William Blair

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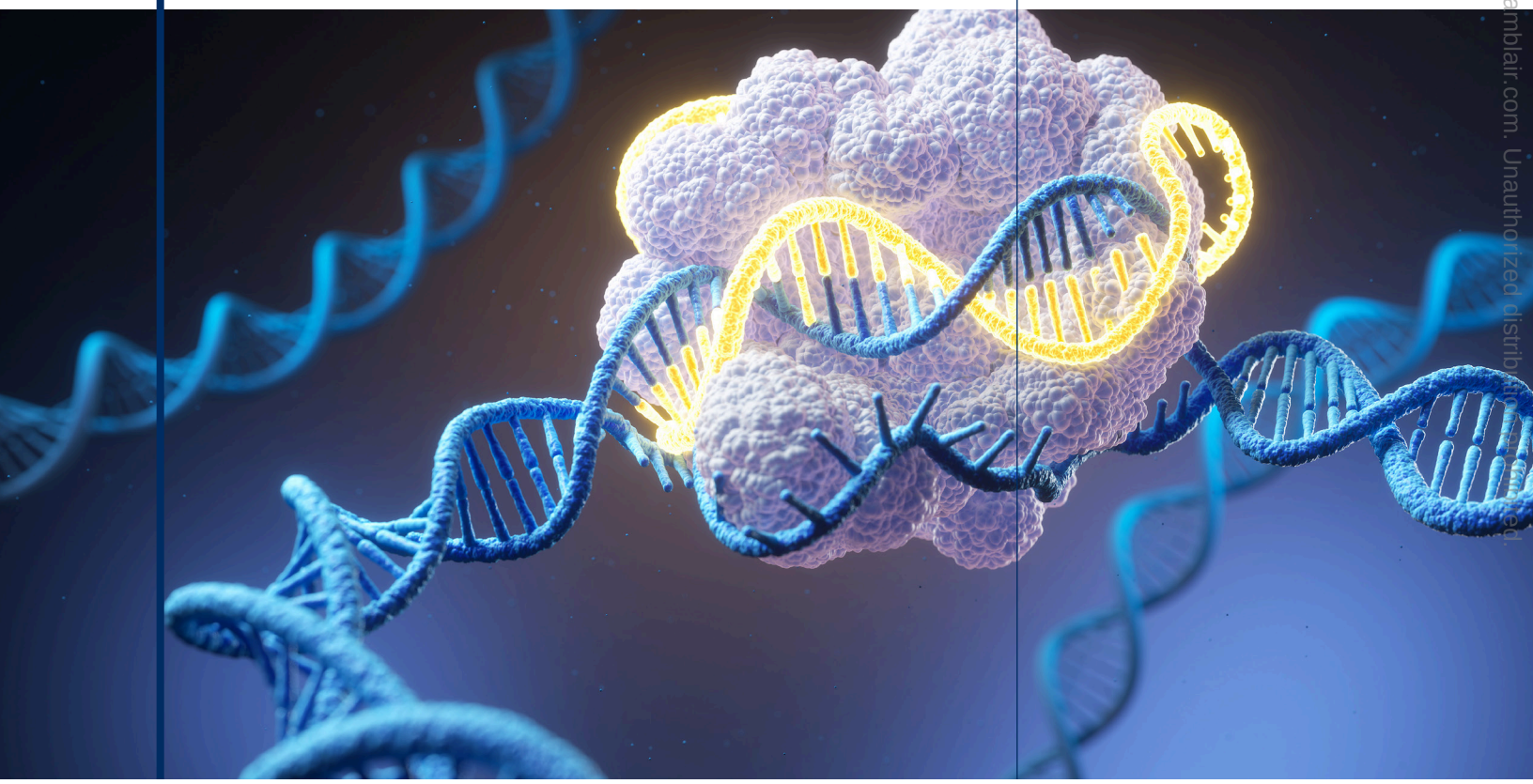
DECODER

CRISPR and Sharper: Gene Editing Technologies Shaping the Next Generation of Therapeutics



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Introduction

In this edition of our DECODER series, we examine gene editing technologies that we believe are on the cusp of clinical impact for patients across a multitude of diseases. In a 2022 edition of our Under the Microscope series, we highlighted advancements in gene editing stemming from the CRISPR technology, a foundational tool in the gene editing landscape. Since then, the first CRISPR-based gene editing therapy was approved in 2023: CRISPR/Vertex's Casgevy, an ex vivo cell therapy treatment for sickle cell disease (SCD) and transfusion-dependent beta thalassemia. Additional therapies based on gene editing have continued to advance in clinical development, and we anticipate initial pivotal Phase III data and potential regulatory approval of the first in vivo gene editing therapy (also CRISPR-based), Intellia's lonvo-z for hereditary angioedema. Several next-gen gene editing technologies beyond those that are CRISPR-based are also in clinical and preclinical development.

Our updated gene editing therapeutics landscape now includes 480 individual gene editing assets from 141 companies across 4 different types of gene editing, explained in depth in this report (see exhibit 1 for a look at the company landscape). *Note that the landscape of gene editing-related assets, financing, and business development deals, as well as clinical data discussed herein is current up to January 1, 2026.*

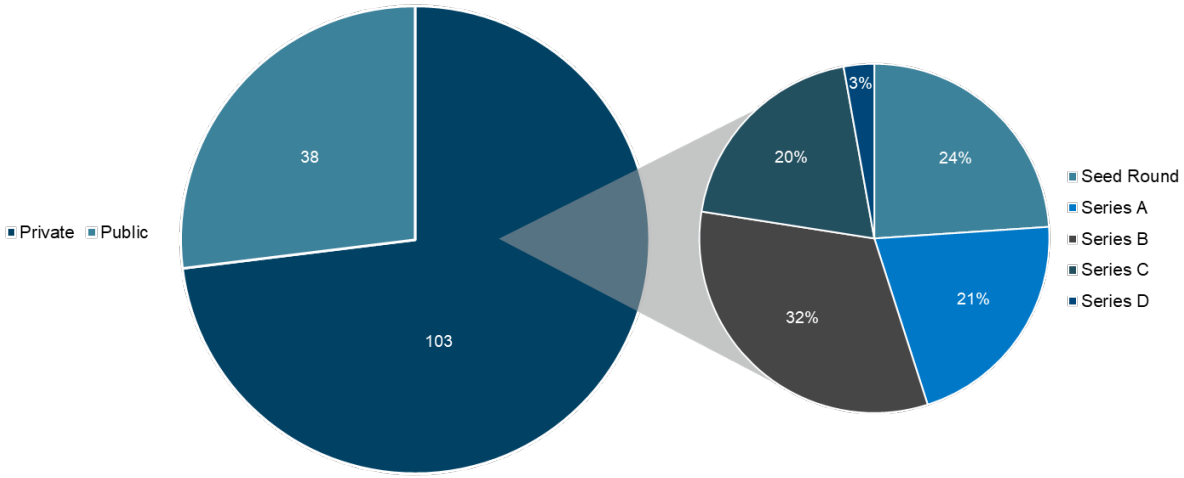
1. ***Nuclease editing –*** Enacting an edit to the genome by cutting both strands of the DNA helix
2. ***DNA base editing –*** Switching the identity of one base (letter) in the genome to another
3. ***DNA writing –*** Writing new DNA into the genome from a template
4. ***RNA editing –*** Editing the RNA copies of a gene (impermanent) instead of the DNA copy (permanent)

Exhibit 1
DECODER
Company Landscape of Gene Editing Assets

Sources: Company materials; William Blair Equity Research

Of the 141 companies, 103 (73%) are private and 38 are public (27%); see exhibit 2 for the private/public landscape. After highs in 2020 and 2021, we view the uptick in financing volume in gene editing technology over the past two years (2024-2025) as evidence of positive momentum in the space, with over $4 billion raised across the public (about $2.75 billion) and private (roughly $1.33 billion) sectors in 2025 (24.5% year-over-year growth). We also view recent business development deals involving gene editing therapeutic assets and companies, including over 25 deals exceeding $1 billion since 2015, as evidence of continued interest in and investment from big pharma.

Exhibit 2
DECODER
Private and Public Company Landscape



Sources: Company materials; BioCentury Inc.; William Blair Equity Research

We view the ability of gene editors to 1) fix mutations that underlie disease, 2) insert new DNA or genes into the genome, and 3) upregulate a target gene's expression as meaningfully differentiated from other genetic medicine approaches. Still, each modality has differing risks related to gene editing, affecting the suitability of a specific asset to a target application/indication. How different gene editing technologies have exposure to off-target editing risk remains a key focus for investors, and we investigate strategies used by different companies (including the use of impermanent RNA editing) to mitigate such risk.

In this report, we walk readers through the design elements of a gene editing asset for clinical development and how to examine an asset based on its modality and application. We have included in-depth technical sections covering each of the four gene editing modalities listed above. Each section includes descriptions of the molecular processes underpinning such editing technologies, how the technology differs from other gene editing modalities with particular emphasis on its ability to enact specific gene editing applications, risks (and mitigation strategies) specific to the technology, and an overview of the most relevant clinical data. We encourage readers to use this report as a compendium to get up to speed on the technical details of a specific gene technology by navigating to the relevant standalone section.

This is an exciting year for the gene editing landscape, with numerous clinical readouts expected (exhibit 3). We believe positive clinical data will be a broader catalyst for this biotechnology industry subsector and generate viable therapies for patients with disorders who have severe unmet need.

Exhibit 3
DECODER
Timeline of Data Readouts for Key Gene Editing Therapeutics Clinical Trials

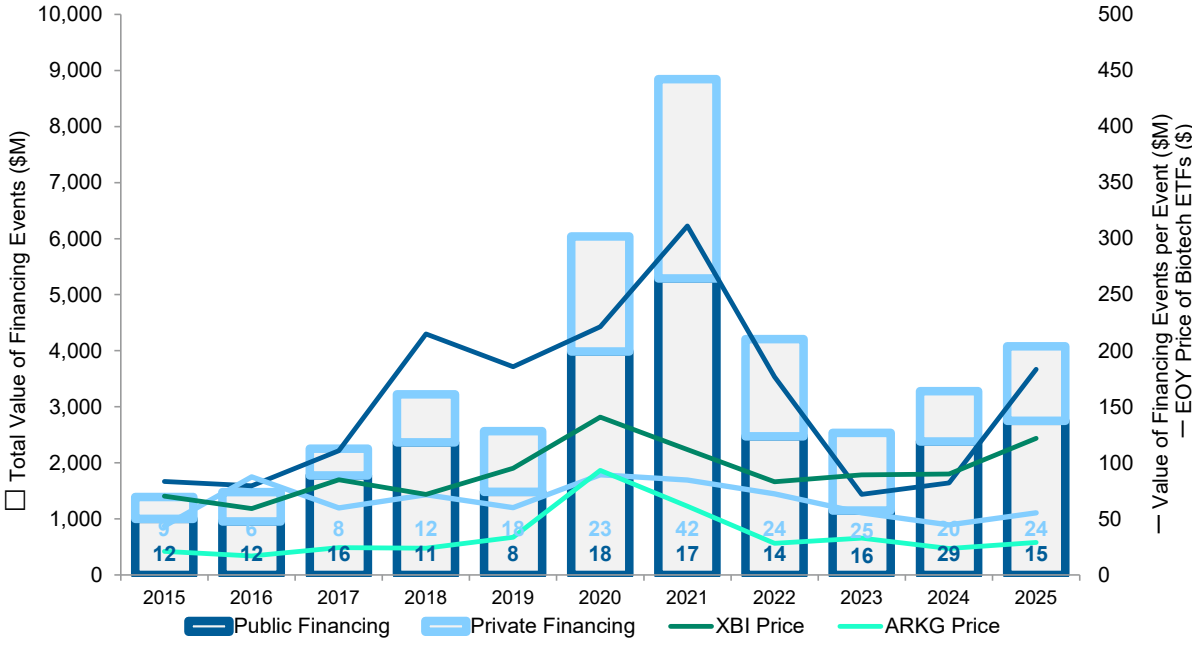
Date	Company	Asset	Editing Type	Indication	Event	Trial
Early 2026	Precision BioSciences	PBGENE-HBV	Nuclease Editing	Viral Infection	Data from the Phase I ELIMINATE-B study	NCT06680232
May 2026	Wave Life Sciences	WVE-006	RNA Base Editing	AATD	MAD data from the 400 mg dose cohort in the Phase Ib/IIa RestorAATion-2 study	NCT06405633
2Q26	Allogene Therapeutics	cema-cel	Nuclease Editing	Oncology	Interim futility data from Phase II ALPHA3 study	NCT06500273
1H26	Allogene Therapeutics	ALLO-329	Nuclease Editing	Autoimmunity	Data from the Phase I RESOLUTION study	NCT07085104
1H26	iECURE	ECUR-506	Nuclease Editing	OTC deficiency	Data from the Phase I portion of the Phase I/II OTC-HOPE study	NCT06255782
1H26	ProQR Therapeutics	AX-0810	RNA Base Editing	Cholangitis	Target engagement data from Phase I study in healthy volunteers	2025-521876-77-00
mid-2026	Intellia Therapeutics	lonvo-z	Nuclease Editing	Hereditary angioedema	Data from the Phase III HAELO study	NCT06634420
2H26	CRISPR Therapeutics	CTX310	Nuclease Editing	Cardiovascular disease	Data from the Phase Ib trial	ACTRN12623000809639
2H26	CRISPR Therapeutics	zugo-cel	Nuclease Editing	Autoimmunity	Data from the Phase I trial	NCT06925542
2H26	CRISPR Therapeutics	zugo-cel	Nuclease Editing	Oncology	Data from the Phase I trial	NCT05643742
2H26	Fate Therapeutics	FT819	Nuclease Editing	Autoimmunity	Data from the Phase I FT819-102 study	NCT06308978
2H26	Fate Therapeutics	FT836	Nuclease Editing	Oncology	Data from the Phase I trial	NCT07216105
2H26	Precision BioSciences	PBGENE-DMD	Nuclease Editing	DMD	Initial data from the Phase I/IIa FUNCTION-DMD study	NCT07429240
2026	Beam Therapeutics	BEAM-302	DNA Base Editing	AATD	Updated data from the Phase I/II trial	NCT06389877
2026	Beam Therapeutics	BEAM-301	DNA Base Editing	GSD1a	Initial data from Phase I/II trial	NCT06735755
2026	Caribou Biosciences	CB-011	Nuclease Editing	Oncology	First-look data from Phase I CaMMouflage study	NCT05722418
2026	Iovance Biotherapeutics	IOV-4001	Nuclease Editing	Oncology	Data from the Phase I/II trial	NCT05361174
2026	Precision BioSciences	PBGENE-HBV	Nuclease Editing	Viral Infection	Additional updates from the Phase I ELIMINATE-B study	NCT06680232
2026	ProQR Therapeutics	AX-0810	RNA Base Editing	Cholangitis	Initial patient data from Phase I trial	2025-521876-77-00
2026	SNIPR Biome	SNIPR001	Nuclease Editing	Bacterial infection	Completion of the Phase Ib trial	NCT06938867
2026	Locus Biosciences	LBP-EC01	Nuclease Editing	Bacterial infection	Completion of the Phase II ELIMINATE study	NCT05488340
2027	Prime Medicine	PM577	DNA Writing	Wilson's disease	Initial data from the Phase I/II trial	NCT07226622*

* prescreening study
Sources: Company reports, AlphaSense

Financing Trends and Business Development

Investment in the gene editing sector has been volatile over the last decade, peaking in 2021 with over $8.8 billion in capital raised by gene editing companies, but appears to be returning to steady growth. We attribute renewed interest to the easing of macro pressures and development of assets into later clinical development with a line of sight to commercial opportunities, rather than relying on platform valuations. In 2025 over $4 billion was raised across the public and private sector (24.5% year-over-year growth). However, an emerging trend of fewer transactions but with a greater amount raised per offering can be observed in the public markets; the 2025 average offering size of $183 million represented 23.4% year-over-year growth, albeit with only half the number of public offerings as in 2024. Private markets appear to be relatively stable over the past few years on this value-per-offering metric, but the total number of deals (24) was actually more than in the public markets (15) for 2025. In our view, with clinical success, this may be a leading indicator for increased M&A or IPO activity as venture funds look for future liquidity events. We also show in exhibit 4 the performance of the smidcap biotech weighted SPDR S&P Biotech ETF (XBI) and the genetic medicines ARK Genomic Revolution (ARKG) ETF relative to these financing trends. The XBI is up about 60% in the past nine months and ARKG is up 41% over the same period. We continue to monitor ARKG performance given that appreciation of this ETF was a strong leading indicator of financing activity in both the public and private markets during the 2020 to 2021 period.

Exhibit 4
DECODER
Financing Events Related to Gene Editing Companies



Value of Financing Events per Event rate calculated based on number of financing events with denoted values (inset numbers)
Financing from 2015 to 2025
Sources: BioCentury Inc.; FactSet; William Blair Equity Research

On business development, in exhibit 5 we have highlighted deals from 2015 to 2025 that have been ascribed total value above $1 billion. Total value includes upfront payments and potential milestone payments in the case of collaborative arrangements. We view recent—defined as 2022 and beyond after the financing peak in 2020-2021—large business development deals as representative of two major trends in the gene editing space:

1. An interest in alternative CRISPR systems beyond Cas9 (e.g., Bayer's collaboration agreement with Mammoth Biosciences, Eli Lilly and Sanofi's collaboration agreements with Scribe, and Chiesi's collaboration agreement with Arbor). See the "CRISPR and Other Nuclease Editing" section of the report (page 27) for more.

2. Gene editing platforms that mitigate some level of associated risk (see the "Assessing and Mitigating Risks Associated With Gene Editing" section of the report [page 24] for more), which include:

    a. Nuclease-based editors that require multiple binding events to trigger DNA cleavage (e.g., Roche's licensing agreement with and subsequent acquisition of Poseida Therapeutics).

    b. Genomic editing platforms avoiding double-stranded DNA breaks (e.g., Pfizer's collaboration agreement with Beam Therapeutics, Bristol Myers Squibb's collaboration agreement with Prime Medicine, and Eli Lilly's acquisition of Verve Therapeutics).

    c. Non-permanent editing modalities focused largely on RNA editing—both RNA base editing and RNA trans-splicing (e.g., GlaxoSmithKline's collaboration agreement with Wave Life Sciences, Roche's collaboration agreement with Ascidian Therapeutics, and Eli Lilly's licensing agreement with Rznomics).

Exhibit 5
DECODER
Prominent Deals Related to Gene Editing Assets (2022-2025)

Date	Companies	Agreement Type	Total Deal Value ($M)	Primary Editing Setting	Primary Editing Type	Indication Focus
October 2025	**Arbor Biotechnologies** X **Chiesi Farmaceutici**	Collaboration Agreement	$2,115	in vivo	Nuclease Editing	Primary Hyperoxaluria Type 1
June 2025	**Verve Therapeutics** X **Eli Lilly**	Acquisition	$1,300	in vivo	DNA Base Editing	Cardiovascular Diseases
May 2025	**Rznomics** X **Eli Lilly**	Licensing Agreement	$1,300	in vivo	RNA Editing	Hearing loss
November 2024	**Poseida Therapeutics** X **Roche**	Acquisition	$1,267	in vitro	Nuclease Editing	Oncology
September 2024	**Prime Medicine** X **Bristol Myers Squibb**	Collaboration Agreement	$3,610	in vitro	DNA writing	Oncology
June 2024	**Ascidian Therapeutics** X **Roche**	Collaboration Agreement	$1,842	in vivo	RNA Editing	Neurological Diseases
July 2023	**Scribe Therapeutics** X **Sanofi**	Collaboration Agreement	$1,240	in vivo	Nuclease Editing	Sickle Cell Disease
May 2023	**Scribe Therapeutics** X **Eli Lilly**	Collaboration Agreement	$1,575	in vivo	Nuclease Editing	Neurological and Neuromuscular Diseases
December 2022	**Wave Life Sciences** X **GlaxoSmithKline**	Collaboration Agreement	$3,695	in vivo	RNA Editing	Multiple
September 2022	**Scribe Therapeutics** X **Sanofi**	Collaboration Agreement	$1,025	ex vivo	Nuclease Editing	Sickle Cell Disease
August 2022	**Gentibio** X **Bristol Myers Squibb**	Collaboration Agreement	$1,900	in vitro	Nuclease Editing	Inflammatory Bowel Disease
August 2022	**Poseida Therapeutics X Roche**	Licensing Agreement	$6,220	in vitro	Nuclease Editing	Oncology
June 2022	**Precision Biosciences** X **Novartis**	Collaboration Agreement	$1,475	in vivo	Nuclease Editing	Sickle Cell Disease & Beta Thalassemia
January 2022	**Century Therapeutics** X **Bristol Myers Squibb**	Collaboration Agreement	$3,150	in vitro	Nuclease Editing	Oncology
January 2022	**Beam Therapeutics** X **Pfizer**	Collaboration Agreement	$1,350	in vivo	Base Editing	Multiple
January 2022	**Mammoth Biosciences** X **Bayer**	Collaboration Agreement	$1,040	in vivo	Nuclease Editing	Multiple

Sources: BioCentury Inc.; FactSet; William Blair Equity Research

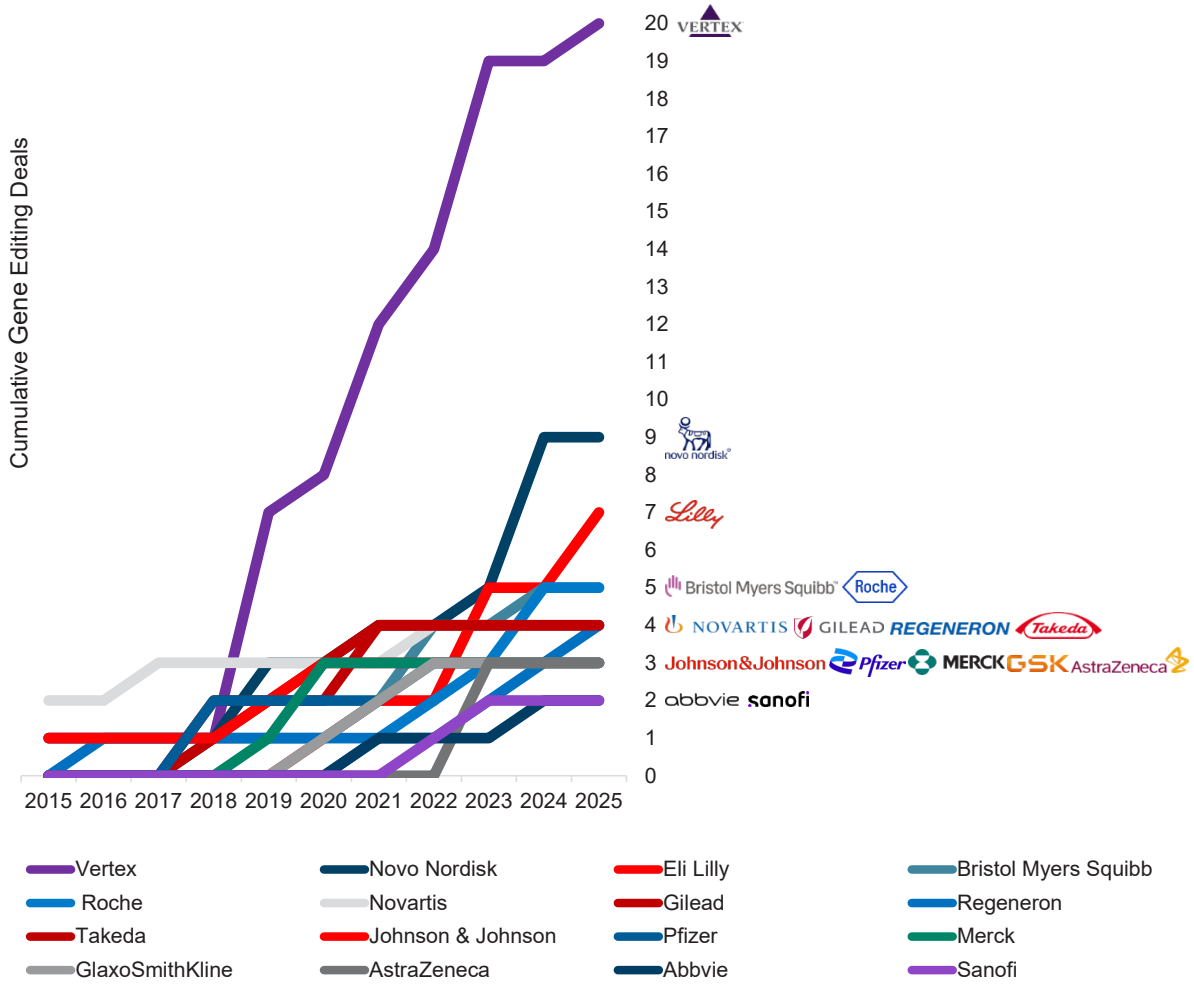
Exhibit 5 (Cont'd)
DECODER
Prominent Deals Related to Gene Editing Assets (2015-2021)

Date	Companies	Agreement Type	Total Deal Value ($M)	Primary Editing Setting	Primary Editing Type	Indication Focus
Spetember 2021	**ProQR Therapeutics** X **Eli Lilly**	Collaboration Agreement	$3,750	in vivo	RNA Editing	Multiple
August 2021	**Shape Therapeutics** X **Roche**	Collaboration Agreement	$3,000	in vivo	RNA Editing	Neurological Diseases
August 2021	**Arbor Biotechnologies** X **Vertex Pharmaceuticals**	Collaboration Agreement	$1,200	in vitro	Nuclease Editing	Type I Diabetes
June 2021	**Shoreline Biosciences** X **Gilead Sciences**	Collaboration Agreement	$2,300	in vitro	Nuclease Editing	Oncology
April 2021	**Obsidian Therapeutics** X **Vertex Pharmaceuticals**	Collaboration Agreement	$1,375	in vivo	Nuclease Editing	Multiple
April 2020	**Fate Therapeutics** X **Johnson & Johnson**	Collaboration Agreement	$3,100	in vitro	Nuclease Editing	Oncology
June 2019	**Crispr Therapeutics** X **Vertex Pharmaceuticals**	Licensing Agreement	$1,175	in vivo	Nuclease Editing	Duchenne's Muscular Dystrophy Myotonic Dystrophy Type I
September 2018	**Fate Therapeutics** X **Ono Pharmaceutical**	Collaboration Agreement	$1,250	in vivo	Nuclease Editing	Oncology
April 2018	**Cellectis** X **Allogene Thearpeutics** X **Pfizer**	Licensing Agreement	$2,800	in vitro	Nuclease Editing	Oncology
February 2016	**Precision Biosciences** X **Baxalta** X **Servier**	Collaboration Agreement	$1,705	in vitro	Nuclease Editing	Oncology
October 2015	**Crispr Therapeutics** X **Vertex Pharmaceuticals**	Collaboration Agreement	$2,625	Multiple	Nuclease Editing	Sickle Cell Disease & Beta Thalassemia Cystic Fibrosis

Sources: BioCentury Inc.; FactSet; William Blair Equity Research

Interest in gene-editing-related business development deals has been seen across large-cap pharma and biotech players. Over the last decade, Vertex has demonstrated the most interest in gene editing deals, steadily accumulating new agreements since its deal with CRISPR Therapeutics in 2015, which included rights to the now-approved Casgevy. We also highlight recent deals from Novo Nordisk and Eli Lilly as evidence of continued interest in the space (exhibit 6).

