MDF Genome Editing Workshop
April 17, 2018
Workshop Report

The Myotonic Dystrophy Foundation (MDF) sought input from a group of experts on the development of a genome editing initiative for myotonic dystrophy type 1 (DM1) in a workshop held on April 17, 2018, in Sausalito, CA. The strategy for MDF is to fund one or more labs to support early-stage genome editing research that targets the dystrophia myotonica protein kinase (*DMPK*) gene. Labs will be selected through a competitive application process (Request for Applications (RFA)) that includes review and selection by a project advisory committee. The advisory committee will include individuals with basic, translational, and clinical development expertise in genome editing technology and/or myotonic dystrophy. The advisory committee will also advise on strategy and content of the RFA and provide oversight and guidance of funded investigator(s) during project conduct.

This report contains a detailed account of those proceedings to guide the MDF in design and implementation of the initiative and to serve as a resource for applicants.

Participants

- Elizabeth Ackermann, Ph.D., Chief Science Officer, MDF
- Dana Carroll, Ph.D., Professor, University of Utah
- Tom Cheever, Ph.D., Program Director, NIAMS/NIH (by phone)
- Irina Conboy, Ph.D., Associate Professor, University of California, Berkeley
- Bruce Conklin, M.D., Professor & Senior Investigator, University of California, San Francisco
- Ed Conner, M.D., Chief Medical Officer and SVP, Sangamo Therapeutics
- Cristina Csimma, PharmD., Senior Advisor, Exonics Therapeutics
- Graeme Fielder, Senior Manager, Audentes Therapeutics
- Kyle Fink, Ph.D., Assistant Adjunct Professor, University of California, Davis
- Kevin Flanigan, M.D., Director, Center for Gene Therapy, Nationwide Children's Hospital
- Woodie Kessel, M.D., MPH, Board Chair, MDF
- Anna Kwilas, Ph.D., Biologist, CBER/FDA (by phone)
- Larry LaBonte, MDF Community Member, donor
- Darren Monckton, Ph.D., Professor, University of Glasgow
- Lianna Orlando, Ph.D., Scientific Program Officer, Muscular Dystrophy Association
- John Porter, Ph.D., Consultant, MDF
- Kathryn Shaw, J.D., MDF Community Member, donor
- Nicolas Wein, Ph.D., PI, Center for Gene Therapy, Nationwide Children's Hospital
- Molly White, Chief Executive Officer, MDF
- Gene Yeo, Ph.D., Professor, University of California, San Diego and Locana Bio

Purpose of Workshop, John Porter

To incentivize and initiate the use of genome editing technology for DM1 and ultimately identify a development candidate that can be moved into IND-enabling activities and clinical testing. Approach is to focus on early stage discovery and development while learning from more

mature programs currently moving into the clinic. The approach is strategy, platform and delivery vehicle agnostic as MDF seeks to learn from this Workshop and the community as to the best path forward independent of being directed by current IP. Focus is on launching a program that considers the best tools and processes to develop a therapy for DM1. The goal is to get to a therapeutic that is not incremental, but has a substantial level of effect.

MDF and Myotonic Dystrophy type 1 (DM1), Lisa Ackermann

The burden of disease was discussed and a video "Challenges of Myotonic Dystrophy" was shown.

Understanding the Target/DM1 Overview, Darren Monckton

The key issues for development of therapeutics for rare diseases are broad availability of diagnostics and patients for trials. Availability of an accurate molecular diagnosis is a critical hurdle for rare diseases—DM1 is in good shape internationally as far as diagnostics. Comment that when the first company takes a therapy to the clinic that results in increased patient engagement with therapy development process.

DM1 exhibits extreme variability in phenotype—variability in symptom patterns, disease progression, etc. contribute towards the variability. CNS issues (cognitive, daytime sleepiness, fatigue) contribute to an increase in already existing muscle loss of use. Most critical need in DM1 is to treat CNS and skeletal/cardiac muscle.

DM1 shows anticipation—typically see a decrease of 20-30 years in time of onset from one generation to the next (correlate is that the lifetime of the disease in a given family ultimately leads to early lethality that ends future generations).

