follow-up data to-date). In the infantile subjects, pre-treatment brain MRIs revealed extensive signal abnormalities in white matter, thalami, caudate and putamen. In the juvenile subjects, baseline brain MRIs ranged from normal to extensive parenchymal volume loss and thalamic atrophy. The surgical procedure was generally well-tolerated, with MRI evidence of accurate targeting and resolution of focal hyperintensities at sites of thalamic injection. Most adverse events (AEs) have been mild or moderate. No AEs led to interruption or discontinuation of the AXO-AAV-GM2 procedure or study withdrawal. As of November 2021, 5 serious adverse events (SAEs) were reported in a single subject who had extensive parenchymal volume loss and disease progression at baseline and succumbed to *C. diff* infection 6 months after dosing. The investigators and independent data safety monitoring board (DSMB) determined 1 SAE of 'neurologic decompensation' to be 'Possibly Related' to AXO-AAV-GM2 and underlying disease, and the fatal SAE of '*C. diff* infection' as 'Unrelated' to AXO-AAV-GM2. Another subject had AEs of AST and ALT elevations that did not require intervention or have associated clinical sequelae. Following review, the DSMB recommended continued enrollment, and FDA agreed with enhanced subject monitoring. **Conclusion:** This is the first report of the Phase 1/2 trial for AXO-AAV-GM2 gene therapy for GM2 gangliosidosis using an innovative combined bithalamic, ICM and IT delivery method. The procedure was well-tolerated, with MRI evidence of accurate bithalamic targeting in all 5 subjects dosed to-date. The death of the subject with the most advanced disease points to a critical window for intervention, during which disease stabilization may be possible.

13. Evolving AAV Capsids with Broad Biodistribution in Deep Brain Structures in Adult Rhesus Macaques

David E. Leib[1], Megan S. Keiser[1], Yong Hong Chen[1], Paul T. Ranum[1], Luis Tecedor[1], Congsheng Cheng[1], Laurence A. Busque[1],

Beverly L. Davidson[1,2]
[1]The Raymond G. Perelman Center for Cellular and Molecular Therapeutics, Children's Hospital of Philadelphia, Philadelphia, PA,[2]Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA

Huntington's disease (HD) is a hereditary neurodegenerative disease that primarily affects an interconnected network of brain areas including the basal ganglia and cerebral cortex. While we and others have developed adeno-associated virus (AAV) gene therapy approaches for HD, current state-of-the-art AAV capsids do not sufficiently target all primate brain areas critical for HD therapy. To overcome this challenge, we performed a screen of tens of millions of peptide-modified AAV capsid variants in rhesus macaques to identify novel variants that transduced multiple basal ganglia and cortical areas following a single focused infusion. The goal of our approach was to take advantage of network connectivity in the brain to aid in AAV biodistribution. After three rounds of selection, next-generation sequencing revealed the top-performing capsid variants. We selected six variants for further validation by packaging one of five reporter transgenes. Mixes of top AAVs were infused unilaterally in two rhesus macaques at relatively low doses (3.5E10-7.0E10 vector genomes each, or 4.6E9-1.3E10 vector genomes per kilogram), and tissue was collected for histology after three weeks. Multiple variants showed robust and widespread transduction in the caudate nucleus, putamen, globus pallidus, and substantia nigra. One capsid variant targeted these brain areas with remarkable efficiency and also layer V/VI projection neurons across multiple cortical areas, including primary motor cortex. Brain biodistribution was corroborated using RNAscope™ fluorescence *in situ* hybridization. To test for AAV transduction outside of the central nervous system, digital droplet PCR was performed on liver samples. Notably, and in contrast to animals receiving intracerebroventricular infusions of a commonly used parental AAV serotype, AAV genomes were below the detection threshold with no transgene expression observed. Taken together, we identified novel AAV capsid variants that efficiently target basal ganglia and cortical projection neurons following focal infusion in rhesus macaques, with delivery at doses orders of magnitude lower than currently in clinical and preclinical use. These capsid variants may be useful for delivery of therapeutic transgenes for HD as well as Parkinson's disease and other disorders affecting the basal ganglia.

