Somatic Cell Genome Editing Program Phase II Planning Workshop

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Purpose

This workshop was designed to explore the current state of the gene therapy field and to identify gaps and opportunities that the Somatic Cell Genome Editing (SCGE) Program could address with targeted efforts during Phase II.

Discussants

External Discussants: Paula Cannon (USC), Murat Cirit (Javelin Biotech), Philip Gregory (bluebird bio), Rachel Haurwitz (Caribou Biosciences), Katherine High (AskBio), Patrick Hsu (UC Berkeley), J. Keith Joung (Harvard/MGH), Anna Kwilas (FDA), Tippi Mackenzie (UCSF), Peter Marks (FDA), Vic Myer (Atlas Venture), William Peranteau (CHOP), Jordan Pober (Yale), Pablo Ross (UC Davis), Zuben Sauna (FDA), Laura Sepp-Lorenzino (Intellia Therapeutics), Megan Sykes (Columbia), Fyodor Urnov (Innovative Genomics Institute), Luk Vandenberghe (Harvard), Amy Wagers (Harvard), J. Fraser Wright (Stanford University), Tim Yu (Boston Children's Hospital and Harvard)

NIH Discussants: Carsten Bonnemann (NINDS), PJ Brooks (NCATS), Marrah Lachowicz-Scroggins (NHLBI), Oleg Mirochnitchenko (OD), Betty Poon (NIAID), Joni Rutter (NCATS), John Tisdale (NHLBI)

Introduction to the Somatic Cell Genome Editing Program

Drs. Joni Rutter and P.J. Brooks, NCATS

The NIH Common Fund's SCGE Program is currently in its first phase (2018 to 2023), which aims to focus on the following gaps and opportunities: (1) informative and predictive animal models, (2) technologies to identify unintended consequences of genome editing, (3) effective delivery vehicles for clinically relevant cells and tissues, (4) safer gene editors, and (5) increased access to advanced technologies. The SCGE Program has launched initiatives to address each of these five areas and aims to continue its progress into Phase II.

Session I: Clinical Trial and Regulatory Innovation

Moderators: Drs. P.J. Brooks, NCATS, and Katherine High, AskBio

Designing Trials for Multiple Indications

Participants discussed whether gene editors can be reapplied to treat multiple indications by changing only the guide RNA. The level of restored expression, and therefore disease burden relief (and perception by the patient), will differ according to the exact guide RNA molecule that is changed.

A company in attendance developed a gene editor platform that specifically targets gene expression in the liver using lipid nanoparticles (LNPs) to induce gene knockouts in the liver. The platform has been found to sufficiently target different areas of the genome using different 20-nucelotide RNAs whose safety profiles and efficacy have been studied in rats and non-human primates (NHPs). However, the off-target profiles of these RNAs can differ and must be assessed robustly. They believe that although one dose may be sufficient, serial dosing is possible with this platform because LNPs are not immunogenic.

Derisking Gene Therapy Trials

Participants emphasized that the possible changes in toxicology and Chemistry, Manufacturing, and Controls (CMC) information, in addition to the high cost of guide RNAs, will complicate facilitation of clinical trials with the same gene editor in multiple indications. Toxicology is largely investigated using model organisms, which may have limited translatability to human toxicology and are expensive. Overall, the components of gene editor studies (particularly, animal models and guide RNAs) are very

expensive, which can prevent researchers from evaluating gene editors and new guide RNAs for rare diseases. Derisking the toxicology and CMC processes such that new guide RNAs can be tested efficiently and inexpensively for rare diseases could be a new avenue for the SCGE Program to pursue. Most sponsor companies will not engage with a researcher who aims to create an antisense oligonucleotide (ASO) to target a rare disease because their trials are more costly and will help only a small subset of patients; however, rare diseases share underlying mechanisms with many other diseases, which may make them more desirable if the shared mechanistic target can be applied to multiple diseases.

Participants suggested development of a collaborative network that would enable researchers to study ultrarare diseases that may not be as attractive for sponsors because of the small number of patients who can be treated. The SCGE Program could select a few projects focused on rare diseases and fund their investigation from preclinical through clinical testing in order to facilitate gene therapy trials for neglected diseases. Participants agreed that such a program would help projects overcome the barriers associated with CMC development.

