Regulatory Changes and Partnerships will Enable Gene Editing Technologies as a Platform-based Therapeutic Approach

CRISPR Cas9-mediated Genomic Editing

It has long been postulated that the genetic code containing the instructions for life could be precisely edited to correct and cure human disease. That dream began the transition towards reality with the breakthrough 2012 publication that first demonstrated use of the CRISPR-Cas9 endonuclease system.[1] Since then, CRISPR-Cas-based genome editing technologies have changed virtually every facet of basic and applied biological research, holding the potential to push both medicine and science into an age of innovation unlike any seen previously.

The CRISPR-Cas9 effector nuclease, from a class 2 bacterial CRISPR system, consists of a small guide RNA (sgRNA) and the Cas endonuclease.[1,2] By modifying and controlling the nucleotide sequence of the guide RNA, the artificial Cas9 system could be programmed to target virtually any DNA sequence. Thus, by utilising the CRISPR-Cas9 system, delivered to a host cell either through viral or nonviral mechanisms, distinct insertions, deletions, or point mutations can be introduced at any loci of interest in the host genome through DNA repair mechanisms.[3]

The Clinical Promise of CRISPR-Cas9 Based Gene Editing

Given the power and utility of CRISPR-Cas9-based genomic editing, it has been utilised extensively in basic as well as translational research to better understand gene function and to ultimately explore clinical targets. Studies utilising both in vitro as well as *in vivo* experimental models have generated compelling and promising results against a vast list of target disease indications.

In the context of cancer, CRISPR-mediated knockouts of genes essential for regulation of the cell cycle and drug resistance have been performed to modulate disease.[4,5] In other studies involving neurological disorders such as Huntington's and Alzheimer's Disease, CRISPR-Cas9-mediated editing was used to suppress the pathogenic expansion of the HTT gene or correct a pathogenic allele of the presenilin 1 gene (PSEN1), respectively.[6,7] Other research areas such as immunology, cardiology, and hematology have all benefited from the application of CRISPR in preclinical models.

Cumulatively, the experimental evidence from preclinical studies suggests that successful genome editing can be accomplished using the CRISPR-Cas9 system, which has in turn facilitated translation and clinical evaluation of this technology.[8] As a result, clinical trials utilising CRISPR technology has exploded with both *ex vivo* and *in vivo* gene editing approaches being explored in multiple therapeutic areas.[9]

Complex Biology and Regulatory Systems Favor Safe Plays

Historical guiding principles in drug development have focused on the underlying pathogenic mechanisms of disease and how to target the biological process to bring forth a favourable clinical outcome. One significant limitation to this approach is that the etiology of a disease can arise from multiple, diverse, and in many instances, multi-faceted underlying biological mechanisms that are impossible to co-target in today's developmental and regulatory framework.

In melanoma, for example, the activating V600E BRAF mutation has been thought to be central in melanomagenesis.[10] Tumors bearing this mutation exhibit sensitivity to downstream MAPK signalling inhibition, representing an attractive therapeutic strategy.[11] However, this mutation is only present in about 50% of clinical cases.[10] Likewise, in frontal temporal dementia (FTD), multiple pathogenic mutations have been described that ultimately result in the onset and subsequent progression of this debilitating disease. Here, genetic abnormalities in C9ORF72, progranulin, and microtubule-associated protein tau are highly prevalent in FTD.[12]

Further, it has now been recognised that FTD is related to and shares clinical, pathological, and genetic features with amyotrophic lateral sclerosis (ALS) as 17 common genes have now been linked with susceptibility to familial forms of ALS and FTD.[12] Thus, a percentage of patients with an ALS diagnosis may exhibit some degree of frontal lobe dysfunction and likewise, a percentage of FTD patients will exhibit motor neuron symptoms associated with ALS.[12] These represent situations where the traditional approach of linking a single biological mechanism to a single disease state is not feasible, which makes development of therapeutics for these indications more complex from a development, and regulatory standpoint.

An even more dire situation exists for rare genetic diseases, where the development cost of a therapeutic is not supported by a favourable business case based on the number of eligible patients. In the case of Dedicator of Cytokinesis 8 (DOCK8) deficiency, which is a combined immunodeficiency, only 230 cases have been described to date.[13] DOCK8 manifests as recurrent infections, autoimmunity, and malignancy through the loss of function of the protein that arise from pathogenic mutations in the DOCK8 gene.[13] While regulatory approval paths for therapeutics to treat rare diseases have been relaxed to incentivise drug developers to address these unmet medical needs, significant investment is still required to develop convincing preclinical data and CMC packages worthy of regulatory review and subsequent approval for clinical evaluation.