Disease mechanism is expansion of CTG repeat sequence in 3' end of *DMPK* gene (5-35 in general population, 50 to thousands in patients). Difficult to assess repeat length—PCR problematic and Southern blots are needed (challenge with Southerns is limited material available from muscle biopsies). Repeat length affects disease severity, but really see a spectrum of symptomology rather than strict classifications based on repeat length. Repeat is unstable in somatic tissues and can show increases over a subject's lifespan.

Number of repeats that are actually inherited has the best prognostic value. Repeat number as determined from peripheral blood is the best predictor of disease. Wieringa group CRISPR paper (van Agtmaal et al., 2017) represented first attempt to remove repeat by genome editing. As long as excision involved cuts from both sides of repeat sequence, good excision resulted. Cutting from just one side this actually increased the instability of repeat. Highlights the somatic cell instability of repeat length.

All DM1 patients have CTG expansion exceeding a threshold length. The vast majority of patients (90-95%) have pure CTG expansions, but remainder have variant repeats—short tracts of CCG among the CTG expansion as one example--that modify the severity of the disease (delayed age of onset by 10-15 years); one idea is that these alterative repeats could be introduced with genome editing to modify the disease. One line of thinking is that the variant tracts result in stabilization of repeat and reduction of somatic expansion of repeat length.

DMPK is in a gene dense region of Chr 19 (14 genes in this 250 kb region) and these genes are close together—this becomes a challenge for genome editing strategies. Little alternative splicing is seen in the *DMPK* gene itself. Large group of CpG islands associated with DMPK that show epigenetic modification in CDM. Little genetic variation is seen around DMPK locus—primary variation is the CTG expansion itself; for normal population, high levels of linkage disequilibrium with 3 haplotype alleles (5 repeats, 7-18 repeats, and 19-40 repeats; most families have A1 haplotype). Participant comment—based on DM1 genetics, best thing to do would be to delete the entire repeat sequence.

Very low degree of SNPs immediately around the *DMPK* CTG repeat; thus a single set of guide RNAs would likely address most patients. Alternatively, don't have many variants that distinguish the mutant chromosome from the normal, so can't use these to target allele specific cuts.

DM1 pathogenic mechanism involves an RNA gain of function. *SIX5* gene is turned off in patients with expanded repeat; likely causes haploinsufficiency resulting in cataracts in *Six5* ko mice. These findings suggest that only a small component of DM1 symptoms can be related to DMPK repeat expansion having an impact on *SIX5*. *Dmpk* knockout mice don't have a severe phenotype, suggesting that reduced DMPK protein function does not play a major role in pathogenesis.

The DMPK mutant transcript is trapped in the nucleus, where it forms hairpin structures; these, in turn, bind Muscleblind (MBNL) protein, sequestering it and rendering non-functional. MBNL is a splicing regulator and, as a result of sequestration, several hundred genes are mis-spliced.

Multiple models have been used in mechanistic and preclinical proof of concept studies. The Six5 knockout, DMPK knockout, MBNL knockouts, and CELF1 over-expresser have been valuable in these studies but don't have value for genome editing studies. The HSALR mouse has insertion of CTG repeat tract under the HSA skeletal muscle promoter, but lacks the human *DMPK* gene context and thus not appropriate for genome editing studies. The DMSXL mouse was derived from a DM1 patient sequence (thus has human genomic environment around *DMPK* locus) with 55 CTG repeats; this was first expanded to 300 repeats and subsequently to >1000 repeats. The DMSXL repeat is somatically unstable and the mouse has a severe, early onset disease phenotype. This model has the advantage of having the intact human DMPK sequence and disease phenotype.

