MCOLN1. Despite successful transgene expression in the brain in NPC mice, and reduction of lysosomal pathology, we observed no rescue in neuromotor phenotype, weight or lifespan of NPC mice, limiting applicability to CNS-targeted TRPML1 activation or lysosomal enhancement as potential treatment for NPC. References: 1. Shen, D., et al., Lipid storage disorders block lysosomal trafficking by inhibiting a TRP channel and lysosomal calcium release. Nat Commun, 2012. 3: p. 731. 2. De Rosa, S., et al., MCOLN1 gene therapy corrects neurologic dysfunction in the mouse model of mucolipidosis IV. Human Molecular Genetics, 2021.

## 262. Growing Clinical Gene Testing Considerations in the Era of RNA and Gene Therapy

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There has been exciting progress in the treatment of many diseases, including both cancer and hereditary disorders, using RNA and gene therapies. The Federal Drug Administration (FDA) has recently approved multiple RNA and gene therapies for neuromuscular diseases, and many are at clinical trial stages including several for other neurologic disorders, such as Alzheimer disease, Parkinson disease, amyotrophic lateral sclerosis, frontotemporal dementia and spinocerebellar ataxia. These RNA and gene therapies function by replacing disease genes, modulating functional products at a gene level, as well as targeting specific mutations (or types of mutations) (table 1).

| Date | Trade Name | Active Ingredient | Disease | Genetic mutation | Function on | Mechanism of action |
|---|---|---|---|---|---|---|
| 2015-07-02 | Orkambi | lumacaftor/ivacaftor | Cystic Fibrosis | F508del; 2 copies | Selected mutation | Chaperone |
| 2016-09-19 | Exondys 51 | eteplirsen | Duchenne muscular dystrophy | DMD exon 51 | Selected mutation | PMO-ASO |
| 2016-12-23 | Spinraza | nusinersen | Spinal muscular atrophy | SMN1 biallelic | Backup gene | ASO (SMN2) |
| 2017-12-19 | LUXTURNA | voretigene neparvovec-rzyl | RPE65 - Retinal dystrophy | RPE65 biallelic | Gene | Gene therapy |
| 2018-02-12 | Symdeko | tezacaftor; ivacaftor | Cystic Fibrosis | F508del; 2 or 1 copies | Selected mutation | Combination |
| 2018-08-10 | Galafold | migalastat | Fabry disease | GLA amendable mutation | Amendable mutation | Chaperone |
| 2018-08-10 | Onpattro | patisiran | Polyneuropathy hATTR | hATTR mutation | Gene | ASO |
| 2018-10-05 | Tegsedi | inotersen | Polyneuropathy hATTR | hATTR mutation | Gene | ASO |
| 2019-05-03 | Vyndaqel | tafamidis meglumine | Transthyretin Amyloid Cardiomyopathy | Wild type or hATTR | Gene | Transthyretin stabilizer |
| 2019-05-24 | ZOLGENSMA | onasemnogene abeparvovec-xioi | Spinal muscular atrophy | SMN1 biallelic | Gene | Gene therapy |
| 2019-10-21 | Trikafta | elexacaftor/ivacaftor/ tezacaftor | Cystic Fibrosis | F508del; 1 copies | Selected mutation | Combination |
| 2019-12-12 | Vyondys 53 | golodirsen | Duchenne muscular dystrophy | DMD exon 53 skipping | Selected mutation | ASO |
| 2020-08-07 | Evrysdi | risdiplam | Spinal muscular atrophy | SMN1 biallelic | Backup gene | Splicing modifier (SMN2) |
| 2020-08-12 | Viltepso | viltolarsen | Duchenne muscular dystrophy | DMD exon 53 skipping | Selected mutation | ASO |
| 2021-02-25 | Amondys 45 | casimersen | Duchenne muscular dystrophy | DMD exon 45 skipping | Selected mutation | ASO |