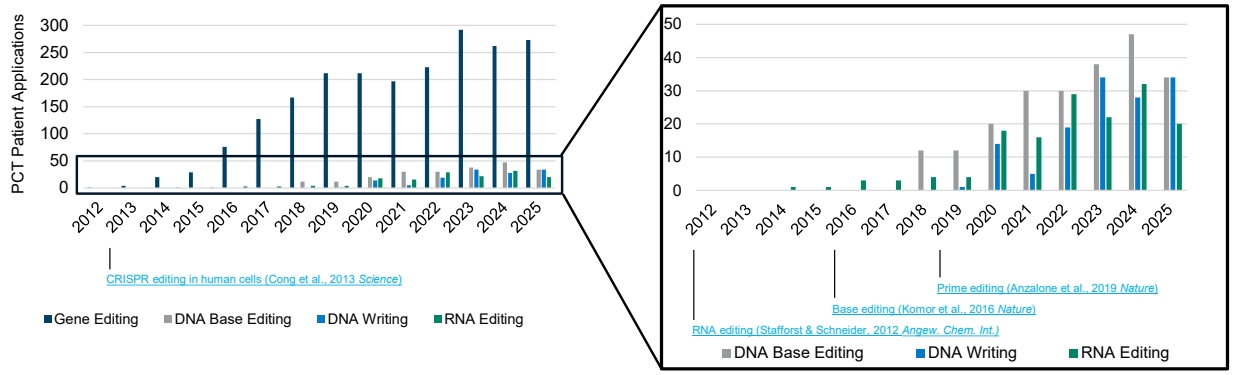
Exhibit 6
DECODER
Cumulative Gene Editing Deals by Large-Cap Pharma



Sources: BioCentury Inc.; William Blair Equity Research

The number of patent applications for gene editing technologies/advancements has also increased (exhibit 7) since the advent of CRISPR technologies for mammalian cell applications in 2013. Across each gene editing modality, a trend emerges where patent application prevalence increases three to four years after a seminal publication on that specific technology. We view recent papers relating to site-specific nonviral gene insertion, through either retrotransposon-based (Zhang et al., 2025 Nature Biotechnology) or integrase-based (Mukhametzyanova et al., 2024 Nature Biotechnology; Fauser et al., 2025 BioRxiv [preprint]) approaches, as indicative of potential next areas where new IP is generated in the near term. On innovation of delivery platforms, nonviral delivery technologies to extra-hepatic tissues remain a major focus for new IP generation in genetic medicines more broadly, but innovation here is outside the scope of this *DECODER* issue.

Exhibit 7
DECODER
Gene Editing Technology Patents

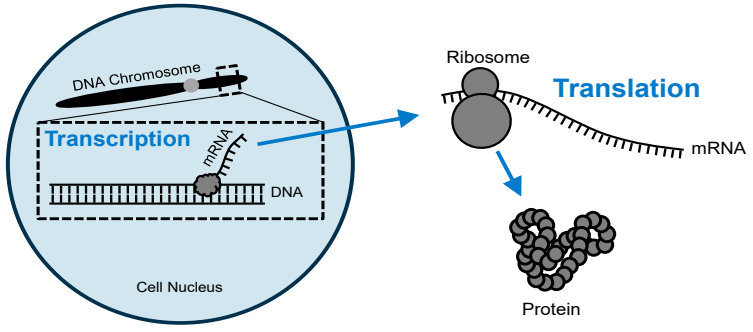


Sources: WIPO; William Blair Equity Research

Gene Editing 101

The genome of a given organism or cell encodes the machinery that carries out functions essential for life. Within the genome, genes are individual, contiguous stretches of DNA that encode cellular machinery. Typically, these machines are proteins, carrying out enzymatic functions within cells. In eukaryotic cells, the genome is packaged as a DNA-protein complex called chromatin. The reading of genes into functional proteins is often dubbed the "central dogma" of molecular biology and is the principal task cells perform. First, during transcription, the DNA sequence of a gene is read out into a molecule of RNA. For RNAs encoding proteins, these RNAs are called messenger RNAs (mRNAs). Messenger RNA molecules are then read by ribosomes, macromolecular machines within cells, which translate the nucleic acid sequence of the RNA into a corresponding sequence of amino acids that make up the functional protein. The various stages of gene transcription, translation, and overall regulation provide opportunities for gene editing therapies to modulate protein abundance, sequence, and functionality (exhibit 8).

Exhibit 8
DECODER
Central Dogma of Molecular Biology



Source: William Blair Equity Research

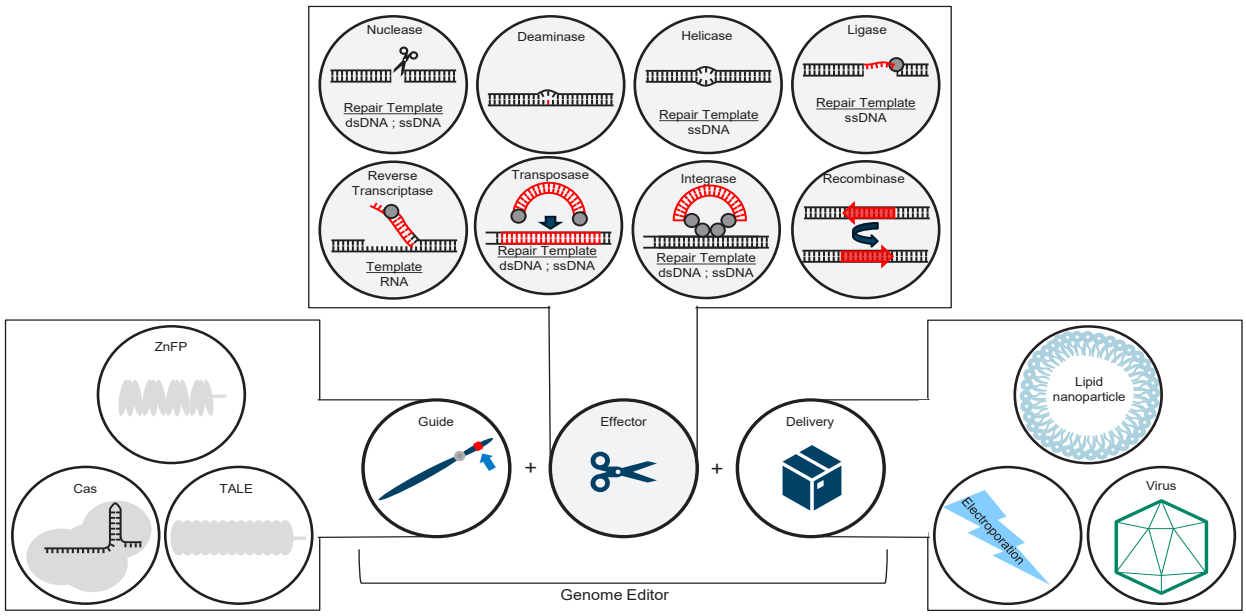
The term "gene editing therapy" refers to a broad class of biologic-based therapeutics that seek to alter the production of specific machinery in cells. For certain applications, therapies may seek to change the amount of production of a gene, either eliminating its expression or boosting its expression above normal levels to acquire benefit. In other instances, gene editing therapies may address a genetic mutation by altering the sequence of that gene, allowing the encoded protein to function properly. In addition, certain gene editing technologies may be used to insert an exogenous gene sequence into the genome of the cell at a specific locus to facilitate its production. It is important to note, however, that the class of gene editing therapies is not limited to those that modify the genome; ***we consider gene editing therapies to be those that alter the sequence of the genetic code at any point along the trajectory of the central dogma to enact therapeutic benefit.***

Because epigenetic editing—which serves to site-specifically alter the physical makeup of chromatin—does not act to alter the sequence of the genome, we consider it outside the umbrella of gene-sequence-based editing covered in this report. Still, because of its ability to change expression of a targeted gene by altering the central dogma (transcription), we deem epigenetic editing an important tool for gene-specific regulation and aim to review its mechanisms and applications in a future report.

Build Your Own Gene Editing Asset

Gene editing therapies focused on editing the genome at the DNA level (genome editing therapies) comprise three main constituent parts: a guide, an effector, and a delivery vehicle. All three components are necessary to enact precise editing within a specific cell type and genomic site of interest: the guide localizes the therapy to a specific gene, the effector enacts the desired edit, and the delivery module permits access of the gene editing cargo into certain cells or tissue types. Though certain considerations are necessary for each component, gene editing application, and desired site of delivery, design of these constituent parts has become well enough developed that they can be largely modular, enabling the efficient combination of a guide, effector, and delivery vehicle for effective therapies.

Exhibit 9
DECODER
Build Your Own Genome Editor



Source: William Blair Equity Research

Guide Template

Gene editing therapies offer the promise of precision medicine by enacting edits to genetic material. However, to mitigate off-target safety risks, such edits must be made precisely to the gene(s) of interest. Therefore, gene editing therapies typically commandeer a guide molecule, which targets the gene editing machinery to the gene(s) of interest and, in part, confers specificity. At both the DNA and RNA level, this is achieved through a gene's unique sequence: by creating a guide molecule that binds to that gene's sequence and no other, the gene editing machinery is specifically targeted to the correct gene(s). It is important to note that these gene editing guides must be programmable, allowing sequence-specific tailoring of the guide to target a gene or genomic site of interest.

In certain instances, that guide molecule is an RNA, which finds its gene target through complementarity. Guide RNAs are often commandeered by protein-based gene editing machinery (encoded by mRNA in most cases); hybridization of the guide RNA with its complementary DNA sequence docks the gene editing machinery at a genomic location, allowing the effector machinery to act on the target site. In the case of genome-based editing, the CRISPR-Cas system is the most common RNA-guided gene editing machinery. In this system, a Cas protein binds to the guide RNA, which identifies its genomic target.

Specific genes may also be targeted via protein-based guide molecules. For example, zinc finger proteins (ZnFPs) may be commandeered for sequence-specific gene editing. Each zinc finger, a 30-amino-acid domain that binds one atom of zinc, binds three nucleotides of DNA. A minimum of three fingers is required to provide adequate affinity to the target DNA sequence, but not all fingers make equal contributions to DNA binding. Therefore, companies such as Sangamo Therapeutics have engineered two-finger ZnFPs, which, when linked with a short linker, provide adequate DNA binding with improved on-target specificity (see Paschon et al., 2019 Nature Communications as an example of Sangamo's ZnFP development).

Alternatively, transcription activator like effector (TALE) proteins, derived from plant pathogenic *Xanthomonas* bacteria, were discovered in 2010 as a DNA binding protein with modular design capable of binding DNA sequences in a customizable manner; repetitive domains within the amino acid sequence of a TALE can be customized to bind to a specific DNA base by altering the 12th and 13th residues of each domain. Unfortunately, the TALE domain that binds guanine exudes less specificity than the other domains, increasing the risk of nonspecific binding in instances of G-A mismatches. Effective TALE proteins typically comprise 11-17 repeats. While effective TALE binding is largely agnostic to specific sequence motifs, it is recommended that a TALE DNA target include at least two to three cytosines or guanines in the target site, as those specific TALE binding domains possess the strongest DNA-binding activity.

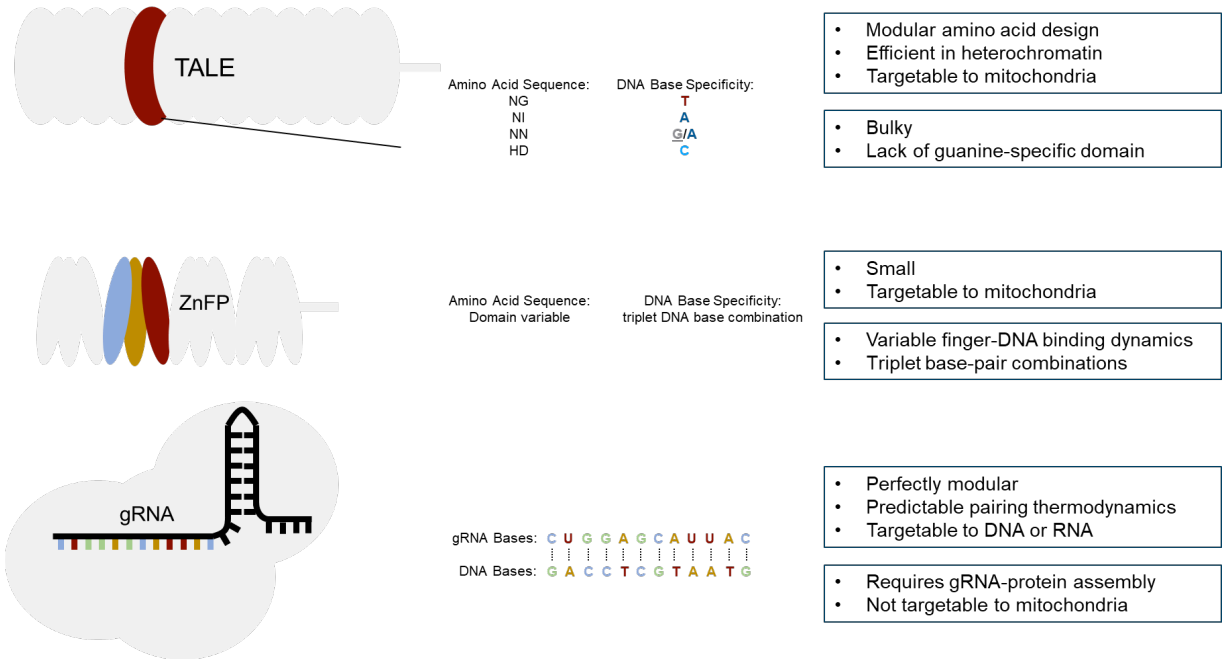
Specific guide selection may be particularly amenable to gene editing in specific contexts, depending on the cell type and gene of interest. For example, RNA guides are uniquely modular, and common algorithms calculating melting temperatures and RNA secondary structures are largely predictive of successful gRNA-target pairs. Contrastingly, affinity and efficacy of protein-based guides with DNA targets may be harder to predict and engineer. In addition, RNA-based guides have few sequence-based restrictions: whereas design of ZnFPs to target novel sequences is potentially more difficult and requires extensive testing to ensure sequence-specific binding and TALEs require a specific GC content in their target sequence for effective binding, RNAs are relatively sequence-unbiased, allowing developers to target most genes in the genome. In the case of CRISPR-based editing, a protospacer adjacent motif (PAM) sequence is necessary for Cas docking. However, that requirement is intrinsic to the Cas protein, not the RNA, and new-age Cas molecules with alternative or less restrictive PAM sites are in ongoing development.

For certain applications, protein-based guides have notable benefits that RNA guides do not possess. For example, protein-based guides may be targeted to subcellular regions that guide RNAs cannot access. This is most notable for gene editing within the mitochondrial genome. A mitochondrion's double membrane and mitochondrial import machinery restrict the delivery of guide RNAs to mitochondria, leaving mitochondrial mutation-based pathologies currently nonamenable to RNA-guided editing technologies. Alternatively, for protein-based editors, peptides that drive mitochondrial import may be appended onto the editor, allowing targeting of mitochondrial genomic mutations. Analogous designs may be applied to other subcellular location targeting – both Precision BioSciences and Primera Therapeutics are developing protein-guided editors to target mutations in the mitochondrial leucine tRNA gene (mt-TL1), which is mutated in multiple primary mitochondrial myopathies. Regarding inefficient mitochondrial guide RNA import, we note recent progress in this area (see a preprint here: Yin, Jarosz, & Ting, 2025 BioRxiv), though we view this barrier as yet to be fully addressed.

Protein-based guides may also increase gene editing efficiencies in heterochromatic DNA regions (regions where chromatin is densely packaged and therefore difficult to access); TALE-based editors have been shown to edit heterochromatic regions more efficiently than CRISPR-based counterparts (Jain et al., 2021 Nature Communications). On the other hand, protein-based guides may be targets of cellular regulation that inhibit their effectiveness. For example, while the immunogenicity of foreign guide RNAs to cells may be reduced by chemically modifying the RNA nucleotides, protein-based guides may be immunogenic through proteosome processing and presentation to the immune system via major histocompatibility complexes (MHC). In addition, proteins may be the target of post-translational modifications, including phosphorylation and/or acetylation of amino acids, which may alter their function or rates of off-target binding. While phosphorylation of TALEs has not been investigated to our knowledge, we note that Serine 11 (S11), adjacent to the

DNA base-specific amino acid pair, is a predicted phosphorylation target of multiple cellular kinases and could have physiological consequence if the TALE were to be post-translationally modified in such a manner. We review benefits and drawbacks of specific gene editing guides in exhibit 10.

Exhibit 10
DECODER
Molecular Guides for Genome Editors



Source: Adapted by William Blair Equity Research from Becker & Boch, *Gene and Genome Editing* (2021)

Effector Domain

The selection of a specific gene editing effector is largely dependent on the desired application. As mentioned above, genome editors may be purposed to alter gene expression, change the genetic sequence, or insert an exogenous gene into the genome. These effectors are proteins or protein domains, which typically already exist in nature, that have been repurposed and/or tuned for these efforts. In most cases, edits to the genome require introduction of a DNA lesion, most often a single-stranded or double-stranded break in the DNA helix, which is then repaired using the effector's or cell's machinery. Though not required for certain applications, a repair template can be used to guide the cell into fixing the genome using a specific sequence, thus introducing a modification encoded by the developer. In the case of DNA-based genome editing, that repair template has classically been a piece of double-stranded DNA (dsDNA) introduced into cells. However, because of advances in single-stranded DNA (ssDNA) synthesis, ssDNA may also be used. As ssDNA templates have been reported to elicit higher repair efficiencies for gene editing, we see ssDNA-template-based genome editing from companies such as Kano Therapeutics as an exciting new frontier. Alternatively, an RNA template can be used by a reverse transcriptase to write ssDNA into the genome, which is then polymerized into dsDNA by cellular machinery.

While introduction of DNA lesions is often the required kick-start for genome editing processes, these lesions can be toxic to cells. For instance, processes for genome repair enacted by cells often require initiation of cellular stress pathways, such as p53-mediated cell cycle arrest, which may alter cellular function. In addition, these repair processes are also imperfect, which may lead to mutated proteins that are toxic to cells. More severely, large-scale DNA lesions open up risk of translocation of large genomic sequences, such as pieces of genes or chromosomes, that could

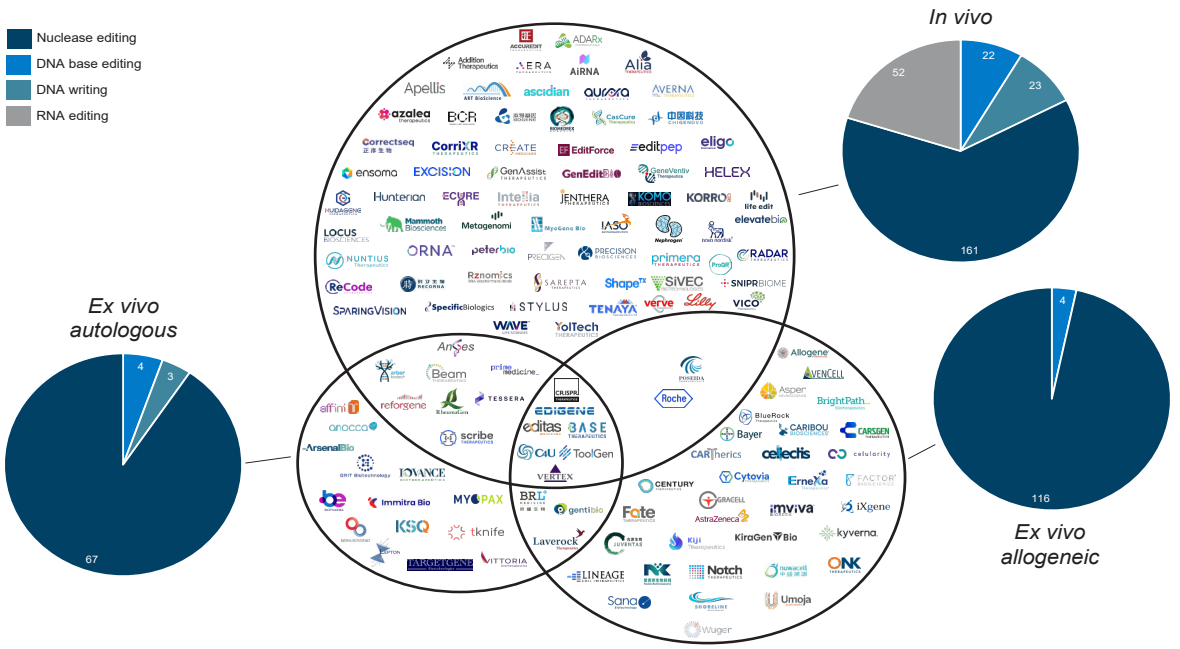
cause malignant transformation. The number and severity (single-stranded versus double-stranded) of these induced DNA lesions influence these cellular outcomes. Moreover, we reason that dsDNA breaks induced by gene editing effectors that participate in the genomic repair process, such as transposases, integrases, and recombinases, likely reduce the likelihood of meaningful genomic toxicities compared to more classic nuclease-based effectors, which exogenously cleave DNA and rely on cellular machinery to fix the break. While classic nuclease-based genome editing effectors remain the most popular due to their long-standing presence in the field, new-age technologies and approaches continue to seek effective, efficient gene editing mechanisms that do not entail risks associated with dsDNA breaks.

Delivery Module

Advent of modalities to deliver biologics to a range of tissues has expanded the potential repertoire of indications targetable by gene editors, particularly for new emerging in vivo applications. In most instances, gene editing machinery is encoded as either DNA or RNA; delivery into cells allows cellular machinery to express the constituent components, creating a functional gene editor that can act on its genetic target. We segment delivery methods into three broad types (exhibit 11).

- ***In vivo delivery:*** Direct administration of the gene editor into patients, requiring the delivery module to penetrate specific tissues and permit access of the gene editing payload into cells.
- ***Ex vivo delivery:*** Delivery of gene editor therapies to cells outside the body.
  - *Ex vivo autologous:* A patient's cells are isolated before delivering the gene editing machinery to those cells and then reintroducing those edited cells into the patient.
  - *Ex vivo allogeneic:* Cells from a third-party source, which have been appropriately genome edited to avoid autoimmune rejection, are introduced into a patient.

Exhibit 11
DECODER
Company Landscape of Gene Editing Assets by Editing Setting



Sources: Company materials; William Blair Equity Research

Both the tissue target of interest and the size of the gene editor payload dictate the delivery method of choice for developers. Lipid nanoparticles (LNPs), popularized as the means of delivery for nucleic-acid-based therapies, including mRNA vaccines, serve as a common delivery method for gene editing therapies. LNPs can deliver both DNA and RNA payloads to cells. Classically designed LNPs comprise four unique lipids: cholesterol, a PEG-lipid, a helper lipid, and a cationic ionizable lipid. Altering both the type and stoichiometry of each of these lipid classes leads to an expansive repertoire of possible LNP formulations. While LNPs present a flexible, non-immunogenic option, certain formulations have triggered adverse events in patients. One example includes the spiking of liver enzymes and thrombocytopenia that was observed after treatment with VERVE-101 in Verve's Phase Ib Heart-1 study (Heart-1 Paused Following Safety Events; Prioritizing VERVE-102; Closer Look at Ionizable Lipid Safety Landscape).

The penetration of LNPs into certain tissues and cell types is primarily dictated by the protein shell that encapsulates the LNP upon administration. This shell can also dictate the pharmacokinetics of the LNP within the body. For classically constructed LNPs delivered intravenously, serum protein apolipoprotein E (ApoE) is the principal component of the LNP's protein shell, which ultimately leads to shuttling of the LNP to the liver, where it is captured and enveloped by LDLR-expressing hepatocytes (LDLR stands for low-density lipoprotein receptor). The propensity of LNPs to deliver their cargo nearly exclusively to the liver limits developers of gene editing tools using classical LNPs to address only a subset of indications that can be assuaged by liver-mediated expression of a functional gene copy.

Viral delivery of a therapeutic DNA payload allows efficient delivery of gene editor payloads to extra-hepatic tissues. Tissue targeting can be modulated via mutation of a virus's capsid protein, which can target specific cell/tissue types; developers can identify a cell/tissue type-specific receptor and screen for a viral capsid protein variant that binds to that receptor. Upon receptor binding, the virus delivers its DNA genome as a payload, which includes the specific gene that a developer has packaged into the viral genome in a specific location.

Current iterations of viral genetic medicine delivery, particularly for in vivo applications, typically use adeno associated viruses (AAVs) as a means of delivery. In wild-type AAVs found in nature, the DNA payload would be integrated into the target cell's genome, and expression of the full slate of viral proteins would allow the virus to replicate itself and infect new cells. However, for gene therapy purposes, developers must use an AAV that cannot replicate, to avoid rampant viral transduction of nontarget tissues. Therefore, the AAV constructs used for therapeutic delivery use AAVs that do not express the *rep* gene, which is necessary for viral replication. However, the *rep* gene is also necessary for integration of the AAV's payload into the target cell's genome. Therefore, an AAV payload delivered as therapy exists as an extra-chromosomal piece of DNA in the transduced cell's nucleus called an episome. We note that the gene payload encoded by developers contains its own regulatory elements (promoter, transcriptional stop sequence, etc.) for independent episomal expression in cells.

While viral delivery of genetic medicines has been a powerful therapeutic tool to date, its limitations leave open opportunities for other (nonviral) delivery options to provide value.