Since there's no mystery of what causes human disease, having a mouse model that mimics disease phenotype is less important—alternatively need the appropriate molecular model to show target engagement and modulation. Overall, having a molecular phenotype and molecular readout is likely the most important factor for a mouse model to be used in a genome editing discovery and development project. One comment though—for FDA, will need an efficacy model to establish a minimally effective dose and phenotypic modulation is important here. Yet, having the starting point of a mouse model that facilitates evaluation of target engagement and modulation is essential for a genome editing effort. Mice that could meet this need are the Thornton/JAX 300 repeat knock in (used CRISPR engineered approach) to be available soon and the MDF/JAX DMPK BAC model under development (700-1200 repeat). Large animal GLP toxicology may not be needed for regulators. Important to work with FDA early though; the CBER pre-pre-IND process is available to sponsors.

The availability of multiple human cell sources—iPSCs, primary cells, and hESC—can facilitate early discovery and optimization of genome editing strategies for DM1.

Opportunities and Challenges in Development of Genome Editing Therapeutics, Ed Conner

Focused on lessons learned from use of ZNF technology for genome editing in a variety of indications at Sangamo. Core strategy is to use diamerized ZNFs to yield an 18 base pair targeting sequence to bind DNA and add function (ZNFs don't cut and delete). Example: the Sangamo Huntington's Disease (HD) program uses ZNF strategy to deliver a gene repressor (i.e., blockade of transcription).

In vivo genome editing presents a series of challenges. AAVs are used as delivery vehicle of choice, but the packaging capacity of AAV (need to get ZNF and transgene) has strict limits. Moreover, most AAVs are highly trophic for liver or other organs —skeletal muscle trophism, as needed for neuromuscular indications, is a challenge. AAV9 is brain trophic and can potentially address CNS issues in DM1, but currently requires either intrathecal administration or a vector with blood-brain barrier permeability. Sangamo (and others) are reengineering the AAV vector capsid to address the delivery issue—can potentially get ubiquitous tissue trophism and delivery. Sangamo and others also are working on BBB permeability for AAV.

AAV immunogenicity also represents a substantive challenge. Segment of the general population has neutralizing antibodies (very high for AAV6). If necessary, re-dosing also represents a challenge for AAV beyond those patients that don't have preexisting immunity for the first dose. Lipid nanoparticles may help address these challenges (initial dosing and re-dosing), but would represent a long-term solution.

In an alternative strategy, Sangamo is testing a key base deletion strategy that results in increased expression of a gene for beta-thalassemia. The program utilizes a high throughput screening approach to determine precision of targeting, specificity, and efficiency of genome editing and optimize these traits. One consideration for this program is the need to follow treated patients for life to understand long-term effects of insertional mutagenesis (long term follow-up is essential for any gene therapy or genome editing program).

Sangamo's ZNF platform approach has used new linkers to yield a 300-fold increase in design options for targeting a particular genomic sequence. For HD, they can't knockout HTT because it serves multiple functions. So have designed a ZNF repressor that is allele specific (optimization program has allowed discrimination of smaller differences between repeat length in normal and mutant allele). This approach gives them the ability to address as many as 80% of HD patients. They are also working on modifying AAV9 to add blood-brain permeability to facilitate systemic administration. Efforts on maturation of the HTS capability are underway to allow more screening options at cellular level.

Overall, focus is that the key genome editing issues are delivery and long-term effects (that the editing technology has come a long way and focus should be on sufficient delivery to minimize immune and off target effects—although some others feel that further advances in editing technology are essential). One advantage of ZNFs is that they are human proteins and thus may not have the problems that long-term expression of Cas9 (a bacterial protein with pre-existing immunity in a large proportion of the human population); humanizing Cas9 is another option that Bruce Conklin is working on.

There is a clear interaction between delivery and genome editing efficiency—to facilitate somatic therapies for diseases like DM1, editing efficiency and delivery have to be really optimized.

Evaluating and Benchmarking Genome Editing Programs, Cristina Csimma

Focused on how to get to a therapy via a process that solicits and triages applications in order to determine which are the best shots on goal. It is crucial to identify and fund the right experiments from the very beginning to triage out those strategies that don't have translational potential (development tractability and business model). How to get into the clinic the fastest is not the right question to ask. Instead, the overall objective has to be getting treatment for largest number of patients.