Taken together, the biological complexity of underlying pathogenic mechanisms that drive disease progression, combined with the sheer cost and economics of drug development, creates an unforgiving and somewhat skewed landscape for the development of novel therapeutics. The

current regulatory and economic structure compels drug developers to select prevalent underlying disease mechanisms in high-impact indications with sufficient case numbers to justify the massive investment required to bring a new drug to market. Thus, substantial therapeutic voids are created, especially prevalent in rare disease syndromes or uncommon genetic mutations in more common disease types, leaving these patients with limited or even no therapeutic options.

CRISPR-Based Therapeutics Represent a Plug-and-Play Opportunity for Rapid and Transferrable Development

CRISPR-based approaches have the potential to change this current regulatory and economic mantra and shift the therapeutic focus from targeting a single underlying mechanism of a disease to identifying the patient-specific genetic abnormality. The CRISPR system itself is a platform-ready technology in that delivery agents and endonuclease can remain constant while only the sgRNA changes based on the sequence to target. Thus, for patients with an FTD/ALS diagnosis, the underlying pathogenic genetic mutation(s) identified would dictate the sequence(s) of the variable sgRNA of the CRISPR-Cas9 cassette. Similarly, the sequence of the sgRNA in melanoma would be dependent on the presence of the activating mutation V600E BRAF or other prevalent, patient-specific upstream mutations that have been identified.

The therapeutic flexibility afforded by CRISPR-Cas9 has already started to appear in novel strategies to address complex and challenging human diseases in both preclinical models, as well as early phase clinical evaluation. In one study, for example, CRISPR-Cas9 was utilised to knockout endogenous T-cell receptor (TCR) genes and exchange patient-specific neoantigen TCRs for the personalized treatment of solid tumours.[14] Similarly, preclinical studies in Alzheimer's Disease showed that the dysregulated Aβ metabolism has the potential to be targeted by CRISPR-Cas9 through the correction of varying patient pathogenic genetic mutations that have been linked to either sporadic or familial forms of the disease.[15]

The potential of a CRISPR-Cas platform system, where most components are conserved and only the sgRNAs are changed to address different or multiple indications, is immense and unprecedented. However, there are still hurdles to address to realise this potential.

Platform-Based Approaches and Industry Partnerships Can Streamline Regulatory Approval

With this CRISPR-Cas9-fueled revolution of highly personalised therapies showing such promise, the regulatory framework governing advanced therapeutics must evolve and change to ensure these novel ideas and technologies have the best chance at clinical translation. Center for Biologics Evaluation and Research (CBER) Director Dr. Peter Marks shares the concern that innovation in cell and gene therapy is outpacing regulatory process, creating a bottleneck for patients who are in desperate need.[16]

As an indication that such an evolution is possible, one need only look to the COVID-19 pandemic and the non-traditional mechanisms used to support development and eventual regulatory approval of diagnostics and vaccines. Operation Warp Speed (OWS) was a three-way partnership between the U.S. Department of Health and Human Services (HHS), industry, and the Department of Defense (DOD) that facilitated vaccine, diagnostics, and therapeutics in response to the COVID-19 pandemic.[17] The cornerstone of OWS was using primarily nonclinical data to move investigational solutions forward through clinical trials and, eventually, product approval in extremely compressed timelines.[17] Marks' vision is to utilise the lessons learned from OWS and apply this concept to rare diseases to facilitate therapeutic translation.

Founded in October of 2021, the Bespoke Gene Therapy Consortium (BGTC), like OWS, is multigroup partnership between the National Institutes of Health (NIH), the U.S. Food and Drug Administration (FDA), life science companies, and nonprofit organisations. The BGTC serves to develop platforms and standards that are intended to facilitate the development and clinical evaluation of gene therapies for rare diseases.[18] Even though Marks is a huge proponent of this approach, he makes his cautionary view apparent that the platform approach intended to facilitate therapeutics development is a "large task," and advises that smaller tasks need to be accomplished to achieve the overarching goal.[19] These smaller tasks are heavily focused in de-risking the clinical development and manufacturing to get novel therapeutics, like CRISPR, over the finish line.[19]

One potential and viable avenue to de-risking multiple aspects of the development process is through strong academic, industry, and regulatory collaborations to bring together and leverage top tier expertise in all aspects of therapeutic development. To illustrate this point, the Innovative Genomics Institute (IGI), an institute composed of California Bay Area's leading scientific research institutions, required assistance with the production of an ultra-low endotoxin cell penetrating Cas9 endonuclease for in vivo gene editing by direct injection into the brain. The system is designed to provide gene editing in the central nervous system for the treatment of neurodegenerative diseases without the common setbacks of viral-based delivery of the CRISPR platform elements.

