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Prenatal Somatic Cell Gene Therapies: Charting a Path Towards Clinical Applications (Proceedings of the CERSI-FDA meeting).

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Abstract:	We are living in a golden age of medicine in which the availability of prenatal diagnosis, fetal therapy, and gene therapy/editing make it theoretically possible to repair almost any defect in the genetic code. Furthermore, the ability to diagnose genetic disorders before birth and the presence of established surgical techniques enable these therapies to be delivered safely to the fetus. Prenatal therapies are generally used in the second or early third trimester for severe, life-threatening disorders for which there is a clear rationale for intervening before birth. While there has been promising work for prenatal gene therapy in preclinical models, the path to a clinical prenatal gene therapy approach is complex. We recently held a conference with the UCSF-Stanford Center of Excellence in Regulatory Science and Innovation (UCSF-Stanford CERSI), researchers, patient advocates, regulatory (members of the FDA), and other stakeholders to review the scientific background and rationale for prenatal somatic cell gene therapy for severe monogenic diseases and initiate a dialogue towards a safe regulatory path for phase 1 clinical trials. This review represents a summary of the considerations and discussions from these conversations.

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CONFLICTS OF INTEREST:

- **Anna L. David:** is the co-Chair of the Maternal Health Project Group of ABPI, the Association of British Pharmaceutical Industry (unpaid position), and consults for Esperare Foundation, Geneva, Switzerland, a private not-for-profit developing a prenatal therapy for a congenital skin disease.
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oligonucleotides targeting SMN-AS1. She receives royalties from Elsevier for the book Spinal Muscular Atrophy: Disease Mechanisms and Therapy (editors, CJ Sumner, S Paushkin, CP Ko; Elsevier, 2017).

- **Tippi C. MacKenzie:** current or past research funding from: Novartis, BioMarin, Ultragenyx. She is SAB of Acrigen (gene editing company).
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ABSTRACT:

We are living in a golden age of medicine in which the availability of prenatal diagnosis, fetal therapy, and gene therapy/editing make it theoretically possible to repair almost any defect in the genetic code. Furthermore, the ability to diagnose genetic disorders before birth and the presence of established surgical techniques enable these therapies to be delivered safely to the fetus. Prenatal therapies are generally used in the second or early third trimester for severe, life-threatening disorders for which there is a clear rationale for intervening before birth. While there has been promising work for prenatal gene therapy in preclinical models, the path to a clinical prenatal gene therapy approach is complex. We recently held a conference with the UCSF-Stanford Center of Excellence in Regulatory Science and Innovation (UCSF-Stanford CERSI), researchers, patient advocates, regulatory (members of the FDA), and other stakeholders to review the scientific background and rationale for prenatal somatic cell gene therapy for severe monogenic diseases and initiate a dialogue towards a safe regulatory path for phase 1 clinical trials. This review represents a summary of the considerations and discussions from these conversations.

1. INTRODUCTION

Monogenic diseases arise from variations in a single gene that cause a constellation of clinical manifestations. They are extremely diverse and can affect the resulting gene product by structure or function. They generally follow Mendelian inheritance patterns and have the potential to be passed onto offspring. Even relatively rare single-gene disorders collectively account for an important public health burden as they are a significant source of childhood morbidity and mortality[1].

In many cases, ultrasound findings (e.g., hydrops fetalis)[2] that suggest an underlying genetic condition or positive family history prompt prenatal genetic testing. Currently, for some of these severe conditions, therapeutic options are limited, and the choices presented to a family are pregnancy termination, if regionally available, or delivery and postnatal treatment, if such treatment exists. These options carry both extensive emotional and financial burden. For subsequent pregnancies, options remain limited to in vitro fertilization with preimplantation genetic diagnosis to prevent an affected pregnancy or early prenatal diagnosis. In families for whom termination is either unavailable or not a viable consideration, a “wait and see” approach with postnatal care, when available, remains the sole option. Thus, there is an unmet medical need to develop strategies to improve outcomes for patients diagnosed with severe genetic conditions before birth.

For prenatally diagnosed anatomic conditions, advances in fetal surgical techniques have been transformative. The current repertoire of fetal surgery comprises open fetal interventions (for example, for the repair of meningomyelocele, in which a randomized clinical trial showed superior outcomes after fetal repair vs. postnatal correction)[3], fetoscopic interventions (such as for twin-to-twin transfusion syndrome (TTTS) or repair of meningomyelocele)[4-6] as well as ultrasound-guided catheter-based procedures. Accessing the umbilical vein (umbilical cord injection)[7,8] to provide blood transfusions has been life-saving for fetuses with isoimmunization[8] and alpha thalassemia major[9].

In contrast, prenatal therapies for genetic conditions are relatively limited. There are currently several ongoing phase 1 clinical trials for in utero treatment of severe genetic conditions: in utero stem cell transplantation for alpha thalassemia major (NCT 02986698, US), in utero enzyme replacement therapy for lysosomal diseases (NCT04532047, US) and in utero stem cell transplantation of mesenchymal stem cells for osteogenesis imperfecta (NCT03706482 / BOOOSTB4 trial Europe). A phase 2 trial of intra-amniotic administration of a recombinant ectodysplasin protein receptor-binding domain for fetuses with X-Linked Hypohidrotic Ectodermal Dysplasia (XLHED) (NCT04980638) has recently launched in Europe[10]. Fetal diagnosis of genetic diseases is expanding rapidly in the era of next-generation sequencing, and many parents are actively seeking opportunities for early diagnosis and fetal therapy. Thus, the opportunity, infrastructure, and technical ability to administer curative therapies such as prenatal somatic cell gene therapy (PSCGT) are currently available, although this has not yet been attempted in human patients. It is important to highlight that PSCGT[11] refers exclusively to gene replacement or editing of somatic cells in fetuses (likely during the late second trimester) and not the gene-editing of pluripotent cells in embryos or germline editing, which can be heritable for future generations. We also do not consider treating diseases that are not severe or fetal/early-onset. Finally, the transplantation of *ex-vivo* edited autologous cells is outside of this discussion because of the important current lack of ability to harvest and expand sufficient numbers of stem cells from the fetus for autologous transplantation.

Recent trials in adults and children have achieved concrete steps to realizing the therapeutic potential of gene therapy or gene editing, with two FDA-approved gene therapies successfully applied for diseases such as retinal blindness (Voretigene Neparvovec-Rzyl)[12] and spinal muscular atrophy (Onasemnogene Abeparvovec)[13], and investigational therapies such as Valoctocogene Roxaparvovec[14] for hemophilia. Numerous other therapies are in the pipeline and more could be developed for conditions relevant to prenatal therapy. The definition of "gene therapy" we are considering in this review include a range of genetically targeted therapies such as:

- Antisense oligonucleotides (ASOs): Short strands of chemically modified nucleotides (15-30 bp) that bind to mRNA to modify levels of the encoded protein or modify splicing.
- Gene-replacement: A missing or defective gene is replaced with a functional transgene, using a viral vector (most commonly adeno-associated virus or lentivirus, either ex-vivo or in vivo).

- Gene editing: DNA is permanently altered to correct a mutation or induce a beneficial effect. Several approaches exist, including CRISPR-Cas9, zinc finger nucleases, and peptide-nucleic acids.
- Gene activation or inhibition: Instead of editing the gene, the expression is upregulated or downregulated. CRISPRa/CRISPRi use modified versions of the CRISPR-Cas9 system.

The relevant considerations for each of these modalities are further discussed in Section 5.

2. BENEFITS OF PRENATAL THERAPY

In 1999, the NIH Recombinant DNA Advisory Committee (RAC) issued a position paper on prenatal gene transfer, outlining requirements for preclinical work in relevant animal models[15]. Since then, research in mouse, sheep, and non-human primate (NHP) models have focused on the safety and efficacy of PSCGT[16,17]. Notably, based on these preclinical studies, PSCGT could address several critical limitations to existing gene therapy strategies. First, immune response to the delivery vector capsid proteins (e.g., Adeno Associated Vector - AAV capsid protein)[18], and/or to transgene-encoded proteins, or to Cas9 nuclease itself can limit postnatal interventions[19]. Such responses could exclude some patients from initial therapy and many patients from future re-administration. In the fetus, the immune system tends towards tolerance to novel antigens; thus, in utero therapy can result in minimal immune response and could tolerize a patient to future therapies after birth. Second, many relevant diseases are progressive (e.g., lysosomal storage disorders), and some patients have significant morbidity even prior to diagnosis in childhood, infancy, or prenatally, particularly for neurologic conditions in which the onset of irreversible damage often starts in utero. In the fetus, therapies can be delivered early in the disease process, providing greater opportunities to curb the development or ameliorate the symptoms. Third, giving a therapy prior to the formation of the blood-brain barrier could allow systemic delivery of reagents that would not penetrate into the brain postnatally; a prenatal approach could also obviate the need for invasive intrathecal injections of therapeutic compounds, which are given postnatally to access the central nervous system. Finally, the cost of emerging gene therapies is astronomical, in part due to the large amounts of vectors necessary to have a clinical effect. Lower amounts of the vector are required in the fetus due to the smaller size. Given the particular importance of immune tolerance, we present more information on this aspect below; the

other benefits of prenatal therapy are covered during the discussion of specific conditions in Section 5 (Preclinical Data on Gene Therapy).

Immune tolerance: Most modalities of gene therapy involve exposure to a novel antigen, including viral capsid antigens, CRISPR-Cas9 proteins, and transgene-encoded or newly expressed missing proteins. The adult immune system would recognize such novel antigens as "foreign" and mount an immune response. In contrast, the immature fetal immune system can be educated to tolerate novel antigens so that they are recognized as "self" and, with persistent expressions, this tolerance should continue upon future exposure. While this phenomenon has been recognized since the pioneering experiments of Billingham, Brent, and Medawar[20], the immune mechanisms that underlie fetal tolerance were only recently elucidated. Fetal naïve T cells are more likely to become regulatory T cells upon exposure to a novel antigen[21,22], a process that may rely on differences in the epigenetic landscape of fetal T cells compared to their adult counterparts[23].

Tolerance to new antigens: The ability to induce tolerance to allogeneic cells and foreign proteins has been demonstrated in multiple settings such as in mice[24,25] and dogs[26] after in utero HSC transplantation. Tolerance to a transgene-encoded protein was further studied in the sheep model of intrauterine administration: intraperitoneal injection of retroviral vector encoding β-galactosidase in fetal sheep was found to lead to the development of post-natal tolerance to the protein product[27]. Administration of a postnatal booster of the protein demonstrated that lymphocytes from transduced animals had significantly lower in vitro stimulation indices and a blunted ability to form an antibody response to the protein than their non-transduced siblings.

Some of the most extensive data on in utero immune tolerance relates to clotting factors, with tolerance to the transgene-encoded protein demonstrated in both mouse[28] and non-human primate[29] models; these are covered in further detail in the section on Hemophilia.

Lack of immune response to viral capsid antigens: In adults, both innate and adaptive immune responses have been demonstrated to be directed against the viral vectors used in gene therapy. This response can result in serious immune responses, even on the first administration of gene therapy, and impaired uptake and rapid destruction of the gene therapy can reduce efficacy. For example, up to 35-80% of individuals can have

antibodies specific to either the viral capsid proteins or other viral gene products to AAV2[30]. Pre-existing immunity to viral vectors is important to detect prior to therapy, given the potential of inducing life-threatening immune responses[31]. Lentiviruses and other retroviruses are less immunogenic but have still been found to be sensitive to inactivation by human complement[32]. Due to the sterile nature of the gestational sac, apart from viral infections, fetuses have almost certainly never encountered the viral vectors commonly used for gene therapy, and the first exposure in utero is, therefore, unlikely to result in a memory response. It is possible that there could be transplacental passage of pre-formed maternal IgG antibodies against the vector capsid, and initial clinical efforts would test for maternal immunity to the proposed vector and exclude such patients. Importantly, fetal administration does not lead to tolerance to the capsid protein in preclinical models since the exposure is transient. This is likely secondary to the lack of effective immune responses in the fetal environment and has been tested in both mouse[28] and NHP[29] models. However, repeated administration with the same vector does not lead to a memory response[29]. Therefore, if the initial fetal exposure does not result in adequate gene expression, the patient could likely be re-dosed with the same vector preparation. Thus, PSCGT can take advantage of a unique window of opportunity during prenatal development to avoid treatment-related immune responses. Importantly, the available data suggest that PSCGT could allow for repeated administration of the therapy postnatally (in the case of waning therapeutic effect) since the fetal administration would not cause sensitization to the vector capsid protein.

3. PRENATAL DIAGNOSIS:

Prenatal diagnosis and management of pregnancies with fetal genetic diseases have evolved rapidly over recent years. Diagnostic genetic testing has shifted from karyotype and chromosomal microarray to include next-generation sequencing with exome and whole-genome sequencing, revealing a vast array of otherwise undetectable fetal genetic diseases[2,33-35].

Early prenatal diagnosis, at the very least, allows couples to prepare for the newborn and coordinate delivery, postnatal care, and insurance coverage. In addition to prenatal ultrasound, the advent of genetic testing enables the identification of genetic conditions that present with ultrasound manifestations (i.e., Hydrops fetalis, a

common pathway in many single-gene disorders)[2] that might be fatal before birth but might be amenable to prenatal molecular therapies, allowing us to expand the scope of fetal interventions to causal medical therapies (intrauterine stem cell transplantation, intrauterine enzyme replacement or protein therapy, or PSCGT).

Diagnosing single-gene disorders remains a challenge, with prenatal genetic screening routinely done for a minority of conditions (e.g., cystic fibrosis). For inherited disorders, such as hemophilia, expanded carrier screening, and family screening can help identify at-risk fetuses. Genetic screening is more challenging for disorders frequently caused by de novo mutations, including many cases of developmental delay[36,37]. The assessment of genetic disorders without an ultrasound finding represents the next frontier in prenatal medicine. Carrier screening is currently available for common severe diseases such as spinal muscular atrophy and cystic fibrosis. For others, invasive and noninvasive prenatal testing and exome/whole genome sequencing are available. Eventually, advances in non-invasive prenatal testing (NIPT) may facilitate such screening, but until then, clinical features during routine prenatal care, including hydrops fetalis[2] and hydrocephalus[38], can help identify some cases. The availability of life-saving fetal treatment could change the equation for some patients who currently decide against prenatal genetic testing.