1. ***Packaging constraints in viral vectors reduce possibility for potential gene therapy payloads.*** Current AAV vectors typically have a limit of about 4,700-5,000 base pairs (bp; 4.7-5.0 kilobases [4.7-5.0 kB]) that may be added to them while still allowing efficient cell transduction. This means that large genes will not fit into an AAV vector, so developers are required to either 1) deliver a truncated form of the therapeutic gene that may not provide all wild-type functionality or 2) accomplish the expression of the desired gene in a different way. We note that gene editing can provide these options in some contexts, as certain gene editing constructs are small enough to fit within an AAV vector. We highlight the patient-specific base

editing of *Cps1* in the context of *Cps1* deficiency as described by Musunuru et al. as one such example; while pathology arising from *Cps1* deficiency could theoretically be addressed via a gene therapy option (delivery of a wild-type copy of *Cps1* to the liver via an AAV vector), the *Cps1* gene is 5,760 base pairs and therefore would not have fit into an AAV vector. Therefore, a gene editing approach (DNA base editing in this case) was used as an alternative method to achieve functional CPS1 expression.

2. ***Potential immune activation to AAV limits ability to redose.*** While an initial dose of AAV can demonstrate a tolerable safety profile by evading a primary immune response from the patient, redosing of AAV potentiates a secondary immune response against AAV machinery, which will be more exaggerated and may lead to tolerability risks. Therefore, patients receiving AAV-based medicines are precluded from receiving a second dose. This may limit patient options for those who want or need a second dose to boost therapeutic efficacy or those who would like to try an alternative AAV-delivered treatment (whether for the same disease or any other application). We note that certain viral gene therapy platforms, such as Krystal Bio's HSV-1-mediated delivery, are amenable to redosing.

3. ***Episomal expression limits utility of genetic medicine in rapidly replicating cell/tissue types.*** Delivery of an AAV payload as an extra-chromosomal episome prevents the DNA payload from being replicated by cellular machinery during cell division, and any episomal copy in the cell's nucleus is randomly distributed between the two daughter cells after replication. This means that as the cells originally carrying the viral payload scale in numbers, the therapeutic gene does not scale along with them. This may be acceptable for applications where cells within a tissue are long-lived and have entered a nonreplicative status, or where only a small amount of therapeutic payload expression across a cell population/tissue is sufficient for therapeutic benefit. However, in cells and tissues that maintain replicative status and/or enact cellular turnover over time (e.g., new cells replace old cells that die off), gene therapy may have a limited window of efficacy. We highlight the blood, liver, and skin as examples of tissues that turn over readily and may not be fit for viral delivery options. We note that viral delivery of CAR genes to blood cells (specifically T cells) for ex vivo autologous CAR T-cell therapy uses lentiviral vectors, which provide genomic integration of the payload to allow CAR expression on every daughter CAR-T cell as the population of CAR-transduced T cells expands. However, lentiviral vectors do not have as tolerable a safety profile as AAV delivery in vivo, and therefore are limited to ex vivo autologous treatments.

The advent of nonviral delivery options provides workable solutions for AAV-associated limitations. Well-established nonviral delivery technologies such as LNPs, mentioned above, and GalNAc conjugation predominantly target hepatocytes, meaning that such vehicles are options only for diseases that pertain to the liver in some fashion (e.g., those that directly affect the liver or are caused by a protein produced by the liver). Thus, many current genetic medicines that target other tissues require viral delivery, and many gene editing therapeutic developers use AAV for such delivery to organs such as the CNS, lung, muscle, and eye. However, rapid advancements in novel lipid chemistries (including LNP nebulization for delivery to the lung) and LNP- or payload-ligand conjugations potentiate extra-hepatic nonviral delivery vehicles with safe dosing profiles in the near term, which we view as a large catalyst for the gene editing space. New-age delivery modalities also use virus-like particles (VLPs; can be thought of as empty viral packaging), which combine the tissue-specific tropism of viruses via assembly of viral capsid proteins but with the payload flexibility (e.g., RNA-based payloads; larger payload size; ability to be redosed) of nonviral delivery options.

We view gene editing technologies delivered via nonviral modalities as providing maximum differentiation from gene therapies, and the combination of nonviral delivery and gene editing boost each other's efficacy and safety profile. Nonviral delivery vehicles may provide a safer means of

providing target cells with the therapeutic payload with fewer constraints on payload size. Still, an inability to transduce target cells with a stably expressing DNA payload means that expression of nonvirally delivered genetic medicines are transiently expressed and require redosing, which can increase the likelihood of delivery-associated adverse events (e.g., reaction to lipids derived from a foreign LNP). Gene editing technologies, particularly genome editing applications, are unique compared to other therapies in their ability to provide one-and-done therapeutic potential despite transient expression in cells, opening a new dosing regime for nonviral delivery vehicles.

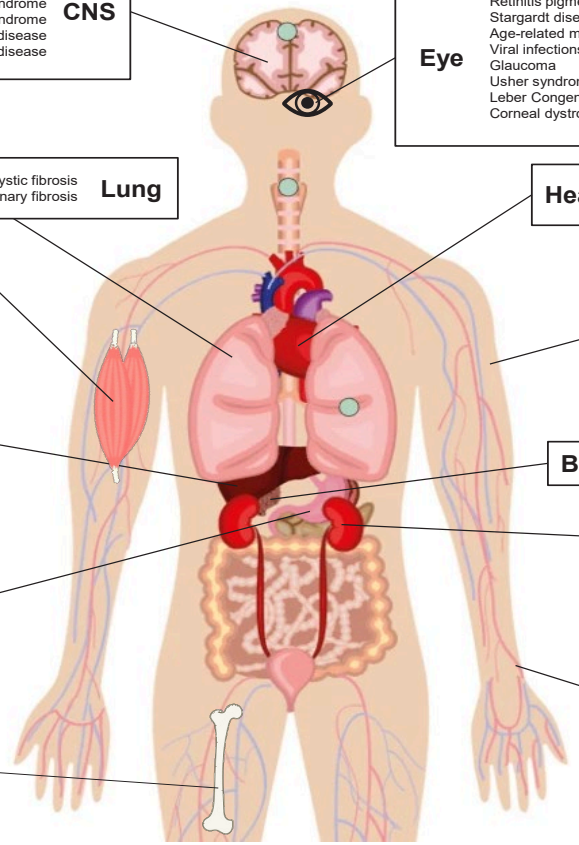
From a payload perspective, we view nonviral means of delivery for gene editors as providing meaningful benefits to the efficacy and safety profiles of gene editors. On efficacy, nonviral delivery methods enable such payloads to include nucleic acid modifications that cannot be synthesized in cells such as RNA-DNA combination guide molecules (e.g., Caribou Biosciences' chRDNA) or novel and/or position-specific RNA modifications. Such modifications may play a meaningful role in the efficacy of the payload and help achieve profiles of on- and off-target editing unattainable by unmodified editors expressed via episomes in cells. On safety, nonviral delivery obviates sustained expression of the gene editor, which, in the case of genome editing, reduces risk for off-target editing by limiting editor expression in target cells.

Virally delivered genetic medicines still have a role in the therapeutic landscape. For now, AAV delivery remains the most modular mechanism for extra-hepatic delivery in vivo, and specific ectopic expression of either a wild-type copy of a gene or a novel gene payload without genomic integration can provide therapeutic benefit. However, we predict the advent of safe extra-hepatic nonviral delivery vehicles will eventually take the place of AAV delivery. In some instances, building off the safety profile, payload size flexibility, and redosability of nonviral options, "nonviral gene therapy" may be a sufficient option. We define nonviral gene therapy as the nonviral delivery of an independent gene expression cassette either through semi-random genomic integration, such as with a nontargeted transposon, or via extrachromosomal DNA expression, such as Engage Bio's tethosome platform, each of which is capable of being expressed nonvirally. In other scenarios, expression of a payload via more targeted genomic integration using gene editing methods such as nuclease-mediated gene integration or gene writing may be more prudent. In either case, such novel platforms allow for sustained payload expression despite nonviral delivery, which we view as a necessary leap to extract maximum value out of new nonviral delivery technologies.

While viable only for ex vivo applications, electroporation, which temporarily increases cell permeability via electrical pulses, can also be used to deliver gene editing cargo. This method can be used to introduce DNA or RNA payloads encoding gene editing machinery, or preassembled RNA-protein complexes ready for editing. However, such electrical pulses can be toxic to cells, as strong electrical pulses, required for administration of large cargo, can lead to damage of the plasma membrane, proteins, or organelles, as well as DNA or RNA. Moreover, weakening of the plasma membrane's integrity permits improper ion exchange, including cellular entry of calcium, which may alter cell signaling pathways. In all cases, such damage can be reparable or irreparable by the cell depending on the extent of cellular injury or dysfunction.

The variety of gene editing delivery tools allows developers to tackle a broad range of diseases across multiple tissue types. In exhibits 12 and 13, we highlight the relevant tissues and diseases targeted by current gene editing assets and the respective types of editing used in different tissues.

Exhibit 12
DECODER
Landscape of Gene Editing Assets by Target Tissue and Disease



CNS
ALS
Huntington's disease
Dravet syndrome
Rett syndrome
Alzheimer's disease
Charcot-Marie-Tooth disease

Eye
Retinitis pigmentosa
Stargardt disease
Age-related macular degeneration
Viral infections
Glaucoma
Usher syndrome
Leber Congenital Amaurosis
Corneal dystrophy

Lung
Cystic fibrosis
Idiopathic pulmonary fibrosis

Heart
Arrhythmogenic cardiomyopathy

Muscle
Duchenne's muscular dystrophy
Myotonic dystrophy
Limb girdle muscular dystrophy
Primary mitochondrial myopathy

Induced Stem Cells
Oncology indications
Endometriosis
Autoimmune indications
Type I diabetes
Acute injury
Stroke
Interstitial lung disease
Parkinson's disease

Liver
ATTR amyloidosis
Cardiovascular disease
Alpha-1 antitrypsin deficiency
Primary Hyperoxaluria Type 1
Phenylketonuria
Glycogen storage disease
Hypertension
Acute Hepatic Porphyria
OTC deficiency
Pompe disease
Citrullinemia
Hereditary angioedema
Hemophilia
Hypertriglyceridemia
Viral infections
Wilson's disease
Cholangitis
MASLD
Obesity

Beta Cells Type I diabetes

Kidney
Polycystic kidney disease
Tubulointerstitial kidney disease

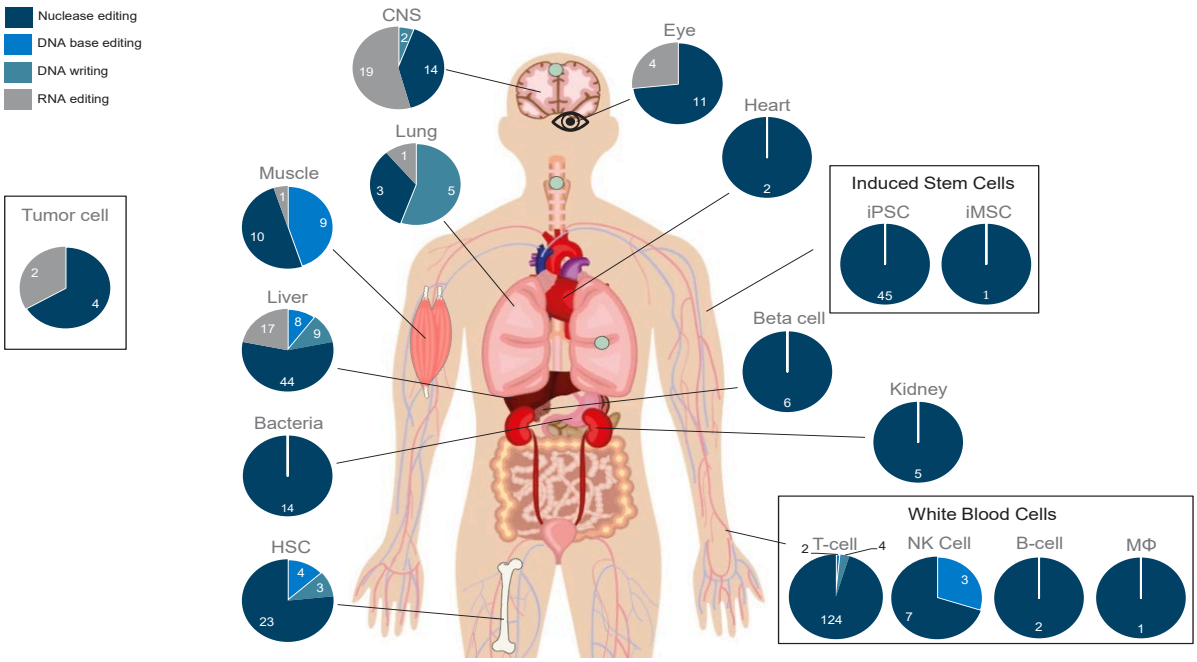
Bacteria
Bacterial Infections
Acne
Parkinson's Disease

White Blood Cells
Oncology indications
Autoimmune indications
Hemophilia
Hypophosphatasia
Aplastic anemia
Type 1 diabetes
Acute injury

HSCs
Congenital neutropenia
Sickle cell disease
Transfusion-dependent beta thalassemia
Chronic granulomatous disease
Oncology indications
Autoimmune indications
Type I diabetes

Sources: Company materials; William Blair Equity Research

Exhibit 13
DECODER
Landscape of Gene Editing Assets by Target Tissue and Editing Type



Sources: Company materials; William Blair Equity Research

Where Does Gene Editing Fit Within Therapeutics Landscape?

The advent of gene editing in human cells and its subsequent therapeutic application for in vivo models of disease sparked a wave of excitement and investment into the sector with the expectation that gene editing technologies would be the means to solve a vast array of human pathologies. However, multiple factors, such as the waning of biotech valuations and funding opportunities in the post-COVID era, as well as real-world assessments of the risk/benefit profiles of current gene editing technologies upon introduction of such assets into the clinic, have forced investors to contemplate the specific role gene editing has to play within the broader therapeutics landscape. Here, we aim to place gene editing within a larger context of developing medicines and suggest where future gene editing therapies may have an outsized utility due to differentiated characteristics.

Gene Editing Is Not Meant for Every Gene

Because of the targeted precision of gene editing and the plug-and-play nature of gene editing guides and effectors, it is tempting to believe that the ability to specifically edit any single locus within the genome may be worthwhile. Moreover, given that genes typically store information for a single product, it may be reasonable to assume that editing a single gene will necessarily precipitate change to a single protein or pathway. However, functional products of genes (proteins, noncoding RNAs, etc.) are often pleiotropic, meaning that they may have multiple cellular functions and influence multiple cell signaling cascades. Further, such diverse functionality may play different roles in different tissues, which may cause unintended side effects from targeting a specific gene with gene editing approaches. This is particularly relevant for delivery methods that may hit multiple organs within the body.

We highlight the significance of genetic pleiotropy due to the frequency with which current gene editing approaches are applied to address multifactorial diseases "indirectly" by editing a single gene. In other words, diseases that may be influenced by multiple genetic variants and/or environmental factors are improved by gene editing of a single gene, even if that gene is not the direct cause of the underlying disease. We highlight *BCL11A* (SCD/beta thalassemia), *KLKB1* (hereditary angioedema), and *PCSK9, APOC3,* and *ANGPTL3* (cardiovascular disease) as notable targets of gene editing-based therapies that aim to address diseases for which the targeted gene is not the direct culprit of disease-causing physiology and is often not even mutated in disease carriers. The utility in these genetic targets is not only in their ability to influence disease physiology when knocked out or mutated, but also that their induced mutation through gene editing causes either no or limited secondary effects that are deleterious. However, the same cannot be said about many genes, which may be effective at addressing a given disease if mutated but would cause side effects that outweigh the disease-alleviating benefits. In turn, this limits the slate of gene editing-targetable diseases to those that are caused by a single mutant gene, or, in the case of a multivariate disease, addressable via editing of a "safely mutatable" gene.

In addition to genetic pleiotropy, certain large-scale gene editing applications such as gene writing and excision may be amenable only to certain genomic locations. Gene expression is influenced by not only sequence-intrinsic properties, but also a gene's location within the 3-dimensional structure of the genome. While there may be many places where large-scale insertions and deletions do not influence neighboring genetic loci, we reason that certain large-scale mutations will have effects on the expression of neighboring genes through alterations to the local 3-dimensional genomic structure. It is therefore important to note that while certain genes and/or genomic loci may be well-qualified targets to tackle disease, their genetic and functional characteristics within the context of the cell may obviate gene editing applications.

More Than a Knockout—Carving a Niche for Genome Editing in an Era of Advanced RNA Therapeutics

Most in vivo genome editing therapeutics in advanced-stage clinical trials currently enact a genetic knockout (ablation of a gene's expression) to treat disease. While such assets have proved potent at target gene suppression in human trials to date, they compete directly with other RNA-based therapeutics (e.g., short interfering RNAs [siRNAs], short hairpin RNAs [shRNAs], and antisense oligonucleotides [ASOs]), which suppress target gene expression through gene-specific RNA engagement and subsequent degradation termed "RNA interference." For more information on mechanisms of RNA interference, see our previous *DECODER:* DECODER – Drugging RNA: Key Chemistry Advancements in the New Era of Titratable Genetic Medicines.

Advancements in the design of RNA interference therapeutics, including target sequence (what location on the target RNA to bind) as well as RNA chemical modifications that aid in prolonging half-life and enhancing affinity with machinery needed to enact RNA interference (e.g., the RISC complex for siRNA/shRNA, RNAse H for ASOs), have improved the potency and dosing frequency required for RNA interference therapeutics. These therapies can now achieve knockdown efficiencies above 90% with dosing intervals ranging from every 6 months for RNAs delivered to peripheral tissues like the liver (e.g., Eli Lilly's lepodisiran, Ionis's ION775) and up to annual dosing for CNS-delivered RNAs (e.g., Biogen/Ionis's salanersen). While genome-editing-based knockouts can undoubtedly compete in this regime of efficacy, we view every-6-month to every-12-month dosing to be a frequency threshold for patients and healthcare providers that would sway them toward RNA interference therapeutics and away from genome editors, despite their one-and-done regime. This is primarily due to their respective risk profiles; while current-generation RNA-based therapeutics have generated laudable safety profiles, genome editors potentiate more risk, including reported liver enzyme elevations, as well as theoretical risks such as off-target editing and genomic rearrangements.

Some gene editing-based knockout therapies can address diseases that RNA interference cannot, owing to the necessity of one-and-done intervention based on delivery limitations. For example, Casgevy, an approved ex vivo gene editing therapy for SCD and transfusion-dependent beta thalassemia, works by knocking out the *BCL11A* gene in the patient's harvested hematopoietic stem cells (HSCs) via a Cas9 nuclease and then reinfusing those cells back into the patient. This treatment process is expensive, time-consuming, and arduous, forcing patients to receive chemotherapy before cell reinfusion. This ex vivo treatment paradigm would not be feasible to perform on a semi-regular (e.g., yearly) basis for an equivalent therapy using *BCL11A*-targeted RNA interference, and therefore gene editing via CRISPR provides the one-and-done paradigm that increases the feasibility of the product. However, we view this competitive advantage as temporary given the rapid advancement in extra-hepatic nonviral delivery technologies. For example, recent advancements in nonviral delivery vehicles targeting HSCs (Shi, Toyonaga, & Anderson, 2023 Nano Letters; Xu et al., 2025 Nature Biomedical Engineering) may eventually allow *BCL11A*-targeting RNA interference therapies, making ex vivo *BCL11A* knockout via gene editing unnecessary.

Given the relative competitiveness of new-age RNA interference, combined with its established safety profile, we view knockout-based applications of gene editing as insufficiently differentiated over the longer term. However, we highlight three applications for gene editing that are sufficiently differentiated to justify their risk/benefit profile.

1. ***Gene correction/mutation.*** While knockdowns can limit the expression of mutated genes whose altered function leads to disease, gene correction addresses the disease while averting potential side effects associated with reduction of that protein's expression in cells and tissues. For diseases where dysfunctional protein is known to be essential in its wild-type form, gene correction is a validated approach. We highlight Serpina1, a gene whose inactivating mutation (Z allele) causes alpha-1 antitrypsin deficiency (AATD), as a popular target for gene editing therapeutics developers seeking to correct Serpina1 back to the wild-type allele (M allele) via various methods of gene correction (DNA base editing, RNA base editing, and DNA writing).

2. ***Gene insertion.*** While certain applications of DNA insertion may be bucketed as gene correction (fixing an encoded genetic deletion), additional applications (e.g., gene insertion) may be used that also provide therapeutic value for both in vivo and ex vivo applications. While mRNA-based gene therapy modalities may nonvirally deliver mRNA copies of a therapeutic gene to patients in vivo for therapeutic applications (e.g., a functional copy of a mutated gene, CAR to immune cells), these mRNA-based gene therapeutics do not have the same extended dosing regimens as RNA interference modalities, since mRNAs do not create long-term interactions with protein machinery and therefore have shorter cellular half-lives. Moreover, DNA insertion strategies via gene editing allow cellular control of gene expression by, for example, providing a specific gene regulator element (e.g., promoter) or dictating the location of DNA insertion in the case of whole gene insertion. Such flexibility is difficult to maintain with constant dosing regiments required for mRNA gene therapeutics.

3. ***Upregulation of gene expression.*** While RNA interference-based therapeutics are potent at silencing genes in a relatively gene-agnostic manner, an equal-and-opposite means of upregulating gene expression via RNA therapeutics has not been developed. Current RNA therapeutics used to upregulate gene expression are primarily composed of splicing modulators, which upregulate expression of their target gene by reducing nonproductive mRNA splicing events. We highlight Ionis's nusinersen (Spinraza) and Stoke's zorevunersen as such examples, and while powerful, such instances of gene upregulation via splicing modulation as a modality are inherently restricted to specific genes in certain diseases. In this context, we also note Camp4's RAP platform, which aims to upregulate target gene transcription via targeting of regulatory

RNA (regRNA) with ASOs. While this approach may be more extendable to genes across the genome than splicing modulators, we believe it limits the degree of gene upregulation due to the complexity of transcriptional control.

Gene editing approaches may be capable of enacting gene upregulation via mutations to, or insertion of, gene regulatory elements. This can be done at the transcriptional level, such as promoters or enhancers, or at a post-transcriptional level, such as UTRs (e.g., Editas's EDIT-401). While these applications are also likely to be gene-dependent, they provide options beyond splicing or regRNA regulation with RNA-targeted therapeutics, which may make additional disease-relevant genes capable of being upregulated with gene editors only (e.g., *LDLR* in the case of EDIT-401). We surmise that such edits capable with gene editing technologies would potentiate more exaggerated gene upregulation that may be required to treat diseases that are more severe than haploinsufficiencies.

Assessing and Mitigating Risks Associated With Gene Editing

In our view, generalist investors tend to see gene editing as a monolithic technology imparting a similar level of risk, namely the potential for permanent off-target edits, across all applications. Such risks are present and concern is warranted, but we hope that our explanations of the molecular compositions, targets, and mechanisms of different gene editing technologies convey not only different use-cases, but also different layers of potential risk. We present multiple variables that may be viewed to alter the relative risk of gene editing applications, in order of our perceived importance.

Note: our discussion of the risks associated with different gene editing technologies is nuanced and incorporates the molecular mechanisms of such modalities. We encourage readers to refer to our in-depth descriptions of such gene editing technologies in subsequent sections of this report for additional context on points made here.

- **DNA editing versus RNA editing:** Risk of off-target editing is heightened in DNA (genomic) editing versus RNA editing given the lifetime of such an edit within the edited cell—DNA edits are permanent whereas RNA edits are finite. In the case of nonviral delivery, however, the one-and-done nature of genomic edits reduces risks associated with the delivery module (only one dose is needed), whereas RNA editing must ensure the tolerability of its delivery vehicle given the requirement for repeat dosing. We view ADAR-mediated RNA editing as an emerging modality that should be a focus for investors interested in base corrections/mutations given 1) its biochemical de-risking based on recent clinical data and 2) its reduced risk for off-target edits in both quantity (sequence-based and chemistry-based advancements of ADAR guide RNAs have reduced off-target and bystander RNA editing) and potential clinical impact (bystander RNA edits are impermanent).

- **Double-stranded DNA breaks versus single-stranded DNA breaks:** The use of nucleases to create double-stranded DNA breaks precipitates a DNA damage response that alters cell signaling drastically (e.g., can induce cell cycle arrest) and potentiates genomic transformation events (large-scale DNA insertions, deletions, translocations, etc.), which may be deleterious to cells or spur malignant transformation. By contrast, gene editing modalities that cause single-stranded DNA breaks (e.g., base editing, DNA writing) induce a more muted DNA repair cascade with a significantly lower risk of genomic transformations and should be preferred for small-scale genomic editing events. For larger-scale applications using exogenous DNA insertion where double-stranded DNA breaks may be required, we view enzymes/machinery that catalyze both the DNA insertion and repair processes, such as transposases and recombinases, as the modalities that pose the least amounts of risk given they avoid cellular double-stranded break repair processes.

- **Exonic versus non-protein-coding edits:** The risk for bystander edits, which we define as off-target edits catalyzed by the editor despite proper guide molecule function, is possible with multiple gene editing modalities/applications. When applied to exons, the protein-coding regions of genes, bystander edits may impact the resultant amino acid sequence of the product protein. By contrast, gene editing targeted to non-protein coding regions, whether within the gene's transcribed RNA product (introns or UTRs) or nontranscribed regions of the genome involved in a gene's transcriptional regulation, may incur bystander edits that can influence expression but will not alter the amino acid sequence/functionality of the gene's protein product. We therefore view such applications targeted to non-protein coding regions of genes as comparatively de-risked. This also applies to potential off-target edits at distal genomic sites. Guide sequences that may have proclivity for off-target editing of exonic sequence(s) due to sequence similarities may be inherently riskier than those whose predicted off-target sites are located at non-protein coding sites.

- **Molecular proofreading:** Risks associated with off-target edits may be mitigated by modalities that require multiple successful sequence-specific events, as the likelihood of multiple events occurring with a non-cognate sequence is low. This is the case for new editors that require multiple binding events to enact a single edit. In addition, we view DNA writing as possessing such proofreading inherently: the requirement for guide binding and RNA-DNA priming for reverse transcription to occur creates a layer of proofreading that we view as de-risking for off-target editing events.