Creating RNAs that meet good manufacturing practice standards can be expensive, partly because of the limited number of manufacturers. Similar to sequencing methods, the price to develop gene therapy materials will likely decrease over time. Participants agreed that standardization across the development and manufacturing pipelines will also help reduce costs.

Regulatory Processes

Historically, ASO reagents have fallen under the review of the Food and Drug Administration's (FDA's) Center for Drug Evaluation and Research, whereas gene editors have been reviewed by FDA's Center for Biologics Evaluation and Research. From FDA's perspective, the use of a single gene editor across multiple indications is difficult because each indication should be assessed according to tailored outcome measures. The FDA is looking carefully at situations in which different guide RNAs might be used for the same gene in the same disease. The differences between RNA therapies (such as ASOs) are important particularly because gene editors could induce more permanent and significant adverse effects after administration (whereas RNA therapies induce transient changes); FDA is standardizing processes to review such applications. Not all gene editors induce permanent genomic changes; some induce only temporary upregulation or downregulation of a particular gene. Increasing the use of such gene editors may help derisk the process of investigating gene therapies for rare diseases and diseases that require modulation of a particular pathway. The SCGE Program is currently funding one project that is investigating such a gene editor.

The general consensus was that the regulatory approval process could be improved to allow researchers to easily submit updated protocols and reagents (e.g., vectors); in many cases, by the time an FDA application is submitted, the researcher has optimized the original protocol, but cannot submit the newer, better vector, which would require a new, lengthy application.

Necessary Considerations and Guidelines

Participants agreed that the gene therapy field is continuously evolving, and thus all gene editor investigations must be completed thoroughly and carefully to ensure that the intended therapy is safe for future patients. Safety considerations are related to the gene editor machinery itself as well as the materials used (such as an ASO). The general consensus was that the SCGE Program should focus on quantifying the level of efficacy (e.g., ability to restore a gene's function by 10, 50, 70, or 100 percent) needed for a particular gene editor for a specific disease, as well as whether that level of efficacy is sufficient to improve patient outcomes. Participants noted that the gene therapy field is still so new that

guidelines on such studies are not available and that developing these guidelines requires improved models as well as clinical trial experiences.

Session II: Prospects for Advancing in utero SCGE

Moderators: Drs. Oleg Mirochnitchenko, NIH, and Katherine High, AskBio

Current State of in utero Gene Therapy

Fetal surgery has been the traditional approach to correct certain conditions that arise in utero, such as spina bifida. However, not all conditions can be corrected using surgery. Although a prioritized concept within the fetal medicine field for decades, gene editing remains in the early stages of execution because of the complexity of fetal diseases. Some ongoing clinical trials are assessing in utero molecular therapies (including stem cell and enzyme replacement therapies) for diseases that are fatal in utero, such as alpha thalassemia. According to FDA regulation, in utero trials can only assess agents that are approved for postnatal use, and the guidelines to facilitate these studies are in initial stages of development.

Postnatal to in utero Translation

Participants emphasized that postnatal treatments may not be appropriate for treating diseases in utero. Whereas postnatal treatments are optimized to address disease that can be prevented or slowed after birth, diseases that require in utero treatments may be too far along at birth and cannot be prevented or slowed at that time. Further, many diseases that would benefit most from in utero therapies cannot be studied after birth because the fetus either did not survive to birth or died shortly thereafter, preventing these patients from being assessed using postnatal therapies.

However, use of FDA-approved postnatal therapies in utero has the advantage of well-established safety profiles. Under FDA restrictions, the SCGE Program could initially investigate gene editing therapies for diseases that are treated postnatally but cause irreversible damage before birth, such as lysosomal storage disorders. Companies aim to study agents and therapies for adult populations and then gradually test them in younger populations and then in utero. Some of these therapies have been tested during postnatal periods when the immune system is immature and less reactive; earlier testing may encounter even fewer immune system complications and provide lifelong tolerance to the affected gene. Participants recommended that the SCGE Program investigate in animal models the ability for gene editors to surpass immune system complications through in utero delivery.