4. APPROPRIATE CONDITIONS FOR PRENATAL THERAPY: There are several prerequisites for a disorder to be appropriate for PSCGT.

a) The benefits of PSCGT, over existing therapies or no therapy, outweigh the risks.

b) The natural history of the disorder is known, allowing treatment efficacy to be evaluated.

c) There is an effective strategy to identify fetuses at risk of the disorder through routine screening (e.g., hydrops identified by ultrasound prompting genetic testing to identify a lysosomal storage disease), known family history, or advances in non-invasive prenatal testing (NIPT).

d) Prenatal molecular diagnosis of the disease is accurate, with a good understanding of genotype/phenotype correlations that affect clinical prognosis to ensure a high probability that the fetus would develop the

disorder, and the therapy will reduce this risk. The expanding use and diminishing costs of prenatal sequencing are resulting in an increased frequency of prenatal molecular diagnoses; therefore, this prerequisite is rapidly becoming easier to achieve. Advances in non-invasive prenatal diagnosis (NIPD) are likely to greatly accelerate this process in the coming years.

e) The technical expertise for performing fetal injections (usually into the umbilical vein) and a multi-disciplinary fetal therapy team experienced with all aspects of the pre-and post-operative care must be available. Please refer to section 7 (Procedural risks of prenatal delivery) regarding relevant procedures and risk assessment.

5. APPROACHES TO GENE THERAPY:

The past decade has seen dramatic advances in nucleic acid therapeutics, both in new technologies and in terms of their clinical impact[39]. Various potential therapeutic modalities (ASO, gene replacement, gene editing) using viral and non-viral delivery methods to treat single-gene disorders are being evaluated and applied in pediatric and adult patients.

Small nucleotide therapeutics, including ASOs, and short interfering RNAs (siRNAs), are often delivered by an intravascular or intrathecal route. They can alter mRNA splicing patterns or modify the cellular concentration of specific proteins. For example, Nusinersen (ASO)[40] is injected intrathecally at three monthly intervals from birth to treat spinal muscular atrophy (SMA) by altering SMN2 splicing patterns to functionally rescue the deficit in SMN1. Gene replacement therapy delivers DNA or RNA constructs into cells to encode deficient proteins, most commonly with AAV vectors for in vivo delivery or lentiviral vectors (LVV) for ex-vivo delivery. Gene editing approaches use nucleases such as CRISPR/Cas9 (**C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats/ **C**RISPR **as**sociated protein **9**) to edit specific sites in the genome; they can also change the extent of mRNA transcription to modify the cellular concentration of specific proteins (e.g., CRISPR dCas9 activation [CRISPRa]). Peptide nucleic acids (PNAs) are DNA analogs that can be designed to bind adjacent to a disease-causing sequence in a chromosome. This recruits endogenous cellular editing pathways to remove and repair the

sequence. Co-administration of a homologous wild-type DNA sequence results in the editing of the disease-causing mutation. Due to the use of the patient's own endogenous nuclease pathways, this is a very high-fidelity method of gene editing. PNA/DNA gene editing reagents have been demonstrated to be most effective in stem and progenitor cells[41].

Delivery modalities: The delivery vector can be viral, often AAV or LVV, viral-like particles (VLP)[42], or synthetic (e.g., lipid nanoparticles - LNP)[39]. For example, Onasemnogene Abeparvovec-Xioi[13] is a method of AAV gene replacement that uses a single intravenous dose before the patient is two years of age to treat SMA by delivering a functional copy of the SMN1 gene to spinal neurons. As methods of nucleic acid therapeutics advance, it is conceivable that therapies could be developed for most single-gene disorders, benefiting millions of affected individuals.

Along with the risks to the fetus, prenatal therapy can also pose a risk to the mother, which is further detailed below in Section 6 (Maternal and Fetal Safety Considerations). To justify this maternal risk, prenatal intervention needs to yield additional benefits over postnatal intervention: prevent fetal lethality (e.g., alpha thalassemia), mitigate prenatal disease onset (e.g., SMA), introduce long-term benefits (e.g., inducing immune tolerance to missing proteins, such as in lysosomal storage disorders), ensure a more complete therapy (e.g., treating a higher fraction of stem cells), or cross the immature BBB to deliver a therapy to the brain.

5. PRECLINICAL DATA ON PRENATAL SOMATIC CELL GENE THERAPY:

Extensive prior studies using mouse, sheep and NHP models have provided detailed information on the safety, benefits, and efficacy of PSCGT. In this section, we will highlight some preclinical studies that address the most pertinent benefits of PSCGT, including immune tolerance, efficacy, and crossing the blood-brain barrier. Many of these studies have focused on relevant disorders that highlight the potential advantages of therapy, such as *immune tolerance* (e.g., hemophilia, lysosomal storage disorders) and *early-onset progressive disease* (e.g., lysosomal storage disorders, spinal muscular atrophy, Duchenne muscular dystrophy, cystic fibrosis, and thalassemia). Assuming the first-in-human use would be a reagent that has been tested in the postnatal

environment, recent successes in postnatal gene therapy trials highlight some potential diseases that could benefit from a prenatal correction along with a significant rationale for treating before birth:

Spinal Muscular Atrophy (SMA) is an early-onset motor neuron disease causing profound muscle weakness. It has historically been the leading inherited killer of infants and young children. Caused by recessive, loss of function mutations of the survival motor neuron 1 gene (*SMN1*)[43], retention of the paralogous, but alternatively spliced *SMN2* gene,[44,45] and reduced expression of the SMN protein[46], SMA was uniquely positioned to take advantage of advances in DNA/RNA-targeting therapeutics. Three drugs that increase SMN expression have been FDA approved since 2016 for the postnatal treatment of SMA patients. The splice-switching ASO, nusinersen, given by intrathecal injection[47], and the oral small-molecule risdiplam[48] both target *SMN2*-derived pre-mRNAs to increase exon 7 inclusion. Onasemnogene abeparvovec[49] is a viral gene replacement therapy delivered once intravenously, which uses AAV9 to deliver full-length SMN cDNA driven by a constitutively active promoter. Each drug has a distinct administration route, biodistribution, and potential toxicities, but clinical trials have repeatedly demonstrated that very early administration prior to overt weakness is substantially more effective than when the drug is initiated after symptom onset[50]. This observation led to the addition of SMA to the Recommended Uniform Screening Panel, and currently, most states in the U.S. and select other countries are screening for SMA in newborns. While this has led to earlier treatment initiation, initial experience with newborn screening suggests that a significant proportion of the most common and severely affected SMA type I patients are developing overt weakness in the first weeks of life, before postnatal treatment can be started[51]. This likely occurs because disease pathologies begin *in utero,* particularly in severely affected patients. Indeed, in humans and in mice with severe SMA, impaired motor axon development is evident by mid-late gestation and is followed by very rapid degeneration neonatally[52]. This degeneration causes a release of the neuronal cytoskeletal proteins, neurofilaments (NFs), into the serum, with the highest levels of NFs evident neonatally in both severe SMA mice[52] and type I SMA patients[53]. The timing of this pathology may be dictated by the expression level of the SMN protein, which is normally relatively high during fetal stages and declines postnatally, suggesting that SMN may be particularly needed for normal fetal motor neuron development[54]. These observations argue that *in utero* initiation of SMN-inducing therapeutics could improve efficacy compared to

postnatally initiated treatment. Two studies have explored the feasibility of *in utero* treatment in severe SMA mice. A risdiplam analog systemically delivered to pregnant dams (with exposure of the fetuses) improved disease outcomes of SMA offspring compared to SMA mice starting treatment postnatally[52]. In another study, AAV9-SMN gene therapy delivered by direct intracerebroventricular (ICV) injection to fetal mice improved survival compared to untreated SMA mice[55]. *In utero* treatment with ASOs delivered by intraamniotic delivery has also been shown to be feasible in mice for other disease indications[56]. Together these studies support proof-of-concept that *in utero* treatment of severely affected SMA infants may be feasible and more efficacious. Going forward, further work is needed to bridge the gap from bench to bedside, including large animal studies to determine ideal routes of administration (ICV, systemic and/or intraamniotic), drug biodistribution, and safety for both the fetus and the mother.

Lysosomal Storage Diseases (LSDs) are inherited metabolic disorders characterized by the accumulation of toxic materials in lysosomes due to deficiencies in an enzyme, activator protein, or transporter. Depending on the LSD, one or multiple organs can be affected. Since these conditions are often inherited and result in ongoing organ damage, there is a good rationale for early therapy. Intracerebral injection of an AAV2/AAV5 vector encoding the α-N-acetylglucosaminidase (*NAGLU*) gene has shown encouraging results in children with Sanfilippo B syndrome[57] A gene therapy approach using AAV vectors is also being investigated in children with Pompe disease.[58] Given the incidence of immune reactions to the missing enzyme in many LSDs, an in utero approach to induce tolerance to the enzyme could improve patient outcomes. Importantly, some LSDs result in in utero or neonatal demise due to the development of nonimmune hydrops fetalis, which could potentially be treated or prevented by in utero therapy. The current trials of gene-editing using zinc-finger nucleases for mucopolysaccharidosis types 1 (NCT02702115) and 2 (NCT03041324) could also be promising for in utero applications once more information is available regarding their safety and efficacy.

Preclinical work for PSCGT has focused on neuronopathic Gaucher disease and Mucopolysaccharidosis type I (MPS1). Massaro et al.[59] used an AAV9 vector, which previously facilitated the neuronal expression of a reporter gene, to treat a transgenic mouse model of Gaucher's disease in which untreated neurodegeneration usually

results in death by two weeks. Fetal intracranial injection of the vector improved neuronal inflammation and, remarkably, the overall survival of the mice. Neonatal mice that were treated with intracranial injection also had improved survival, but to a lesser extent than PSCGT, highlighting the benefit of fetal therapy. More recently, mice with MPS1 were successfully treated with prenatal base editing[60]. Mucopolysaccharidosis type I (MPS-IH, Hurler syndrome) is an LSD with a predominant G-to-A (W402X) disease-causing mutation in the IDUA gene. The pathology begins before birth, affecting multiple organs, and infants present by 6 months of age with hepatosplenomegaly, abdominal wall hernias, musculoskeletal abnormalities, retinal and neurocognitive degeneration, and cardiac disease. Treatment options, including enzyme replacement therapy and hematopoietic cell transplantation, are limited, and many children die by ~10 years of age from cardiorespiratory complications. Studies in the Idua-W392X MPS-IH mouse model, which recapitulates W402X MPS-IH disease in humans, demonstrated the feasibility of in utero CRISPR-mediated base editing to correct the disease-causing mutation[60]. Specifically, the mutation was efficiently corrected in hepatocytes and cardiomyocytes with low-level corrective editing in the brain. This was associated with a reduction in glycosaminoglycans in multiple organs, improved cardiac and musculoskeletal phenotype, and improved survival. There is currently an ongoing phase 1 clinical trial of prenatal enzyme replacement therapy for 8 different LSDs (NCT04532047), which include MPS1 and Neuronopathic Gaucher disease. A recent survey of families who have affected children noted that most parents would opt for participation in a clinical trial of a prenatal molecular therapy, including enzyme replacement or gene therapy[61].

Hemoglobin disorders (Alpha or Beta Thalassemia, Sickle Cell Disease): One potential advantage of prenatal therapy for disorders affecting hematopoietic stem cells (HSCs) is the possibility of accessing these stem cells in the fetal liver, before they migrate to the bone marrow. Indeed, it has been demonstrated that systemic delivery of nanoparticles results in tremendous uptake in fetal liver, with successful delivery into HSC[11]: fetal delivery of NPs loaded with PNA/DNA gene editing reagents results in 6-8% editing of bone marrow HSCs. In a mouse model of beta-thalassemia, a single fetal dose of NP/PNA/DNA gene editing corrected anemia, normalized reticulocyte count and peripheral blood smear, normalized spleen size, and architecture, and improved survival compared to affected untreated mice. These results are encouraging for other

hemoglobinopathies (such as alpha thalassemia, in which there is an added rationale for correcting a disease that can be fatal in utero) as well as other beta-hemoglobinopathies such as sickle cell disease.

The hemophilias: A (HA) and B (HB), are the most frequent inheritable coagulation defects, and are caused by mutations in the genes coding FVIII and FIX proteins, respectively. Current treatments greatly increase the quality of life and have lengthened the life expectancy for those affected, but the need for lifelong treatment and associated high cost are far from ideal. In addition, 30% of persons affected by HA have a significant risk of treatment failure due to FVIII inhibitor induction. The safety and efficacy profiles of several AAV vectors for HA[62] and HB[63] are quite encouraging for future in utero applications of this strategy. Despite effective prenatal diagnosis, the availability of a safe and effective intrauterine treatment would motivate prenatal hemophilia screening[64].

There have been numerous preclinical studies of PSCGT using AAV vectors. For example, prenatal plus postnatal re-administration of AAV-1 resulted in long-term expression of hFIX[28,65]. More importantly, the treated mice did not have a cellular or humoral immune response to hFIX, whereas all mice injected at the adult stage developed antibodies to hFIX[28]. A study of PSCGT using AAV in fetal sheep demonstrated that hFIX was present at earlier time points post-therapy. However, hFIX levels dropped as fetal liver and lamb weights increased, and lambs mounted an antibody response after injection of hFIX protein and Freund's adjuvant[66].