- **RNA-based versus protein-based editing effectors:** While most gene editing therapeutics are delivered to target cells entirely as RNA, most of such editors are, at least in part, mRNA, which is subsequently translated into a protein-based effector that performs the enzymatic gene editing reaction. Inherent in the expression of such foreign proteins is that they will be presented to the patient's immune system and may engender an immune response. While there is no definitive clinical data demonstrating such a phenomenon to our knowledge, such mechanisms have been postulated as linked to liver enzyme spikes in patients receiving in vivo gene editors that occurred weeks after treatment, which likely rules out that such liver enzyme spikes are being caused by the delivery vehicle (e.g., LNPs). We again highlight ADAR-mediated RNA editing, which to our knowledge is the sole gene editing modality that is delivered as a noncoding RNA payload and is therefore not translated into a foreign protein and avoids this potential issue.

FDA Guidelines for Clinical Development of Gene Editors

The FDA has published guidelines for the development of cell and gene therapy (CGT) products, which include gene editing therapeutics. The FDA updated its guidelines in September 2025, releasing three related documents:

- Innovative Designs for Clinical Trials of CGT Products in Small Populations
- Postapproval Methods to Capture Safety and Efficacy Data for CGT Products
- Expedited Programs for Regenerative Medicine Therapies for Serious Conditions

In our view, these updates confirm the FDA's continued commitment to enabling CGT development, especially for rare diseases, while tightening expectations around evidence generation and long-term safety follow-up.

The focus is on allowing informative clinical trials to be completed in rare diseases where numbers of available patients may be too small for efficient trial enrollment or statistical powering, while also ensuring that safety is a top priority for drug development. The FDA is willing to consider externally controlled (historical or real-world data from patients who did not receive the investigational treatment as the comparator group) and Bayesian trial designs (incorporate clinical data external to the trial in analyses) to expand trial flexibility without requiring large trials. We note this in the context of Regenxbio's AAV-delivered gene therapy RGX-121 for Hunter syndrome, which recently received a complete response letter (CRL) from the FDA regarding use of an external control for its study (see our note: RGX-121 CRL in Hunter Syndrome; FDA Questioning Single-Arm Trials and Surrogate Endpoints Triggering Peer Volatility). These case studies have caused investor concerns about regulatory risk despite FDA guidance documents in place.

1. In addition to guidelines on trial design, the FDA has also offered recommendations for post-marketing commitments. These guidelines were originally outlined in 2020 (see the guidelines here). For genome editing therapeutics, these have been open-label long-term safety follow-up studies lasting up to 15 years. These studies should include:

2. A plan to monitor for delayed adverse events based on the off-target editing noted in preclinical studies (e.g., in vivo, in vitro, and in silico analyses of indels). For example, if the off-target activity involves a tumor suppression gene in liver cells, developers may propose a monitoring plan for evaluation of occurrence of liver cancer as part of the long-term follow-up observation.

3. A monitoring plan regarding the adverse events from the specific organ system that the genome editing targets; it may include history and physical examination, general and specific laboratory tests, and imaging studies. If direct monitoring of the target tissue is not ethical or feasible, such as the brain tissue, an alternative plan for monitoring of the product's effects may be implemented.

4. A method to quantitate the relationship between the off- and on-target editing activity.

A plan to monitor nontarget organ/tissue safety if the gene editing asset is administered via systemic administration.

Long-term follow-up studies for therapies using AAV vectors were recommended for up to 5 years. While genome editing therapeutics using AAV delivery will still be subject to 15-year follow-up studies, these 5-year follow-up study guidelines may apply to developing RNA editing therapies that use AAV for delivery.

Long-term follow-up studies should require patient follow-up with a healthcare professional once a year for the first five years of the study. For therapies using a viral vector, testing of persistent vector sequences must be performed annually until the vector sequence is undetectable. Moreover, any follow-up may be longer for pediatric patients. Post-approval collection of real-world evidence, including healthcare records, data registries, and decentralized data collection, can add data to studies with small sample sizes, lack of comparators, and low completion rates.

CRISPR and Other Nuclease Editing

What Is a Nuclease?

DNA nucleases are enzymes that cleave phosphodiester bonds of DNA, thus disrupting the DNA structure at that active site. Though a large class of DNA nucleases are *exo*nucleases, which cleave DNA bases from the ends of a DNA polymer, genome editing applications typically use *endo*nucleases, which can cleave DNA in the middle of the DNA polymer. The breadth, structural diversity, and alternative catalytic mechanisms of different DNA nucleases make it difficult to categorize all DNA nucleases in a comprehensive manner. However, gene editing techniques tend to use specific types of nucleases for DNA cleavage.

Nuclease choice tends to depend on the desired guide used. Protein-guided, sequence-specific endonucleases, called meganucleases, recognize specific DNA sequences, which trigger double-stranded DNA cleavage. Natural meganucleases, such as the I-sceI and I-creI meganucleases, are derived from the intronic regions of lower organisms and can be used for genome editing in mammalian cells. We note that the ARCUS nuclease, developed by Precision BioSciences, is an engineered form of the I-creI enzyme, derived from an intron of the gene encoding the 23S ribosomal RNA of *Chlamydomonas reinhardtii.* Meganucleases may also be engineered by fusing the catalytic domain of an endonuclease to a protein-based TALE or ZnFP guide to form TALE nucleases (TALENs) or zinc finger nucleases (ZnFNs). The endonuclease domain of an engineered meganuclease is typically derived from a class of DNA endonucleases called restriction endonucleases. Specifically, developers of TALENs and ZnFNs often use the catalytic domain of type IIS restriction endonucleases. For example, the TALEN developed by Cellectis and the CRISPR-guided meganuclease Cas-CLOVER from Poseida Therapeutics (acquired by Roche) use the catalytic domain from the type IIS restriction enzyme FokI.

Nucleases may also be guided by a guide RNA. Specifically, the CRISPR-Cas system uses a Cas nuclease, which pairs with a guide RNA to locate its sequence target in the genome, permitting site-specific endonuclease activity. The modularity of RNA guides paired with the specific nuclease activity of Cas nucleases makes CRISPR-Cas the most common tool used in gene editing therapeutics, which we expand on below.

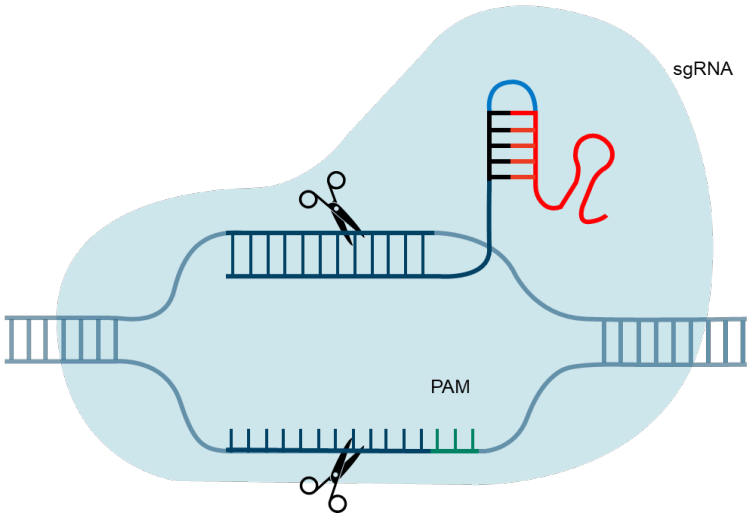
CRISPR 101

Analogous to the adaptive immune system of vertebrates, which possesses the capacity to generate cellular clones encoding receptors to specific pathogens, bacteria and archaea may also neutralize foreign invaders (i.e., viruses, plasmids, bacteriophages) in a pathogen-specific manner. In addition, in both systems, such adaptive immunity harnesses a memory component, which allows the immune system to more effectively ward off foreign entities it has seen before. For many prokaryotes and archaea, this adaptive immune system involves the genomic encoding and expansion of an array of clustered regularly interspaced short palindromic repeats (CRISPR), which are repetitive stretches of 23-55 nucleotides separated by protospacer sequences.

Upon infection of a bacterium, DNA derived from the pathogen's genome is inserted into the CRISPR array, serving as a protospacer between repeats. In this system, one unit of the repeat and the coupled protospacer are transcribed as a unit and lead to two RNAs—a tracrRNA and crisprRNA. Both transcribed RNAs are functionally linked with a Cas protein, a bacterially encoded nuclease; the tracrRNA serves as a scaffold to maintain the RNA-Cas interaction, while the crisprRNA encodes a tracrRNA/Cas binding component as well as the protospacer. Upon infection, the protospacer element of the crisprRNA serves as a guide to localize the Cas nuclease to the foreign genome, leading the Cas to dock at the pathogenic genome via gRNA/DNA sequence complementarity. Cas docking and activity also rely on a protospacer adjacent motif (PAM) site, a specific, short DNA sequence. PAM sequences may be specific to different Cas isoforms. The PAM site is located on the nontarget DNA strand—the strand that is not hybridizing with the

gRNA. The Cas nuclease can then enact its nuclease activity, cleaving the foreign DNA about 3-4 bp upstream of the PAM site, thus neutralizing the pathogen by damaging the foreign genomic material (exhibit 14).

Exhibit 14
DECODER
CRISPR-Cas System



Source: Adapted from Gomez-Escribano et al., 2025 *eBioMedicine*

After recognition of the CRISPR array as a potential genome editing tool occurred in 2012 (Jinek et al., 2012 Science; Cong et al., 2013 Science), modifications to the RNA guide component were engineered, which combined the tracr and crispr RNAs into a single guide RNA (sgRNA) for programmable, site-specific DNA cleavage (Mali et al., 2013 Science). Today's sgRNA guides are typically designed with gene-specific spacer sequences of about 20 bp in length. In addition, genome editing applications in eukaryotic cells typically require appending of a nuclear localization signal (NLS), a peptide that directs entry into the nucleus, onto the Cas for more efficient access to the genome.

Types of Cas Nucleases

Cas nucleases in nature

Phylogenetic analyses of Cas proteins spread across the prokaryotic and archaeal trees of life have classified Cas proteins into six types distributed into two separate classes: class 1 Cas systems, which comprise Cas types I, III, and IV, use a multi-protein complex to achieve nuclease activity, while class 2, composed of Cas types II, V, and VI, use single nuclease proteins.

The initial discovery of CRISPR as a gene editing tool was accomplished with Cas9, a type II Cas, from *Streptococcus pyogenes* (spCas9). Other naturally occurring Cas9 orthologs from other organisms have also been tested and provide certain utilities. For example, the Cas9 from *Staphylococcus aureus* (saCas9) is slightly smaller than spCas9, making it easier to package into AAV viral vectors. Cas9 variants may also provide meaningful differences to developers for their alternative enzymatic utility. Such is the case with the Cas9 from *Streptococcus canis,* which recognizes an "NNG" PAM sequence, as opposed to the "NGG" PAM sequence recognized by spCas9 (N could be any of the four DNA bases). Cas9 families remain the most used Cas system in the gene editing space currently.

Cas12 is another class 2 family of Cas nucleases used for gene editing. The Cas12 family is a type V Cas with an alternative nuclease domain to that of Cas9. Many Cas12 families have been identified to date (Cas12a-k) and are used for genome editing. Cas12a (Cpf1) derived from *Acidaminococcus species* (asCas12a), *Eubacterium rectale* Cas12a (erCas12a; MAD7), and *Lachnospiraceae bacterium* (lbCas12a) are the most common type V Cas used for genome editing. Notably, Cas12a proteins, along with certain other Cas12 families, can process their crRNA themselves and do not require a tracrRNA for DNA cleavage, which reduces complexities associated with expressing a separate tracrRNA or engineering an sgRNA. However, most other Cas12 families possess a tracrRNA, although Cas12c and Cas12d, also known as "CasY," use an alternative tracrRNA called scoutRNA, which is required for its nuclease activity (Harrington et al., 2020 Molecular Cell). Cas12 systems also rely on PAM sequences disparate from Cas9; for example, the PAM sequence for as/lbCas12a is "TTTN."

Discovery of Cas12 families of reduced size has garnered new interest and utilization. For example, Cas12e, also known as "CasX," which was discovered in *Planctomycetes* (plmCas12e/CasX), is about 1,000 bp shorter than Cas12a (Liu et al., 2019 Nature). Cas proteins from the Cas12f family, formerly called "Cas14," can be even smaller. In the most commonly used Cas12f family, Cas12f1, Cas proteins range from 1,446 to 2,181 bp. However, other Cas12f variants have been identified as small as 1,077 bp (Harrington & Burstein et al., 2018 Science). The most recent Cas12 family discovered, Cas12j, also known as CasPHI/Casϕ, which was discovered within the genome of huge bacteriophages, is also relatively compact, though not as small as Cas12f, and requires no tracrRNA (Pausch et al., 2020 Science).

While class 2 CRISPR systems are predominantly used in gene editing therapeutics because of their single-protein design and predictable nuclease activity, class 1 systems are also used for specific gene editing purposes. Specifically, Cas3 is a type I Cas family that associates with a multiprotein complex, Cascade, which uses a guide RNA to localize Cas3 to its genomic target. Cas3 has both nuclease and helicase (DNA unwinding) activity, which allows it to separate DNA into its constituent strands and translocate along the DNA, upwards of thousands of nucleotides, while degrading it. Therefore, as opposed to class 2 Cas systems, which enact "DNA cutter" activity, Cas3 can be thought of as a "DNA shredder."

Genomic shredding is mostly being used for developing therapies targeting bacterial or viral genetic material. LBP-EC01 from Locus Biosciences, a Cas3-encoding bacteriophage-based therapy for the treatment of E. coli infections, is the most advanced Cas3-based therapy in development. We note that CRISPR Cas3 systems have recently also been applied to long deletions of human genes, showing fewer off-target effects than Cas9 when compared directly to Intellia's NTLA-2001 gene editor at the TTR gene (Ishida et al., 2026 Nature Biotechnology).

Along with discoveries of naturally occurring CRISPR systems from the academic setting, multiple biotechnology companies are innovating discovery platforms for naturally occurring CRISPR systems that might be amenable to therapeutic applications. For example, discovery of smaller Cas nucleases may allow easier design and more efficient therapeutic delivery. Alternatively, Cas proteins with different or more flexible PAM recognition motifs may allow specific genomic locations, down to specific disease-causing genotypes, to be targeted by CRISPR-based or -guided gene editing applications. For example, the "NGG" PAM requirement of spCas9 occurs once in every 16 randomly chosen genomic loci, on average, which limits its targeting scope (Hu et al., 2018 Nature). Discovery of naturally occurring Cas variants that display hyper-specificity to their genomic targets (whether genome-wide or at a specific genomic locus) would also be therapeutically relevant by mitigating off-target editing. These, or other factors, motivate discovery of novel CRISPR effectors by biotechnology companies in-house.

Examples of sponsors iterating on novel Cas variants

Metagenomi has used alignment with the RuvC-HNH-RuvC protein domain structure, the nuclease structure of Cas9, to identify multiple Cas9 families, including the Cas9c2 family (renamed by Metagenomi as MG33), as well as three distinct Cas9d families (MG34, MG102, and MG144). This analysis also led to the identification of other nucleases with RuvC and HNH catalytic domains, including multiple HEARO nucleases (MG35), which have homology to Cas9 nucleases (Goltsman et al., 2022 Nature Communications). Metagenomi has referred to these Cas9 family nucleases as SMall Arginine-Rich sysTems (SMART). Metagenomi continues to search for new CRISPR systems by collecting samples from diverse climates and geographies and uses an AI-enabled screening platform to screen, analyze, and identify new CRISPR nucleases and other effectors. To date, Metagenomi has discovered upwards of 1.43 million predicted CRISPR loci.

Arbor Biotechnologies has contributed to the expansion of the known Cas12 family. A paper from Arbor published in Science identified and characterized Cas12c, -g, -h, and -i. Using a combination of metagenomic data curation and high-throughput in silico screening, Arbor has discovered nine proprietary gene editors.

Mammoth Biosciences is also leveraging discovery of Cas12-type nucleases for genome editing. Mammoth was founded by the team that used metagenomics to discover and characterize the Cas12f family proteins (Harrington & Burstein et al., 2018 Science) and is using Cas12f and Cas12j family proteins for varying gene editing therapeutics. In addition, Mammoth is using a protein discovery platform with proprietary AI algorithms to search for novel CRISPR systems with the aim of harnessing smaller Cas proteins for therapeutic purposes.

Life Edit, a subsidiary of ElevateBio focusing on next-generation gene editing, has screened more than 8 billion proteins to identify potential protein sequences homologous to RNA-guided nucleases, or ones that are predicted to have similar structure, using AI. To enable gene editing at a broader swath of the genome, Life Edit is identifying nucleases with diverse PAM sequences, including those that are rich in Ts instead of Gs, from non-pathogenic microbes. Life Edit has four proprietary nucleases (LEG14, LEG95, LEG98, LEG145), which are used for multiple gene editing applications.

Similarly, EmendoBio's OMNI technology platform has provided a suite of novel nucleases derived from metagenomic searches capable of covering 86% of the genome with differential PAM targeting.

Cas nuclease engineering

Discovery of novel CRISPR systems and Cas nucleases lays a foundation for expanding the RNA-guided genome editing toolbox. Further complexity and utility can be added by taking naturally derived Cas proteins and engineering specific synthetic mutations.

For example, certain genome editing applications are not compatible with double-stranded DNA cleavage by the Cas, and instead necessitate DNA nicking—the cleavage of only one DNA strand. For Cas9, development of Cas9 nickases (nCas9) is well documented: mutation of the 10th amino acid, an aspartic acid (D10), which resides in the first (N-terminal) RuvC domain, to an alanine (D10A) induces nCas9-mediated nicking at the target DNA strand (the strand hybridizing with the gRNA). Alternatively, mutation of histidine 840 (H840A) within the HNH nuclease domain results in an nCas9 that cleaves the nontarget strand (for more information, see the following resource here). While nCas9 nickases are commonplace, a Cas12 nickase has only recently been discovered using asCas12a by mutating arginine 1226 (R1226A) within the nuclease domain sandwiched between the two endmost (C-terminal) RuvC domains. Similar to the mutation of the HNH domain in Cas9, which also lies between Cas9's two C-terminal RuvC domains, asCas12a R1226A cleaves

the nontarget DNA strand (Kim et al., 2024 Scientific Reports). Further characterization of the asCas12a R1226A mutant, its translatability to other Cas12 family Cas nucleases, and its utility to relevant gene editing applications will need to be elucidated further.

Developers may not want the Cas protein to enact any nuclease activity itself, instead using it purely as an RNA-guided shepherd to the appropriate genomic locus and using other effector components for the actual gene editing process. For Cas9, adding D10A and H840A mutations, or their equivalents, in the same Cas renders the protein nuclease dead ("dead Cas9" [dCas9]). Similarly, mutation of either of the RuvC domains in Cas12 proteins renders a catalytically dead mutant.

In addition to generalizable Cas variants, specific mutations may be engineered into naturally derived Cas nucleases to alter or improve functionality. The methodologies by which developers engineer hyper-functional variations from a starting protein are largely out of the scope of this piece, though we highlight a key resource on this topic here. Certain techniques, such as saturated mutagenesis screens, are primarily capable of testing utility of different amino acids within a concentrated section of a protein's sequence without changing the protein's global size or structure. Alternatively, techniques such as minimization by iterative size exclusion and recombination (MISER), which comprehensively test the functionality of different deletions within proteins, may be useful to developers of CRISPR-based therapies by increasing the potential to reduce the size of a Cas-based gene editor. MISER has already been applied to Cas9 and demonstrated tolerance of spCas9 to deletions in specific domains (Shams et al., 2021 Nature Communications). We highlight some engineered Cas proteins below.

After the discovery of CRISPR as a gene editing tool, modifications to Cas9 were enacted to improve its functionality. An initial study showed that improvements to Cas9 specificity could be made by dampening the potency of spCas9 to unwind the double-stranded DNA helix, which is ultimately required for DNA cleavage. Mutations in the groove that stabilizes the nontarget strand upon DNA-gRNA hybridization accomplished this, resulting in a spCas9 mutant enhanced spCas9 ("espCas9") highly intolerant of mismatches between the gRNA and DNA target (Slaymaker and Gao et al., 2016 Science). Biophysical investigations of spCas9 function led to targeted mutagenesis of the noncatalytic REC3 domain in spCas9, resulting in a hyper-accurate Cas9 variant ("HypaCas9"; Chen et al., 2017 Nature). Cas9 variants with less restrictive PAM requirements have also been engineered, including "xCas9," a Cas9 that recognizes a broad range of PAM sequences while maintaining hyper-specificity compared to spCas9 (Hu et al., 2018 Nature). Distinct from hyper-accuracy, mutations in Cas9 that induce hyperactivity have also been elucidated (Vos et al., 2022 Nature Communications). Recently, a Cas9 ("HyperDriveCas9") that combines mutations that endow hyper-accuracy and hyperactivity was developed (Vos et al., 2024 Cell Reports Methods).

Examples of sponsors engineering Cas nucleases for enhanced activity

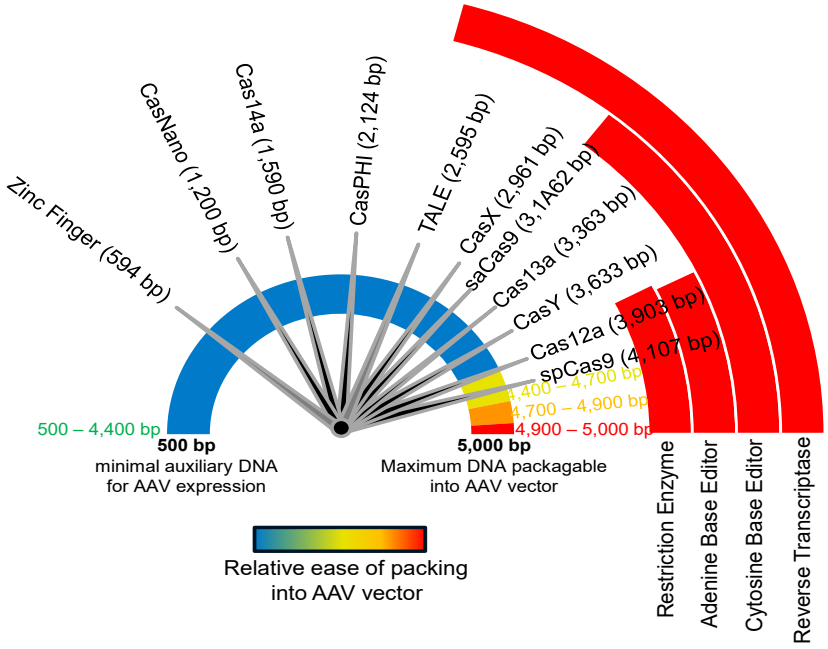
While Cas9 maintains its stronghold with current CRISPR-based therapeutics, many biotech companies are centering their developing assets on Cas12-based variants. Along with discovery of multiple Cas12 families, Arbor applied semi-rational engineering of Cas12i2 with structure-based amino acid substitutions, which markedly improved its DNA endonuclease efficiency in mammalian cells. This modified Cas12i2-based nuclease (ABR-001) demonstrated genome editing rates in line with spCas9 while reducing off-target editing. ABR-001 has also been demonstrated to be compatible with AAV-mediated in vivo delivery (McGaw et al., 2022 Nature Communications).

Subsequent investigations have found Cas12i2 nuclease activity to be specific, but with low editing efficiency. Therefore, hfCas12max was developed as a Cas12i variant with hyper-specificity and activity (Zhang et al., 2022 Protein & Cell). Currently, hfCas12max is being used by HuidaGene for gene editing therapeutic efforts. Scribe Therapeutics is using CasX (Cas12e) as the foundation for creating variants, leading to its proprietary X-editing technology.

Multiple companies are using more recently discovered compact Cas variants for their gene editing therapeutic assets. Building off the metagenomic discovery of multiple Cas12 families, Mammoth is developing Cas12f-based ("NanoCas") and Cas12j-based products for its gene editing applications.

The dCas12f-based systems are particularly well used in epigenetic editing therapies. For example, Epic Bio is pioneering epigenetic applications with "CasMINI," a variant of a Cas12f family member from uncultured archaea. CasMINI was engineered through an iterative screen of mutants in the Cas's DNA binding pocket, which has previously been demonstrated to improve Cas activity by roughly 200-fold over wild-type (Xu et al., 2021 Molecular Cell). CasMINI was also demonstrated to be functional in multiple gene editing contexts. Lastly, Epitor has developed the smallest publicly known Cas for therapeutic purposes, "CasNano" (not to be confused with Mammoth's NanoCas), a Cas12f-based nuclease 1,200 bp in length. For size comparisons of different naturally occurring and engineered Cas nucleases, see exhibit 15.

Exhibit 15
DECODER
AAV Delivery Packaging Constraints for Genome Editors



All reported lengths reflect actual protein lengths. Placement on dial adds an additional 500 bp to reflect auxiliary DNA required for AAV expression.

Lengths of TALE and zinc finger constructs are estimated for 18 base pair target sequences.

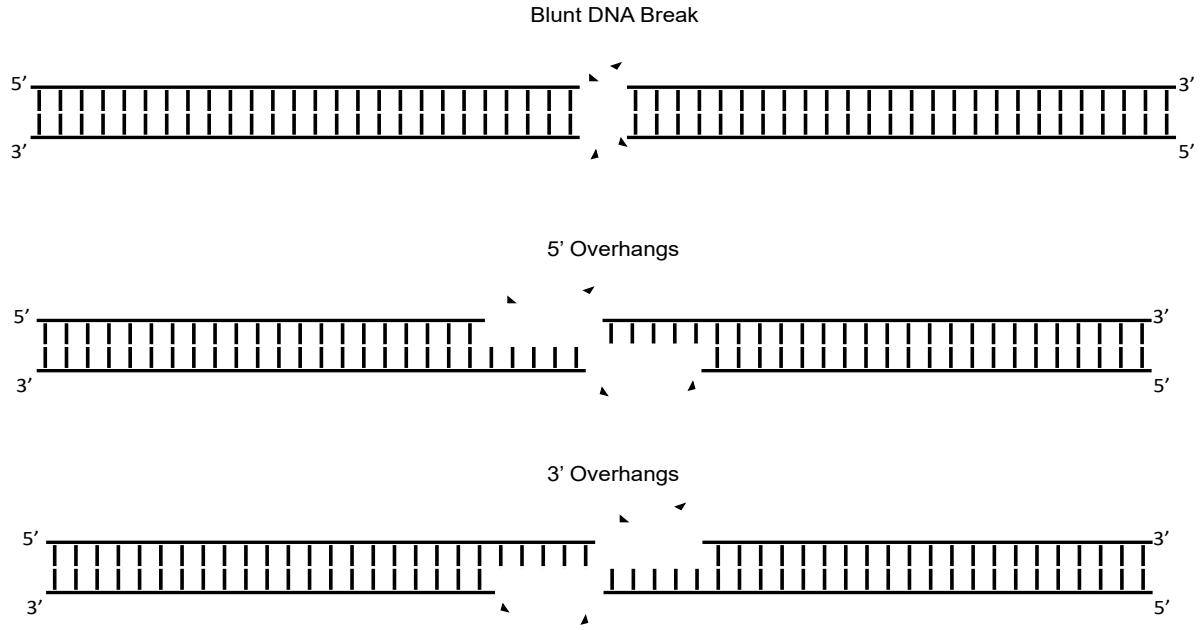
Sources: Cappelluti et al. 2024 *Nature* ; William Blair Equity Research

Cellular Response to Nuclease-Mediated DNA Breaks—Method of DNA Repair and Editing Matters

While they are an integral part of gene editing technologies, Cas proteins and other nucleases do not themselves *edit* the sequence of the genome. Instead, in cases where the nuclease is used for gene editing, the physical action imparted onto the genome is a DNA break at its docked location. Potential changes to the surrounding DNA sequence that ensue are not enacted by the nuclease itself, but are instead introduced during the cell's response to DNA damage, which serves to fix the break caused by the nuclease.