DM1 is at the confluence of a building environment for rare disease drug development and recent gene therapy advances. Small company involvement in rare diseases has increased the opportunities for DM1 to get industry engagement in drug discovery and development and to move therapy programs faster. Innovation tends to happen in biotechnology companies, not large pharma. The diligence process for an MDF RFA has to be whether the applicant and study design are doing appropriate diligence to attract subsequent company investments. No one is going to do this on their own—it is critically important to leverage relationships between patient organizations, academia, industry and government (NIH/FDA). Venture philanthropy strategies are important in the respect that foundation can participate in early de-risking and triaging in order to guide programs.

Need to ask the key questions early and often. Is the preclinical data showing relevance to clinical disease? Need to take the industry approach of walking away when something doesn't work. Academia has been way ahead of industry in terms of gene therapy and has made key contributions to genome editing. The right attitude in launching a drug discovery and development effort is that it's not about the science, but rather about the strategy. Having a fundable team, preclinical data that support disease rationale and credible business model (can't think purely in the lab-based model—need to focus on data and milestones) is the path forward.

Important to consider manufacturing issues early in the development of a genome editing program, as scale-up of production can be rate-limiting for pre-IND activities and early stage clinical trials. For this class of therapies, the manufacturing process is the product. For the product, must have high quality, sufficient yield and low COGs. Need to build in as much de-risking as possible into program, so that early stage go/no-gos ensure that the program doesn't go down a rabbit hole. Because of the importance of product availability, it is critical to engage a manufacturing consult to make sure that the approach is scalable.

The preclinical program requires high quality, clearly interpretable and reproducible data. Conduct (or certainly repeat) every killer experiment in independent CRO to maximize de-risking. Important to ensure that preclinical program is set up to inform clinical trial design.

Clinical considerations have to be taken into account early. It is important to differentiate the program to be launched from others (patient has to perceive that there is an improvement, not simply an endpoint result that is signaled by a p value alone). For DM1, need to understand how observed splicing improvements correlate with long-term clinically meaningful benefit. Need to monitor genomic region—understanding whether other genes in DMPK region are altered (or

may be altered by the intervention). Need to determine whether clinical practice is consistent with ability to run the trial. Community education is a key issue with high risk therapies such as genome editing—plan for this early. Important to ask which disease domains can be targeted in DM (indexes that measure multiple organ system impact will likely be important in DM1); also, ask what is time to clinical impact?

Early clinical proof-of-concept studies have to be designed to facilitate transition to pivotal study. From the beginning, ask, how far can MDF take a project on its own—MDF can manage the incubation phase, but after that?

Regarding the RFA. In reviewing these applications, have to not only look at the science, but ask multiple other questions; need a review team with expertise focused on these multiple considerations. Each study proposed needs to have an actionable outcome that can be milestoned (need results-oriented milestones, not simply activity-based milestones).

Definition of a Cure Discussion

The stated purpose of the MDF genome editing initiative is to develop a cure for DM1. The consensus of the discussion defined a cure as a therapeutic that halts or slows progression of disease. The key questions then become: how to evaluate candidate therapeutics at each stage of development and how does each experiment inform us about the potential success/failure of the program. Bottom line: need to insure that candidate genome editing therapeutic has the potential to exert a clinically meaningful level of effect on the symptomatology of DM1 and need to avoid engaging in programs that are not tractable for clinical development and commercialization.

Discussion #1: Genome Editing Strategy/Optimization

- Among the current and evolving genome-editing strategies, what options should be considered for DM1? How should expanded repeat DMPK target properties shape development of a genome-editing program (repeat sequence, repeat instability, sequence variation at locus, allele specificity, etc.)?

Key advantage of DM1 is that the repeat is in a non-coding region—using genome editing to remove the repeat would result in removal of the pathogenic mechanism. Suppressing expression of the repeat or of DMPK might be better (avoids cutting DNA and potential downsides there), but potentially requiring multiple treatments over time and thus not a cure. Need to assess early on whether editing out the CTG repeat sequence can be efficient—if this can't be done efficiently, that's a no go.