Following these proof-of-concept studies in rodent and sheep models, the efficacy of PSCGT with a therapeutic transgene was demonstrated in a preclinical NHP model, where a single intravenous injection of scAAV-LP1-hFIXco in NHP fetuses late in gestation produced sustained, clinically relevant levels of hFIX with liver-specific expression and a non-neutralizing immune response[67]. In a long-term follow-up of this trial, four of six PSCGT-treated animals continued to express hFIX at therapeutic levels (3.9%–120.0%) for more than 6 years of monitoring. These studies also indicated that low-frequency random genome-wide hepatic integration of AAV occurred without evidence of hot spots[68]. PSCGT performed at earlier time points of gestation in NHP using AAV5-hFIX or AAV8-hFIX, demonstrated that animals that received AAV5 exhibited long-term hFIX expression,

but at subtherapeutic levels. By contrast, AAV8 recipients had sustained hFIX expression in the therapeutic range. PSCGT did not sensitize the animals to AAV capsid, as anti-AAV antibodies remained below the positive threshold, and animals did not develop antibodies against hFIX. Nevertheless, when PSCGT was performed at earlier time points of gestation, linear amplification-mediated-PCR analysis demonstrated random integration of AAV sequences in hepatocytes, with no events occurring in or near oncogenic hotspots[29].

While direct delivery of vectors is promising, the use of cells as vehicles for gene delivery allows for copy number and integration-site analyses during product development, which would eliminate the possibility of off-target effects. In utero transplantation of sheep fetuses with human placental cells (PLC) transduced with a LVV encoding a bioengineered high-expression FVIII transgene (mcoET3) resulted in curative and sustained plasma levels of FVIII for at least 3 years after treatment, despite the exponential growth of the sheep with no cellular or humoral response to the human cells or to the FVIII transgene, and no evidence of any lentiviral-related or procedural toxicity in any tissue[69]. Thus, PSCGT has the potential to change the treatment paradigm, providing an early cure for HA and HB, without sensitizing the recipient to the viral capsid or to the cells that deliver the transgene, and preclude the development of antibodies against FVIII or FIX protein.

Monogenic lung diseases such as Cystic Fibrosis (CF): The rationale for prenatal therapy for CF is that the disease begins in utero, with manifestations seen in neonates (impaired male reproductive system, abnormal gastrointestinal development leading to meconium ileus, destruction of the exocrine pancreas.) The transition from in utero to postnatal life, during which adequate lung function is required for survival, highlights the potential benefit of prenatal gene editing for numerous monogenic lung diseases. For example, surfactant protein B deficiency is associated with neonatal respiratory failure and death within months of birth without a lung transplant. Alternative prenatal delivery routes, including intraamniotic/intratracheal or intravascular, provide ways to potentially target different pulmonary cells in developing fetal lungs. In studies of fluorescent reporter mice, in utero intraamniotic injection of viral vectors carrying SpCas9 has been shown to efficiently target pulmonary epithelial cells, including alveolar type 2 cells, the cell population of interest in genetic surfactant protein deficiencies[70]. The application of this approach to the mouse model of surfactant protein C deficiency

highlights the therapeutic potential of in utero pulmonary cell gene editing. The Sftpc^{I73T} mouse model carries a gain-of-function mutation recapitulating a disease-causing mutation in children's interstitial lung disease (18hild), and mice with this mutation die within hours of birth from respiratory failure. In utero CRISPR-mediated nonhomologous end-joining (NHEJ) excised the mutant gene, resulting in improved lung morphology, function, and overall survival[70]. For CF, in particular, experiments using peptide nucleic acids delivered using nanoparticles have given promising results.[71] The next steps in optimizing this strategy include demonstrating if improved uptake yields improved editing and testing of these agents in larger animal models.

7. MATERNAL AND FETAL SAFETY CONSIDERATIONS:

Potential risks of PSCGT have been assessed in large animal (sheep and NHP) models and include the following:

1) The genome of the ***viral vector can integrate*** with the human genome, even with the ssDNA AAV vectors. Viral integration is a common consequence of viral infections, to the extent that about 8% of the human genome[72,73] is composed of viruses that integrated during human evolution; however, each integration carries the risk of disrupting the function of an important component of the genome. The most recent NHP study to examine the extent of integration after PSCGT with AAV vectors, observed integration into somatic cells, but integration into hot spots has not been seen, with no evidence of oncologic consequences in long-term follow up[29].

2) Gene therapy, particularly gene editing, can also act outside of the target gene. These ***"off-target" effects*** may include editing of other regions of the genome, altering the transcription levels of other genes, or altering the splicing behavior of other genes. These risks are usually specific to the particular therapy (e.g., guide RNA or ASO) and can be evaluated in cellular models.

3) ***Germline Transduction:*** one of the earliest studies of whether PSCGT could achieve therapeutically meaningful levels of delivery of an exogenous gene to the hematopoietic system by directly injecting an amphotropic MMLV-based vector into fetal sheep[74,75] resulted in efficient transduction of long-term repopulating hematopoietic stem cells and lifelong presence of transgene-expressing cells within the peripheral blood and bone marrow of the recipients. Polymerase Chain Reaction (PCR) analysis of DNA isolated from tissues of these

animals showed that all tissues analyzed harbored the provirus, including the reproductive tissues, raising the possibility that this approach to PSCGT might place the germline at risk.

As a result of these findings, prevention of these undesired "off-target effects" and increasing the safety of this approach to PSCGT became the focus. Subsequent work showed that by altering the gestational age at which the vector is administered, it is possible to dramatically affect which tissues are transduced and the levels of transduction within those tissues[76]. These changes in tissue tropism as a function of gestational age were the result of developmental changes in the levels of the amphotropic receptor PiT2 (both RNA and protein) expressed on these tissues during gestation[77]. Collectively, these findings raise the exciting possibility that it may be feasible to target desired tissues by simply pseudotyping the vector with an envelope whose receptor is expressed on the desired tissue/cell type and is absent from non-target tissues like the germline.

Off-target germline editing is of concern due to the unintended changes becoming heritable (Section 10: Ethical and Regulatory Considerations for Prenatal Gene Therapy). An important caveat to these studies[74,75] was that the DNA used for PCR analysis was isolated from unfractionated testes tissue. As such, hematopoietic cells within the vasculature of the tissue and multiple types of somatic cells were present within the samples and could have accounted for the PCR positivity. Consequently, the PCR data on the reproductive tissue did not prove conclusively that the germline itself is actually at risk following PSCGT. In the first studies to explore this issue, wild-type MMLV were injected into mouse embryos and showed that not only did the provirus integrate into the germline, but the provirus was then passed to subsequent generations in a Mendelian fashion as part of the permanent genome[74,78-80]. These studies thus provided the first definitive evidence that retroviruses can, in fact, transduce the germline and that this information is passed to subsequent generations. However, those studies used wild-type, replication-competent virus and were performed in early embryos, so they likely grossly overestimated the risk of germline alteration in the context of PSCGT. Nevertheless, myriad studies have now shown that, under the right experimental conditions, germ cells from both small and large animals are susceptible to transduction with viral vectors and that this technology can be used to create transgenics[81-86].

To rigorously address whether the nascent germline is in fact at risk as a result of prenatal gene therapy and determine the frequency at which this might occur, a multi-pronged approach has been used in a highly translational sheep model[87]. Breeding studies were performed, in which female or male offspring after prenatal gene therapy were bred with naïve partners. While the number of resultant offspring was limited because of the use of sheep and the 5-month gestation period, proviral DNA was never detected by PCR in any of the offspring of any of the breeding pairs. The second method used to assess modification of the fetal germline following prenatal gene therapy was to perform PCR on DNA isolated from sperm cells purified using various methods. Performing provirus-specific PCR on OviPure-isolated sperm cells consistently yielded positive results. However, subsequent RT-PCR on the "purified" sperm cell population revealed that the cells all contained hematopoietic contaminants, as all samples contained $CD45^+$ cells. As such, no definitive conclusions could be drawn about whether the germ cells had been modified. However, provirus-specific PCR performed on both forensically purified sperm and on sperm cells purified by fluorescence-activated cell sorting also yielded positive results, providing the first evidence that the male germline is, in fact, at risk following prenatal gene therapy with amphotropic MMLV vectors. IHC studies of sections of the testes with an antibody to the transgene product (NPT II) confirmed transduction of the germ cells and expression of the vector-encoded transgene[87]. However, the incidence of germline alteration was exceedingly small, and alterations were only seen in male recipients. Of the 19 males analyzed, only 6 were PCR-positive for proviral DNA in the purified sperm. Of these 6, only 2 had transgene-positive germ cells in the tissue sections examined, and the incidence of germline modification in these experiments was ~1 in 6250[87].

To evaluate the factors that determine the risk to the fetal germline, detailed IHC studies were done on the testes of fetal sheep at various stages of gestation using markers for germ cells[88]. In that study, germ cells expressing SSEA1 were shown to be migratory and uncompartmentalized until about 70 days of gestation. The germ cells then began expressing SSEA3 as they began the process of compartmentalization within the forming sex cords. Upon compartmentalization, the germ cells began uniformly expressing SSEA4, and continued to do so throughout gestation. These studies used MMLV-based vectors, which require cell division for genomic integration to occur. Staining with Ki67 showed that the male germ cells do not undergo significant cell cycling

until they have become compartmentalized within the sex cords, and they would thus be presumed to be protected from viral vectors by the blood testes barrier. These studies collectively revealed several aspects of male germ cell behavior which likely impact the risk of inadvertent modification following PSCGT: 1) most male germ cells are not, in fact, compartmentalized during early to mid-gestation, and they may thus be unprotected/at risk; 2) male germ cells appear to be largely quiescent until they compartmentalize, so vectors requiring cell division will not transduce them very efficiently; 3) Sertoli cells that form the barrier of the sex cord will likely take the majority of the vector "hit", and 4) germ cells will be most vulnerable to viral vectors (and environmental mutagens) during passive compartmentalization, as cell cycling begins prior to complete closure of the sex cords. It is important to note that these studies were not performed with a vector that is currently considered for clinical use. Thus, the specific risks of a product considered for prenatal therapy should be assessed in the appropriate models to follow ICH guidelines to address the risk of germline integration.

For vectors that do not require cell division for integration, studies conducted in NHPs using LVV showed that the male germline was unaltered, but transduction of female germ cells occurred (also at very low levels), which was confirmed by laser dissection of individual germ cells followed by vector-specific PCR[89]. More recent studies using AAV vectors to perform PSCGT in NHPs[29,68,90] showed the presence of vector and transgene product within the reproductive tissues, often at a fairly high copy number. However, no in-depth studies were done to ascertain whether the germline itself was modified and, if so, at what frequency? Further studies are needed to address this question.

Maternal risks: Maternal safety is a critical consideration in any fetal therapy. Of particular concern is possible maternal exposure to the viral vectors infused into the fetus. An NHP study of PSCGT using AAV5 and AAV8 vectors examined maternal tissues for exposure to the virus and determined that although the virus was detected in the maternal blood early after fetal injection, it resolved by 72 hours[67]. This study also carefully examined maternal oocytes in 7 dams and did not detect any evidence of viral integration into these cells. Exposure to a viral vector may result in maternal immune responses to the capsid protein (it is likely that early trials would exclude mothers with pre-existing antibodies, which could cross the placenta) or to the recombinant protein. We

think that the latter is unlikely when this is an unmodified human protein, since the mother should already be producing – and therefore tolerant to – the recombinant protein. Clinical protocols would include monitoring for the presence of the gene therapy in the maternal blood and for evidence of an immune response.

7. PROCEDURAL RISKS OF PRENATAL DELIVERY:

Any surgical intervention carries a procedural risk. For fetal surgery, the procedures have become much safer after decades of experience with in-utero access. Here, we detail the procedural risks of intravascular and intracerebroventricular injections, the two most relevant routes of delivery.

Intravascular: a systemic injection into the bloodstream is achieved using the umbilical vein, which is a routine procedure for performing fetal blood transfusions for common indications such as Rh alloimmunization, or cordocentesis (also known as percutaneous umbilical cord sampling, PUBS), for diagnosis and therapy. PUBS was first used in 1964[91,92] via a hysterotomy with extrauterine umbilical transfusion. The percutaneous ultrasound-guided fetal blood sampling was developed in 1983[93] and is still the method used today for cordocentesis. Common indications include severe fetal anemia in alloimmunizations. One main limitation of the procedure is gestational age (>18 weeks) because of the technical challenge posed by the size of the umbilical vein. The preferable approach is the ultrasound/doppler-guided puncture of the umbilical vein at the placental cord insertion; in some cases, the umbilical vein (UV) is accessed as it traverses the fetal liver instead of within the umbilical cord. Complications are related to the injury of the umbilical vessels, leading to fetal bradycardia (arterio-spasm) and possible bleeding. These interventions are performed by a multidisciplinary team and take place under ultrasound guidance using a 20–22-gauge spinal needle with a Quincke point. To prevent uterine contractions, tocolytics such as indomethacin or terbutaline are used. Fetal analgesia is also given to prevent fetal pain when the intrahepatic UV is transfused. In rare cases, especially for posteriorly located placentas, fetal paralytic drugs (such as vecuronium bromide) minimize fetal movement. Complications can arise, and the procedural risks for each fetal access is reported to be 1.2% per procedure, with a procedure-related perinatal loss rate per fetus of 1.8%[94]. These risks could be higher for fetuses who are already compromised from their systemic anemia. The overall survival rate of severely anemic fetuses undergoing blood transfusions (often greater than one transfusion per pregnancy) is greater than 90%[95]. As such, cordocentesis is a well-established

procedure in maternal-fetal medicine, is safe in high-volume centers, and has the potential to easily deliver intravascular fetal therapy, including in utero gene and cell therapy. Some therapies may be more effective if given at an earlier stage of development, in which case intraperitoneal or intracardiac injection may be appropriate when access to the UV is not possible. Thus, there are well-established methods for fetal access that can be employed to deliver prenatal somatic cell gene therapies. However, a low but non-zero risk of procedural complications that can result in preterm delivery or, in rare cases, fetal demise must be discussed with each family.