Different nucleases catalyze different types of DNA breaks, which are important for the type of DNA repair process and also any directional insertion of genetic material in the editing process. "Blunt DNA breaks" are characterized as a clean split of the DNA strands with no overhanging bases on each end; all bases on each "DNA half" maintain base pairing with the partner strand. In contrast, because nucleases catalyze the phosphodiester cleavage of each DNA strand independently, cuts to DNA can also occur that are staggered—a number of complementary bases are split between the separate DNA halves, leaving those bases "overhanging" with no bound complementary bases from the partner strand. Because these two resulting DNA halves have some affinity to one another since their overhangs are complementary, these cuts are known as "sticky" DNA breaks. Sticky DNA breaks can derive from overhangs on the 5' end of the DNA halves (5' overhangs) or the 3' end (3' overhangs); see exhibit 16. For CRISPR-mediated DNA cleavage, Cas9 nucleases create blunt DNA breaks, while Cas12 nucleases create sticky cuts with 5' overhangs. For meganucleases, customizability of the resulting DNA cut can be engineered by swapping in different nuclease effectors. For example, I-creI meganucleases create 3' overhangs, whereas the FokI restriction endonuclease creates 5' overhangs. While FokI restriction endonuclease is the typical restriction endonuclease used in ZFNs and TALENs, catalytic domains of other restriction endonucleases that catalyze alternative DNA breaks could also be commandeered for specific gene editing purposes.

Exhibit 16
DECODER
Types of Double-Stranded DNA Breaks



Source: William Blair Equity Research

A cell's ensuing response to the nuclease-induced DNA break determines the outcome and global efficiency of genome editing. To repair a break faithfully so as to reduce the introduction of mutations, cells may use a nucleotide template for repair, which is discovered through complementarity (homology-directed repair [HDR]); opening of the double-stranded DNA helix into its single stranded DNA components allows a separate DNA molecule to anneal on both sides of the break, enabling the cell to fill in the gap. In natural DNA breaks, this template strand may be provided by the other chromosomal gene copy or, more typically, the homologous chromosome, which is present during phases of the cell cycle after the cell has replicated its genome.

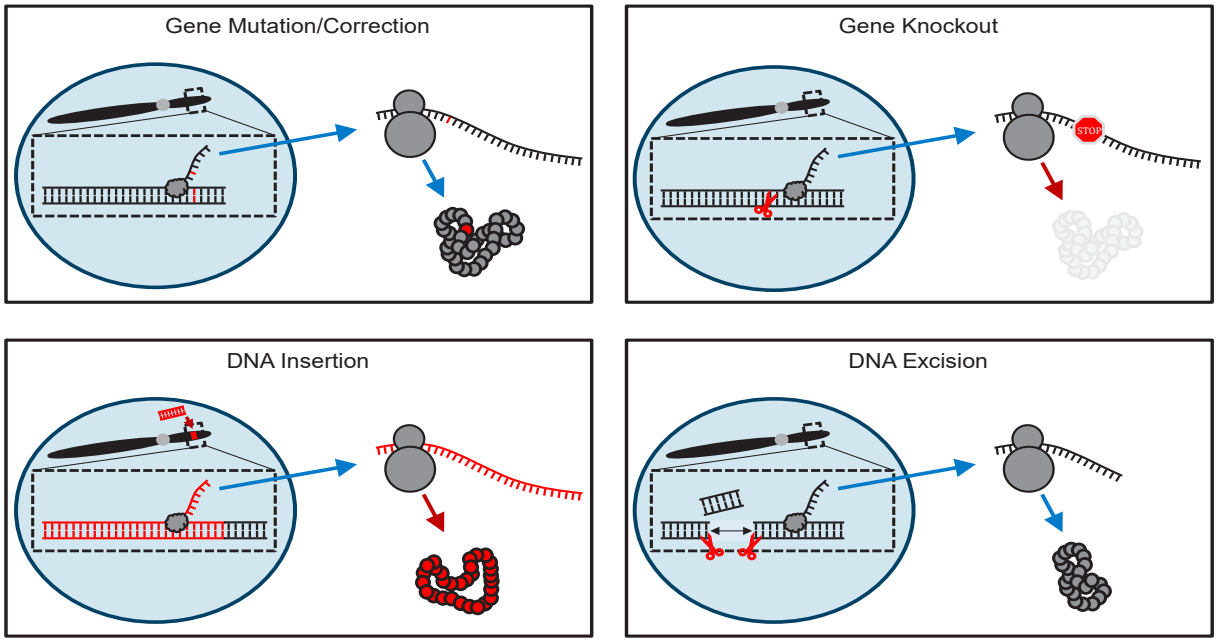
However, most cells in the body are terminally differentiated and are not replicating and therefore are not copying their DNA. Moreover, cells that are actively dividing spend only a minority of time in a state with replicated DNA. Therefore, the cell requires DNA damage responses that do not rely on a template to mend the break. Such "end joining" means of DNA repair include nonhomologous end joining (NHEJ) and microhomology-mediated end joining (MMEJ), which anneal the broken DNA strands back together with little resection (chewing back of the naked DNA ends) involved. Repair via end joining mechanisms is typically more error prone than HDR and can lead to *in*sertions and *del*etions (indels) within the genome compared to the original DNA sequence before the DNA break occurred. These indels are the gene editing imparted by nuclease-based gene editors and are an intended consequence for therapeutic applications. While indels induced by end-joining-based DNA repair may be semi-random in sequence outcome from DNA locus to DNA locus (the type [insertion or deletion] and/or size [number of base pairs] of the indel may vary depending on where in the genome the nuclease performs a DNA break), the specific sequence outcome at a given DNA locus may be fairly consistent, and even predictable based on the DNA sequence surrounding the cut site (Shen & Arbab et al., 2018 Nature; Allen et al., 2018 Nature Biotechnology).

The rate of unfaithful (indel-inducing) DNA repair depends on multiple factors, including the cell type involved and the genomic location of the break (Allen et al., 2018 Nature Biotechnology). The faithfulness of the DNA repair is also influenced by the type of break induced, with staggered cuts leading to more faithful, homology-based repair mechanisms (HDR, MMEJ), whereas blunt cuts favor an NHEJ-led response, which can introduce mutations (So & Martin, 2019 PLoS Genetics). In addition, the efficiency of the nuclease can influence the faithfulness of DNA repair, with more efficient nucleases precipitating repair that is more error-prone, potentially because both alleles of the target gene have a greater likelihood of being cut simultaneously (Kosicki et al., 2022 Nature Communications). Faulty DNA repair can lead to large-scale mutations in the genome, such as large-scale genomic deletions, inversions, or translocations of DNA/chromosomes into improper genomic locations (for more information about potential genomic rearrangements potentiated by nuclease activity, see this article). Such alterations may impart oncogenic properties and pose serious risk to the health of the cell and/or tissue. Therefore, the choice of nuclease used for gene editing therapeutics can significantly affect the resulting cellular response and resulting genotype when considering gene editing techniques for therapeutic applications.

Applications for Nuclease-Based Genome Editing

Nuclease-based editors allow multiple types of editing, and given their prominence in the field are often the go-to methodology for enacting a number of well-used genome editing outcomes (see exhibit 17 for examples of common genome editing applications). We note that other types of gene editing technologies may provide an easier or more effective way of enacting such genome editing outcomes, and we highlight such strengths and weaknesses as part of this report.

Exhibit 17
DECODER
Prominent Applications of Genome Editing

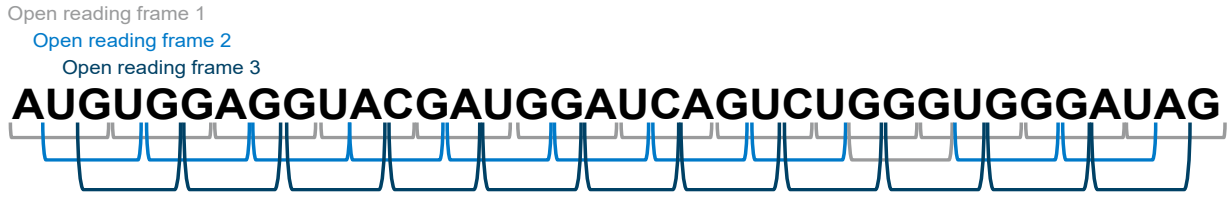


Source: William Blair Equity Research

Gene knockout

To date, the most common application for genome editing is a genetic knockout, which eliminates expression of a gene's functional protein product. For nuclease-based editing, knockouts are enacted via administration of a targeted DNA break, resulting in a DNA damage response. Developers may target a number of different genomic locations within a gene to enact a gene knockout. For example, targeting a nuclease to the protein-coding region of a gene will elicit indels into the edited gene. When reading a transcribed gene's mRNA sequence into protein, ribosomes translate the mRNA sequence into a sequence of amino acids via triplet-RNA-base segments (codons); each codon corresponds to a specific amino acid. Therefore, a single mRNA can, in theory, be translated in three unique sets of codons, called "reading frames," which would each yield unique amino acid sequences (exhibit 18). If indels caused by the DNA damage response result in the addition/subtraction of a number of DNA bases that is not divisible by 3, the reading frame will be thrown off, leading to the mRNA bases downstream of the indel to assemble as different codons, which will change the resultant amino acid sequence. Alternatively, even if the reading frame is maintained, a mutation may cause modifications to the amino acid sequence at that site, which hampers the protein's activity. Lastly, mutations may lead to a premature "Stop" codon ("UAA," "UAG," or "UGA") within the reading frame, which will induce the ribosome to terminate the protein's production prematurely. The resultant mutant protein either will be degraded by the cell or exist in a dysfunctional state, though we note the potential (however unlikely) for a resultant protein with an alternative amino acid sequence to generate unintended consequences, including gains of function that may lead to alternative cellular signaling or toxicities. Because modifications to the amino acid sequence are incurred at and downstream of the site of the indel or mutation, developers attempting to knock out a gene by targeting the gene's protein-coding sequence typically design the gene editor to cut near the beginning of the gene so that a larger percentage of the protein product is mutated, which raises the probability that the protein will be dysfunctional.

Exhibit 18
DECODER
Theoretical Reading Frames Within mRNA Sequence



Source: William Blair Equity Research

Genes may also be knocked out via targeting a nuclease not within the protein-coding gene itself, but to a genomic sequence that regulates the gene's transcription. Such gene regulatory elements, such as promoters and enhancers, recruit proteins, such as transcription factors, in a sequence specific manner to promote transcription of a gene. If the nuclease-induced DNA damage response leaves mutations in the sequence that abrogate transcription factor recruitment, expression of the gene may be lost. Given that the gene regulatory element is not part of the transcribed mRNA product, considerations of reading frame do not apply, meaning indels of any number of DNA bases may preclude transcription. In addition, as certain transcriptional regulatory elements are used only by certain cell types, editing of such elements in a mixed population of cells, or in stem cells that may differentiate into multiple cell types, can allow developers to enact a gene knockdown in specific cell population(s), making the gene editing therapy more targeted. This strategy is employed by Crispr Therapeutics/Vertex's Casgevy, the first FDA-approved gene editing therapy, for the treatment of sickle cell anemia and beta thalassemia. Casgevy delivers Cas9 to HSCs to create a DNA lesion within the erythroid-specific enhancer of the *BCL11A* gene. Mutation of the enhancer's sequence precludes binding of the transcription factor GATA1 at that site, which suppresses *BCL11A* expression and allows gamma-globin production specifically in erythrocytes. While editing of a gene's noncoding regulatory element(s) represents an effective means of controlling a targeted protein's expression, such strategies require additional *a priori* knowledge of the underlying transcriptional circuitry of the regulatory element and target gene, including whether mutation of that regulatory element is sufficient to eliminate expression of the target gene, or whether abrogating the regulatory element's function will have transcriptional effects on other genes in the genome.

Genomic excision, fragmentation, and degradation

In addition to genetic knockout strategies employing a single nuclease to make a single cut at a single genomic locus, certain gene editing therapeutics use nuclease activity at multiple genomic sites at the same time to enact specific aims. For example, introducing a cut at two distal sites on the same chromosome can induce a genomic deletion between the cut sites, particularly when end-joining mechanisms of DNA repair are used. This has the potential to address multiple disease applications resulting from unwanted stretches of DNA. For example, Excision BioTherapeutics is using a CRISPR-based approach to excise viral genes that are integrated into the human genome upon infection. This excision-based strategy makes it more unlikely for the DNA repair machinery to fix the break faithfully and restore viral function. In addition, recombination events within human genes can also lead to pathological outcomes via expansion of repetitive DNA sequences within a gene's coding region. Excision of these expanded repeats can address these diseases. Lastly, specific regions of genes, including exons and untranslated regions (UTRs), may be excised for therapeutic benefit. We highlight EDIT-401 from Editas, which excises the 3' UTR of LDL receptor (LDLR) to boost LDLR expression and LDL uptake in familial hypercholesterolemia.

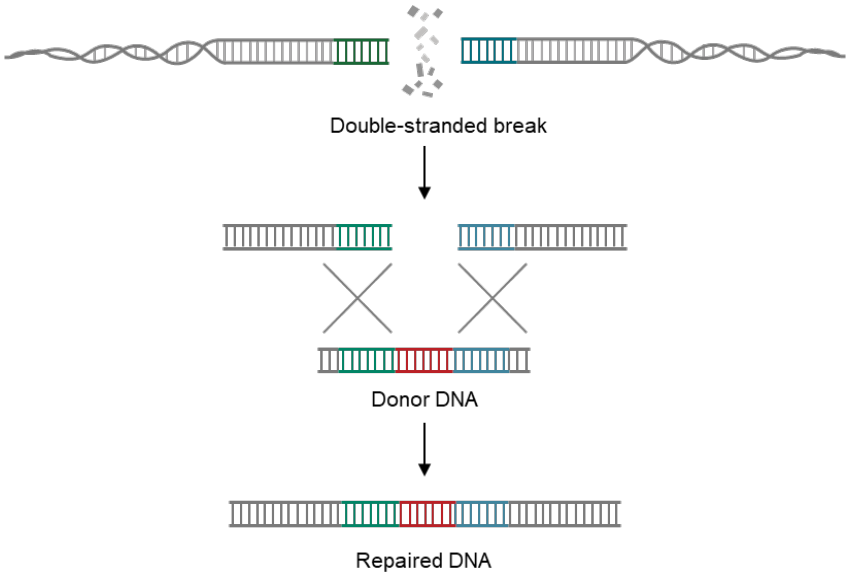
Genomic fragmentation by spreading multiple cuts across the genome, if done to a proper extent, can also cause cellular death. Part of the cell signaling cascade disseminated during the DNA damage response involves cessation of the cell cycle and other cellular activities until the break is repaired. However, if DNA damage is incurred to a significant enough extent, the cell will undergo regulated cellular death (apoptosis). Such a mechanism is commandeered by multiple chemotherapies to fight proliferating cancer cells, albeit with limited specificity on the malignant cells, leading to unwanted side effects. Alternatively, gene editing may be able to create enough cuts in a cancer genotype-specific manner as to cause cell death in malignant cells while sparing healthy cells. Such is the strategy behind CINDELA, the proprietary therapeutic platform of CasCure Therapeutics.

In addition to targeted genomic cuts, DNA degradation can also serve to eliminate pathogenic DNA in a more nonspecific manner. This application is primarily accomplished with Cas3, a class 1 CRISPR system that enacts both helicase and nuclease activity to degrade extended stretches of DNA. To date, Cas3 has been used in the advent of CRISPR-based antibiotic therapies, including the degradation of both bacterial and viral genomes. Also, we note that because the human mitochondrial genome has a severely limited DNA damage response compared to the nuclear genome, a single DNA cleavage event of mtDNA can spur mitochondrial genome degradation. This is particularly relevant to Precision BioSciences' mitochondrial genome degrading therapeutic PBGENE-PMM, which induces degradation of mutant mitochondrial DNA via a single cut in the MT-TL1 gene by its ARCUS meganuclease.

Homology-based gene correction and insertion

Upon introduction of a DNA lesion administered by a nuclease, the cell will attempt to repair the break back to the original DNA sequence as faithfully as possible. To determine what the repaired sequence should be, the cell can use HDR, which recruits instances of sustained homology in the genome, such as a sister chromatid or homologous chromosome; this sequence can anneal with the broken DNA strands on either side of the break during repair and serve as a template for the polymerization of new DNA at the site of damage. This HDR strategy can be commandeered for gene editing purposes; by inducing a DNA break while concomitantly introducing a ssDNA or dsDNA repair template, cells can be prompted to use that template in the repair process. We note that ssDNA templates are used more and considered safer due to their lower rates of random integration into the target genome. Such repair templates typically have sustained homology on either side of the insertion site (homology arms). These arms are typically about 200-500 bp (each) in size, although shorter homology arms (roughly 50 bp) have also been successful. Optimal homology arm length may be dictated by the length of the insert, with larger genomic additions requiring more homology to facilitate insertion. Between the homology arms, genetic edits can be encoded (exhibit 19). These edits may be as minor as swapping out a base pair within a gene's sequence to adding an entire gene into the genome. Homology-independent means of DNA integration have also been engineered (Suzuki et al., 2016 Nature). For gene editing therapeutics, AAV is typically used to deliver an ssDNA repair template, though novel ssDNA synthesis, including that from Kano Therapeutics, may soon revolutionize the preferred method of choice for delivering repair templates to cells.

Exhibit 19
DECODER
Homologous Recombination-Based Gene Insertion



Sources: AAT Bioquest

In instances where a repair template is used to edit a gene's sequence, the nuclease is targeted near the site of the desired insertion, allowing integration of the repair template in the proper location. Such repair strategies have varying rates of efficiencies depending on the size of the insertion; they also run the risk of introducing indels that may throw off the reading frame upon nuclease activity, even if the repair template is used. For therapies using a nuclease/repair template to introduce an entire gene into the genome, developers want to minimize the size of the repair template, whether for packaging restraints relating to delivery or to maximize gene insertion efficiency. Therefore, unlike gene therapies, gene editing repair templates do not encode transcriptional regulatory sequences (promoters, enhancers, etc.). As a result, developers typically insert these full gene payloads into actively transcribed genes to ensure the insert's expression. Some design the nuclease to deliver the gene insert into one of a target gene's exons, portions of the transcribed mRNA that are spliced together during pre-mRNA processing to encode the protein product. This serves to simultaneously insert the desired payload into the genome while knocking out the targeted gene. Such a strategy is often used by developers of gene-edited chimeric antigen receptor (CAR) T-cell therapies who wish to simultaneously insert their CAR payload to drive its expression at levels commensurate with T-cell receptor (TCR) expression in T cells, while simultaneously knocking out the endogenously expressed TCR by inserting the CAR gene within an exon of the *TRAC* locus. In addition, genes can be inserted into "safe harbor" genomic loci, which are transcribed but their perturbation is largely inconsequential to human health. The *PCSK9* gene is an example of a safe harbor locus used to insert genes, and is used by iECURE for multiple gene insertion therapies in clinical development.

To express the gene as a standalone protein, with no amino acids from the targeted exon appended onto the protein, developers may use a 2A ribosome skipping sequence, such as a P2A, T2A, or E2A sequence, which are short sequences (about 65 bp) that induce the ribosome to skip formation of a peptide bond. When placed within an mRNA, the ribosome will translate the mRNA sequences on either end of the 2A site as separate polypeptides (more information about 2A ribosome skipping

sequences can be found in this article). Therefore, by placing a 2A site at the beginning of the gene insert, the gene, despite being appended to the mRNA exons from the target locus, will be translated as a wild-type protein product.

Gene editing therapeutic developers also use gene insertion strategies to insert a functional copy of a gene into the genome to supplement the cell/tissue with a functional gene copy. This is done when one or both endogenous gene copies are mutated, leading to disease. While insertion of a functional gene copy into the locus of the mutated gene is an ideal strategy in theory, as it would serve to knock out the mutated protein and replace it with a wild-type copy, this is rarely done in practice; the low efficiency of successful gene insertion (typically 5%-10% for whole gene insertion strategies) means that successful insertion of a gene into its endogenous locus would achieve only 2.5%-5% of total functional protein production compared with a healthy individual expressing two healthy copies of the gene, assuming no compensatory mechanisms by the cell/tissue to boost expression of the single functional gene copy inserted. Therefore, to maximize expression of the inserted gene upon genome integration, gene inserts are introduced into gene loci that are highly transcribed. One such example is the albumin gene (*Alb*), which is a highly transcribed gene that is expressed/produced by the liver, a common site of delivery for current gene editing therapeutics. Albumin is an essential protein for bodily function, and therefore insertion strategies targeting the *Alb* locus attempt to reduce potential knockout of albumin that may be caused upon nuclease cleavage at that site. To achieve this, the nuclease is targeted to an intron, a portion of the transcribed RNA that does not encode for the protein and is "spliced out" during pre-mRNA processing. Because introns are not part of the translated mRNA that encodes the protein, any mutations that arise after nuclease cleavage are inconsequential to synthesis of a functional protein. To ensure that the inserted gene is translated and is not spliced out as an intron during pre-mRNA processing, splice sites must be added to the inserted gene to append it as an exon. Therefore, regardless of the design for intronic gene insertion, the efficiency of expression of the inserted gene will be affected by not only the rate of successful genomic insertion, but also the rate of reconfigured splicing; "canonical" splicing between the endogenous exons flanking the targeted intron of the targeted locus will prevent expression of the inserted gene. In addition, as with exon insertion strategies, 2A ribosome skipping sequences can be commandeered to ensure synthesis of a standalone wild-type protein product.

Multiple schemes for intronic insertion of genetic payloads have been employed for therapeutic purposes. For example, to maximize successful insertion events, Intellia Therapeutics' gene insertion scheme, which is being used by Regeneron to insert a functional *F9* gene into the genome for treatment of hemophilia B, places two copies of the *F9* gene into its repair template—one in the forward direction and one in the reverse direction in case of an inversion caused during the insertion process—into intron 1 of the *Alb* gene. In addition, a splice site has been placed at the beginning of each gene insert copy (5' end) to allow for successful splicing with exon 1. Because both the forward and reverse copy of the gene are inserted into the genome, a poly-A signal/transcriptional stop sequence is also added to the 3' end of each gene copy to prevent the reverse copy, and the remaining part of *Alb*, to be transcribed. This allows a functional mRNA to be transcribed with exon 1 of *Alb* appended to it. While the first exon of *Alb* is only 26 amino acids, we expect a 2A site is included to allow translation of the inserted gene in its wild-type form. However, we note that the first exon of *Alb* also contains the first base of the encoded H27 codon, and therefore additional bases must be added after the splice site of the encoded insert to maintain the proper reading frame. This insertion strategy into the intron of *Alb* mitigates albumin knockouts or mutations in the albumin protein upon nuclease activity from Intellia's Cas9, but prevents albumin expression in any instance where the repair template has been inserted. Because of the relatively low efficiency of gene insertion and the robust albumin expression by hepatocytes, this strategy can be employed, and the company has articulated extensive preclinical testing to ensure that the loss of functional *Alb* gene copies in the gene-edited hepatocytes has little effect on total serum albumin levels in nonhuman primates.

Alternatively, other intron-based gene insertion schemes attempt to insert a gene of interest into an intron while maintaining expression of the functional protein encoded by the target locus. While Intellia's approach encodes both a forward and reverse gene sequence within its insert, Cellectis encodes only the forward sequence, which is flanked by 2A sites at both the 5' end and 3' end of the gene. In addition, the insert re-encodes the first exon of the targeted locus. Therefore, during translation, three unique proteins are translated from a single mRNA: 1) the peptide encoded by exon 1 of the target locus, 2) the inserted gene of interest, and 3) the full-length protein within the targeted locus. This allows the gene of interest to be inserted while maintaining expression of the gene in the targeted locus. While not detailed by the company, two distinct splice sites on either end of the insert must be present to splice the insert with exon 1 and exon 2 of the targeted locus.

In comparing these intronic gene insertion strategies, we note that Cellectis uses a TALEN with a FokI catalytic domain, which induces a staggered cut, compared to the blunt cut from Intellia's Cas9-based approach. Because staggered cuts favor homology-directed repair pathways more than blunt cuts, we speculate this could be a motivating factor driving these alternative approaches. Multiple strategies, including specific mutations in Cas9 that trigger staggered cuts (Chauhan et al., 2023 PNAS) as well as Cas9 fusion proteins with protein domains that inhibit NHEJ repair (Jayavaradhan et al., 2019 Nature Communications), have been used to improve faithful gene insertion using Cas9-based approaches.

Non-homology-based gene correction and insertion

In addition to using the cell's innate DNA damage machinery to resolve nuclease-induced DNA damage and facilitate homology-based insertions, other exogenous enzymes may be used for genome editing, which both enact nuclease activity and help resolve the integration of DNA into the genome. Such dual actions, in our view, may provide safer, more well-regulated strategies for genomic manipulation, if harnessed appropriately, than canonical nuclease-only-based efforts.