Molecular-based therapeutics generally require a clinical genetic diagnosis to determine eligibility, and for mutation targeting drugs, only patients affected by "amenable" mutations (a term from chaperone therapy) will benefit. The rapid progress in RNA and gene therapy development and approval has resulted in challenges for clinicians and clinical diagnostic laboratories. We increasingly need clinical genetics testing to identify and molecularly characterize patients for clinical trial enrollment and access to approved drugs. Multiple pharmaceutical-sponsored genetic testing programs were launched to increase access. Early diagnosis is essential with a growing interest in presymptomatic diagnosis for best clinical outcome via presymptomatic treatment. State run newborn screening (NBS) programs provide opportunities for early diagnosis and intervention for all families regardless of socioeconomic status. Yet, NBS on spinal muscular atrophy (SMA) was introduced two years after first FDA approved drug by Recommended Uniform Screening Panel (RUSP) in 2018., and is not available to all states as of spring 2022. Although pharmaceutical-sponsored programs and newborn screening (NBS) have helped with both access and early diagnosis, these programs are not available for several hereditary conditions that will benefit from molecular-based therapeutics. The availability of approved therapies also brings critical considerations for the clinical genetic testing laboratories to support treating specialty clinics. A central system resource to disseminate information regarding the availability of novel therapies, update clinical diagnostic algorithms, and further build evidence-based review and guidelines is needed to ensure patients receive appropriate care. In summary, RNA and gene therapy are in a race to the clinic. Joint efforts from medical and genetic communities are needed to increase genetic testing access and early diagnosis to unlock the utmost value.

## 263. Endogenous Human SMN1 Promoter-Driven Gene Replacement Improves the Efficacy and Safety of AAV9-Mediated Gene Therapy for Spinal Muscular Atrophy (SMA) in Mice

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Zolgensma®, an FDA-approved scAAV9 ubiquitously expressing a human *SMN1* cDNA transgene under cytomegalovirus enhancer/chicken β-actin promoter (*CMVen/CB-hSMN1*), is a significant breakthrough for treating SMA. Nonetheless, a high dose of the vector is required, leading to liver damage and hematologic complications in most patients. Severe toxicities were also found in nonhuman primates and piglets with AAV9-like vectors expressing *hSMN1*. Here, we hypothesize that restoration of physiological levels of hSMN1 expression in the appropriate cell types with the lowest effective dose of vector may be essential for the next generation SMA gene therapy. By optimizing the coding sequence of *hSMN1* (*co-hSMN1*) under *CMVen/CB* promoter, we increased hSMN1 protein expression by ~8 fold over the published Zolgensma® construct in Neuro2a cells. However, when we treated postnatal day 1 SMNdelta7 mice with this potent vector rAAV9-*CMVen/CB-co-hSMN1* at 3.3 x10e14 GCs/kg by facial vein administration, all treated animals (n = 6) died earlier than the untreated SMA mice. H&E staining of liver sections at day 8 revealed severe liver damage in both SMA mice and their healthy littermates treated with this vector, suggesting that the early lethality was likely to be associated with hepatic overexpression of *hSMN1*. In an attempt to achieve physiologically regulated hSMN1 expression, we created a second generation (2nd gen) scAAV9 vector expressing co-*hSMN1* from an endogenous *hSMN1* promoter (i.e., scAAV9-SMN1p-co-*hSMN1*) and injected neonatal SMA mice with either 3.3x10e14 GCs/kg (High dose, n = 9) or 1.1 x10e14 GCs/kg (Low dose, n = 12). We also dosed another group of SMA mice with 3.3x10e14 GCs/kg (high dose only, n = 13) of scAAV9-*CMVen/CB-hSMN1* harboring the same expression cassette as used in Zolgensma® as the benchmark vector. This side-by-side comparison study revealed that our novel 2nd gen vector has therapeutic potential overcoming the current SMA treatment limitations, which is supported by the following key findings. 1) All the mice injected with the high dose 2nd gen vector survived until the full 90-day study period compared with 60 days median survival from benchmark vector-treated mice. 2) The high dose 2nd gen vector-treated female mice gained more body weight than the benchmark vector

group starting from Day 25, while the low dose group showed similar body weights as the high dose benchmark vector. The average body weights of the treated male mice were equal in all three groups. 3) The high dose 2nd gen vector-treated mice have a faster righting response than benchmark vector-treated mice (Day 3-7 *vs* Day 7-13). In addition, the low dose group, also had an earlier righting response (Day 3-9) than the high dose benchmark vector group. 4) Rotarod tests of surviving mice at Days 30 and 80 showed comparable performances in all three groups. 5) At Day 60, 67% of mice treated with the benchmark vector developed ear necrosis, while none of the high dose 2nd gen vector-treated mice had ear necrosis at Day 90. In the low dose group, 33% of mice showed ear necrosis at Day 90. 6) The structures of neuromuscular junctions in the 2nd gen vector-treated mice were restored to that of wild-type mice, better than those of benchmark-treated SMA mice. 7) Less liver hSMN1 expression was detected from 2nd gen vector-treated healthy mice than the benchmark vector at Day 3 and Day 8, suggesting its potential to reduce hSMN1 expression associated liver toxicity. In summary, our novel *hSMN1* AAV gene therapy vector consisting of the endogenous SMN1 promoter and codon-optimized *hSMN1* has improved potency and safety profile as compared to the Zolgensma® vector, holding promise for clinical applications.