Intracerebroventricular/intraparenchymal: In the post-natal setting, direct delivery of gene therapy to the central nervous system (CNS) is feasible and safe with early evidence of efficacy. For example, an adenoviral vector encoding a functional copy of the L-amino acid aromatic decarboxylase (AADC) gene was delivered using convection enhanced delivery to the midbrain of children with this disease[96]. Imaging and clinical measures were consistent with gene expression and restoration of function of at least a portion of the physiologic pathway. Direct access to the fetal ventricular system in humans has been demonstrated in a few reports for children with a prenatal diagnosis of ventriculomegaly[97]. Although specific issues such as diagnosis of the fetal condition, selection of therapeutic agent, and route of delivery (intraparenchymal, intraventricular, or intracisternal) still need to be addressed, therapeutic delivery in fetuses is technically feasible and offers the advantage of early treatment of diseases, particularly those with a progressive impact on function.

The intraamniotic injection is another promising delivery modality that should be considered due to its low technical risk. For example, serial injection of a therapeutic protein into the amniotic fluid was demonstrated to treat patients with X-linked hypohidrotic ectodermal dysplasia and is now undergoing a phase 2 clinical trial[10]. Intra-amniotic injection of splice switching ASOs in a mouse model has shown therapeutic benefit in a model of deafness[98] and deserves further investigation in other diseases.

8. BALANCING SAFETY AND INNOVATION: Conducting clinical trials of novel somatic-cell gene therapies in utero raises a plethora of challenges, primarily because of safety concerns for the mother and fetus. The paucity

of such trials has led to an absence of the customary safety reporting frameworks, such as standardized definitions and severity grading for maternal and fetal adverse events (AEs). This makes it even more challenging to trial novel therapies in pregnancy.

AEs are important signals in clinical trials. Although they may not necessarily have a causal relationship with the investigational drug, AE assessment allows swift and responsible communication of safety data between study investigators, sponsors, and regulators. AEs should be recorded in medical records and reported to the sponsor and other relevant authorities, after which a decision is made as to whether they meet the regulatory definition of 'serious' and are directly related to the administration of the investigational drug, classified as a serious adverse reaction or SAR. AEs are then graded, which permits comparison of safety signals between clinical trials, whereas for novel prenatal therapies such as we are considering here, it allows decisions around dose-escalation to be rendered more objectively. AE severity is recorded using standard grading criteria, with limitations regarding perinatal conditions[99], but maternal and fetal adverse event terminology (MFAET 1.1)[100] is now available that provides standard definitions and grading specifically for maternal and fetal AEs. This makes a detailed assessment of safety possible, adding to the condition-specific severity grading schemes available for clinical trials of vaccination in pregnancy via the GAIA project[101], the Division of AIDS terminology[102], and the Clavien-Dindo classification for surgical complications[103].

Fetal AEs need to be diagnosed in utero because severe ones can potentially cause a detrimental effect before birth. Consideration of the different fetal organ systems that might be affected by a therapeutic intervention set out new fetal AE definitions that have been adopted by the Medical Dictionary for Regulatory Activities (MedDRA)[104]. The fetal AE grading system is based on Common Terminology Criteria for Adverse Events (CTCAE) criteria, ranging from mild grade 1 with minimal effect on the fetus through grade 5, which is fetal death. For example, fetal development of pericardial, pleural, or peritoneal fluid collection after a prenatal intervention might be grade 2, moderate when it is not life-threatening, but would be graded 4 or life-threatening if the presence of fluid was detected in at least two fetal compartments, called hydrops. In addition, AE severity is graded independently for the pregnant woman and fetus so that a more complete picture of the effect of prenatal

interventions can be understood. For example, anemia in pregnancy might be classified as grade 3 severe for the mother, necessitating a maternal blood transfusion, but could result in fetal compromise requiring urgent delivery, classified as a grade 4 fetal life-threatening event.

Also relevant to prenatal somatic-cell gene therapies is the Neonatal Adverse Events Severity Scale (NAESS)[105], which classifies neonatal AEs into 5 grades (mild, moderate, severe, life-threatening, or death). Severity is defined by the effect of the AE on age-appropriate behavior, basal physiologic functions, and healthcare changes in response to the AE. NAESS was developed through a Delphi consensus process to integrate neonatal terminology and definitions into wider dictionaries undertaken by the International Neonatal Consortium[106,107].

MFAET fills a vital gap in fetal translational medicine research and supports the development of somatic cell gene therapies for the fetus. It is undergoing regular review to align it with ongoing changes in regulatory terminology at MedDRA and CTCAE[108].

Additional Discussion Highlights: There are several other important safety considerations that prompted discussion during the meeting as detailed below:

Endpoint analysis and reporting: The primary goal of the initial first in human application of PSCGT will be not only to demonstrate safety but also to assess preliminary efficacy in patients for whom a given treatment holds a prospect of direct benefit. Since many of the components are likely to be shared PSCGT approaches for different disorders (e.g., vectors, CRISPR-Cas9, mode of delivery), there will be substantial benefits in sharing relevant data, most importantly safety data. We propose that a set of standardized measures be developed for all applications of PSCGT with the aim of evaluating potential immune reaction, fetal health, and maternal health, adverse events, presence of the gene therapy in the maternal circulation, and degree of viral integration in the fetal genome when biopsy material is available. A sampling of the placenta, amniotic fluid, umbilical cord, and cord blood at delivery is useful for routine assessment of these parameters.

Alongside evaluating the safety, molecular and clinical efficacy should also be assessed; however, these will often be specific to single disorders. Assessments of molecular efficacy could include RNA levels, protein levels, or DNA variant mosaic frequency. It is important to consider "natural history" not only in the context of untreated disease but also in the context of disease treated with available therapies, including benefits and risks (and disease outcomes) after post-natal treatment with available therapies vs. prenatal intervention with investigational therapy.

Outcome measures may include measures of clinical benefit such as mortality (e.g., SMA), requirements for adjuvant therapy (e.g., ventilator use in SMA, blood transfusions in thalassemias), adverse event frequency (e.g., seizures), and developmental milestones (e.g., ability to walk at a certain age in SMA), or biomarkers and candidate surrogate measures such as factor IX levels in hemophilia, hemoglobin levels in thalassemias and observations on imaging studies (e.g., degree of hydrops in lysosomal storage disorders). In these evaluations, the workshop participants advocated to consider aspects that are most important to families, as recently reported for the hemophilia community[109].

A stepwise approach: Both established and emerging technologies, including gene editing using CRISPR, may enable more effective correction of genetic disorders in the prenatal period; however, at this time, their off-target effects are less well understood. As such, the workshop participants discussed a stepwise approach to the development and testing of PSCGT for the prenatal treatment of genetic diseases. This approach includes an initial implementation of therapies that are already approved for postnatal use (for example, gene replacement using AAV vectors). This stepwise approach would enable the facilitated development of novel therapies for fetal applications while taking careful and rigorous consideration of the risks and benefits.

8. REGULATORY CONSIDERATIONS FOR PRECLINICAL STUDIES- THE FDA PERSPECTIVE:

Preclinical studies for gene therapy products are conducted to characterize biological activity, the potential for efficacy in the target disease, and safety profile at the intended clinical dose-level range[110]. Preclinical studies to support the initiation of a clinical trial for a PSCGT product are expected to address the same goals while

focusing on the unique considerations that arise from investigational product administration during pregnancy to treat the fetal condition. Given the often-complex procedures required for the delivery of gene therapy products to the fetus, the safety of the administration methods and the delivery devices used are also important considerations.

The diversity of gene therapy products and their therapeutic applications necessitates a preclinical program tailored to the specific product or product class. However, some general factors should be considered when designing preclinical studies for investigational products intended to treat fetal conditions. For example, proof-of-concept and biodistribution studies[111,112] can provide information about the bioactive dose of the gene therapy product following administration, the involved organs and tissues in the fetus and the mother, and level of replication/expression. Toxicology studies can elucidate potential adverse findings attributed to the vector and the expressed transgene, help determine the dose levels at which the toxicities become dose-limiting, the potential for immune activation, on- and off-target events, germline transmission, potential for mutagenesis, and any effects on reproduction. The information obtained from preclinical studies provide support for determining a starting clinical dose that is potentially safe and effective, as well as the dose-escalation scheme and safety monitoring plan for the clinical trial participants[113].

A single “perfect” animal species for performing preclinical studies for PSCGT does not exist. The choice of species depends on the affirmation of biological activity of the specific gene therapy product in the animal. The use of an animal model of human disease, if available and feasible, is desirable. When designing a preclinical program, several assessments can be combined in a definitive study for evaluation of multiple bioactivity and safety outcomes. For example, a proof-of-concept study performed in a pregnant animal model of disease can be expanded to include assessments of the biodistribution, organ and organ-system toxicity, and other safety parameters for a comprehensive evaluation of the product’s effects. Overall, for any chosen product and disease considerations, it is important that scientifically sound principles, current state-of-the art technology, and the 3R’s principles (Reduction, Refinement, and Replacement) be applied when selecting animal models and establishing a preclinical program for PSCGT.

10. ETHICAL AND REGULATORY CONSIDERATIONS FOR PRENATAL GENE THERAPY: Efforts at in utero research and therapy are always complicated by the need to keep both the pregnant woman and the fetus in mind when evaluating risks and benefits. In addition, the role of a parenting partner and/or a genetic father can be ambiguous, particularly with respect to who may give consent for a procedure. Furthermore, federal protections for human research subjects appear to limit the permissible degree of risk to the developing fetus, which also has implications for which of the interested adults may consent to the intervention.

For interventions involving somatic-cell genome editing, risk management[114,115] will require long-term follow-up of resulting children to determine the efficacy of the intervention and, because of systemic administration, to monitor for evidence of off-target activity in the tissues and organs of interest, as well as in other parts of the developing body. A special concern is possible off-target effects on developing gametes, which could affect not just the child who received the edits while in utero but also subsequent generations. Such long-term, even possibly multi-generational follow-up is a task that many investigators, clinicians, and even commercial sponsors may find logistically and financially daunting. In addition, because consent can be withdrawn at any time by the responsible parents or guardians (as well as by the child, once the age of majority is reached), there may well be a significant loss to follow-up among those enrolled in the trials.

Consent and risk tolerance should be viewed in the context of the provisions set forth in 45 CFR part 46, Subpart B, which prohibit anything greater than minimal risk to the fetus if research does not hold out the prospect of direct medical benefit to the fetus (or the pregnant woman herself). In addition, consent is also required from the 'father,' unless he is unavailable or incompetent. This adds complications when the pregnant woman and prospective father disagree, as well as when the intended co-parent is not the father[116].

If the research is permissible, then informed consent will require several topics to be discussed. These include alternative post-natal therapeutic interventions; the possibility that even if successful, there might be a miscarriage, a stillbirth, or a child born with serious health problems; the local standards and applicable hospital

policies for withholding and withdrawing care in cases of severely impaired neonates; and whether late-term abortion is available in case the in-utero therapy goes badly awry.

In 2021, the International Society for Stem Cell Research issued a revised set of global guidelines for regenerative medicine research and clinical care, including, for the first time, a specific reference to in utero genome editing. The guideline states: "Clinical research involving in utero stem-cell-based interventions or genome editing involves risks to both the pregnant woman and the future child, and it should be undertaken only when it offers the prospect of a benefit greater than that of post-natal interventions, does not pose an excessive risk to the pregnant woman, and where there is an institutional capacity for autopsy (in the case of miscarriage or stillbirth) or follow-up (in the case of live birth)"[117].

Gene therapies could offer potentially curative treatment for previously untreatable diseases. As more gene therapies emerge, it is reasonable to expect the overall spending in the class to rise. Nevertheless, making transformative medicine accessible poses unique challenges for providers and payers. As such, concerns about the cost of gene therapy, variable insurance coverage, and insurance policies possibly limiting access to these new therapies despite their potential benefits are gaining terrain worldwide.

Estimating the number of prospective patients treated by gene therapy combined with mathematical models to estimate the cost and the potential financial impact of gene therapy could allow for informed financial decisions about the future of this essential therapeutic class[118]. It also aids the establishment of support strategies to counteract the difficulties some gene therapy patients may face (insurance coverage, access to care, childcare, and financial support during recovery). Current practices around cost-effectiveness calculations and values might need to be adjusted to reach a consensus on how to reward the value created by these therapies. Estimates based on the relation between the cost-effectiveness threshold and the value-based price could warrant the development of innovative payment models as an example of a potential solution for the issue[119].

Furthermore, democratizing information (accurate, actionable, and understandable data) on gene therapies and ensuring transparency as well as a concerted effort from all stakeholders (policymakers, healthcare providers,

insurance companies, and patients)[120] may help identify existing systems that can be leveraged or adapted to facilitate effective dissemination of treatments in an equitable manner.

11. CONCLUSIONS: Critical steps are being taken toward more accurate prenatal diagnosis, characterizing the unique fetal phenotypes of genetic diseases, understanding the genetic variants capable of causing severe fetal disease, correlating genetic variants with postnatal outcomes, and implementing preclinical and clinical studies to investigate additional emerging in utero therapies.

As preclinical studies are translated to human clinical trials[20,21], and drugs approved for postnatal use are being used in the prenatal setting, it is important to review their associated clinical effects, risks, and benefits of treating prenatally, with a clear justification for the prenatal use (more beneficial and/or less risky when compared to the postnatal use), as well as ethical, legal and social implications[121] and the importance of patient involvement in the research and development of clinical trials. Familiarity with postnatal approaches increases the receptiveness to innovative fetal molecular treatments and the likelihood of pursuing fetal therapies in a trial or clinical setting[61].

Prenatal gene therapy to target the somatic cells of a fetus is a novel and promising strategy to improve the survival and comorbidities[122] in patients with a spectrum of monogenic disorders. The increasing number of gene therapy trials available in the postnatal setting, and the benefits shown by in-utero applications in preclinical trials, pave the way for future clinical trials in the prenatal setting. The attention to these conditions and opportunities to inform the medical community about clinical trials offer hope to these families, their children, and future generations.

Multidisciplinary discussions about the merits and risks of this approach with patients, patient advocacy groups, and clinical and bioethics experts define a responsible roadmap for achieving in utero gene therapy in humans. Importantly, further research in this space must be guided by bioethical principles with close involvement of patient stakeholders and consideration of personal utility as viewed by individuals who have experienced pregnancy with a fetal genetic disease.