Given the extremely sensitive application of injecting a Cas9 directly into the central nervous system, the IGI approached Aldevron for assistance with the endonuclease manufacturing project. Aldevron, an industry leader in Cas9 expression and purification, applied their extensive expertise to this project to identify and optimise a high expression method to produce a high yielding, ultra-low endotoxin Cas9 protein. The time lapse, from initial discussion with the IGI until Aldevron delivered the final product to IGI for evaluation, was less than 30 days. This collaboration led to the landmark publication of Stahl *et al.*[20]

During the study, the authors noted that the production of an ultra-low endotoxin Cas9 protein manufactured by Aldevron prevented microglial cell response and reduced humoral immunity at 21 days post injection.[20] Collectively, the transient Cas9 RNPs demonstrated equivalent editing of neurons when compared to Cas9 delivered by AAV-9 and thus injection-based delivery of minimally immunogenic genome editing is a viable alternative to virus-mediated genome editing.[20]

CASE STUDY:
ENABLING NOVEL CRISPR-CAS9 DELIVERY THE CHALLENGE

In July 2022, Dr. Jennifer Doudna, Dr. Elizabeth Stahl, and the team at Innovative Genomics Institute (IGI) approached Aldevron for support of a collaborative research program entitled, "Correction of Neurological Disease via Allele Specific Excision of Pathogenic Repeats." In this effort, an interdisciplinary team of biomedical scientists and clinicians from UC-Berkeley's IGI, UCSF, and the Ohio State University endeavoured to use CRISPR-based genome editing to advance therapeutic strategies to address two devastating diseases: Huntington's disease and amyotrophic lateral sclerosis.

In vivo editing of somatic cells promises to be the next wave of therapies for many genetic diseases. However, the delivery of these therapies remains a significant challenge. Recombinant adeno-associated virus (AAV) serotype 9 has greatly succeeded in gene therapy. Yet, many drawbacks remain, including costly customisation and manufacturing, immunogenicity, and limited cargo capacity. Additionally, crossing the blood-brain barrier with gene editing modalities remains an issue.

The IGI-led team is developing a non-viral delivery system that can effectively edit neurons with minimal immunogenicity to treat diseases such as HD and ALS. The Doudna laboratory already had a cell-penetrating Cas9 variant that showed promise. However, evidence of dose-limiting immune response due to high endotoxin levels from their in-house Cas9 endonuclease production led the group on a journey to find an improved manufacturing process for their delivery system.

The IGI-led team needed a custom, highly pure, ultra-low endotoxin nuclease. And they needed it quickly.

The Action

The IGI team needed a tag-less custom Cas9 variant for its cell penetrating functionality. The Aldevron stable of "off-the-shelf" products were not an option. Relying on 10+ years of experience in custom protein manufacturing, the Aldevron team got to work.

Once the construct was designed and synthesized, Aldevron applied our expertise in Cas9 technology. We identified a high expression method and optimised our purification method for this construct. Knowing the goal of ultra-low endotoxin, the team included in-process endotoxin testing on fractions after first and second-step chromatography. In-process testing is a gold standard with FDA's process analytical technology (PAT) initiative. This allowed us to identify the best quality product to move forward.

From initial discussion to delivery of final research-grade product was < 30 days.*

The Results

Over the following year, the IGI-led research team conducted experiments on mice. They recently published results in Molecular Therapy, where they concluded:

These transient Cas9 RNPs showed comparable editing of neurons and reduced adaptive immune responses relative to one formulation of Cas9 delivered using AAV serotype 9. The production of ultra-low endotoxin Cas9 protein manufactured at scale further improve innate immunity. We conclude that injection-based delivery of minimally immunogenetic CRSPR genome editing RNPs into the CNS provides a valuable alternative to virus-mediated genome editing.[20]

Figure S10A in the paper describes the large reduction of endotoxin in the Cas9 endonuclease using the Aldevron-produced protein versus the lab standard. It displayed 0.035 EU/mg as compared to 0.2 EU/mg, enabling dosage below the 0.2 EU/kg/hr FDA threshold for intrathecal delivery.[21] This study showed the translational potential of an ultra-low endotoxin RNP as a cost-effective method.