## 264. Therapeutic Effect of IL21 Blockage by Gene Therapy in Experimental Autoimmune Encephalomyelitis

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The pathogenic role of the interleukin 21 (IL21) in different autoimmune diseases, such as multiple sclerosis (MS), has been extensively studied. However, its pleiotropic nature makes it a cytokine that may exhibit different activity depending on the immunological stage of the disease. In this study, we developed a gene therapy strategy to block the interaction between IL21 and its receptor (IL21R) by using adeno-associated vectors (AAV) encoding a new soluble cytokine receptor (sIL21R) protein. We tested this strategy in a murine model of experimental autoimmune encephalomyelitis (EAE), obtaining different clinical effects depending on the time at which the treatment was applied. Although the administration of the treatment during the development of the immune response was counterproductive, the preventive administration of the therapeutic vectors showed a protective effect by reducing the number of animals that developed the disease, as well as an improvement at the histopathological level and a modification of the immunological profile of the animals treated with the AAV8.sIL21R. The beneficial effect of the treatment was also observed when inducing the expression of the therapeutic molecule once the first neurological signs were established in a therapeutic approach with a Doxycyline (Dox)-inducible expression system. All these clinical results highlight the pleiotropicity of this cytokine in the different clinical stages and its key role in the EAE immunopathogenesis.

## 265. Optimizing MRI-Guided Intra-Striatal AAV Dose Administration in Non-Human Primates

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Direct parenchymal infusion enables precision delivery of AAV vectors to deep subcortical structures, such as putamen and caudate, which are difficult to transduce by AAVs dosed via intra-CSF routes of administration. To ensure maximal coverage with minimum reflux or leakage, we conducted a study in non-human primates (NHP) to optimize intra-striatal dosing procedure. We report the evaluation of infusion volume, flow rate and cannula trajectories in three cynomolgus monkeys of approximately 2.5 years of age. The monkeys underwent bilateral intra-striatal convection-enhanced delivery (CED) using the Clearpoint system with real-time MRI guidance. Gadolinium was co-administered to visualize test article distribution in the brain with regular MRI scans taken during infusion. The animals were monitored for 10 days after dose administration and examined postmortem for tolerability of the dosing procedure. We were able to arrive at optimal dose volumes and flow rates that result in the dosing solution to distribute mostly within the targeted structures with minimal reflux or leakage. Coverage ranged from an estimated 26-38% and 30-56% in the caudate and putamen, respectively. Furthermore, the dosing procedure was well tolerated, and no animals showed adverse clinical signs or remarkable histopathology findings at injection sites in the brain beyond those attributable to the cannula penetration.

## 266. Evolved AAV Capsids for Gene Therapy of CLN2 Disease

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CLN2 disease is a childhood neurodegenerative disease due to deficiency of TPP1, a lysosomal enzyme amenable to cross-correction. Currently, enzyme replacement therapy (ERT) to the brain is the standard of care for patients, which requires treatment every two weeks directly to the CSF via a permanent Omaya reservoir. Gene therapy is an attractive alternative to ERT. Previously we showed that AAV-targeting of ependymal cells, which line the brain ventricles, allows for recombinant enzyme secretion into the CSF with dramatic extension of life span and clinical benefit in a canine model of CLN2 deficiency. For clinical translation of this approach, however, more efficient capsids are required for better transduction of the target cells. Here, we evolved AAV capsids for improved ependyma cell targeting *in vivo*. We identified peptide modified variants (PM-AAVs) that when delivered to mouse CSF provided levels of expression surpassing the current gold standard for mouse ependyma, AAV4. Our PM-AAVs resulted in up to 1000-fold increase in TPP1 levels in multiple brain areas, extended lifespan, and improved phenotypes at doses significantly lower than is required for AAV4, which could never achieve these levels of correction