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Prenatal Somatic Cell Gene Therapies: Charting a Path Towards Clinical Applications (Proceedings of the CERSI-FDA meeting).

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CONFLICTS OF INTEREST:

- **Anna L. David:** is the co-Chair of the Maternal Health Project Group of ABPI, the Association of British Pharmaceutical Industry (unpaid position), and consults for Esperare Foundation, Geneva, Switzerland, a private not-for-profit developing a prenatal therapy for a congenital skin disease.
- **Stephan J. Sanders:** receives research funding from BioMarin Pharmaceutical Inc.
- **Charlotte J. Sumner:** has been a consultant to Avexis, Novartis, Ionis Pharmaceuticals, Biogen, PTC Therapeutics, Roche, Genentech, Cytokinetics, Sarepta, Nura Bio, Argenx, Biomarin, Scholar Rock, GenEdit, Epirium, Capsigen, and Atalanta. She received research grants from Ionis Pharmaceuticals and Argenx, and currently receives grant support from Roche. CJS is a coholder of 2 pending patent applications (BIOL0274USA and BIOL0293WO) with Ionis Pharmaceuticals on antisense

oligonucleotides targeting SMN-AS1. She receives royalties from Elsevier for the book Spinal Muscular Atrophy: Disease Mechanisms and Therapy (editors, CJ Sumner, S Paushkin, CP Ko; Elsevier, 2017).

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- **Larissa Lapteva and Evi Strubble:** no conflicts of interest. The opinions presented in this article are those of the authors and do not necessarily represent the views or policies of the FDA.
- **Nalin Gupta:** Consultant for Encoded Therapeutics and Sana Therapeutics.

ABSTRACT:

We are living in a golden age of medicine in which the availability of prenatal diagnosis, fetal therapy, and gene therapy/editing make it theoretically possible to repair almost any defect in the genetic code. Furthermore, the ability to diagnose genetic disorders before birth and the presence of established surgical techniques enable these therapies to be delivered safely to the fetus. Prenatal therapies are generally used in the second or early third trimester for severe, life-threatening disorders for which there is a clear rationale for intervening before birth. While there has been promising work for prenatal gene therapy in preclinical models, the path to a clinical prenatal gene therapy approach is complex. We recently held a conference with the UCSF-Stanford Center of Excellence in Regulatory Science and Innovation (UCSF-Stanford CERSI), researchers, patient advocates, regulatory (members of the FDA), and other stakeholders to review the scientific background and rationale for prenatal somatic cell gene therapy for severe monogenic diseases and initiate a dialogue towards a safe regulatory path for phase 1 clinical trials. This review represents a summary of the considerations and discussions from these conversations.

1. INTRODUCTION

Monogenic diseases arise from variations in a single gene that cause a constellation of clinical manifestations. They are extremely diverse and can affect the resulting gene product by structure or function. They generally follow Mendelian inheritance patterns and have the potential to be passed onto offspring. Even relatively rare single-gene disorders collectively account for an important public health burden as they are a significant source of childhood morbidity and mortality[1].

In many cases, ultrasound findings (e.g., hydrops fetalis)[2] that suggest an underlying genetic condition or positive family history prompt prenatal genetic testing. Currently, for some of these severe conditions, therapeutic options are limited, and the choices presented to a family are pregnancy termination, if regionally available, or delivery and postnatal treatment, if such treatment exists. These options carry both extensive emotional and financial burden. For subsequent pregnancies, options remain limited to in vitro fertilization with preimplantation genetic diagnosis to prevent an affected pregnancy or early prenatal diagnosis. In families for whom termination is either unavailable or not a viable consideration, a “wait and see” approach with postnatal care, when available, remains the sole option. Thus, there is an unmet medical need to develop strategies to improve outcomes for patients diagnosed with severe genetic conditions before birth.

For prenatally diagnosed anatomic conditions, advances in fetal surgical techniques have been transformative. The current repertoire of fetal surgery comprises open fetal interventions (for example, for the repair of meningomyelocele, in which a randomized clinical trial showed superior outcomes after fetal repair vs. postnatal correction)[3], fetoscopic interventions (such as for twin-to-twin transfusion syndrome (TTTS) or repair of meningomyelocele)[4-6] as well as ultrasound-guided catheter-based procedures. Accessing the umbilical vein (umbilical cord injection)[7,8] to provide blood transfusions has been life-saving for fetuses with isoimmunization[8] and alpha thalassemia major[9].

In contrast, prenatal therapies for genetic conditions are relatively limited. There are currently several ongoing phase 1 clinical trials for in utero treatment of severe genetic conditions: in utero stem cell transplantation for alpha thalassemia major (NCT 02986698, US), in utero enzyme replacement therapy for lysosomal diseases (NCT04532047, US) and in utero stem cell transplantation of mesenchymal stem cells for osteogenesis imperfecta (NCT03706482 / BOOOSTB4 trial Europe). A phase 2 trial of intra-amniotic administration of a recombinant ectodysplasin protein receptor-binding domain for fetuses with X-Linked Hypohidrotic Ectodermal Dysplasia (XLHED)X (NCT04980638) has recently launched in Europe[10]. Fetal diagnosis of genetic diseases is expanding rapidly in the era of next-generation sequencing, and many parents are actively seeking opportunities for early diagnosis and fetal therapy. Thus, the opportunity, infrastructure, and technical ability to administer curative therapies such as prenatal somatic cell gene therapy (PSCGT) are currently available, although this has not yet been attempted in human patients. It is important to highlight that PSCGT[11] refers exclusively to gene replacement or editing of somatic cells in fetuses (likely during the late second trimester) and not the gene-editing of pluripotent cells in embryos or germline editing, which can be heritable for future generations. We also do not consider treating diseases that are not severe or fetal/early-onset. Finally, the transplantation of *ex-vivo* edited autologous cells is outside of this discussion because of the important current lack of ability to harvest and expand sufficient numbers of stem cells from the fetus for autologous transplantation.

Recent trials in adults and children have achieved concrete steps to realizing the therapeutic potential of gene therapy or gene editing, with two FDA-approved gene therapies successfully applied for diseases such as retinal blindness (Voretigene Neparvovec-Rzyl)[12] and spinal muscular atrophy (Onasemnogene Abeparvovec)[13], and investigational therapies such as Valoctocogene Roxaparvovec[14] for hemophilia. Numerous other therapies are in the pipeline and more could be developed for conditions relevant to prenatal therapy. The definition of "gene therapy" we are considering in this review include a range of genetically targeted therapies such as:

- Antisense oligonucleotides (ASOs): Short strands of chemically modified nucleotides (15-30 bp) that bind to mRNA to modify levels of the encoded protein or modify splicing.
- Gene-replacement: A missing or defective gene is replaced with a functional transgene, using a viral vector (most commonly adeno-associated virus or lentivirus, either ex-vivo or in vivo).

- Gene editing: DNA is permanently altered to correct a mutation or induce a beneficial effect. Several approaches exist, including CRISPR-Cas9, zinc finger nucleases, and peptide-nucleic acids.
- Gene activation or inhibition: Instead of editing the gene, the expression is upregulated or downregulated. CRISPRa/CRISPRi use modified versions of the CRISPR-Cas9 system.

The relevant considerations for each of these modalities are further discussed in Section 5.

2. BENEFITS OF PRENATAL THERAPY

In 1999, the NIH Recombinant DNA Advisory Committee (RAC) issued a position paper on prenatal gene transfer, outlining requirements for preclinical work in relevant animal models[15]. Since then, research in mouse, sheep, and non-human primate (NHP) models have focused on the safety and efficacy of PSCGT[16,17]. Notably, based on these preclinical studies, PSCGT could address several critical limitations to existing gene therapy strategies. First, immune response to the delivery vector capsid proteins (e.g., Adeno Associated Vector - AAV capsid protein)[18], and/or to transgene-encoded proteins, or to Cas9 nuclease itself can limit postnatal interventions[19]. Such responses could exclude some patients from initial therapy and many patients from future re-administration. In the fetus, the immune system tends towards tolerance to novel antigens; thus, in utero therapy can result in minimal immune response and could tolerize a patient to future therapies after birth. Second, many relevant diseases are progressive (e.g., lysosomal storage disorders), and some patients have significant morbidity even prior to diagnosis in childhood, infancy, or prenatally, particularly for neurologic conditions in which the onset of irreversible damage often starts in utero. In the fetus, therapies can be delivered early in the disease process, providing greater opportunities to curb the development or ameliorate the symptoms. Third, giving a therapy prior to the formation of the blood-brain barrier could allow systemic delivery of reagents that would not penetrate into the brain postnatally; a prenatal approach could also obviate the need for invasive intrathecal injections of therapeutic compounds, which are given postnatally to access the central nervous system. Finally, the cost of emerging gene therapies is astronomical, in part due to the large amounts of vectors necessary to have a clinical effect. Lower amounts of the vector are required in the fetus due to the smaller size. Given the particular importance of immune tolerance, we present more information on this aspect below; the

other benefits of prenatal therapy are covered during the discussion of specific conditions in Section 5 (Preclinical Data on Gene Therapy).

Immune tolerance: Most modalities of gene therapy involve exposure to a novel antigen, including viral capsid antigens, CRISPR-Cas9 proteins, and transgene-encoded or newly expressed missing proteins. The adult immune system would recognize such novel antigens as "foreign" and mount an immune response. In contrast, the immature fetal immune system can be educated to tolerate novel antigens so that they are recognized as "self" and, with persistent expressions, this tolerance should continue upon future exposure. While this phenomenon has been recognized since the pioneering experiments of Billingham, Brent, and Medawar[20], the immune mechanisms that underlie fetal tolerance were only recently elucidated. Fetal naïve T cells are more likely to become regulatory T cells upon exposure to a novel antigen[21,22], a process that may rely on differences in the epigenetic landscape of fetal T cells compared to their adult counterparts[23].

Tolerance to new antigens: The ability to induce tolerance to allogeneic cells and foreign proteins has been demonstrated in multiple settings such as in mice[24,25] and dogs[26] after in utero HSC transplantation. Tolerance to a transgene-encoded protein was further studied in the sheep model of intrauterine administration: intraperitoneal injection of retroviral vector encoding β-galactosidase in fetal sheep was found to lead to the development of post-natal tolerance to the protein product[27]. Administration of a postnatal booster of the protein demonstrated that lymphocytes from transduced animals had significantly lower in vitro stimulation indices and a blunted ability to form an antibody response to the protein than their non-transduced siblings.

Some of the most extensive data on in utero immune tolerance relates to clotting factors, with tolerance to the transgene-encoded protein demonstrated in both mouse[28] and non-human primate[29] models; these are covered in further detail in the section on Hemophilia.

Lack of immune response to viral capsid antigens: In adults, both innate and adaptive immune responses have been demonstrated to be directed against the viral vectors used in gene therapy. This response can result in serious immune responses, even on the first administration of gene therapy, and impaired uptake and rapid destruction of the gene therapy can reduce efficacy. For example, up to 35-80% of individuals can have

antibodies specific to either the viral capsid proteins or other viral gene products to AAV2[30]. Pre-existing immunity to viral vectors is important to detect prior to therapy, given the potential of inducing life-threatening immune responses[31]. Lentiviruses and other retroviruses are less immunogenic but have still been found to be sensitive to inactivation by human complement[32]. Due to the sterile nature of the gestational sac, apart from viral infections, fetuses have almost certainly never encountered the viral vectors commonly used for gene therapy, and the first exposure in utero is, therefore, unlikely to result in a memory response. It is possible that there could be transplacental passage of pre-formed maternal IgG antibodies against the vector capsid, and initial clinical efforts would test for maternal immunity to the proposed vector and exclude such patients. Importantly, fetal administration does not lead to tolerance to the capsid protein in preclinical models since the exposure is transient. This is likely secondary to the lack of effective immune responses in the fetal environment and has been tested in both mouse[28] and NHP[29] models. However, repeated administration with the same vector does not lead to a memory response[29]. Therefore, if the initial fetal exposure does not result in adequate gene expression, the patient could likely be re-dosed with the same vector preparation. Thus, PSCGT can take advantage of a unique window of opportunity during prenatal development to avoid treatment-related immune responses. Importantly, the available data suggest that PSCGT could allow for repeated administration of the therapy postnatally (in the case of waning therapeutic effect) since the fetal administration would not cause sensitization to the vector capsid protein.

3. PRENATAL DIAGNOSIS:

Prenatal diagnosis and management of pregnancies with fetal genetic diseases have evolved rapidly over recent years. Diagnostic genetic testing has shifted from karyotype and chromosomal microarray to include next-generation sequencing with exome and whole-genome sequencing, revealing a vast array of otherwise undetectable fetal genetic diseases[2,33-35].

Early prenatal diagnosis, at the very least, allows couples to prepare for the newborn and coordinate delivery, postnatal care, and insurance coverage. In addition to prenatal ultrasound, the advent of genetic testing enables the identification of genetic conditions that present with ultrasound manifestations (i.e., Hydrops fetalis, a

common pathway in many single-gene disorders)[2] that might be fatal before birth but might be amenable to prenatal molecular therapies, allowing us to expand the scope of fetal interventions to causal medical therapies (intrauterine stem cell transplantation, intrauterine enzyme replacement or protein therapy, or PSCGT).

Diagnosing single-gene disorders remains a challenge, with prenatal genetic screening routinely done for a minority of conditions (e.g., cystic fibrosis). For inherited disorders, such as hemophilia, expanded carrier screening, and family screening can help identify at-risk fetuses. Genetic screening is more challenging for disorders frequently caused by de novo mutations, including many cases of developmental delay[36,37]. The assessment of genetic disorders without an ultrasound finding represents the next frontier in prenatal medicine. Carrier screening is currently available for common severe diseases such as spinal muscular atrophy and cystic fibrosis. For others, invasive and noninvasive prenatal testing and exome/whole genome sequencing are available. Eventually, advances in non-invasive prenatal testing (NIPT) may facilitate such screening, but until then, clinical features during routine prenatal care, including hydrops fetalis[2] and hydrocephalus[38], can help identify some cases. The availability of life-saving fetal treatment could change the equation for some patients who currently decide against prenatal genetic testing.