High efficiency editing has been obtained (B. Conklin) for multiple cell types of importance for DM1—neurons, skeletal muscle, and heart (with non-homologous end joining (NHEJ). Some controversy here (I. Conboy) as to whether efficient editing can be obtained via induction of double strand breaks.

One concern is the gene-rich *DMPK* locus (see D. Monckton talk). Deletion of CTG repeats will have to be precise and not impact other genes in region (or regulatory regions of these genes). Will require in silico and experimental validation of off target effects on neighboring genes. RFP

must note that whether allele specificity is needed and what effects the selected platform and strategy may have upon DMPK and adjacent genes will have to be considered in research plan

Base editing strategies using AAV delivery could work for DM1, but would have to work out which base to target. Necessary to target very long repeats though. With germline editing, would not have to change many repeats, but there are ethical and regulatory issues at this time that would preclude germline editing—not feasible at this point. Have to consider specificity of approach toward *DMPK* CTG repeats and avoid other CTG repeats in genome.

Delivering Cas9 protein instead of nucleotide sequence has advantage of exposure time at target tissue—protein will have a shorter half-life and thus less susceptible to generating off target edits. Could use RNP complexes to deliver Cas9 protein—this approach would likely avoid issue of humoral immunity to Cas9 (with AAV delivery of Cas9, some cell types may present Cas9 antigen on surface and activate cell mediated immune responses).

Delivery and distribution issues. Direct injection into muscle not viable strategy—far too much muscle mass to treat, thus requires systemic delivery via AAV or alternative. More regulatory experience available for AAV9 vector and heart, skeletal muscle, and brain are targeted. Need an AAV vector to be regulated (deliver once and then turn off after editing is complete) in order to avoid long target exposure that could lead to off target editing.

DM1 is autosomal dominant—raises possibility of allele-specific targeting (rather than bi-allelic approach). Both are likely potential directions since some studies show that DMPK protein may not have an essential role.

Is targeting upstream of *DMPK* a potential strategy? Unlikely to be effective since expansion is in non-coding region and may still be transcribed and generate splicopathy that is characteristic of DM1.Targeting an early exon might turn off transcription and thus have a positive effect. Targeting DMPK promoter could reduce expression of adjacent Six5 gene, which likely could be problematic although full consequences are currently unknown.

Using deactivated Cas9 in DM1 cell and animal models has been effective in binding the repeat and shutting down expression (Pinto et al., 2017). Unclear if impact on adjacent genes at the DMPK locus has been evaluated. Comment was made that exon skipping strategy has been implemented in Duchenne, but whether that could have value to turning off translation of repeat sequence in DM1 is unclear.

Targeting the expanded repeat RNA is another option. Cas13 proteins have been shown to bind and result in digestion of toxic RNA (Batra et al., 2017). Approach has the advantage that the longer the repeat sequence, the more efficient on-target suppression of translation becomes. Also addresses disease mechanism without resulting in permanent change in somatic cell DNA and the potential consequences that may have.

The Sangamo approach and experience with other indications suggests that the ZNF platform strategy may also be applicable in DM1.

Appeared to be a consensus (even with very long repeats, > 1000) on cutting out the entire repeat sequence, sparing the DMPK promoter. Potential damage to the other allele may be mitigated by data indicating dispensability of DMPK protein (although that likely needs to be verified further). Ability to use ZNF arrays to improve specificity is unclear for long repeat sequences that need to be edited here. For allele specificity, would need to avoid normal repeat

lengths and cut only those exceeding a threshold level—potentially difficult. Agreement that would have to use adjacent sequences and cut outside the expanded repeat region to remove the entire sequence.

Prior data using CRISPR/Cas9 in DM1 models (van Agtmaal et al., 2017) showed that cutting from one side of the CTG repeat increased the instability of remaining repeats—thus a dual cutting strategy is required for DM1. Dual cut generating blunt ends has been shown to result in efficient repair. One concern is that the regulators don't know what to do with dual cut strategies yet. A key concern that has to be overcome is whether dual AAV transfection is necessary or whether necessary sgRNA and Cas9 can be packaged into a single AAV vector. If packaging for a dual cut in a single vector is not possible, that is problematic for the approach—many cells might be transfected by one vector only, receive single cuts and have their repeat sequence further destabilized with the potential consequence of accelerated pathogenic process in those cells. B. Conklin has done dual cuts as a single approach for spinal cord and eye delivery, so dual cut approach may be feasible. Delivery of dual sgRNAs and Cas9 might be facilitated by the recent development of smaller Cas9s. Key issue then would become delivery (nanoparticle delivery is a potential option where vector carrying capacity would be moot, but nanoparticle is in early stages of development and likely not feasible as a clinical strategy for some time).