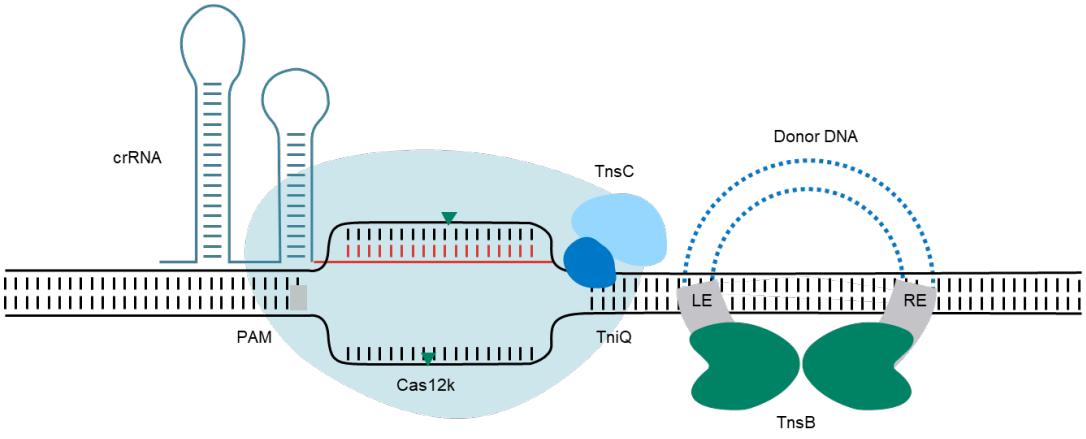
Transposons are mobile genetic elements that take harbor within host genomes. The mobility of transposons stems from a transposase, an enzyme encoded within the transposon that enacts nuclease activity to snip out its parent transposon sequence from the genome through recognition of the transposon's inverted terminal repeats (ITRs) and then reintegrate the transposon sequence back into the genome at a different location. There are many transposons in nature, but the most commonly used transposons for biotechnological purposes are sorted into three main superfamilies: the piggyBac superfamily, which hosts the piggyBac transposase; the Tc1/*mariner* superfamily, which hosts the Sleeping Beauty transposase; and the *hAT* superfamily, which hosts the Tol2 and TcBuster transposases. Using the transposase's genomic integration activity, developers can deliver a transposase to cells along with a DNA oligomer flanked by the transposase's cognate ITR sequence, which will spur the transposase to integrate that piece of DNA into the genome. Transposases can handle large DNA cargos—the piggyBac transposase can integrate up to 100 kb—which allows large insertions into the genome to be accomplished. Unfortunately, transposons provide little specificity for sites of integration: piggyBac transposases integrate near transcriptional start sites and prefer a TTAA sequence, Sleeping Beauty and TcBuster transposases integrate at TA dinucleotide sequences, and Tol2 transposases provide even less defined specificity (for more information about transposons, see this article). Therefore, variability regarding effects on gene expression of the host cell, as well as levels of expression of the genetic payload, are inherent in these systems.

When used in isolation, transposases provide great tools to insert gene cassettes semi-randomly into the genome, but do not provide gene-specific editing capabilities, owing to their lack of insertion specificity. Therefore, such applications do not fit within our definition of genome editing. However, combination of the cargo capacity and integration efficiency of transposons with the gene targeting specificity of guide molecules may allow proper harnessing of transposon-based genetic manipulations at a gene-specific level. Multiple companies have engineered transposon-guide

fusions to impart specificity onto transposase-mediated gene insertion. Integra Therapeutics has developed a proprietary FiCAT system, which fuses piggyBac transposase to a Cas9 with full nuclease activity. The developers demonstrate that administration of a double-stranded break executed by the Cas9 enhances the efficiency of site-specific payload integration by the transposase (Pallares-Masmitja et al., 2021 Nature Communications). In addition, Poseida Therapeutics (acquired by Roche) is developing site-specific integration with its proprietary super piggyBac transposase by coupling it with undisclosed sequence-specific DNA binders.

Developers are also using guided transposon systems that have already developed in nature—integration of transposon elements within CRISPR systems in bacteria have evolutionarily created such targeted gene integration systems. Such CRISPR-associated transposons (CASTs) use the Tn7 and Tn7-like transposons for integration. These transposons have naturally coupled with multiple CRISPR systems. Most CAST systems use class 1 CRISPR systems, whereby the Cascade protein complex is used to direct the transposon to a genomic site. CAST systems are also used in class 2 CRISPR systems and use Cas12k as their guide. Cas12k, unlike other type V Cas proteins, is a pseudonuclease, meaning it does not enact nuclease activity. Therefore, Cas12k acts like a dCas to shuttle the transposon to the proper genomic locus. Because of their compact size, these Cas12k-integrated CAST systems (type V-K CASTs) are likely the most useful for gene editing therapy applications (exhibit 20). Find more information about CAST systems in this article. We note that Metagenomi lists over 1,000 CAST systems, discovered through its CRISPR system discovery platform, as part of its gene editing toolbox and has developed a construct that improves site-specific integration by 50-fold compared to the wild-type system. In addition, recent work used a continuous evolution platform to develop a CAST system (evoCAST), which boasts 420-fold improvements in site-specific payload integration (Witte et al., 2025 Science). While CAST systems currently trail other methods in integration efficiency, we see potential for continued development of type V-K CRISPR systems given their built-in programmability and specificity, with multiple protein components in the CAST system that may be further optimized to yield improved results.

Exhibit 20
DECODER
CRISPR-Cas12K Associated Transposon (CAST) System

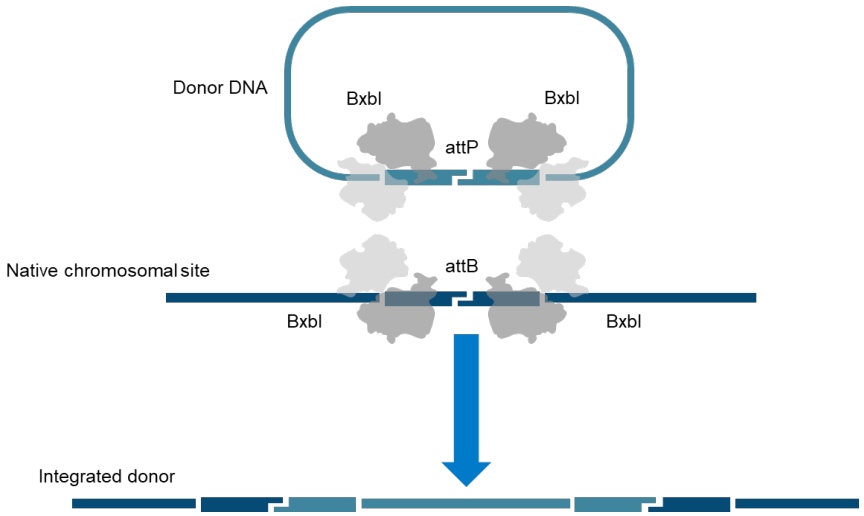


Source: Rozov & Deineko, 2025 *Molecular Biology*

In addition to transposon systems, recombinases are a class of enzymes that can perform genetic excisions, inversions, or translocations, depending on the recombinase and the orientation of recombinase sites, which are DNA sequences that are recognized by the recombinase to enact its activity. For site-specific genomic insertions, large serine recombinases (LSRs), also known as "integrases," are being investigated for gene editing therapeutics. The LSR Bxb1, which derives from

the Bxb1 bacteriophage, has been identified as a potential enzyme ideally suited for transgene insertion into human cells in a therapeutic context because of its 1) low off-target integration activity, 2) ability to reduce initiation of single- and double-strand DNA break responses due to inherent ligation activity, and 3) unidirectional DNA integration mechanism, thereby ensuring proper orientation of the transgene payload. For transgene insertion, Bxb1 excises its DNA donor through recognition of an attachment phage (attP) site and facilitates directional DNA donor integration via recognition of an attachment bacterial (attB) site within the target genome. Both the attP and attB sites are semi-palindromic in nature with a Bxb1 complex binding to each "half-site" independently; a Bxb1 tetramer (one Bxb1 on each half-site of the attP and attB site, respectively) facilitates transgene insertion into the genome (exhibit 21). We note that during the actual recombination reaction, each DNA end is covalently attached to the active site of one of the Bxb1 monomers and the DNA ends are re-ligated to new DNA strands at the end of the reaction. This means that though a double-stranded genomic break occurs during recombinase-mediated insertion, the cellular DNA damage response is not recruited, which significantly limits possible risks of unwanted genomic transformations compared to other nuclease-based insertion methods.

Exhibit 21
DECODER
Large Serine Recombinase (Integrase)-Based Gene Insertion



Source: Sangamo company materials

Structure-based investigations into the interaction between Bxb1 and the attB site have led to insights into the basis for how the integrase recognizes the attB site in a sequence-specific manner. This has motivated engineering-based efforts to create Bxb1 mutants that may possess a cognate attB site chosen by developers that is located within the human genome. As with other transgene insertion modalities detailed above, the attB insertion site may be located in a gene's intron or exon, with safe-harbor sites often used as a desired locale for Bxb1-mediated transgene insertion. We highlight preclinical work from Sangamo Therapeutics, Seamless Therapeutics, and Stylus Medicine, among others, which are using integrase engineering efforts to insert desired payloads into cells at specific genomic sites in vivo. In addition, as demonstrated by Sangamo's modular integrase (MINT) platform (see more from the company's preprint here), the efficiency of site-specific Bxb1 integration may be increased by appending a protein-based guide to Bxb1 to direct the integrase to its desired location.

In addition to Bxb1, other recombinases employ nuclease activity to enact genetic manipulation, which may correct mutated DNA sequences in a therapeutic setting. For example, an engineered zinc-finger guided recombinase capable of performing genetic inversions may be capable of correcting inverted gene sequences. Most prevalently, inversion of the *F8* gene, which is caused by an intrachromosomal recombination event of two homologous regions on the X-chromosome, leads to hemophilia (Lansing et al., 2022 Nature Communications; Mukhametzyanova et al., 2024 Nature Biotechnology). This work is being furthered by Seamless Therapeutics.

Nuclease-based strategies to integrate short DNA sequences (fewer than 100 bp) using DNA ligases are also being engineered without the need for introduction of double-stranded breaks. In this strategy, termed ligase-mediated programmable genomic integration (L-PGI), a Cas-based nickase is used to open the double-stranded DNA helix while a splint molecule hybridizes to the Cas's guide RNA, the DNA proximal to the nick, as well as a piece of donor DNA to be inserted into the genome. A DNA ligase, a DNA repair enzyme that can seal the sugar phosphate backbone between two DNA strands, is appended to the nickase and catalyzes the integration of the donor DNA into the genome. This technique was being developed by Tome Biosciences (this company is no longer in operation, to our knowledge).

Mitigation of Off-Target Nuclease Activity

Effective nuclease-based gene editing therapeutics must display precise, on-target effects, as off-target cuts could engender unwanted mutations or other DNA damage-based genomic rearrangements that could risk malignancy. Therefore, developers of such therapeutics must employ the utmost care to limit additional DNA cuts made by their targeted nuclease. Below are a few strategies employed to reduce off-target nuclease activity.

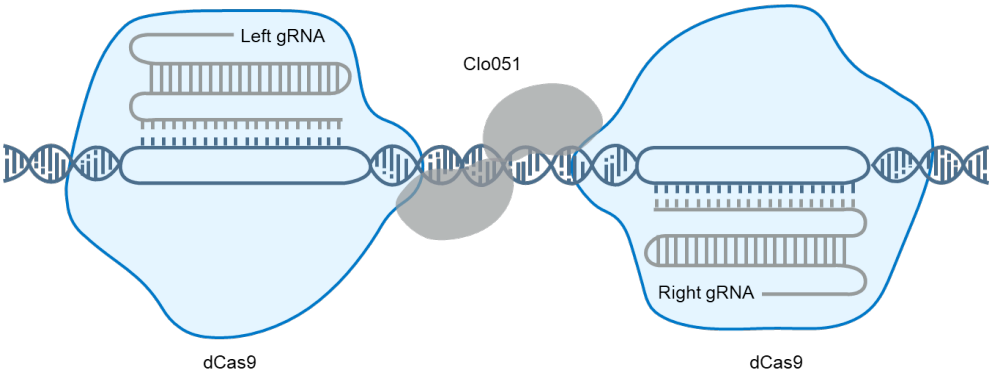
Guide design

While certain nuclease-based gene editing applications require activity at an exact position within the genome, other applications allow for that nuclease event to occur within a larger window, such as a gene, exon, or intron. Therefore, developers may have multiple options for the specific sequence the guide can be targeted to. In these instances, developers must ensure that the genomic sequence being targeted is unique within the genome, and the more dissimilar the targeted sequence is to any other sequence in the genome, the less likely the guide will have proclivity to bind to other genomic locations and spur unwanted nuclease activity. Guides can tolerate multiple mismatches when binding off-target sites, but providing hard-and-fast rules regarding the number of mismatches needed within the off-target site to hinder nuclease activity is difficult because the location of mismatches within the guide binding site influences such effects; generally, the closer the mismatch between the guide and the genomic target is to the nuclease's catalytic site, the more influential that mismatch will be on abrogating nuclease activity. For CRISPR-based guides, gRNA mismatches proximal to the PAM site will have an outsized effect on limiting Cas docking and activity. Optimal guide development typically relies on both in silico tools to predict a guide's off-target activity throughout the rest of the genome and experimental tools to identify genomic sites of off-target nuclease activity when a given guide-nuclease construct is introduced to cells. For an introduction to such methods, see this article. It is important to note that such analyses must take into consideration natural human genetic variation, as specific single nucleotide polymorphisms (SNPs) present in the genomes of certain human populations or individuals may promote or deter on-target or off-target binding sites. In addition, we note that for nuclease-based applications that require a single nuclease cut with no DNA donor sequence required (e.g., protein knockout applications), off-target sites located in noncoding genomic regions or intronic regions within other genes may be less deleterious, as potential indels created will not impact the gene expression profile of the cell. Nonetheless, we still deem the mitigation of any off-target nuclease activity by gene editing therapeutics as paramount to achieving clinical success and regulatory approval.

Require multiple DNA binding events

While employing a single nuclease to enact a desired modification improves efficiency and reduces size-related complexities for delivery, a single nuclease, if capable of enacting its activity upon DNA binding, can act spuriously. On the other hand, gene editing strategies that require two independent gene editors to bind in order to enact a single gene editing event significantly reduce the probability of off-target activity. For example, the FokI restriction enzyme requires dimerization for nuclease activity. Therefore, developers employing FokI-based meganucleases typically attach a single FokI monomer to two independent guides specific for two proximal DNA sites. Nuclease activity, therefore, occurs only when both FokI monomers are brought into proximity upon each meganuclease binding to its cognate DNA sequence. We highlight Poseida Therapeutics' Cas-CLOVER, which, instead of using the nuclease activity inherent in Cas9, employs a Clo51 nuclease monomer attached to dCas9 for nuclease activity. A Clo51 dimer, and thus nuclease activity, is allowed only when two dCas9 molecules, each guided by a unique sgRNA for proximal DNA sites, bind (exhibit 22). Two separate effectors can increase design-related hurdles regarding accommodation of two separate guide-based binding events. For example, as CRISPR-based guides require a PAM sequence to bind, considerations for a specific cut site must accommodate PAM sites proximal enough to allow proper activity, or the design of two unique Cas variants with different PAM sites, for gene editing to occur. This complexity is increased with ZnFNs due to difficulties with ZnFP design for a given DNA sequence.

Exhibit 22
DECODER
Poseida Therapeutics' Cas-CLOVER System



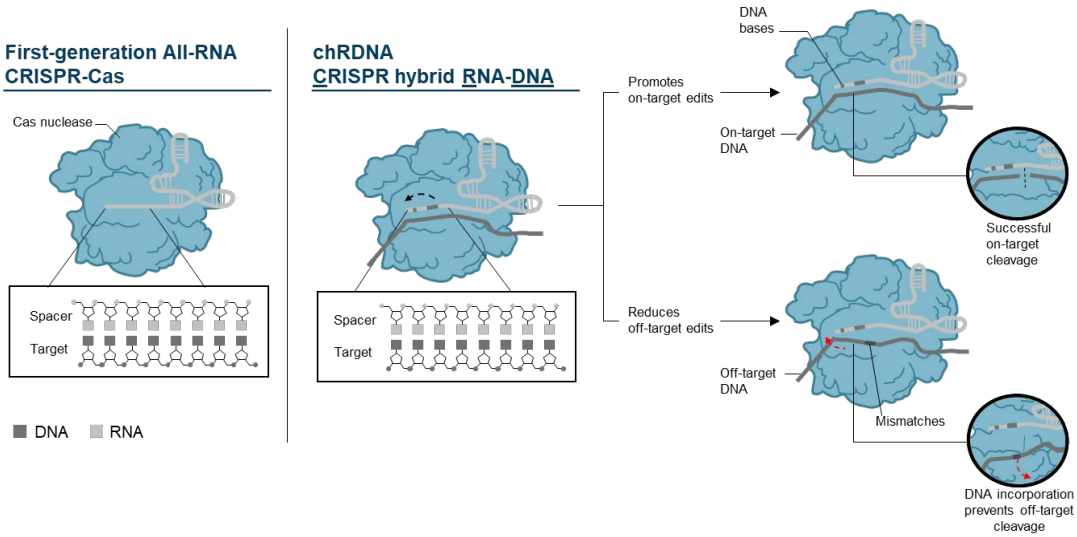
Source: Poseida Therapeutics company materials

Reducing DNA affinity

While DNA binding is required for activity of all DNA nucleases and is often necessary for the enzyme's "search" functionality where it scans and identifies its cognate DNA sequence, reducing the guide's affinity for DNA can improve the stringency of activity; this allows proper DNA binding and conformational changes within the protein necessary for cleavage to occur only when the guide's exact cognate sequence is found. Multiple strategies can be employed to reduce DNA binding affinity. For protein-based guides, point mutations can be made to reduce the affinity for the cognate sequence by adding a TALE/ZnFP domain that does not precisely match its DNA target. Alternatively, for TALENs, given the relative low binding affinity of A- and T-binding TALEs, using a more TA-rich sequence will aid in the avoidance of the TALEN for other near-cognate sequences. For CRISPR-based approaches, modification to the Cas itself, whether creating changes in the DNA binding domain, as was executed with CasMINI, or the PAM recognition motif, will alter the Cas's affinity for DNA. Changes to the gRNA can also be executed to reduce DNA binding affinity, including shortening of the gRNA or adding chemical modifications to the gRNA bases or backbone, which can improve specificity (for more information about chemical modifications to CRISPR guide RNAs,

see this article). Alternatively, gRNA-DNA pairing can be perturbed by interspersing cognate DNA bases within the gRNA sequence. This CRISPR hybrid RNA-DNA (chRDNA, pronounced "chardonnay") is used by Caribou Biosciences (exhibit 23). We note that such complex modifications to RNA are more difficult to manufacture and cannot be executed if using AAV-based delivery, which requires expression of the guide RNA in the viral genome.

Exhibit 23
DECODER
Caribou Biosciences chRDNA System



Source: Caribou company materials

Chromatin-specific DNA binding

In addition to sequence specificity, novel gene editors are using chromatin accessibility as an additional dimension of regulation for editing activity. Chromatin, the DNA-protein amalgam that regulates the genome, including 3-dimensional genome organization and gene-specific transcription, can exist in different states depending on how the cell wishes to regulate that genomic locus. Areas of the genome that are transcriptionally inactive typically exhibit a tightly packed chromatin structure, called heterochromatin, whereas euchromatin represents a sparser chromatin structure, allowing transcription to happen more freely. Eterna Therapeutics has developed a proprietary gene editor, NoveSlice, which is selective for nuclease activity in euchromatic regions. This feature potentially limits the number of off-target sites in the genome that are targetable by the nuclease.

Shorten editor half-life

For most gene editing therapies, their greatest utility lies in their one-and-done nature; gene editors alter the underlying genetic issue to rescue the cells and tissues affected with a single treatment. In addition, the timescale of these enzymatic reactions is relatively quick. However, dosing requires a balancing act of administering enough gene editor such that not only does it get into enough cells, but also there is enough within one cell so that at least one editor can access and edit the genomic locus of interest. However, too much editor in a cell will raise the statistical likelihood that an off-target editing event is enacted. This is particularly pertinent to AAV-based delivery of gene editors, which typically expresses the editor off a constitutively active transcriptional

promoter. Therefore, in these contexts, mechanisms to either stop the expression of the editor or severely limit the half-life of the editor within the cell are needed to raise the bar for accessing the genome so that an unwanted edit is not made eventually. Certain protein domains can be appended to an editor to limit its half-life in cells. For example, a PEST amino acid sequence—named because of the abundance of proline (P), glutamic acid (E), serine (S), and threonine (T) amino acids within it—triggers rapid protein degradation by the cell's proteosome and is commonly used by developers. Alternatively, such strategies can be tailored to control editor expression in a small-molecule-dependent manner. Methods to limit the half-life of ZnFPs by appending a protein moiety that could regulate its destruction via a small molecule have been explored (Pruett-Miller et al., 2009 PLoS Genetics). Such a strategy has been built on by Obsidian Therapeutics, which can append its proprietary Drug Responsive Domain (DRD) to a protein, which induces its degradation by the cell's proteosome. Only by adding a small molecule can the protein maintain expression in the cell for function. The company has recently applied this technology to gene editor(s).

Strategies to exogenously modulate an AAV-expressed editor can also be done at the transcriptional or mRNA level. While Neurogene does not have a gene editing asset currently, we highlight its proprietary EXpression Attenuation via Construct Tuning (EXACT) AAV technology, which allows controlled expression of its encoded genetic payloads from its AAV plasmids. In addition, AAV plasmids can be designed to include the native nuclease site within it such that when the nuclease is expressed, it will cleave the AAV and prevent further expression of the nuclease from occurring. Such a strategy is being explored by Precision BioSciences. In addition, RNA aptamer-based engineering from MeiraGTx has demonstrated small-molecule-controlled modulation of gene editor expression via modulation of its mRNA splicing.

Nuclease-Based Genome Editing Business and Clinical Updates

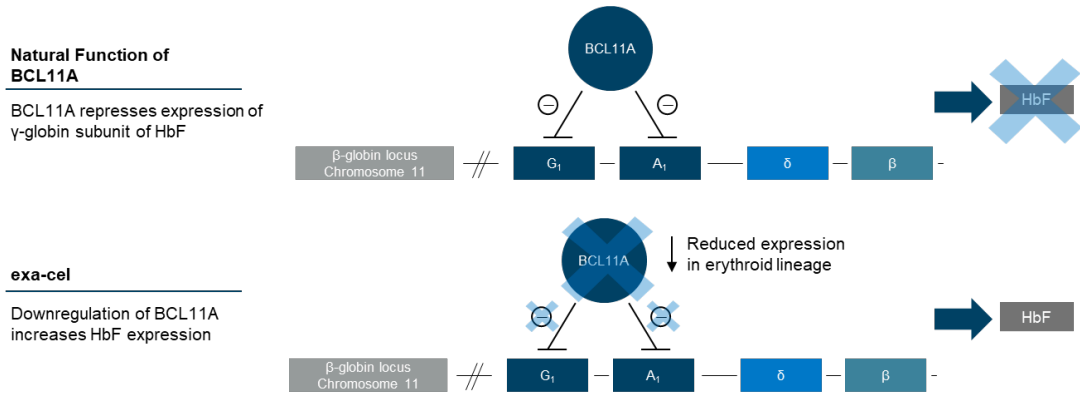
CRISPR Therapeutics AG (CRSP)
Location: Boston

Technology
CRISPR uses CRISPR/Cas9 technology in combination with a sgRNA in its current clinical-stage therapies. For delivery of its primary therapeutic assets, which target hemoglobinopathies and liver-mediated diseases, CRISPR uses electroporation and LNPs, respectively, to promote delivery of its gene editing machinery. Although not discussed below, the company is also developing CAR-T therapies containing multiple gene edits for the treatment of cancer and autoimmune diseases; additional liver-targeted, LNP-delivered therapies; and gene edited islet cells for the treatment of type 1 diabetes.

Key gene editing assets

Casgevy (exa-cel; CTX-001) is an autologous, CRISPR/Cas9-edited, hematopoietic stem cell therapy for the treatment of transfusion-dependent beta-thalassemia (TDT) and sickle cell disease (SCD). The therapeutic uses CRISPR/Cas9 and the SPY101 gRNA to disrupt the GATA1 transcription factor binding domain of the *BCL11A* gene. Inactivation of the *BCL11A* gene or its enhancer enables the reactivation of fetal hemoglobin (HbF) through a single target (exhibit 24). Therefore, the edited cells lead to sustained generations of HbF-containing, functional red blood cells without the potential for graft-versus-host disease, a severe risk for allogeneic hematopoietic stem cell transplantation.

Exhibit 24
DECODER
Mechanism of Action of Casgevy



Source: CRISPR Therapeutics company presentation

In 2015, CRISPR entered a strategic four-year research collaboration with Vertex Pharmaceuticals aimed at the discovery and development of gene editing treatments using the CRISPR/Cas9 technology, under which Vertex was granted exclusive rights to license up to six CRISPR/Cas9-based treatments that emerge from the collaboration. As part of the collaboration, Vertex made an upfront commitment of $105 million to CRISPR, including $75 million in cash and a $30 million equity investment. Under the terms of the agreement, discovery activities were to be conducted primarily by CRISPR, and the related expenses would be fully funded by Vertex. Vertex was to fund 100% of the development expenses of licensed treatments and, for each of the treatments in-licensed for development, Vertex would pay future development, regulatory, and sales milestones of up to $420 million as well as royalty payments on future sales. In 2017, Vertex selected Casgevy as the first treatment to be developed as a part of the collaboration, with research-and-development costs and profits worldwide to be split equally between CRISPR and Vertex.

In 2021, CRISPR and Vertex announced that they had amended their collaboration agreement to allow Vertex to lead the worldwide development, manufacturing, and commercialization of Casgevy and any future hemoglobinopathy programs in the collaboration. Under the revised agreement, costs, revenues, and profits related to Casgevy and other hemoglobinopathy programs were to be split 60:40 between Vertex and CRISPR. In addition, CRISPR received an upfront payment of $900 million and was eligible for a $200 million payment upon the first regulatory approval of Casgevy for either indication.

Casgevy was approved by the FDA in December 2023 for the treatment of adolescents and adults with SCD and January 2024 for the treatment of adolescents and adults with TDT based on positive interim results from the single-arm, Phase I/II/III CLIMB-THAL-111 and CLIMB-SCD-121 trials.