4. APPROPRIATE CONDITIONS FOR PRENATAL THERAPY: There are several prerequisites for a disorder to be appropriate for PSCGT.

a) The benefits of PSCGT, over existing therapies or no therapy, outweigh the risks.

b) The natural history of the disorder is known, allowing treatment efficacy to be evaluated.

c) There is an effective strategy to identify fetuses at risk of the disorder through routine screening (e.g., hydrops identified by ultrasound prompting genetic testing to identify a lysosomal storage disease), known family history, or advances in non-invasive prenatal testing (NIPT).

d) Prenatal molecular diagnosis of the disease is accurate, with a good understanding of genotype/phenotype correlations that affect clinical prognosis to ensure a high probability that the fetus would develop the

disorder, and the therapy will reduce this risk. The expanding use and diminishing costs of prenatal sequencing are resulting in an increased frequency of prenatal molecular diagnoses; therefore, this prerequisite is rapidly becoming easier to achieve. Advances in non-invasive prenatal diagnosis (NIPD) are likely to greatly accelerate this process in the coming years.

e) The technical expertise for performing fetal injections (usually into the umbilical vein) and a multi-disciplinary fetal therapy team experienced with all aspects of the pre-and post-operative care must be available. Please refer to section 7 (Procedural risks of prenatal delivery) regarding relevant procedures and risk assessment.

5. APPROACHES TO GENE THERAPY:

The past decade has seen dramatic advances in nucleic acid therapeutics, both in new technologies and in terms of their clinical impact[39]. Various potential therapeutic modalities (ASO, gene replacement, gene editing) using viral and non-viral delivery methods to treat single-gene disorders are being evaluated and applied in pediatric and adult patients.

Small nucleotide therapeutics, including ASOs, and short interfering RNAs (siRNAs), are often delivered by an intravascular or intrathecal route. They can alter mRNA splicing patterns or modify the cellular concentration of specific proteins. For example, Nusinersen (ASO)[40] is injected intrathecally at three monthly intervals from birth to treat spinal muscular atrophy (SMA) by altering SMN2 splicing patterns to functionally rescue the deficit in SMN1. Gene replacement therapy delivers DNA or RNA constructs into cells to encode deficient proteins, most commonly with AAV vectors for in vivo delivery or lentiviral vectors (LVV) for ex-vivo delivery. Gene editing approaches use nucleases such as CRISPR/Cas9 (**C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats/ **C**RISPR **as**sociated protein **9**) to edit specific sites in the genome; they can also change the extent of mRNA transcription to modify the cellular concentration of specific proteins (e.g., CRISPR dCas9 activation [CRISPRa]). Peptide nucleic acids (PNAs) are DNA analogs that can be designed to bind adjacent to a disease-causing sequence in a chromosome. This recruits endogenous cellular editing pathways to remove and repair the

sequence. Co-administration of a homologous wild-type DNA sequence results in the editing of the disease-causing mutation. Due to the use of the patient's own endogenous nuclease pathways, this is a very high-fidelity method of gene editing. PNA/DNA gene editing reagents have been demonstrated to be most effective in stem and progenitor cells[41].

Delivery modalities: The delivery vector can be viral, often AAV or LVV, viral-like particles (VLP)[42], or synthetic (e.g., lipid nanoparticles - LNP)[39]. For example, Onasemnogene Abeparvovec-Xioi[13] is a method of AAV gene replacement that uses a single intravenous dose before the patient is two years of age to treat SMA by delivering a functional copy of the SMN1 gene to spinal neurons. As methods of nucleic acid therapeutics advance, it is conceivable that therapies could be developed for most single-gene disorders, benefiting millions of affected individuals.

Along with the risks to the fetus, prenatal therapy can also pose a risk to the mother, which is further detailed below in Section 6 (Maternal and Fetal Safety Considerations). To justify this maternal risk, prenatal intervention needs to yield additional benefits over postnatal intervention: prevent fetal lethality (e.g., alpha thalassemia), mitigate prenatal disease onset (e.g., SMA), introduce long-term benefits (e.g., inducing immune tolerance to missing proteins, such as in lysosomal storage disorders), ensure a more complete therapy (e.g., treating a higher fraction of stem cells), or cross the immature BBB to deliver a therapy to the brain.

5. PRECLINICAL DATA ON PRENATAL SOMATIC CELL GENE THERAPY:

Extensive prior studies using mouse, sheep and NHP models have provided detailed information on the safety, benefits, and efficacy of PSCGT. In this section, we will highlight some preclinical studies that address the most pertinent benefits of PSCGT, including immune tolerance, efficacy, and crossing the blood-brain barrier. Many of these studies have focused on relevant disorders that highlight the potential advantages of therapy, such as *immune tolerance* (e.g., hemophilia, lysosomal storage disorders) and *early-onset progressive disease* (e.g., lysosomal storage disorders, spinal muscular atrophy, Duchenne muscular dystrophy, cystic fibrosis, and thalassemia). Assuming the first-in-human use would be a reagent that has been tested in the postnatal

environment, recent successes in postnatal gene therapy trials highlight some potential diseases that could benefit from a prenatal correction along with a significant rationale for treating before birth:

Spinal Muscular Atrophy (SMA) is an early-onset motor neuron disease causing profound muscle weakness. It has historically been the leading inherited killer of infants and young children. Caused by recessive, loss of function mutations of the survival motor neuron 1 gene (*SMN1*)[43], retention of the paralogous, but alternatively spliced *SMN2* gene,[44,45] and reduced expression of the SMN protein[46], SMA was uniquely positioned to take advantage of advances in DNA/RNA-targeting therapeutics. Three drugs that increase SMN expression have been FDA approved since 2016 for the postnatal treatment of SMA patients. The splice-switching ASO, nusinersen, given by intrathecal injection[47], and the oral small-molecule risdiplam[48] both target *SMN2*-derived pre-mRNAs to increase exon 7 inclusion. Onasemnogene abeparvovec[49] is a viral gene replacement therapy delivered once intravenously, which uses AAV9 to deliver full-length SMN cDNA driven by a constitutively active promoter. Each drug has a distinct administration route, biodistribution, and potential toxicities, but clinical trials have repeatedly demonstrated that very early administration prior to overt weakness is substantially more effective than when the drug is initiated after symptom onset[50]. This observation led to the addition of SMA to the Recommended Uniform Screening Panel, and currently, most states in the U.S. and select other countries are screening for SMA in newborns. While this has led to earlier treatment initiation, initial experience with newborn screening suggests that a significant proportion of the most common and severely affected SMA type I patients are developing overt weakness in the first weeks of life, before postnatal treatment can be started[51]. This likely occurs because disease pathologies begin *in utero,* particularly in severely affected patients. Indeed, in humans and in mice with severe SMA, impaired motor axon development is evident by mid-late gestation and is followed by very rapid degeneration neonatally[52]. This degeneration causes a release of the neuronal cytoskeletal proteins, neurofilaments (NFs), into the serum, with the highest levels of NFs evident neonatally in both severe SMA mice[52] and type I SMA patients[53]. The timing of this pathology may be dictated by the expression level of the SMN protein, which is normally relatively high during fetal stages and declines postnatally, suggesting that SMN may be particularly needed for normal fetal motor neuron development[54]. These observations argue that *in utero* initiation of SMN-inducing therapeutics could improve efficacy compared to

postnatally initiated treatment. Two studies have explored the feasibility of *in utero* treatment in severe SMA mice. A risdiplam analog systemically delivered to pregnant dams (with exposure of the fetuses) improved disease outcomes of SMA offspring compared to SMA mice starting treatment postnatally[52]. In another study, AAV9-SMN gene therapy delivered by direct intracerebroventricular (ICV) injection to fetal mice improved survival compared to untreated SMA mice[55]. *In utero* treatment with ASOs delivered by intraamniotic delivery has also been shown to be feasible in mice for other disease indications[56]. Together these studies support proof-of-concept that *in utero* treatment of severely affected SMA infants may be feasible and more efficacious. Going forward, further work is needed to bridge the gap from bench to bedside, including large animal studies to determine ideal routes of administration (ICV, systemic and/or intraamniotic), drug biodistribution, and safety for both the fetus and the mother.

Lysosomal Storage Diseases (LSDs) are inherited metabolic disorders characterized by the accumulation of toxic materials in lysosomes due to deficiencies in an enzyme, activator protein, or transporter. Depending on the LSD, one or multiple organs can be affected. Since these conditions are often inherited and result in ongoing organ damage, there is a good rationale for early therapy. Intracerebral injection of an AAV2/AAV5 vector encoding the α-N-acetylglucosaminidase (*NAGLU*) gene has shown encouraging results in children with Sanfilippo B syndrome[57] A gene therapy approach using AAV vectors is also being investigated in children with Pompe disease.[58] Given the incidence of immune reactions to the missing enzyme in many LSDs, an in utero approach to induce tolerance to the enzyme could improve patient outcomes. Importantly, some LSDs result in in utero or neonatal demise due to the development of nonimmune hydrops fetalis, which could potentially be treated or prevented by in utero therapy. The current trials of gene-editing using zinc-finger nucleases for mucopolysaccharidosis types 1 (NCT02702115) and 2 (NCT03041324) could also be promising for in utero applications once more information is available regarding their safety and efficacy.

Preclinical work for PSCGT has focused on neuronopathic Gaucher disease and Mucopolysaccharidosis type I (MPS1). Massaro et al.[59] used an AAV9 vector, which previously facilitated the neuronal expression of a reporter gene, to treat a transgenic mouse model of Gaucher's disease in which untreated neurodegeneration usually

results in death by two weeks. Fetal intracranial injection of the vector improved neuronal inflammation and, remarkably, the overall survival of the mice. Neonatal mice that were treated with intracranial injection also had improved survival, but to a lesser extent than PSCGT, highlighting the benefit of fetal therapy. More recently, mice with MPS1 were successfully treated with prenatal base editing[60]. Mucopolysaccharidosis type I (MPS-IH, Hurler syndrome) is an LSD with a predominant G-to-A (W402X) disease-causing mutation in the IDUA gene. The pathology begins before birth, affecting multiple organs, and infants present by 6 months of age with hepatosplenomegaly, abdominal wall hernias, musculoskeletal abnormalities, retinal and neurocognitive degeneration, and cardiac disease. Treatment options, including enzyme replacement therapy and hematopoietic cell transplantation, are limited, and many children die by ~10 years of age from cardiorespiratory complications. Studies in the Idua-W392X MPS-IH mouse model, which recapitulates W402X MPS-IH disease in humans, demonstrated the feasibility of in utero CRISPR-mediated base editing to correct the disease-causing mutation[60]. Specifically, the mutation was efficiently corrected in hepatocytes and cardiomyocytes with low-level corrective editing in the brain. This was associated with a reduction in glycosaminoglycans in multiple organs, improved cardiac and musculoskeletal phenotype, and improved survival. There is currently an ongoing phase 1 clinical trial of prenatal enzyme replacement therapy for 8 different LSDs (NCT04532047), which include MPS1 and Neuronopathic Gaucher disease. A recent survey of families who have affected children noted that most parents would opt for participation in a clinical trial of a prenatal molecular therapy, including enzyme replacement or gene therapy[61].

Hemoglobin disorders (Alpha or Beta Thalassemia, Sickle Cell Disease): One potential advantage of prenatal therapy for disorders affecting hematopoietic stem cells (HSCs) is the possibility of accessing these stem cells in the fetal liver, before they migrate to the bone marrow. Indeed, it has been demonstrated that systemic delivery of nanoparticles results in tremendous uptake in fetal liver, with successful delivery into HSC[11]: fetal delivery of NPs loaded with PNA/DNA gene editing reagents results in 6-8% editing of bone marrow HSCs. In a mouse model of beta-thalassemia, a single fetal dose of NP/PNA/DNA gene editing corrected anemia, normalized reticulocyte count and peripheral blood smear, normalized spleen size, and architecture, and improved survival compared to affected untreated mice. These results are encouraging for other

hemoglobinopathies (such as alpha thalassemia, in which there is an added rationale for correcting a disease that can be fatal in utero) as well as other beta-hemoglobinopathies such as sickle cell disease.

The hemophilias: A (HA) and B (HB), are the most frequent inheritable coagulation defects, and are caused by mutations in the genes coding FVIII and FIX proteins, respectively. Current treatments greatly increase the quality of life and have lengthened the life expectancy for those affected, but the need for lifelong treatment and associated high cost are far from ideal. In addition, 30% of persons affected by HA have a significant risk of treatment failure due to FVIII inhibitor induction. The safety and efficacy profiles of several AAV vectors for HA[62] and HB[63] are quite encouraging for future in utero applications of this strategy. Despite effective prenatal diagnosis, the availability of a safe and effective intrauterine treatment would motivate prenatal hemophilia screening[64].

There have been numerous preclinical studies of PSCGT using AAV vectors. For example, prenatal plus postnatal re-administration of AAV-1 resulted in long-term expression of hFIX[28,65]. More importantly, the treated mice did not have a cellular or humoral immune response to hFIX, whereas all mice injected at the adult stage developed antibodies to hFIX[28]. A study of PSCGT using AAV in fetal sheep demonstrated that hFIX was present at earlier time points post-therapy. However, hFIX levels dropped as fetal liver and lamb weights increased, and lambs mounted an antibody response after injection of hFIX protein and Freund's adjuvant[66].