The SCGE Program is funding a project at the Oregon Health and Science University that will develop NHP reporters for eventual use in in utero testing of gene therapies.

Biodistribution and Delivery

The general consensus was that understanding the biodistribution of gene editing agents is critical for implementing gene therapies during fetal development. FDA will be concerned about biodistribution of a gene editor. Gene editors offer the advantage of leaving marks within the genome that can be readily identified by next generation sequencing and reporter methods (which can be used to assess biodistribution).

Fetal therapies can be administered very early (13 weeks gestation), but the timing of administration must be tailored to the trajectory and development of the disease of interest. Knowledge of family history enables researchers and clinicians to test fetuses in order to detect a condition as early as possible and administer necessary therapies. Participants agreed that more projects should focus on

how the distribution of gene editors is affected by different delivery methods and whether the delivery method must be paired to specific periods of fetal development. There is not sufficient data on different routes of administration in small and large animal models; however, some experiments in NHPs have shown that gene editors can broadly distribute. In addition, participants emphasized the need to assess adeno-associated virus (AAV) vectors and LNPs for their behavior during in utero administration. One of the very important aspects of in utero genome editing that should be addressed is a maternal safety.

The blood–brain barrier is not fully formed in utero, and thus gene editors will be less likely to encounter central nervous system tissues if administered after birth. Another significant advantage of in utero genome editing is a low dose of the delivery systems due to the small size of the fetus/targeted organs. Participants noted that gene editors could be administered intrafetally or intraventricularly. Sheep are commonly used to investigate in utero therapies because the fetuses are similar in size to humans and sheep do not experience preterm births. In contrast, pigs commonly experience preterm labor and the fetuses are more difficult to access. As an advantage, a significant number of disease models in pigs can be used at the preclinical stage. Guinea pigs have the most similar placenta to humans and therefore may be the best model to test placental transfer of gene editors into maternal circulation.

Pilot Study of in utero Gene Therapies

Participants suggested that the SCGE Program initiate a pilot study in animal models to evaluate gene therapy for a few indications that likely require in utero intervention (i.e., diseases that have progressed too far at birth to be alleviated or prevented at or after birth) and share data with FDA to assess whether in utero gene therapy approval is warranted. Participants endorsed this suggestion and recommended the following indications as possible foci: Tay-Sachs, alpha-thalassemia, retinal dystrophy, epilepsy, spinal muscular atrophy, and congenital muscular dystrophies.

Participants agreed that, in any SCGE Program in utero testing study, rigorous assessments should be made in large animal models to assess distribution in the fetal genome, whether the gene editor transfers to the maternal tissues, delivery optimization to specific fetal tissues and cells, and whether the gene editor induces germline mutations.

Future Directions

Participants agreed that treating a disease before it takes full effect in the fetus or infant is an important goal for the gene therapy field. Participants emphasized the need to evaluate whether redosing is required during later stages of fetal development or later in the individual's life and whether each of those stages requires different dose concentrations.

Participants discussed the possibility of sequencing fetal genomes early in development to identify actionable mutations that would most benefit from gene editing corrections; however, testing all 4 to 6 million pregnancies that occur in the United States each year may be financially infeasible. Instead of sampling the fetus directly, these sequences could be generated using fetal DNA found in maternal serum samples, which may be more financially feasible.

Session III: Gaps and Opportunities in Basic Development and Discovery

Moderators: Drs. Betty Poon, NIAID, and Vic Myer, Atlas Venture

Enzyme Development

Participants agreed that the field of gene editing has expanded significantly since the development of CRISPR methods, which has helped to address the early-on limitations regarding enzyme availability.

Thus, enzyme development is not as high priority as it was even a few years ago. Researchers have developed and made available many enzymes and optimized these reagents to enable targeted editing; however, reagents for specialized approaches, such as base, prime, or transcriptional editing, or with optimized activity remain a development priority.