14. Preliminary Safety, Tolerability and Efficacy of Direct Epicardial Administration of Encoberminogene Rezmadenovec to Ischemic Myocardium in Patients with Refractory Angina: Six Month Phase 1 Data

Thomas J. Povsic[1], Jay H. Traverse[2], Timothy D. Henry[3], Todd K. Rosengart[4], Ronald G. Crystal[5], Howard C. Dittrich[6], Elizabeth A. Tarka[6], Nahush A. Mokadam[7]
[1]Duke University Medical Center, Durham, NC,[2]Minneapolis Heart Institute, Minneapolis, MN,[3]The Christ Hospital, Cincinnati, OH,[4]Baylor College of Medicine, Houston, TX,[5]Weill Cornell Medicine, New York, NY,[6]XyloCor Therapeutics, Wayne, PA,[7]The Ohio State University Wexner Medical Center, Columbus, OH

Rationale: Safety concerns in gene therapy trials have generally been associated with high viral particle loads administered systemically. Encoberminogene rezmadenovec (XC001) is a replication-deficient adenoviral serotype 5 (Ad5) vector expressing multiple isoforms of vascular endothelial growth factor (VEGF) including isoforms -121, -165 and -189. This construct shown to have superior angiogenesis when compared to vectors expressing single VEGF isoforms is currently being studied in the Phase 1/2 EXACT (Epicardial Delivery of XC001 Gene Therapy for Refractory Angina Coronary Treatment) Trial (NCT04125732) where epicardial administration of the vector allows for lower doses yielding high gene expression in target tissue compared with systemic administration. In preclinical toxicology studies in human equivalent doses up to $1x10^{12}$ viral particles of XC001, biodistribution analysis demonstrated high levels of vector in the heart relative to liver and other organs.
Objective: Phase 1 of the EXACT trial is a first-in-human, multicenter, open-label, single arm dose escalation study to evaluate safety, tolerability, and preliminary efficacy of epicardial injections of encoberminogene rezmadenovec to ischemic myocardium in refractory angina patients.
Methods: Twelve subjects with refractory angina and Canadian Cardiovascular Society (CCS) Class 2-4 without revascularization options underwent mini thoracotomy with one-time administration of XC001 in increasing doses per cohort (n=3/cohort; $1x10^{9}$, $1x10^{10}$, $4x10^{10}$, and $1x10^{11}$ viral particles, respectively) divided in 15 injections of 0.1 ml each across the left ventricular free wall with emphasis on ischemic zones. Anti-Ad5 neutralizing antibody (nAb) titers less than 1:320 were required for eligibility and titers were measured post-dosing through twelve months. Safety and tolerability were monitored via adverse event (AE) and serious adverse event (SAE) reporting as well as laboratory and electrocardiographic parameters. Preliminary key efficacy evaluation was change from baseline to six months post-treatment in exercise capacity.
Results: Over the 6 months of follow up, there were 17 SAEs in 7 subjects, and none were related to the study drug. Six SAEs in 4 subjects were related to the mini-thoracotomy procedure and none of these were unexpected or resulted in patient death. Eleven SAEs were related to either the underlying disease process or other causes. Three AEs related to study drug were reported in 2 subjects in the highest dose group and these events were fever, fatigue, and lip swelling. As expected, all treated subjects developed sustained nAbs. Improvements from baseline to month 6 in Total Exercise Duration (minutes) [mean (median)], were 0.6 (0.9), 0.5 (0.7), 1.1 (1.1), and 2.0 (2.2) for dose cohorts 1-4, respectively.
Conclusion: In the Phase 1 portion of this Phase 1/2 Study treating refractory angina, direct epicardial administration of encoberminogene rezmadenovec appears to be well-tolerated and safe at all tested doses. Preliminary efficacy evaluation in a small sample size suggests a possible dose-response and therapeutic potential with this therapy. Based on observed safety, tolerability, and preliminary efficacy with $1x10^{11}$ viral particle dose, continued investigation of the direct epicardial approach at this dose in the Phase 2 expansion portion of this trial has begun.