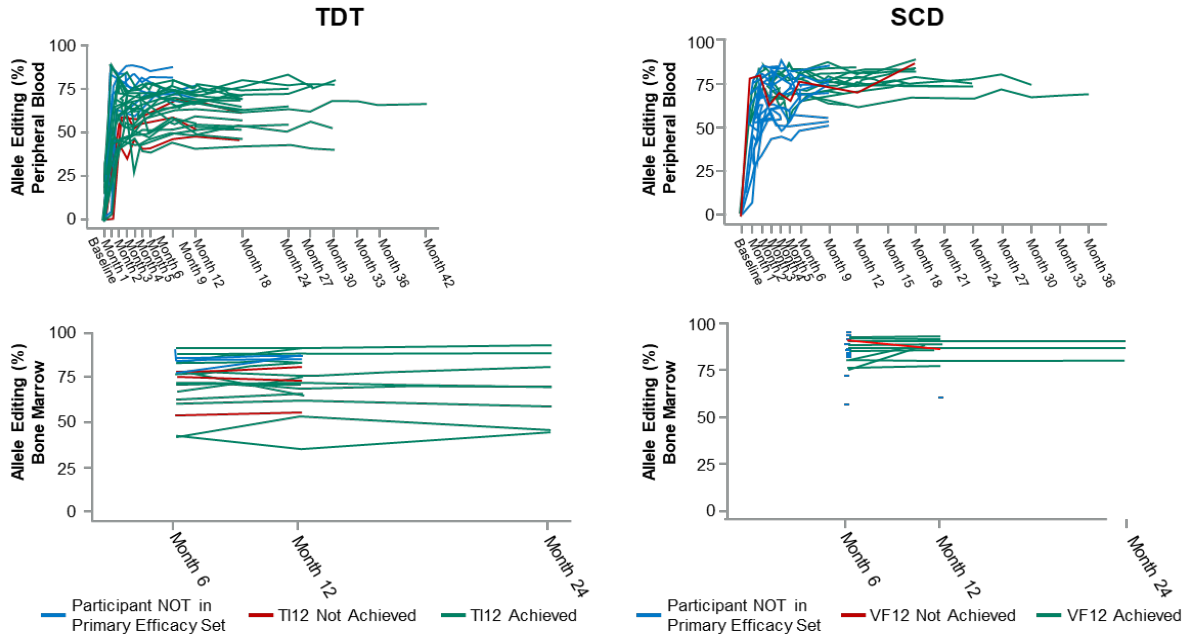
CLIMB-SCD-121 included 44 patients who had been treated with exa-cel, with a mean follow-up of 20.1 months (ranging from 0.8 to 48.1 months), including 12 adolescent patients. By month 6, it was estimated that 37% of patients' CD34+ cells had the intended genetic modification. The mean proportion of Hb comprised by HbF was 43.9% at month 6 and was maintained thereafter. Increases in total Hb were observed as early as month 3 and continued to increase through month 6. Consistent with the increase in HbF levels, the mean proportion of circulating erythrocytes expressing HbF (F-cells) at month 3 was 70.1% and continued to increase over time to 94.0% at month 6, with levels remaining stable thereafter, indicating sustained pan-cellular expression of HbF. The primary efficacy set (PES) comprised patients with at least 16 months of follow-up. In the PES cohort, 97% (29 out of 30) of patients achieved freedom from vaso-occlusive crises (VOCs) for at least 12 consecutive

months (VF12), including 6 adolescent patients, and 100% (30/30) of patients achieved freedom from hospitalizations related to VOCs for at least 12 consecutive months (HF12), with a mean duration of VOC freedom of 22.4 months and a maximum duration of 45.5 months. It is important to note that the patient who did not achieve VF12 achieved HF12 and had a complex set of comorbidities, including a history of chronic pain. In addition, one patient who achieved VF12 experienced a VOC 22.8 months following exa-cel infusion in the setting of a parvovirus infection, but this patient has since fully recovered from the infection and has been VOC-free since.

Fifty-two patients with TDT received Casgevy in CLIMB-THAL-111, 35 of whom were included in the primary efficacy set (patients followed ≥16 months), with a median follow-up of 20.4 months. Similar to SCD, increases in mean total Hb and HbF levels were observed as early as month 3 after infusion and continued to increase to 12.2 g/dL and 10.9 g/dL, respectively, at month 6. After month 6, levels of total Hb and HbF were maintained, with HbF constituting ≥88% of total Hb. Consistent with the increase in HbF levels, the mean proportion of circulating erythrocytes expressing HbF (F cells) at month 3 was 73.8% and continued to increase over time to 95.9% at month 6, with levels remaining stable thereafter, indicating sustained pan-cellular expression of HbF. In addition, the proportion of edited *BCL11A* alleles was stable over time in bone marrow CD34+ cells and in peripheral blood nucleated cells. Thirty-two patients (91.4%) in the primary efficacy set achieved the primary endpoint of transfusion-independence for at least 12 consecutive months (TI12), with an average duration of 22.5 months and a maximum duration of 45.1 months. Of the three patients who did not achieve TI12, one had reductions in annualized RBC transfusion volume of 83.9%, and the other two have been transfusion-free for 7.3 months and 4.0 months, respectively, starting 60 days after the last transfusion, highlighting a strong reduction in transfusion burden despite not achieving TI12.

In exhibit 25, we show the BCL11A editing reported in TDT and SCD patients.

Exhibit 25
DECODER
***BCL11A* Editing Efficacy of Casgevy in SCD and TDT Patients**



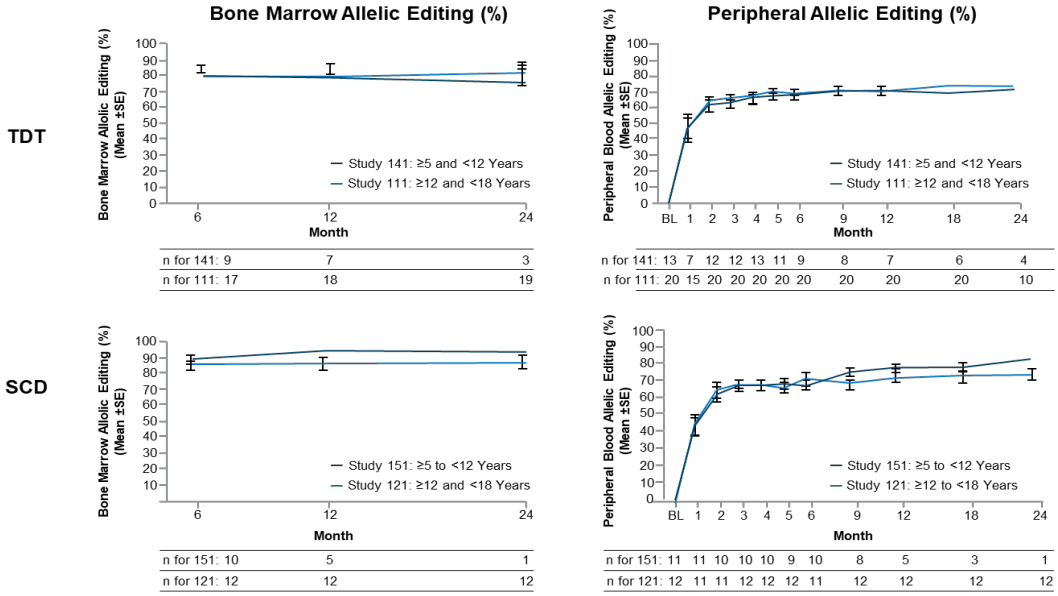
Bone marrow allele editing figures include patients who have at least 12 months of follow-up whereas the blood allele editing figures include all patients.
BCL11A, B-cell lymphoma/leukemia 11A; SCD, sickle cell disease; TDT, transfusion dependent β-thalassemia

Source: CRISPR Therapeutics company presentation

Before the approval of Casgevy, the FDA hosted an advisory committee meeting focused on the potential for off-target editing with exa-cel in the target SCD population due to the presence of genetic variations. Overall, the committee viewed the in silico analysis conducted by the companies as detailed, with lenient thresholds, and the additional application of GUIDE-seq enabled substantial genetic variation to be considered, thereby being a sufficient investigation of the potential off-target effects of exa-cel. One member noted there could be room for deeper sequencing and discussed the use of whole genome sequencing of patients before and after exa-cel, but others did not see this approach as necessary and noted that potential off-target editing edits would likely be too low for this type of detection. Another suggestion was to incorporate prescreening for the CPS1 variant and to retrospectively look to see if any patients treated with exa-cel possess the variant and what, if any, the consequences were. The committee supported the use of monitoring patients for 15 years post-treatment, as the companies outlined, and suggested indel frequency and clonal dominance be tracked over time (for more details, see: The Only Thing Spooky About Exa-cel AdCom Was How Technical It Was; Approval for SCD Expected by December 8 PDUFA).

At the American Society of Hematology (ASH) 2025 conference, Vertex and CRISPR presented initial data in 5- to 11-year-olds who were treated with Casgevy, including 11 patients with SCD and 13 with TDT. Twelve of the 13 TDT patients had stopped red blood cell transfusions as of the last follow-up (range: 2.3 to 22.5 months), with 100% (6/6) of patients with enough follow-up achieving TI12. For the SCD patients, no patients experienced a VOC following Casgevy treatment (range: 3.2 to 24.1 months), with 100% (4/4) of patients with enough follow-up achieving VF12 and HF12. Efficacy, HbF expression, and bone marrow allelic editing in 5- to 11-year-olds appeared similar to older adolescents (12- to 17-year-olds); see exhibit 26.

Exhibit 26
DECODER
Efficacy of Casgevy in Pediatric Patients with SCD or TDT



Source: CRISPR Therapeutics company presentation

Exa-cel demonstrated a similar safety profile to that in CLIMB SCD-121 (mainly related to myeloablative conditioning), although no adverse events related to exa-cel were observed in CLIMB SCD-151, whereas 28% were observed in CLIMB SCD-121. One pediatric patient death from exa-cel was

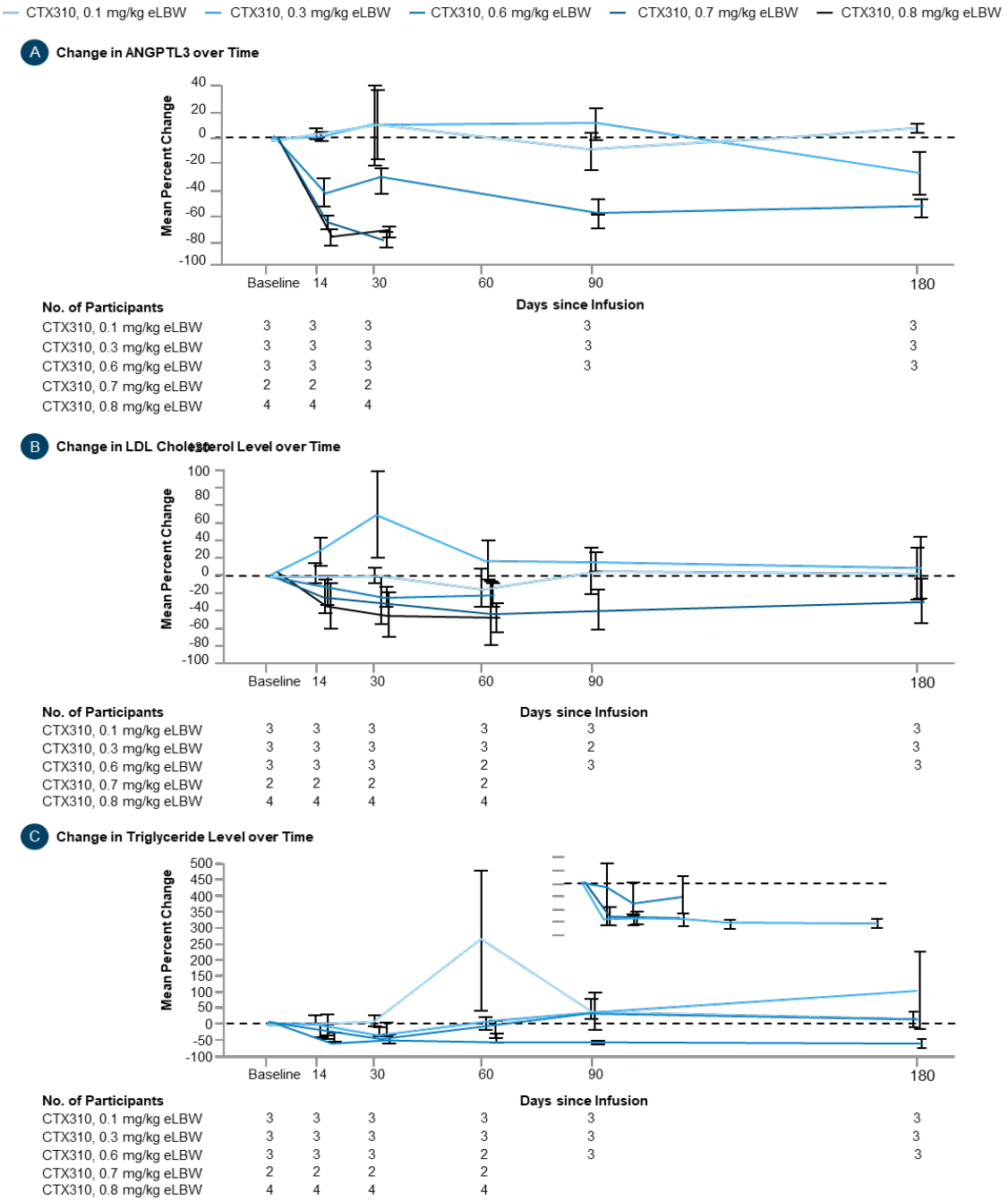
observed in the CLIMB TDT-141 study, which was determined to be from vaso-occlusive disease (VOD) due to the high-dose busulfan preconditioning regimen. No HSC-related malignancies were observed across the -151 and -141 studies.

CTX310 is CRISPR's first-in-class in vivo CRISPR-Cas9 gene editing therapy designed to induce a permanent loss-of-function mutation in the ANGPTL3 gene, which inhibits lipoprotein and endothelial lipases. Naturally occurring ANGPTL3 loss-of function variants are associated with lifelong reductions in LDL cholesterol and triglycerides, as well as reduced cardiovascular risk.

Results from the Phase I trial were published in The New England Journal of Medicine and presented at the 2025 American Heart Association (AHA) conference. The Phase I trial evaluated single intravenous doses of CTX310 ranging from 0.1 to 0.8 mg/kg (lean body weight) in 15 adults with dyslipidemia refractory to standard therapy. Participants included individuals with homozygous familial hypercholesterolemia (HoFH), heterozygous familial hypercholesterolemia (HeFH), severe hypertriglyceridemia (sHTG), and mixed dyslipidemia. Eligibility required triglyceride levels above 150 mg/dL (median: 192 mg/dL) and/or LDL-C above 100 mg/dL (mean: 155 mg/dL), despite background standard of care per local guidelines. Most participants were receiving statins and/or ezetimibe, and 40% had clinical atherosclerotic cardiovascular disease (ASCVD).

At day 30 post-infusion, mean ANGPTL3 changes were 9.6% (0.1 mg/kg), 9.4% (0.3 mg/kg), -32.7% (0.6 mg/kg), -79.7% (0.7 mg/kg), and -73.2% (0.8 mg/kg). These were associated with mean LDL cholesterol reductions of 49% (range: -19.5% to -86.6%) and triglyceride reductions of 55% (range: -37.9% to -83.7%) at day 60 in the 0.8 mg/kg group, respectively. In participants with elevated baseline triglycerides (above 150 mg/dL), mean reductions of 60% were observed at therapeutic dose levels (exhibit 27). In addition, two participants on background PCSK9 inhibitors achieved LDL-C reductions exceeding 80%. Additional lipid parameters also improved, including apolipoprotein B, or ApoB (-33.4%), and non-HDL cholesterol (-49.8%) in the highest-dose group. Exploratory analyses showed reductions in apolipoprotein CIII (ApoCIII) and remnant cholesterol, supporting CTX310's broad impact on lipid metabolism. Variability in dose-response relationships across the broader trial population is likely the result of heterogeneity in baseline lipid profiles and clinical backgrounds.

Exhibit 27
DECODER
Efficacy of CTX-310 in Patients With HoFH, HeFH, sHTG, or Mixed Dyslipidemia



Source: Laffin et al., 2025 *NEJM*

One participant, a 50-year-old male with HeFH and a history of coronary artery bypass grafting and multiple myocardial infarctions, achieved a 77% reduction in LDL-C and a 53% reduction in triglycerides, reaching LDL and triglyceride levels of 59 mg/dL and 142 mg/dL, respectively, at day 180. Another participant, a 53-year-old male with sHTG and a history of post-percutaneous

coronary intervention and coronary artery bypass grafting, experienced a 68% reduction in triglycerides and a 76% reduction in LDL-C, achieving LDL and triglyceride levels of 24 mg/dL and 345 mg/dL, respectively, on day 90. Both patients were on multiple background lipid-lowering therapies. These individual responses support CRISPR's plans for advancing CXT310 into a Phase Ib study in sHTG and mixed dyslipidemia.

Overall, the safety profile observed in the trial was consistent with other LNP therapies, with mostly mild to moderate adverse events. One participant with elevated transaminases at baseline experienced a transient grade 2 liver enzyme elevation peaking on day 4 and resolving by day 14, without any rise in bilirubin or other liver function abnormalities. One participant experienced an allergic reaction that resolved within 24 hours with supportive care. Infusion-related reactions occurred in three participants—two at the 0.6 mg/kg dose and one at 0.8 mg/kg—and all were classified as grade 2 and resolved without complications. Two serious adverse events occurred: a spinal disc herniation and a sudden death 179 days after treatment in the lowest-dose cohort (0.1 mg/kg), both deemed unrelated to CTX310.

Intellia Therapeutics, Inc. (NTLA)
Location: Cambridge, Massachusetts

Technology
Intellia uses CRISPR technology derived from Streptococcus pyogenes Cas9 (spCas9). An sgRNA is used to guide the spCas9 to the therapeutic's targeted genomic locus. For delivery of its primary therapeutic assets, which target liver-mediated diseases, Intellia has designed a proprietary LNP, iterated on a version originally in-licensed from Novartis, to promote delivery of its gene editing machinery to hepatocytes. Though not mentioned below, Intellia has also established gene-editing-based collaborations with multiple companies regarding gene editing of T cells, and we note that the company has presented previous data highlighting its T-cell editing platform via LNP-mediated delivery, though we do not know if Intellia's T-cell delivery modality is being used for such collaborations.

Key gene editing assets

Nexiguran ziclumeran (*nex-z;* NTLA-2001) is a CRISPR spCas9-based asset targeting the TTR gene for the treatment of ATTR amyloidosis, which includes ATTR-cardiomyopathy (ATTR-CM) and ATTR-polyneuropathy (ATTR-PN), being developed in collaboration with Regeneron. Nex-z serves to knock out TTR in target cells and is encoded for the second exon of TTR. Nex-z was granted regenerative medicine advanced therapy (RMAT) designation by the FDA for the treatment of ATTR-CM and ATTR-PN, which gives Intellia the ability to participate in additional interactions with the FDA, potential for accelerated approval pathways, and priority review (see our note: Nex-z Receives FDA's RMAT Designation; Opens Expedited Regulatory Path, Focus on MAGNITUDE Execution).

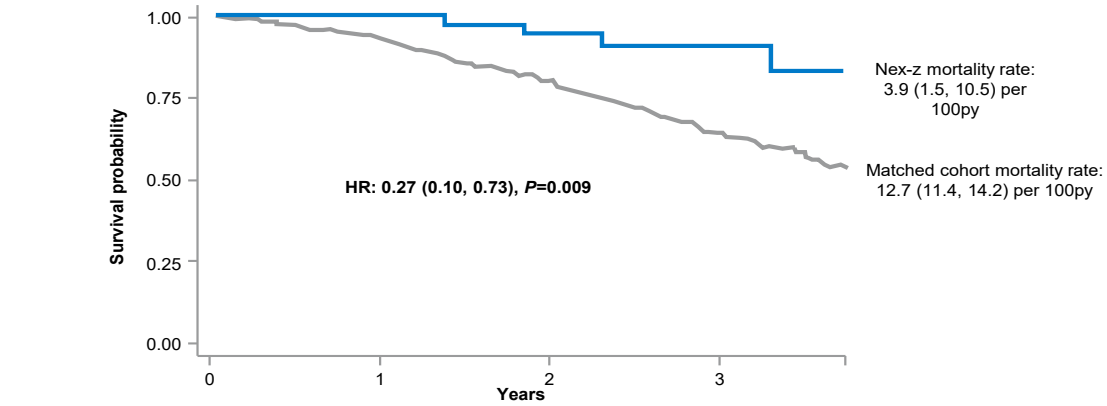
In 2016, Intellia and Regeneron announced a collaboration agreement for six years whereby Regeneron has the exclusive right to develop Intellia's CRISPR-based products against up to 10 targets. An upfront payment of $75 million was made and Regeneron further purchased $50 million in Intellia stock. The collaboration was extended for an additional two years in October 2023 in exchange for a $30 million payment to Intellia. In 2018, the companies entered a co-development/co-commercialization agreement for nex-z, whereby Regeneron shares in about 25% of worldwide development costs and commercial profits and has an option to enter a co-promotion agreement in the U.S.

An open-label Phase I study of nex-z enrolled both ATTR-CM and ATTR-PN patients (NCT04601051). Results from the ATTR-CM (Fontana et al., 2024 NEJM) and ATTR-PN (Gillmore et al., 2025 NEJM) patient cohorts at follow-up times of 18 months (median) and 27 months

(mean) post-treatment, respectively, were each published in *The New England Journal of Medicine.* In the study, ATTR-CM patients (n=36) were given a single administration of 0.7 mg/kg (n=9), 1.0 mg/kg (n=3), or 55 mg (part 2 expansion cohort; n=24) nex-z. Measurements of serum TTR protein levels showed an 89% reduction at day 28 and a 90% reduction at month 12. On secondary measures at month 12, patients achieved an additional 5 meters in the 6-minute walk test (predicted to decline over this time) and the median change in the Kansas City Cardiomyopathy Questionnaire (KCCQ) was an increase of 8 points. In addition, nex-z elicited an improvement in NYHA class in 47% of patients, with 44% remaining stable and 8% worsening (see our note: AHA Update: Nex-z Phase I Trial Shows TTR-CM Stabilization; Nucresiran Data Competed for TTR Knockdown Rapidity and Magnitude).

A more recent patient follow-up of the Phase I trial in ATTR-CM patients, which extended out to a median 26 months of follow-up (maximum of 36 months [n=9]), showed continued stabilization of NT-proBNP, hs-Troponin T, and 6-minute walk test performance, and 81% of patients demonstrated either no change or improvement in NYHA class at 24 months. An additional analysis comparing nex-z treatment in ATTR-CM patients to an external ATTR-CM patient natural history cohort demonstrated that nex-z improved all-cause mortality (HR: 0.27, p=0.009; exhibit 28). Though preliminary, and caveats pertaining to cross-trial comparisons are applicable, we view these efficacy data as on par with the TTR silencer class of molecules in ATTR-CM, such as Alnylam's Amvuttra (see our note: AHA Update: Phase I Study of Nex-z Shows Stabilization of Clinical Endpoints in TTR-CM; Clinical Hold on MAGNITUDE Looms).

Exhibit 28
DECODER
All-Cause Mortality in TTR-CM Patients After Nex-z Treatment Compared to Matched External Control



Source: NTLA AHA 2025 presentation

In ATTR-PN, patients were administered either 0.1 mg/kg (n=3), 0.3 mg/kg (n=3), 0.7 mg/kg (n=3), or 1.0 mg/kg (n=6) in part 1 of the study; in part 2, 16 patients received a 55 mg dose (total n=36). Across patients receiving higher than 0.1 mg/kg (n=33), nex-z elicited a 90% reduction in serum TTR at day 28 and a 92% reduction at month 24. Observed reductions in the serum TTR level were sustained through month 36 in all 12 patients who had completed three years of follow-up at the time of the data cutoff. Nex-z also reduced levels of neurofilament light chain (NfL), a biomarker of neuronal damage. On clinical-based scoring, nex-z elicited numerical reductions in the Neuropathy Impairment Score (NIS) and NIS+7 as well as the Norfolk Quality of Life–Diabetic Neuropathy Score.

Intellia is currently conducting the Phase III MAGNITUDE study in ATTR-CM patients (NCT06128629) and the Phase III MAGNITUDE-2 study in ATTR-PN patients (NCT06672237). The MAGNITUDE studies are ongoing and aim to enroll about 1,200 patients and roughly 50 patients in MAGNITUDE and MAGNITUDE-2, respectively, randomizing participants 2:1 to receive nex-z or a placebo control. In October 2025, Intellia announced that it was voluntarily pausing the MAGNITUDE studies after a patient experienced liver enzyme and bilirubin elevations triggering Hy's Law and eventually died. The patient (male) was in his early 80s and had a high body mass index (BMI). This was the fourth instance of liver enzyme elevations reported in association with nex-z dosing to date (two have occurred in the Phase I study in ATTR-CM patients [both grade 2: one occurring on day 1 and one occurring on day 22 after dosing] and two incidents [grade 4] have been reported in the Phase III MAGNITUDE study). We note that the Hy's Law incident was the only symptomatic instance to date. The FDA formally placed the MAGNITUDE and MAGNITUDE-2 studies on clinical hold (see our notes: Hy's Law Case Triggers MAGNITUDE Pause; Confident in Efficacy but Downgrading to Market Perform as We Await Clarity and Third-Quarter Earnings; Unfortunate Patient Death, Awaiting FDA Clinical Hold Letter, Presence at ACAAI and AHA Meetings).

A subsequent 8-K filing from the company provided additional context related to the Hy's Law and fatality incident in the MAGNITUDE study; notably, the cause of death was septic shock induced by perforation of a duodenal ulcer, which caused a sizable hole in the patient's intestine with free air. Such an incident provides a reasonable explanation for the patient's abdominal pain, which triggered the hospital visit whereupon elevated liver enzymes were detected and Hy's Law was triggered. It is important to note that liver damage was identified during the hospital visit, and we agree with management's interpretation that the liver damage was likely due to nex-z treatment and unrelated to the ulcer/sepsis. In our follow-up with management, it noted that the patient's ulcer was not known about until the aforementioned hospital visit and that knowledge of the ulcer would likely have precluded the patient from entering the MAGNITUDE study (see our note: 2026 Priorities; Additional Context on Patient Death in MAGNITUDE Study; Data From Phase III HAELO Study in HAE Midyear).