Issue was raised of whether performing genome editing using HDR is off the table. Response was no, and that it might be an ideal approach. However, real question for use in DM1 is need to edit post-mitotic cells.

- What is the plus/minus on the reagent platform options available for DM1 (CRISPR, dCas9, homing nucleases, TALENs, ZNFs, RNPs, etc.)?

Agreement that the editing platform doesn't matter as they all can make the necessary double strand breaks. Platform choice will be impacted by delivery issues (and TALENs are harder to package).

Proteins packaged in RNPs raise different manufacturing issues. GMP material is easier to make and scale-up than for viral vectors. Although scalable, in a disease requiring whole body delivery such as DM1, production costs of viral vectors become considerably less.

- What characteristics should preclinical DM1 efficacy models and endpoints have? Adequacy of current models?

With development of high-risk therapies such as genome editing, FDA will require animal efficacy. Suggestion that an animal model endpoint that directly correlates to the disease is essential. Ideal would be to use humanized animal model but if not available could use a mouse model with a surrogate species-specific drug to support starting clinical dose.

Comment that human muscle/organ chips (such as those developed by NCATS) can be used to focus studies on a specific tissue (I. Conboy). While such cultures may help with optimization of DMPK gene targeting, they do not provide necessary data on dose ranging. FDA representative suggested that organ chips may be a long way off in filing niche occupied by animal models.

Suggestion that having a rodent and non-rodent model would be valuable (B. Conklin). FDA suggested that many biologics go into clinical trial without two-species data, but this depends on nature of the product and associated risks.

- What strategy(ies) would you prioritize for genome editing in a repeat expansion disorder like DM1? What other issues have not been discussed yet?

Immunosuppressants have a role in gene therapy and genome editing candidate therapeutics that will use AAV delivery. This strategy is applicable in patients with existing immunity to a specific AAV serotype and for potential re-dosing (if necessary) in all patients. May be an important issue in the future but moot at this time since those with existing immunity are being excluded from trials and re-dosing is not on the table yet.

Overall, it is virtually essential to have the human locus in a mouse model as a driver to genome editing in DM1. At the start of a genome-editing project, the mouse is not essential as considerable optimization can be done in available human cell lines. The DMSXL mouse, as well as the Thornton/Jax mouse, are options for the DM1 genome editing project as the BAC transgenic development and validation is completed at Jackson Labs.

Discussion #2: Choice of Delivery Vehicle

- How would you approach choice of a delivery vehicle/route of delivery in the multi-systemic disease target environment of DM1? What is the plus/minus of different delivery vehicles (AAV, nanoparticles, others)?

Use of RNP conjugates (Cas9 protein and sgRNAs) is a potentially good delivery approach, particularly when dual cut strategy is necessary for DM1, but is developing technology and potentially pretty far off. Non-integrating lentivirus is also an option, but less regulatory experience with these. Considerable biologic therapy development and regulatory experience with AAVs makes that the vehicle of choice for the DM1 program. Will need primate safety data though as part of IND-enabling activities.

Eric Olson data in Duchenne shows good level of effect in skeletal muscle, heart, and diaphragm (disagreement among workshop participants regarding level of effect achieved in those studies). While that data is in pediatric population, treating adults has raised more questions (sufficient vector production, etc.). Audentes and others are working on these issues and they are likely to be resolved by the time DM1 candidate moves to the clinic. Agreement was reached among participants that *at this time*, AAV is the most advanced vehicle and would be the best choice.