Following these proof-of-concept studies in rodent and sheep models, the efficacy of PSCGT with a therapeutic transgene was demonstrated in a preclinical NHP model, where a single intravenous injection of scAAV-LP1-hFIXco in NHP fetuses late in gestation produced sustained, clinically relevant levels of hFIX with liver-specific expression and a non-neutralizing immune response[67]. In a long-term follow-up of this trial, four of six PSCGT-treated animals continued to express hFIX at therapeutic levels (3.9%–120.0%) for more than 6 years of monitoring. These studies also indicated that low-frequency random genome-wide hepatic integration of AAV occurred without evidence of hot spots[68]. PSCGT performed at earlier time points of gestation in NHP using AAV5-hFIX or AAV8-hFIX, demonstrated that animals that received AAV5 exhibited long-term hFIX expression,

but at subtherapeutic levels. By contrast, AAV8 recipients had sustained hFIX expression in the therapeutic range. PSCGT did not sensitize the animals to AAV capsid, as anti-AAV antibodies remained below the positive threshold, and animals did not develop antibodies against hFIX. Nevertheless, when PSCGT was performed at earlier time points of gestation, linear amplification-mediated-PCR analysis demonstrated random integration of AAV sequences in hepatocytes, with no events occurring in or near oncogenic hotspots[29].

While direct delivery of vectors is promising, the use of cells as vehicles for gene delivery allows for copy number and integration-site analyses during product development, which would eliminate the possibility of off-target effects. In utero transplantation of sheep fetuses with human placental cells (PLC) transduced with a LVV encoding a bioengineered high-expression FVIII transgene (mcoET3) resulted in curative and sustained plasma levels of FVIII for at least 3 years after treatment, despite the exponential growth of the sheep with no cellular or humoral response to the human cells or to the FVIII transgene, and no evidence of any lentiviral-related or procedural toxicity in any tissue[69]. Thus, PSCGT has the potential to change the treatment paradigm, providing an early cure for HA and HB, without sensitizing the recipient to the viral capsid or to the cells that deliver the transgene, and preclude the development of antibodies against FVIII or FIX protein.

Monogenic lung diseases such as Cystic Fibrosis (CF): The rationale for prenatal therapy for CF is that the disease begins in utero, with manifestations seen in neonates (impaired male reproductive system, abnormal gastrointestinal development leading to meconium ileus, destruction of the exocrine pancreas.) The transition from in utero to postnatal life, during which adequate lung function is required for survival, highlights the potential benefit of prenatal gene editing for numerous monogenic lung diseases. For example, surfactant protein B deficiency is associated with neonatal respiratory failure and death within months of birth without a lung transplant. Alternative prenatal delivery routes, including intraamniotic/intratracheal or intravascular, provide ways to potentially target different pulmonary cells in developing fetal lungs. In studies of fluorescent reporter mice, in utero intraamniotic injection of viral vectors carrying SpCas9 has been shown to efficiently target pulmonary epithelial cells, including alveolar type 2 cells, the cell population of interest in genetic surfactant protein deficiencies[70]. The application of this approach to the mouse model of surfactant protein C deficiency

highlights the therapeutic potential of in utero pulmonary cell gene editing. The Sftpc^{I73T} mouse model carries a gain-of-function mutation recapitulating a disease-causing mutation in children's interstitial lung disease (18hild), and mice with this mutation die within hours of birth from respiratory failure. In utero CRISPR-mediated nonhomologous end-joining (NHEJ) excised the mutant gene, resulting in improved lung morphology, function, and overall survival[70]. For CF, in particular, experiments using peptide nucleic acids delivered using nanoparticles have given promising results.[71] The next steps in optimizing this strategy include demonstrating if improved uptake yields improved editing and testing of these agents in larger animal models.

7. MATERNAL AND FETAL SAFETY CONSIDERATIONS:

Potential risks of PSCGT have been assessed in large animal (sheep and NHP) models and include the following:

1) The genome of the ***viral vector can integrate*** with the human genome, even with the ssDNA AAV vectors. Viral integration is a common consequence of viral infections, to the extent that about 8% of the human genome[72,73] is composed of viruses that integrated during human evolution; however, each integration carries the risk of disrupting the function of an important component of the genome. The most recent NHP study to examine the extent of integration after PSCGT with AAV vectors, observed integration into somatic cells, but integration into hot spots has not been seen, with no evidence of oncologic consequences in long-term follow up[29].

2) Gene therapy, particularly gene editing, can also act outside of the target gene. These ***"off-target" effects*** may include editing of other regions of the genome, altering the transcription levels of other genes, or altering the splicing behavior of other genes. These risks are usually specific to the particular therapy (e.g., guide RNA or ASO) and can be evaluated in cellular models.

3) ***Germline Transduction:*** one of the earliest studies of whether PSCGT could achieve therapeutically meaningful levels of delivery of an exogenous gene to the hematopoietic system by directly injecting an amphotropic MMLV-based vector into fetal sheep[74,75] resulted in efficient transduction of long-term repopulating hematopoietic stem cells and lifelong presence of transgene-expressing cells within the peripheral blood and bone marrow of the recipients. Polymerase Chain Reaction (PCR) analysis of DNA isolated from tissues of these

animals showed that all tissues analyzed harbored the provirus, including the reproductive tissues, raising the possibility that this approach to PSCGT might place the germline at risk.

As a result of these findings, prevention of these undesired "off-target effects" and increasing the safety of this approach to PSCGT became the focus. Subsequent work showed that by altering the gestational age at which the vector is administered, it is possible to dramatically affect which tissues are transduced and the levels of transduction within those tissues[76]. These changes in tissue tropism as a function of gestational age were the result of developmental changes in the levels of the amphotropic receptor PiT2 (both RNA and protein) expressed on these tissues during gestation[77]. Collectively, these findings raise the exciting possibility that it may be feasible to target desired tissues by simply pseudotyping the vector with an envelope whose receptor is expressed on the desired tissue/cell type and is absent from non-target tissues like the germline.

Off-target germline editing is of concern due to the unintended changes becoming heritable (Section 10: Ethical and Regulatory Considerations for Prenatal Gene Therapy). An important caveat to these studies[74,75] was that the DNA used for PCR analysis was isolated from unfractionated testes tissue. As such, hematopoietic cells within the vasculature of the tissue and multiple types of somatic cells were present within the samples and could have accounted for the PCR positivity. Consequently, the PCR data on the reproductive tissue did not prove conclusively that the germline itself is actually at risk following PSCGT. In the first studies to explore this issue, wild-type MMLV were injected into mouse embryos and showed that not only did the provirus integrate into the germline, but the provirus was then passed to subsequent generations in a Mendelian fashion as part of the permanent genome[74,78-80]. These studies thus provided the first definitive evidence that retroviruses can, in fact, transduce the germline and that this information is passed to subsequent generations. However, those studies used wild-type, replication-competent virus and were performed in early embryos, so they likely grossly overestimated the risk of germline alteration in the context of PSCGT. Nevertheless, myriad studies have now shown that, under the right experimental conditions, germ cells from both small and large animals are susceptible to transduction with viral vectors and that this technology can be used to create transgenics[81-86].

To rigorously address whether the nascent germline is in fact at risk as a result of prenatal gene therapy and determine the frequency at which this might occur, a multi-pronged approach has been used in a highly translational sheep model[87]. Breeding studies were performed, in which female or male offspring after prenatal gene therapy were bred with naïve partners. While the number of resultant offspring was limited because of the use of sheep and the 5-month gestation period, proviral DNA was never detected by PCR in any of the offspring of any of the breeding pairs. The second method used to assess modification of the fetal germline following prenatal gene therapy was to perform PCR on DNA isolated from sperm cells purified using various methods. Performing provirus-specific PCR on OviPure-isolated sperm cells consistently yielded positive results. However, subsequent RT-PCR on the "purified" sperm cell population revealed that the cells all contained hematopoietic contaminants, as all samples contained $CD45^+$ cells. As such, no definitive conclusions could be drawn about whether the germ cells had been modified. However, provirus-specific PCR performed on both forensically purified sperm and on sperm cells purified by fluorescence-activated cell sorting also yielded positive results, providing the first evidence that the male germline is, in fact, at risk following prenatal gene therapy with amphotropic MMLV vectors. IHC studies of sections of the testes with an antibody to the transgene product (NPT II) confirmed transduction of the germ cells and expression of the vector-encoded transgene[87]. However, the incidence of germline alteration was exceedingly small, and alterations were only seen in male recipients. Of the 19 males analyzed, only 6 were PCR-positive for proviral DNA in the purified sperm. Of these 6, only 2 had transgene-positive germ cells in the tissue sections examined, and the incidence of germline modification in these experiments was ~1 in 6250[87].

To evaluate the factors that determine the risk to the fetal germline, detailed IHC studies were done on the testes of fetal sheep at various stages of gestation using markers for germ cells[88]. In that study, germ cells expressing SSEA1 were shown to be migratory and uncompartmentalized until about 70 days of gestation. The germ cells then began expressing SSEA3 as they began the process of compartmentalization within the forming sex cords. Upon compartmentalization, the germ cells began uniformly expressing SSEA4, and continued to do so throughout gestation. These studies used MMLV-based vectors, which require cell division for genomic integration to occur. Staining with Ki67 showed that the male germ cells do not undergo significant cell cycling

until they have become compartmentalized within the sex cords, and they would thus be presumed to be protected from viral vectors by the blood testes barrier. These studies collectively revealed several aspects of male germ cell behavior which likely impact the risk of inadvertent modification following PSCGT: 1) most male germ cells are not, in fact, compartmentalized during early to mid-gestation, and they may thus be unprotected/at risk; 2) male germ cells appear to be largely quiescent until they compartmentalize, so vectors requiring cell division will not transduce them very efficiently; 3) Sertoli cells that form the barrier of the sex cord will likely take the majority of the vector "hit", and 4) germ cells will be most vulnerable to viral vectors (and environmental mutagens) during passive compartmentalization, as cell cycling begins prior to complete closure of the sex cords. It is important to note that these studies were not performed with a vector that is currently considered for clinical use. Thus, the specific risks of a product considered for prenatal therapy should be assessed in the appropriate models to follow ICH guidelines to address the risk of germline integration.

For vectors that do not require cell division for integration, studies conducted in NHPs using LVV showed that the male germline was unaltered, but transduction of female germ cells occurred (also at very low levels), which was confirmed by laser dissection of individual germ cells followed by vector-specific PCR[89]. More recent studies using AAV vectors to perform PSCGT in NHPs[29,68,90] showed the presence of vector and transgene product within the reproductive tissues, often at a fairly high copy number. However, no in-depth studies were done to ascertain whether the germline itself was modified and, if so, at what frequency? Further studies are needed to address this question.

Maternal risks: Maternal safety is a critical consideration in any fetal therapy. Of particular concern is possible maternal exposure to the viral vectors infused into the fetus. An NHP study of PSCGT using AAV5 and AAV8 vectors examined maternal tissues for exposure to the virus and determined that although the virus was detected in the maternal blood early after fetal injection, it resolved by 72 hours[67]. This study also carefully examined maternal oocytes in 7 dams and did not detect any evidence of viral integration into these cells. Exposure to a viral vector may result in maternal immune responses to the capsid protein (it is likely that early trials would exclude mothers with pre-existing antibodies, which could cross the placenta) or to the recombinant protein. We

think that the latter is unlikely when this is an unmodified human protein, since the mother should already be producing – and therefore tolerant to – the recombinant protein. Clinical protocols would include monitoring for the presence of the gene therapy in the maternal blood and for evidence of an immune response.

7. PROCEDURAL RISKS OF PRENATAL DELIVERY:

Any surgical intervention carries a procedural risk. For fetal surgery, the procedures have become much safer after decades of experience with in-utero access. Here, we detail the procedural risks of intravascular and intracerebroventricular injections, the two most relevant routes of delivery.

Intravascular: a systemic injection into the bloodstream is achieved using the umbilical vein, which is a routine procedure for performing fetal blood transfusions for common indications such as Rh alloimmunization, or cordocentesis (also known as percutaneous umbilical cord sampling, PUBS), for diagnosis and therapy. PUBS was first used in 1964[91,92] via a hysterotomy with extrauterine umbilical transfusion. The percutaneous ultrasound-guided fetal blood sampling was developed in 1983[93] and is still the method used today for cordocentesis. Common indications include severe fetal anemia in alloimmunizations. One main limitation of the procedure is gestational age (>18 weeks) because of the technical challenge posed by the size of the umbilical vein. The preferable approach is the ultrasound/doppler-guided puncture of the umbilical vein at the placental cord insertion; in some cases, the umbilical vein (UV) is accessed as it traverses the fetal liver instead of within the umbilical cord. Complications are related to the injury of the umbilical vessels, leading to fetal bradycardia (arterio-spasm) and possible bleeding. These interventions are performed by a multidisciplinary team and take place under ultrasound guidance using a 20–22-gauge spinal needle with a Quincke point. To prevent uterine contractions, tocolytics such as indomethacin or terbutaline are used. Fetal analgesia is also given to prevent fetal pain when the intrahepatic UV is transfused. In rare cases, especially for posteriorly located placentas, fetal paralytic drugs (such as vecuronium bromide) minimize fetal movement. Complications can arise, and the procedural risks for each fetal access is reported to be 1.2% per procedure, with a procedure-related perinatal loss rate per fetus of 1.8%[94]. These risks could be higher for fetuses who are already compromised from their systemic anemia. The overall survival rate of severely anemic fetuses undergoing blood transfusions (often greater than one transfusion per pregnancy) is greater than 90%[95]. As such, cordocentesis is a well-established

procedure in maternal-fetal medicine, is safe in high-volume centers, and has the potential to easily deliver intravascular fetal therapy, including in utero gene and cell therapy. Some therapies may be more effective if given at an earlier stage of development, in which case intraperitoneal or intracardiac injection may be appropriate when access to the UV is not possible. Thus, there are well-established methods for fetal access that can be employed to deliver prenatal somatic cell gene therapies. However, a low but non-zero risk of procedural complications that can result in preterm delivery or, in rare cases, fetal demise must be discussed with each family.