Immunogenicity and Redosing

Participants agreed about the need to study immunogenicity when developing editors, particularly when redosing is considered. Upon redosing, the immune system may attack the gene editor, and thus second doses may need to be immunologically distinct from the first doses to ensure that the immune system does not become primed to a specific editor. Investigation of methods to enhance immune shielding of gene editor reagents is critical.

The field aims to dose patients one time and not require redosing. However, single doses are not always sufficient to reach the necessary efficacy; thus, redosing may be necessary and the field should investigate methods to effectively redose without significant immunogenicity.

Zinc Finger Platforms

Participants discussed whether the field should reprioritize other materials with less immunogenicity potential, such as zinc finger nuclease (ZFNs), even though ZFNs cannot perform all gene editing types, particularly base editing. ZFN sequences are significantly smaller than those of Cas9 (600 base pairs compared to 4,000 base pairs), which benefits delivery and reduces manufacturing needs; thus, a consortium effort to develop zinc finger–based gene editor models or other non-CRISPR platforms may benefit the gene therapy field.

Delivery Methods

Participants emphasized the need for gene editing platforms to provide efficacious enzyme activity and to be delivered to the target tissue or cell type effectively. Whereas the enzyme development aspect of gene editors has flourished, the development of effective delivery techniques, with the appropriate level and specificity of editing, has lagged and can result in negative in vivo studies, despite having an optimized enzyme. Polymeric nanoparticles and normothermic perfusion can be used to test whether gene editors can effectively target specific cell types.

Participants agreed that studies to develop optimized delivery methods should be a priority for the SCGE Program and that every new delivery method should be tested for safety in both in vitro and in vivo models. Participants suggested investing more funds into developing more reliable pharmacokinetic assays for use in safety assessments, particularly dosing calculations.

In vivo and in vitro Models

The field of gene therapy should also investigate how in vitro and in vivo models translate to human delivery and safety. Participants agreed that human organoid models pose a unique opportunity to overcome the known translatability issues related to animal models (including that NHP genomes are not identical to human genomes). Organoid models may not be able to model delivery as well as animal models because the organismal context is lost.

Reversibility and Permanence

During the first session, participants discussed methods that induce permanent genomic changes and those that induce transient changes. Most gene editors induce permanent changes, unless researchers aim to eventually perform an additional gene edit to reverse the genomic change. Altering gene

expression, instead of genomic sequence, offers the potential for reversibility without requiring permanent editing of the genome.

Participants generally agreed on the need for experiments that determine the level of the gene edit that persists over time following a transient gene edit exposure in NHPs. Such experiments could reveal the relationship between duration of the exposure and potency of the gene edit and ways to enhance that relationship for clinical benefit.

Standards for Discovery and Validation

Validation assay sensitivity is limited by the error rate of next generation sequencing methods, and thus, although sufficient, current techniques can be improved upon.

Session VI: IND-Enabling Preclinical Tools

Moderators: Drs. P.J. Brooks, NCATS, and Fyodor Urnov, Innovative Genomics Institute

Interrogating Genetic Diversity

Regarding gene editor data package submissions, participants considered whether FDA requires one or both of the following components: (1) long-term follow-up data (in order to assess genetic diversity) and (2) innovative assay development and assay data that enable evaluation of the impact of genetic diversity. Most sequencing projects do not use genetically diverse cohorts, which may lead some gene therapy studies to not address diversity properly.

The cost of whole genome sequencing is decreasing rapidly and is quite small in comparison to the overall clinical development costs; participants discussed whether whole genome sequencing should become routinely incorporated into gene editor studies. Several participants expressed caution at this idea, noting the difficulties in accurately predicting off-target editing. FDA currently requires long-term follow-up assessments but has discussed the possibility of requiring sequencing of subjects (which may become more feasible as the costs of sequencing methods continue to decrease). Genetic diversity can also impact an individual's immune system reactivity in response to gene editor machinery. Even within individuals, genomic sequences can vary from cell to cell.