- Is it feasible to target multiple organ systems with genome editing technology or should a program in DM focus on specific organ(s)/tissue(s)? If seeking broad bioavailability does germ line editing become a concern?

The SMA gene therapy (AveXis) delivered intrathecally was originally delivered systemically (IV) to target skeletal and cardiac muscle—AAV, particularly with further modifications, can potentially target multiple organ systems. Issue then may become whether that opens the approach up to gonad bioavailability and germline editing. FDA wasn't sure how unintended germline editing might be viewed, although intentional germline targeting was not permitted at this time. To minimize off-target editing, Cas9 protein needs to have short periods of expression and then downregulated—ways to do this with AAV vectors (auto-methylated promoters is one approach). Key factors in off-target editing are concentration and temporal properties of exposure at target tissue. Whole body delivery, while addressing the multi-organ system

involvement seen in DM1, raises concerns about tissue specific regulation and germline exposure.

Overall consensus was that nanoparticle and RNP delivery of Cas9 conjugates may well hold potential as future therapeutics, delivery of genome editing reagents using an AAV vector is the strategy of choice for the MDF program.

- How should exposure/bioavailability be assessed in early stage program development (measuring/benchmarking drug level at target)?

In order to establish preclinical proof of concept, one component is showing that the candidate therapeutic accesses and modulates the molecular target. With genome editing, traditional pharmacokinetic studies do not apply. Because the pathogenic mechanism is so well understood for DM1, measurement of splicing correction in target tissue is a solid surrogate of drug availability at target. Splicing changes can serve as an effective, early milestone, but do not replace impact upon functional measures as required by regulatory agencies. Measurements of myotonia (by EMG) or other physiological/functional measures should augment a splicing biomarker. Ultimately, regulatory approval of a BLA will require modulation of an endpoint(s) that is/are clinically meaningful to the patient.

Discussion #3: Safety and Efficacy Evaluations

- How do we design a program to best develop confidence in the safety and efficacy of a candidate genome-editing product? What do we know about off-target concerns with current and evolving genome editing strategies? How to define and quantify specificity and off-target editing? What predictive toxicology assays should be considered for a genome-editing program?

Specificity is a key issue in determining on-target versus off-target genome editing. Genome-wide measurements will provide a clear indicator of off-target editing. The Workshop consensus was that such assessments are not problematic to undertake. A NIST consortium has focused on qualification assays for various stages of development of genome editing products and offers considerable advice on detection of off-target editing (https://www.nist.gov/programs-projects/nist-genome-editing-consortium).

The NIST Consortium identified several issues to be considered in safety evaluations:

  - Cleaving the desired target in the genome - specificity issue
  - Adverse effects of genomic DNA cleavage at on- and off-target site
  - Adverse effects of gene mutation introduced by endogenous DNA repair activities
  - Overall chromosome instability
  - Inadequate assembly of a donor gene in the genome
  - Adverse impact of the vector delivery system (e.g., insertional mutagenesis potential)
  - Factors underlying risks of germ line editing--biodistribution to gonads an immediate no go

Off-target editing is a function of concentration and exposure time of genome editing reagents in the target tissue. Unregulated expression of Cas9 with AAV vectors can have dramatic off-target

consequences. A key safety issue is to design reagent delivery using vectors that can be turned off. While nanoparticle delivery is another option that addresses these safety issues (delivery short half-life RNAs), nanoparticle delivery is not as advanced and may not be available for use in genome editing for some time.

In evaluating off-target editing, a focus on secondary targets may not be of benefit. The key here is not simply an assessment of the frequency of unintended editing of a specific target, but the identity/nature/role of the off-target gene/genes that are involved.

Given the gene-rich environment at the *DMPK* locus, it is essential to evaluate the impact of an editing strategy on the neighboring genes. One strategy to identify or exclude such effects is to make knockout mouse models of each of the five genes in the vicinity of *DMPK* and assess functional consequences.

Currently unclear what percentage of cells will need to be edited to have an impact in DM1.