Intracerebroventricular/intraparenchymal: In the post-natal setting, direct delivery of gene therapy to the central nervous system (CNS) is feasible and safe with early evidence of efficacy. For example, an adenoviral vector encoding a functional copy of the L-amino acid aromatic decarboxylase (AADC) gene was delivered using convection enhanced delivery to the midbrain of children with this disease[96]. Imaging and clinical measures were consistent with gene expression and restoration of function of at least a portion of the physiologic pathway. Direct access to the fetal ventricular system in humans has been demonstrated in a few reports for children with a prenatal diagnosis of ventriculomegaly[97]. Although specific issues such as diagnosis of the fetal condition, selection of therapeutic agent, and route of delivery (intraparenchymal, intraventricular, or intracisternal) still need to be addressed, therapeutic delivery in fetuses is technically feasible and offers the advantage of early treatment of diseases, particularly those with a progressive impact on function.

The intraamniotic injection is another promising delivery modality that should be considered due to its low technical risk. For example, serial injection of a therapeutic protein into the amniotic fluid was demonstrated to treat patients with X-linked hypohidrotic ectodermal dysplasia and is now undergoing a phase 2 clinical trial[10]. Intra-amniotic injection of splice switching ASOs in a mouse model has shown therapeutic benefit in a model of deafness[98] and deserves further investigation in other diseases.

8. BALANCING SAFETY AND INNOVATION: Conducting clinical trials of novel somatic-cell gene therapies in utero raises a plethora of challenges, primarily because of safety concerns for the mother and fetus. The paucity

of such trials has led to an absence of the customary safety reporting frameworks, such as standardized definitions and severity grading for maternal and fetal adverse events (AEs). This makes it even more challenging to trial novel therapies in pregnancy.

AEs are important signals in clinical trials. Although they may not necessarily have a causal relationship with the investigational drug, AE assessment allows swift and responsible communication of safety data between study investigators, sponsors, and regulators. AEs should be recorded in medical records and reported to the sponsor and other relevant authorities, after which a decision is made as to whether they meet the regulatory definition of 'serious' and are directly related to the administration of the investigational drug, classified as a serious adverse reaction or SAR. AEs are then graded, which permits comparison of safety signals between clinical trials, whereas for novel prenatal therapies such as we are considering here, it allows decisions around dose-escalation to be rendered more objectively. AE severity is recorded using standard grading criteria, with limitations regarding perinatal conditions[99], but maternal and fetal adverse event terminology (MFAET 1.1)[100] is now available that provides standard definitions and grading specifically for maternal and fetal AEs. This makes a detailed assessment of safety possible, adding to the condition-specific severity grading schemes available for clinical trials of vaccination in pregnancy via the GAIA project[101], the Division of AIDS terminology[102], and the Clavien-Dindo classification for surgical complications[103].

Fetal AEs need to be diagnosed in utero because severe ones can potentially cause a detrimental effect before birth. Consideration of the different fetal organ systems that might be affected by a therapeutic intervention set out new fetal AE definitions that have been adopted by the Medical Dictionary for Regulatory Activities (MedDRA)[104]. The fetal AE grading system is based on Common Terminology Criteria for Adverse Events (CTCAE) criteria, ranging from mild grade 1 with minimal effect on the fetus through grade 5, which is fetal death. For example, fetal development of pericardial, pleural, or peritoneal fluid collection after a prenatal intervention might be grade 2, moderate when it is not life-threatening, but would be graded 4 or life-threatening if the presence of fluid was detected in at least two fetal compartments, called hydrops. In addition, AE severity is graded independently for the pregnant woman and fetus so that a more complete picture of the effect of prenatal

interventions can be understood. For example, anemia in pregnancy might be classified as grade 3 severe for the mother, necessitating a maternal blood transfusion, but could result in fetal compromise requiring urgent delivery, classified as a grade 4 fetal life-threatening event.

Also relevant to prenatal somatic-cell gene therapies is the Neonatal Adverse Events Severity Scale (NAESS)[105], which classifies neonatal AEs into 5 grades (mild, moderate, severe, life-threatening, or death). Severity is defined by the effect of the AE on age-appropriate behavior, basal physiologic functions, and healthcare changes in response to the AE. NAESS was developed through a Delphi consensus process to integrate neonatal terminology and definitions into wider dictionaries undertaken by the International Neonatal Consortium[106,107].

MFAET fills a vital gap in fetal translational medicine research and supports the development of somatic cell gene therapies for the fetus. It is undergoing regular review to align it with ongoing changes in regulatory terminology at MedDRA and CTCAE[108].

Additional Discussion Highlights: There are several other important safety considerations that prompted discussion during the meeting as detailed below:

Endpoint analysis and reporting: The primary goal of the initial first in human application of PSCGT will be not only to demonstrate safety but also to assess preliminary efficacy in patients for whom a given treatment holds a prospect of direct benefit. Since many of the components are likely to be shared PSCGT approaches for different disorders (e.g., vectors, CRISPR-Cas9, mode of delivery), there will be substantial benefits in sharing relevant data, most importantly safety data. We propose that a set of standardized measures be developed for all applications of PSCGT with the aim of evaluating potential immune reaction, fetal health, and maternal health, adverse events, presence of the gene therapy in the maternal circulation, and degree of viral integration in the fetal genome when biopsy material is available. A sampling of the placenta, amniotic fluid, umbilical cord, and cord blood at delivery is useful for routine assessment of these parameters.

Alongside evaluating the safety, molecular and clinical efficacy should also be assessed; however, these will often be specific to single disorders. Assessments of molecular efficacy could include RNA levels, protein levels, or DNA variant mosaic frequency. It is important to consider "natural history" not only in the context of untreated disease but also in the context of disease treated with available therapies, including benefits and risks (and disease outcomes) after post-natal treatment with available therapies vs. prenatal intervention with investigational therapy.

Outcome measures may include measures of clinical benefit such as mortality (e.g., SMA), requirements for adjuvant therapy (e.g., ventilator use in SMA, blood transfusions in thalassemias), adverse event frequency (e.g., seizures), and developmental milestones (e.g., ability to walk at a certain age in SMA), or biomarkers and candidate surrogate measures such as factor IX levels in hemophilia, hemoglobin levels in thalassemias and observations on imaging studies (e.g., degree of hydrops in lysosomal storage disorders). In these evaluations, the workshop participants advocated to consider aspects that are most important to families, as recently reported for the hemophilia community[109].

A stepwise approach: Both established and emerging technologies, including gene editing using CRISPR, may enable more effective correction of genetic disorders in the prenatal period; however, at this time, their off-target effects are less well understood. As such, the workshop participants discussed a stepwise approach to the development and testing of PSCGT for the prenatal treatment of genetic diseases. This approach includes an initial implementation of therapies that are already approved for postnatal use (for example, gene replacement using AAV vectors). This stepwise approach would enable the facilitated development of novel therapies for fetal applications while taking careful and rigorous consideration of the risks and benefits.

8. REGULATORY CONSIDERATIONS FOR PRECLINICAL STUDIES- THE FDA PERSPECTIVE:

Preclinical studies for gene therapy products are conducted to characterize biological activity, the potential for efficacy in the target disease, and safety profile at the intended clinical dose-level range[110]. Preclinical studies to support the initiation of a clinical trial for a PSCGT product are expected to address the same goals while

focusing on the unique considerations that arise from investigational product administration during pregnancy to treat the fetal condition. Given the often-complex procedures required for the delivery of gene therapy products to the fetus, the safety of the administration methods and the delivery devices used are also important considerations.

The diversity of gene therapy products and their therapeutic applications necessitates a preclinical program tailored to the specific product or product class. However, some general factors should be considered when designing preclinical studies for investigational products intended to treat fetal conditions. For example, proof-of-concept and biodistribution studies[111,112] can provide information about the bioactive dose of the gene therapy product following administration, the involved organs and tissues in the fetus and the mother, and level of replication/expression. Toxicology studies can elucidate potential adverse findings attributed to the vector and the expressed transgene, help determine the dose levels at which the toxicities become dose-limiting, the potential for immune activation, on- and off-target events, germline transmission, potential for mutagenesis, and any effects on reproduction. The information obtained from preclinical studies provide support for determining a starting clinical dose that is potentially safe and effective, as well as the dose-escalation scheme and safety monitoring plan for the clinical trial participants[113].

A single “perfect” animal species for performing preclinical studies for PSCGT does not exist. The choice of species depends on the affirmation of biological activity of the specific gene therapy product in the animal. The use of an animal model of human disease, if available and feasible, is desirable. When designing a preclinical program, several assessments can be combined in a definitive study for evaluation of multiple bioactivity and safety outcomes. For example, a proof-of-concept study performed in a pregnant animal model of disease can be expanded to include assessments of the biodistribution, organ and organ-system toxicity, and other safety parameters for a comprehensive evaluation of the product’s effects. Overall, for any chosen product and disease considerations, it is important that scientifically sound principles, current state-of-the art technology, and the 3R’s principles (Reduction, Refinement, and Replacement) be applied when selecting animal models and establishing a preclinical program for PSCGT.

10. ETHICAL AND REGULATORY CONSIDERATIONS FOR PRENATAL GENE THERAPY: Efforts at in utero research and therapy are always complicated by the need to keep both the pregnant woman and the fetus in mind when evaluating risks and benefits. In addition, the role of a parenting partner and/or a genetic father can be ambiguous, particularly with respect to who may give consent for a procedure. Furthermore, federal protections for human research subjects appear to limit the permissible degree of risk to the developing fetus, which also has implications for which of the interested adults may consent to the intervention.

For interventions involving somatic-cell genome editing, risk management[114,115] will require long-term follow-up of resulting children to determine the efficacy of the intervention and, because of systemic administration, to monitor for evidence of off-target activity in the tissues and organs of interest, as well as in other parts of the developing body. A special concern is possible off-target effects on developing gametes, which could affect not just the child who received the edits while in utero but also subsequent generations. Such long-term, even possibly multi-generational follow-up is a task that many investigators, clinicians, and even commercial sponsors may find logistically and financially daunting. In addition, because consent can be withdrawn at any time by the responsible parents or guardians (as well as by the child, once the age of majority is reached), there may well be a significant loss to follow-up among those enrolled in the trials.

Consent and risk tolerance should be viewed in the context of the provisions set forth in 45 CFR part 46, Subpart B, which prohibit anything greater than minimal risk to the fetus if research does not hold out the prospect of direct medical benefit to the fetus (or the pregnant woman herself). In addition, consent is also required from the 'father,' unless he is unavailable or incompetent. This adds complications when the pregnant woman and prospective father disagree, as well as when the intended co-parent is not the father[116].

If the research is permissible, then informed consent will require several topics to be discussed. These include alternative post-natal therapeutic interventions; the possibility that even if successful, there might be a miscarriage, a stillbirth, or a child born with serious health problems; the local standards and applicable hospital

policies for withholding and withdrawing care in cases of severely impaired neonates; and whether late-term abortion is available in case the in-utero therapy goes badly awry.

In 2021, the International Society for Stem Cell Research issued a revised set of global guidelines for regenerative medicine research and clinical care, including, for the first time, a specific reference to in utero genome editing. The guideline states: "Clinical research involving in utero stem-cell-based interventions or genome editing involves risks to both the pregnant woman and the future child, and it should be undertaken only when it offers the prospect of a benefit greater than that of post-natal interventions, does not pose an excessive risk to the pregnant woman, and where there is an institutional capacity for autopsy (in the case of miscarriage or stillbirth) or follow-up (in the case of live birth)"[117].

Gene therapies could offer potentially curative treatment for previously untreatable diseases. As more gene therapies emerge, it is reasonable to expect the overall spending in the class to rise. Nevertheless, making transformative medicine accessible poses unique challenges for providers and payers. As such, concerns about the cost of gene therapy, variable insurance coverage, and insurance policies possibly limiting access to these new therapies despite their potential benefits are gaining terrain worldwide.

Estimating the number of prospective patients treated by gene therapy combined with mathematical models to estimate the cost and the potential financial impact of gene therapy could allow for informed financial decisions about the future of this essential therapeutic class[118]. It also aids the establishment of support strategies to counteract the difficulties some gene therapy patients may face (insurance coverage, access to care, childcare, and financial support during recovery). Current practices around cost-effectiveness calculations and values might need to be adjusted to reach a consensus on how to reward the value created by these therapies. Estimates based on the relation between the cost-effectiveness threshold and the value-based price could warrant the development of innovative payment models as an example of a potential solution for the issue[119].

Furthermore, democratizing information (accurate, actionable, and understandable data) on gene therapies and ensuring transparency as well as a concerted effort from all stakeholders (policymakers, healthcare providers,

insurance companies, and patients)[120] may help identify existing systems that can be leveraged or adapted to facilitate effective dissemination of treatments in an equitable manner.

11. CONCLUSIONS: Critical steps are being taken toward more accurate prenatal diagnosis, characterizing the unique fetal phenotypes of genetic diseases, understanding the genetic variants capable of causing severe fetal disease, correlating genetic variants with postnatal outcomes, and implementing preclinical and clinical studies to investigate additional emerging in utero therapies.

As preclinical studies are translated to human clinical trials[20,21], and drugs approved for postnatal use are being used in the prenatal setting, it is important to review their associated clinical effects, risks, and benefits of treating prenatally, with a clear justification for the prenatal use (more beneficial and/or less risky when compared to the postnatal use), as well as ethical, legal and social implications[121] and the importance of patient involvement in the research and development of clinical trials. Familiarity with postnatal approaches increases the receptiveness to innovative fetal molecular treatments and the likelihood of pursuing fetal therapies in a trial or clinical setting[61].

Prenatal gene therapy to target the somatic cells of a fetus is a novel and promising strategy to improve the survival and comorbidities[122] in patients with a spectrum of monogenic disorders. The increasing number of gene therapy trials available in the postnatal setting, and the benefits shown by in-utero applications in preclinical trials, pave the way for future clinical trials in the prenatal setting. The attention to these conditions and opportunities to inform the medical community about clinical trials offer hope to these families, their children, and future generations.

Multidisciplinary discussions about the merits and risks of this approach with patients, patient advocacy groups, and clinical and bioethics experts define a responsible roadmap for achieving in utero gene therapy in humans. Importantly, further research in this space must be guided by bioethical principles with close involvement of patient stakeholders and consideration of personal utility as viewed by individuals who have experienced pregnancy with a fetal genetic disease.

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