Aldevron is looking forward to continued support of this research, including producing cGMP grade material for later phases of this study.

* Timelines vary. This is an atypical result. Contact Aldevron to discuss a more accurate timeline for your project.

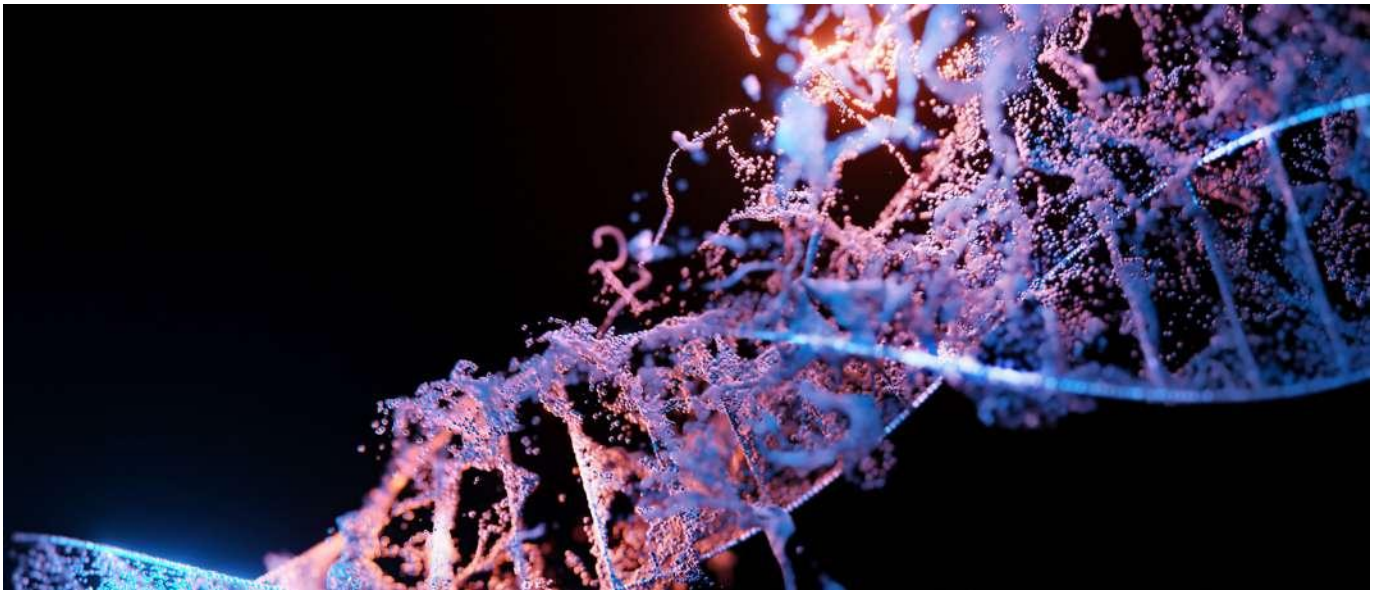
Concluding Remarks

CRISPR-Cas9-mediated gene editing has the potential to personalise therapeutics like never seen before. Through simply modifying the sgRNA sequences in the system and keeping the enzyme and delivery method the same, platform CRISPR-Cas9 can expand the reach of druggable targets to levels not seen in modern science and medicine. Thus, the genomic platform-based therapeutic approach has the potential to fundamentally change the status quo approach from treating the disease to treating the patient.

The realisation of this dream, however, is a significant task. Close partnerships and collaboration are absolutely required between academia, industry, and regulators to coalesce the expertise needed to ensure translational success of this as well as other nascent technologies. An established CDMO, such as Aldevron, can play in instrumental role both in manufacturing and in regulatory support for Cas9 enzymes and RNP complexes.

With a recently announced partnership with Integrated DNA Technologies (IDT), Aldevron can now support gRNA ordering to be complexed with either stocked nucleases or custom-manufactured enzymes. To deliver a therapeutic CRISPR RNP** Aldevron uses a variety of in-house methods to quantify the amount of complexed RNP, free-Cas9 and free-gRNA, during cGMP manufacturing. In addition, our proprietary release panels are compliant with 21CFR210-211 and designed to meet current draft guidance from the FDA regarding gene editing, which provides consistency of the final product.

** Aldevron provides RNPs only to customers who are duly licensed, including to make and have made RNPs, for their intended use.



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