Participants agreed that the necessity of genetic sequencing should be viewed in the field's current context. Currently, sequencing the genomes of every subject in a clinical trial is not financially feasible, and the information obtained from those analyses cannot reveal the functional consequences of a given gene therapy's off-target effect. Different areas of the genome are more efficiently targetable by gene therapies, whereas some areas are difficult to reach; genomic sequencing cannot provide information on this factor of gene therapy research. The benefit of a given method must outweigh the risks and off-target effects of the gene editor. However, genomic sequencing provides a limited view into the possible efficacy and safety profile of a gene editor.

Methods

Participants emphasized the need to develop scalable assays prospectively before the need for specific assays arise instead of reactively developing assays in response to an immediate need. These assays could be used to generate actionable information, for example, connecting a patient with the right gene therapy.

Assays must also be developed to look at off-target effects in other tissues and cells. These assays could use humanized mice and machinery components. Humanized mice are a vital resource but have their limitations, for example when the mice have only a humanized immune system.

Biobank

Participants recommended the establishment of a national gene editing registry and biobank that would collect samples and information across the lifespan of individuals who have been administered gene therapies.

Unintended Consequences of Gene Editing

Participants agreed that better methods are needed to assess off-target effects that could result in the worst-case scenario effects (e.g., mutations that cause cancer) and that every study should robustly assess and validate assay results to identify detrimental off-target effects. Evaluating the mutational susceptibility of specific cell populations in a given indication should be required to determine whether the gene editor will induce more harm than benefit before it is translated to the clinic.

Session V: Issues in Immunogenicity

Moderators: Drs. Marrah Lachowicz-Scroggins, NHLBI, and Paula Cannon, USC

Lack of Immunogenicity Data and Tools

Participants emphasized the lack of published data related to the immunogenicity of gene editor machinery and the need to better understand immunogenicity, particularly in relation to the immune components at play in different indications. Currently, all information related to immunogenicity is speculation, and thus the true challenges associated with immunogenicity are not fully known (making mitigation strategies difficult to conceptualize). Participants agreed that the best method to begin addressing immunogenicity is to use antibody assays; however, the sensitivity of these assays and the animal models used to evaluate immunogenicity may not be currently optimized to provide the level of information needed to move the field forward.

Current knowledge about gene editors and the immune system is primarily focused on adaptive immune system responses, whereas the effect of the innate immune system is largely unknown. Participants agreed that the field does not currently possess assays that can effectively assess the innate immune system responses to a gene editor and whether those responses change over time or in response to redosing.

Addressing Immunogenicity Challenges

Participants recommended addressing immunogenicity by improving current gene editing machinery to consist of less immunogenic vectors and by assessing the optimal level of immune suppression required to allow specific gene editor/vector combinations to be effective. A company in attendance has used histamine and dexamethasone to suppress the immune system during redosing and recommended that the SCGE Program perform experiments to assess the level of immune suppression required across multiple gene editor methods.

T-cell and B-cell assays have improved significantly in recent years, enabling more informative immune response assessments. Studying secondary lymphoid organ samples from treated animal models would support more comprehensive evaluation of the immune responses to gene editors and vectors.

Some patients may have a primed immune system that may more robustly attack a gene editor or vector after an initial dose, which complicates redosing. Participants agreed that experiments are needed to assess the immune response effects after transient gene editor exposures, noting that the experiments could be completed in animal models (likely pigs) with humanized immune systems and investigate multiple delivery methods. In addition, knockout animal models could be incorporated into these experiments to assess particular aspects of the immune system in relation to the response to gene editor machinery and correct sequences. The biobank resources discussed previously could provide the tissues needed to assess T-cell responses after gene editor administration.

Tolerance

Immune responses can be viewed in two phases: (1) an early innate immunity response to the vector and enzymes in the gene editor machinery and (2) a later T-cell-driven response to the corrected gene sequence. The general consensus highlighted the need to develop tolerance-inducing approaches that prevent the T-cell response from occurring and impacting the product of the gene corrected by the gene editor. Participants recommended humanizing each of the gene editor machinery components to help induce tolerance to the adaptive immune response. Participants agreed with this recommendation but noted that immune responses can be damaging to the eventual corrected gene product and thus immune responses to the machinery are not the only responses to consider.