Genome editing using viral vectors for delivery will require a standard set of toxicology studies currently in use for gene therapy product development. These include long-term toxicology studies. Funding should be available to support such long-term studies, including use of blinded pathologists to verify toxicology data. Preliminary toxicology studies should be an early part of any program to detect obvious issues prior to moving toward expensive GLP toxicology studies.

- What are the immune response concerns (e.g., vector, Cas9, etc.) for a genome editing program and how to factor these into program design and conduct? Additional issues from whole-body treatment of adults with DM1?

Immune response issues for AAV vector serotype as well as the demonstrated potential for pre-existing Cas9 immunity (both cell-mediated and humoral) are important considerations for design and conduct of genome editing discovery and development programs. The ZNF platform relies on human proteins and thus does not have the same immune concerns as bacterial Cas9. Modified Cas9s are under development and thus represent still another option.

Early consideration (at preclinical stages) should be given to the patient population that will be engaged in early stage clinical trials. Risk-benefit calculations are different when planning pediatric versus adult subject trials.

- What are the traits of appropriate cell and animal models for activity/specificity screens and proof of concept studies? To what extent can preclinical studies inform efficacy, specificity, and safety? What types of studies provide the best information?

Cell models have to utilize derivatives that are of sufficient maturity to express the gene and repeat expansion to be of utility. NIH rigor and reproducibility standards should apply to all studies.

Discussion #4: Brainstorming on Strategy for Incentivizing Research in Genome Editing for DM1

- Does the proposal to develop an RFA make sense? Other strategies to consider? What are the prerequisites (knowledge and resources) for launch of a genome editing program for DM1? What elements should be included in an early stage genome editing program

for DM1? Evaluation criteria: what does a rigorously designed study look like? What are the critical steps, go/no-go milestones, and time line for a genome editing program for DM1?

RFA approach is logical/appropriate. A very specific risk-benefit outcome is desired here—need to decide desired benefits and acceptable risks and use these to guide the discovery and development effort. Because of the absence of de novo mutations, a meaningful cure for DM1 has the potential to virtually eliminate the disease.

RFA should ensure flexibility for applicants. Will require a considerable outreach effort to attract applicants with appropriate expertise. RFA should have well fleshed out goals to identify programs with the potential to move forward. Include information on the tools available—mice, cell lines, and other resources—in order to facilitate applications from those not already in DM space. Need to ensure that applicants address IP issues and attempts to attract VC funding and industry partners, including plans to protect IP generated during the course of an MDF award. MDF should actively seek to develop partnerships that will be essential to clinical development and commercialization. Program planned here has similarities to venture philanthropy programs that some foundations have launched.

Can learn from NIH Common Fund genome editing initiatives. Six, very focused RFAs—NIH did a workshop, understood what was needed and clearly explained exactly what they would fund. MDF should look at potential to attract funding partnerships from other triplet repeat expansion disorders, where efficacy and safety measures may be similar.

Consortium, along the lines of NIH center grants, was one suggestion for the MDF effort. Concern here is that funding level doesn't fit with funding multiple PIs working together. Consortium approach would be more plausible if funding level was on NIH center grant level ($5M). Consortium approach also may dilute the intent to develop champions for genome wide editing in DM1.

Availability of animal models to assess molecular and phenotypic level of effect will be essential for this initiative. Will need to understand what level of splicing changes is associated with phenotypic improvement.

Workshop participants suggested that rigidity on page limits and other logistics that characterize NIH initiatives not be used here. Since genome editing product production is a key issue at every stage of preclinical and clinical development, suggestion was made to utilize CMC consultants in assessing applications.

Milestones for funded projects can be broadly written as long as endpoints are well defined and quantifiable and should provide for clear go/no-go decision making.

MDF should have "shelfing"/"clawback" provisions in all contracts to recover progress and IP for abandoned programs.

The Workshop included a long discussion of applicability of germline editing for DM1 (well-defined genetic mechanism, autosomal dominant, germline expansion/anticipation well known for disease). Arguments are moot at this point since there are regulatory and ethical barriers to germliine editing that may not be resolved for some time. FDA representative ruled out intentional germline editing, but had not been asked before about unintentional germline editing—although that's also likely to be a no-go.

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