Recently, the FDA lifted the clinical holds on both the MAGNITUDE and MAGNITUDE-2 studies. The lifts from the FDA include updated protocol amendments to include increased patient monitoring for liver enzyme elevations, including the ability to dose with a short-term regimen of steroids to address liver enzyme spikes, as well as more stringent enrollment criteria to screen out those with severely elevated liver enzymes; this includes patients with NASH and/or autoimmune hepatitis. In addition, amendments to the MAGNITUDE study include exclusion of ATTR-CM patients with an ejection fraction (measure of cardiovascular function) less than 25%, which management noted represents a midsingle-digit percentage of the ATTR-CM patient population enrolled in the MAGNITUDE study to date (see our notes: MAGNITUDE-2 Clinical Trial Hold Lifted for ATTR-PN Clearly Positive; Awaiting News on MAGNITUDE Hold in ATTR-CM and Clinical Hold on Nex-z MAGNITUDE Study in ATTR-CM Lifted; Upgrading to Outperform Ahead of HAELO HAE Data Midyear).

Lonvoguran ziclumeran (*lonvo-z;* NTLA-2002) is a CRISPR spCas9-based asset targeting the *KLKB1* gene for the treatment of hereditary angioedema (HAE). Lonvo-z serves to knock out *KLKB1* in target cells to eliminate expression of prekallikrein (protein encoded by *KLKB1*), and based on our analysis of published patent literature, we believe the sgRNA for lonvo-z is encoded to target exon 10 of *KLKB1.* Like nex-z, lonvo-z has also received RMAT designation from the FDA.

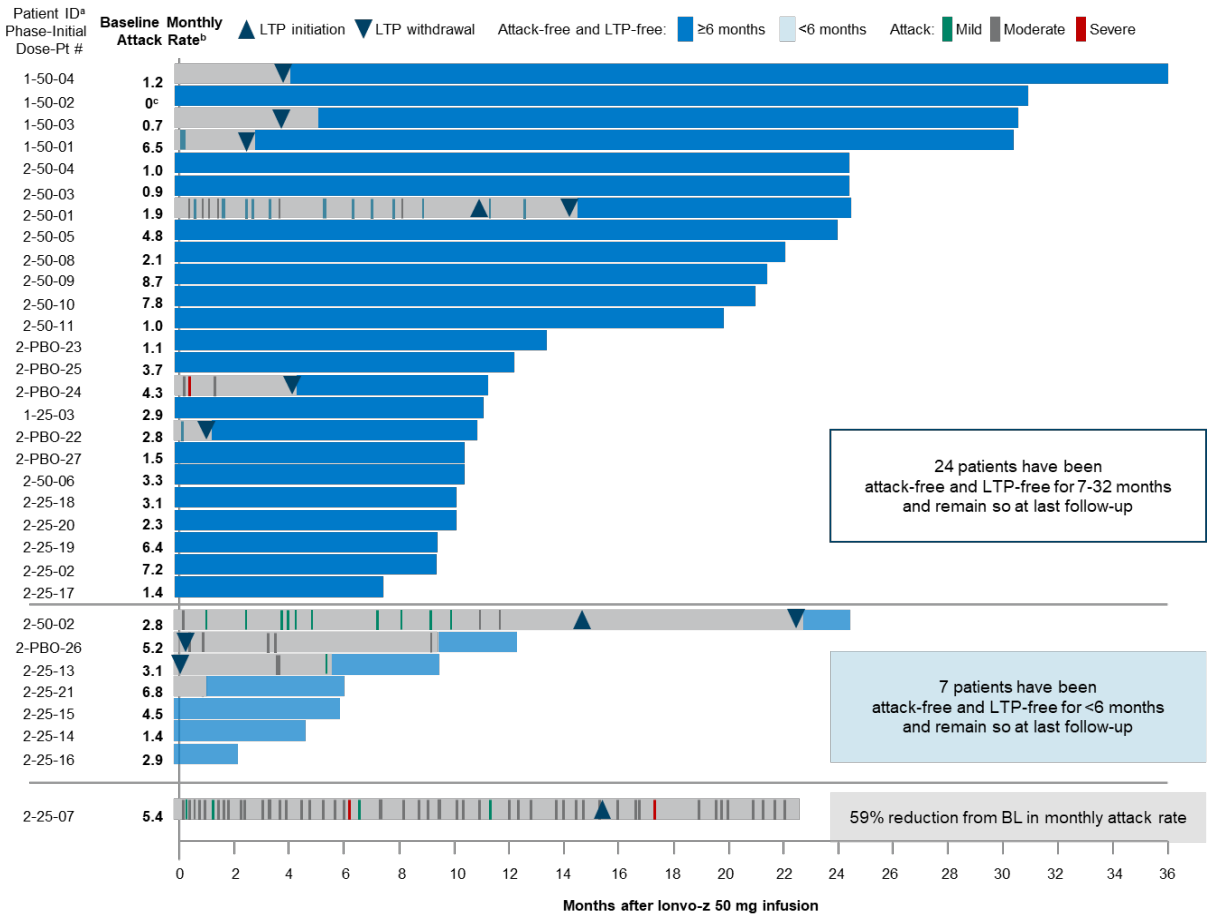
Lonvo-z is being investigated in an ongoing Phase I/II clinical trial in HAE patients for attack prophylaxis (NCT05120830). In the Phase I portion of the trial, HAE patients were randomized to receive either 25 mg (n=10) or 50 mg (n=11) lonvo-z or a placebo control (n=6). At the end of the primary observation period (16 weeks), lonvo-z elicited significant reductions in kallikrein protein: 55% in the 25 mg cohort and 86% in the 50 mg cohort. This corresponded with a reduction in HAE attacks, particularly in the 50 mg dose cohort, which experienced a 77% reduction

in HAE attacks, including 73% (8/11) of patients who were attack-free. While these initial data were indicative of lonvo-z's efficacy in knocking out *KLKB1,* the lack of correlation between kallikrein reductions and HAE attack rate reductions was troubling for investors (see our note: NTLA-2002 Response Heterogeneity in HAE Raises Some Questions; Still Impressive for Majority; BLA Submission 2026).

A longer-term data update was recently presented; it included initiation of the Phase II portion of the study, in which patients receiving either placebo or 25 mg lonvo-z in the Phase I portion were given the option to receive a 50 mg dose of lonvo-z, while patients receiving an initial 50 mg dose in the Phase I portion had extended follow-up. Data from patients receiving a 50 mg dose (n=32; 2 patients receiving a 25 mg dose in Phase I did not elect to receive a 50 mg dose) showed robust kallikrein reductions out to year 2 post-dosing (89% reduction; n=9) as well as robust HAE attack rate reductions by 6 months, which fell from 3.4 attacks per month to 0.2 attacks per month at 6 months (94% reduction; n=30) and 0 per month at 26 months (100% reduction; n=9).

On a patient-specific basis, 31 of 32 patients treated with 50 mg of lonvo-z were attack-free for a sustained period up to the data cutoff, with 24 of 32 patients attack-free for more than 6 months while concomitantly off their background long-term prophylaxis treatment. Of the patients (n=7) who were attack-free as of the data cutoff but for less than 6 months, we note that 1 patient was attack-free for more than 6 months but was on concomitant long-term prophylactic treatment (and has since stopped) and 3 have been attack-free since lonvo-z dosing but have not accrued 6 months of time post-dose. In addition, 10 of 32 patients have stopped long-term prophylaxis treatment after receiving lonvo-z, and of the 3 patients who started long-term prophylaxis since being dosed with lonvo-z, 2 subsequently stopped. On attack severity, only 2 patients have experienced a severe attack since lonvo-z dosing, with 1 of those patients experiencing a cluster of moderate to severe attacks within the first two months of dosing, but subsequently stopped long-term prophylactic treatment around month 4 and has been attack-free for over 6 months. One patient was a nonresponder who initiated on long-term prophylaxis treatment and experienced 2 severe HAE attacks (1 before and 1 after long-term prophylaxis treatment). The majority of the patient's attacks were considered moderate, and management noted that despite the continued attacks, the patient's monthly attack rate has fallen by 59% (exhibit 29), though we remain curious about the patient's kallikrein levels after dosing, which have not been disclosed to our knowledge.

Exhibit 29
DECODER
Patient-Specific HAE Attacks After 50 mg Lonvo-z



Source: NTLA ACAAI 2025 company presentation

On safety, we note one grade 2 AST elevation. This signal occurred within four days of receiving the 50 mg dose. While this time frame is consistent with LNP-related reactions, we note that this patient was in the 25 mg → 50 mg dose cohort, so this response could be a memory-based immune response triggered by a second dose. Management noted overall, however, that the safety profile of 50 mg lonvo-z after receiving a 25 mg dose was consistent with the global 50 mg safety profile. In addition, one serious adverse event was reported (pulmonary embolism), which occurred in a patient with a plethora of comorbidities and therefore cannot be conclusively linked to lonvo-z dosing. For more information on the latest data update from lonvo-z, see our note: Lonvo-z Impressive With Sustained HAE Attack Suppression; Safety From Nex-z Main Focus for Stock.

Intellia is currently conducting the Phase III HAELO study (NCT06634420) of lonvo-z in 60 HAE patients randomized 2:1 to receive lonvo-z or a placebo control. After the primary observation (28 weeks), patients will have the option to cross over to receive the opposite treatment administered in part 1. Patients will be followed for a total of 104 weeks. While we view data from the Phase II portion of the Phase I/II trial as a positive read-through to the HAELO study, we view the increase in HAE attack reductions from the Phase I portion to the Phase II portion to be related to not only the time on treatment, but also the cessation of dosing a placebo control. Intellia management has pointed out that a placebo may cause increases in patient-reported HAE attacks, as such patients

do not know if they are on active therapy and therefore are more paranoid about minor symptoms being a sign of an HAE attack. Therefore, we do not expect the HAE attack reduction in the Phase III HAELO study to be as dramatic as was observed in the Phase II portion, and this trend has also been observed in development programs of other HAE prophylactic therapies.

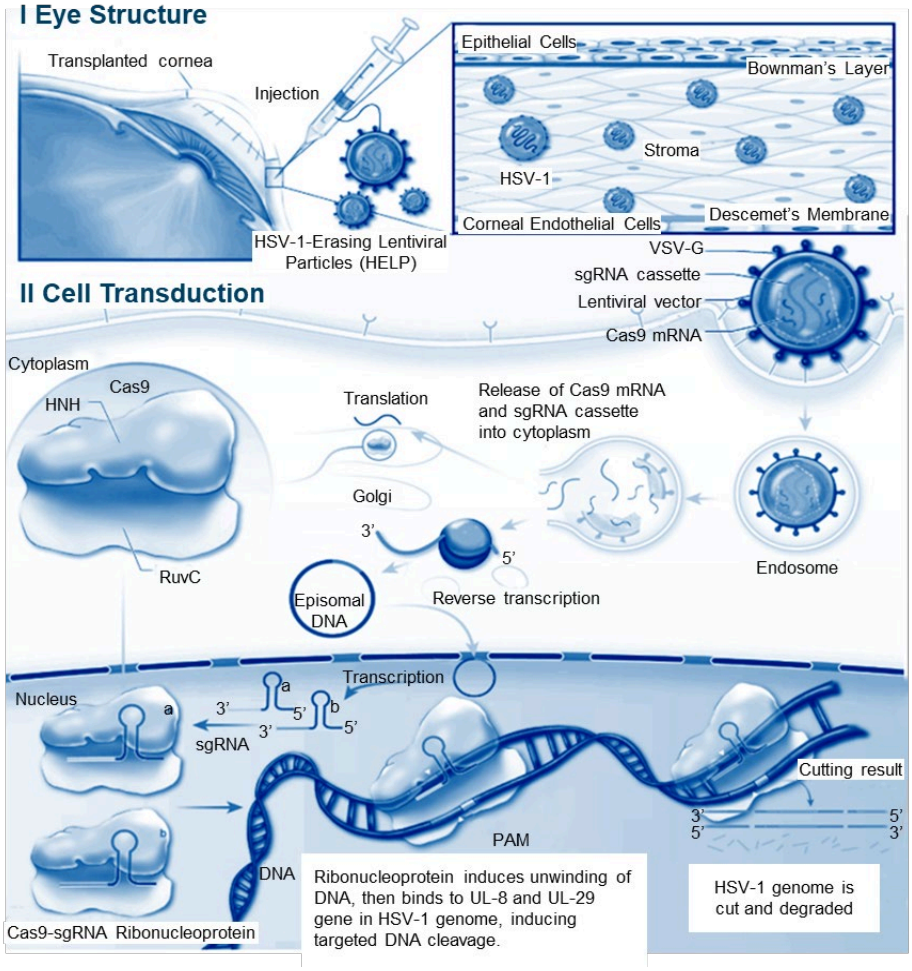
BDgene Therapeutics (Private)
Location: Shanghai, China

Technology
For its gene editing-based assets, BDgene uses CRISPR technology derived from *Streptococcus pyogenes Cas9* (spCas9). Single guide RNAs are used to guide the spCas9 to the therapeutic's targeted genomic loci. For delivery of its gene editors, the company uses its BDmRNA delivery technology, which uses a lentivirus-based virus-like particle (VLP). BDmRNA technology facilitates rapid expression and subsequent degradation of the gene editing payload (degraded within 72 hours) to reduce the potential for off-target editing activity.

Key gene editing assets

BD111 is a CRISPR spCas9-based therapy for the treatment of herpes simplex virus (HSV) keratitis, which is caused by HSV-1 infection in the cornea of the eye. BD111 is a lentiviral VLP-delivered payload containing a spCas9-encoding mRNA and an RNA-based sgRNA-encoding lentiviral cassette, which is delivered via intracorneal injection. After the VLP transduces target cells in the cornea, the mRNA components are translated by the cell into spCas9 protein as well as the protein components of the lentiviral cassette, which include the lentiviral reverse transcriptase responsible for reverse transcribing the cassette into DNA. While lentiviruses typically integrate into the host genome via an integrase, the encoded lentiviral integrase in BD111's VLP has a mutation that prevents DNA integration and forces the reverse transcribed sgRNA-encoding DNA cassette to be expressed extra-chromosomally (on an episome). The sgRNA cassette expresses two distinct sgRNAs, which are transcribed from the episome and trigger spCas9 cleavage at two genes essential for HSV-1's life cycle: *UL8* and *UL29.* In preclinical studies of BD111 treatment to HSV-1–infected healthy human corneas, sequencing of the *UL8* and *UL29* loci revealed spCas9-induced indel formation from both sgRNAs (exhibit 30).

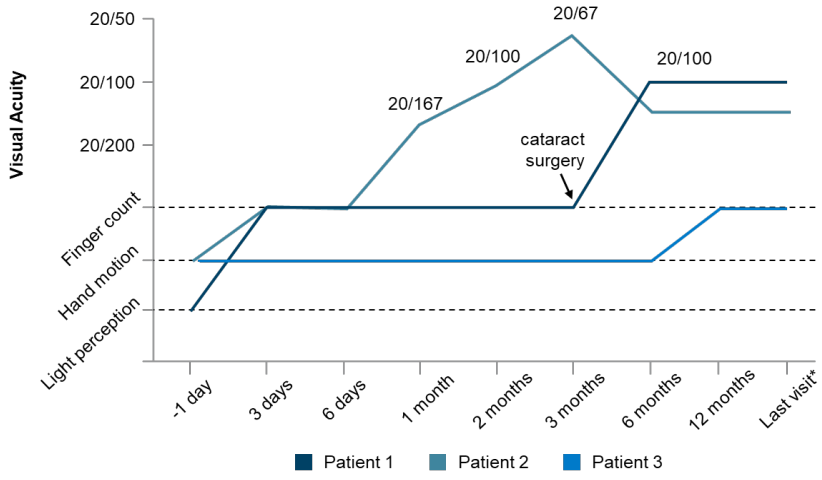
Exhibit 30
DECODER
Mechanism of Action for BDgene's BD111



Source: Wei et al., 2023 *Molecular Therapy*

A small open-label Phase I trial was conducted in China (NCT04560790) that enrolled three patients with refractory HSV keratitis in which BD111 was administered concomitantly with corneal transplantation. Patients' visual acuity baselines ranged from light perception (n=1) to hand motion (n=2). All three patients showed improvement of visual acuity within 12 months of treatment, with one patient (patient 2) capable of detecting only hand motion at baseline achieving peak visual acuity of 20/67 at 3 months. Patient 1 was capable of light perception at baseline and advanced to 20/100 vision after cataract surgery at 3 months. Patient 3 did not show improvements to visual acuity until month 12, when the patient progressed from detecting hand motion to finger counting. No patient saw a decrease in visual acuity after treatment (exhibit 31).

Exhibit 31
DECODER
Visual Acuity of HSV Keratitis Patients After Concomitant Corneal Transplantation and BD111 Treatment



Source: Wei et al., 2023 *Molecular Therapy*

On measurement of off-target editing, which was measured by detection of DNA integrations via GUIDE-seq, BD111 did not elicit significant changes in DNA integrations in patients' epithelial cellular DNA. suggesting that the therapy was mainly confined to the viral genome. On general safety, the ocular adverse events (AEs) deemed moderate were neurotrophic keratitis and cataract, which were both resolved (see publication of trial results: Wei et al., 2023 Molecular Therapy). BDgene is currently conducting a Phase I (NCT06474416) and Phase II (NCT06474442) study of BD111 in HSV keratitis patients with no previous corneal transplant. Both studies are comparing BD111 with an active HSV keratitis therapy arm (ganciclovir eye gel + valacyclovir tablets + prednisolone acetate eye drops for three weeks).

Editas Medicine, Inc. (EDIT)
Location: Cambridge, Massachusetts

Technology
Editas's platform consists of CRISPR technologies that work in tandem with proprietary and engineered Cas9 and Cas12a enzymes. For delivery of its primary therapeutic assets, Editas uses electroporation and LNPs to promote delivery of its gene editing machinery. However, it previously evaluated the delivery of its gene editing technology using AAV viral vectors as well. Below we discuss clinical data generated by two of the company's therapeutic products that have since been discontinued and highlight that the company is now focused on advancing in vivo gene editing therapies for hyperlipidemia and hemoglobinopathies, as well as ex vivo engineered CAR-T therapies containing multiple gene edits for the treatment of autoimmune diseases and oncology.

Key gene editing assets

EDIT-101 used an AAV5 vector to deliver DNA encoding a *Staphylococcus aureus* Cas9 (SaCas9) nuclease expressed with the photoreceptor-specific GRK1 promoter and two highly specific guide RNAs for the treatment of Leber congenital amaurosis 10 (LCA10) that was caused by a specific damaging variant in intron 26 of *CEP290*. *CEP290*-associated LCA10 is a rare retinal dystrophy

condition characterized by profound vision loss due to disorganized outer segments of rod and cone photoreceptors and early death of rods in the midperipheral retina. EDIT-101 was designed to remove the IVS26 mutation and restore expression of full-length *CEP290*.

The Phase I/II single-arm, open-label BRILLIANCE study of EDIT-101 enrolled three cohorts of adults who were treated at a low dose (6x1011 vg/mL), midsize dose (1x1012 vg/mL), or high dose (3x1012 vg/mL) of EDIT-101 and a pediatric cohort that was treated at a midsize dose (1x1012 vg/mL) of EDIT-101. Positive responses were defined as clinically meaningful improvements in the best-corrected visual acuity (BCVA), and improvement in two or more other endpoints including the full-field stimulus threshold (FST), visual function navigation (VFN), and the PRO vision-related quality of life (PRO:QoL); see exhibit 32. Results have subsequently been published in The New England Journal of Medicine. In patients who had an appropriate follow-up of 3 or more months, 3 of 14 (21%) patients demonstrated clinically meaningful improvements in BCVA and in two other endpoints.

Exhibit 32
DECODER
Efficacy of EDIT-101 in Patients With Leber Congenital Amaurosis 10 (LCA10)

Patient	Gender	Age	Zygosity	Baseline BCVA in Study Eye	BCVA	FST (red)	VFN	PRO: VFQ/CVFQ±
Cohort 1 – Adult Low-dose								
Subject 1	F	50	CH	3.5	-0.3	-0.01	-2	-13
Subject 2	M	42	CH	3.9	0	NA	+3	+13
Cohort 2 – Adult Mid-dose								
● Subject 1	F	54	H	2.7	-1.3	-0.22	+2*[a]	+10
Subject 2	M	20	CH	1.4	-0.2	-0.79	+7[a]	-4
Subject 3	F	19	CH	0.6	0	+0.07	0	+5
Subject 4	F	63	CH	0.9	0	+0.54	-1	+7
Subject 5	F	17*	CH	3.9	0	-0.60	-1	+15
Cohort 3 – Adult High-dose								
Subject 1	F	28	CH	2.3	+0.6	-0.25	-5	-23
Subject 2	F	38	CH	1.0	+0.2	+0.50	+2	-2
Subject 3	F	36	CH	3.9	0	-1.07	+3	+6
Subject 4	M	35	CH	2.0	0	+0.11	-3	-8
Subject 5	F	59	CH	2.9	-0.7	-1.01	-2	+13
Cohort 4 – Pediatric Mid-dose								
● Subject 1	M	14	H	3.9	-1.0[a]	-1.09	+1	+3
Subject 2	M	9	CH	1.2	-0.2	-0.38	+2	0

Clinically or statistically meaningful improvement ● Homozygous CEP290 IVS26 mutation

Source: Editas Medicine company presentation

EDIT-101 was well tolerated; most events were mild (77%) or moderate (22%) in severity. Forty-one percent of the patients in the study reported an adverse event related to the surgical procedure. One patient reported a severe ocular adverse event at six months post-treatment (exhibit 33). EDIT-101 was discontinued in early 2023 as the company reprioritized its pipeline to focus on blood disorders.

Exhibit 33
DECODER
Safety of EDIT-101 in Patients With Leber Congenital Amaurosis 10 (LCA10)

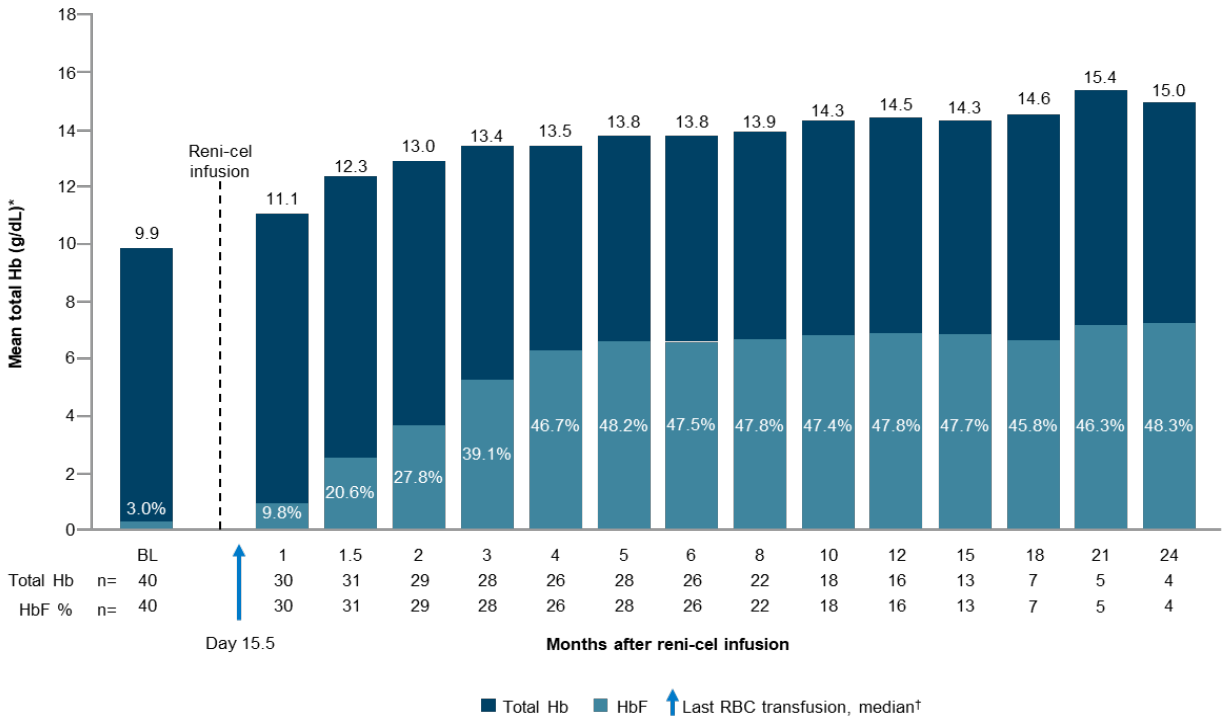
System Organ Class Preferred Term	All Patients (N = 14) n (%)	Adult Low-dose Cohort 1 (N =2) n (%)	Adult Mid-dose Cohort 2 (N = 5) n (%)	Adult High-dose Cohort 3 (N = 5) n (%)	Pediatric Mid-dose Cohort 4 (N = 2) n (%)
ANY TEAE RELATED TO EDIT-101	7 (50%)	1 (50%)	3 (60%)	3 (60%)	-
EYE DISORDERS (TOTAL)	7 (50%)	1 (50%)	3 (60%)	3 (60%)	-
Anterior chamber cell	1 (7.1%)	-	-	1 (20.0%)	-
Anterior chamber inflammation	2 (14.3%)	1 (50%)	1 (20.0%)	-	-
Conjunctival edema	1 (7.1%)	1 (50%)	-	-	-
Conjunctival hyperemia	1 (7.1%)	1 (50%)	-	-	-
Photophobia	1 (7.1%)	-	1 (20.0%)	-	-
Posterior segment of eye anomaly	1 (7.1%)	1 (50%)	-	-	-
Retinal cyst	1 (7.1%)	-	-	1 (20.0%)	-
Retinal degeneration	1 (7.1%)	-	-	1 (20.0%)	-
Retinal deposits	1 (7.1%)	-	-	1 (20.0%)	-
Retinal drusen	1 (7.1%)	-	1 (20.0%)	-	-
Retinal Infiltrates	1 (7.1%)	1 (50%)	-	-	-
Retinal pigment epithelial tear	1 (7.1%)	1 (50%)	-	-	-
Retinal pigment epitheliopathy	1 (7.1%)	-	1 (20.0%)	-	-
Subretinal hyperreflective exudation	1 (7.1%)	-	1 (20.0%)	-	-
Visual Impairment	2 (14.3%)	-	-	2 (40.0%)	-
Vitreal cells	2 (14.3%)	1 (50.0%)	-	1 (20.0%)	-

Source: Editas Medicine company presentation

EDIT-301 (reni-cel) was an ex vivo autologous hematopoietic stem cell therapy for the treatment of SCD and TDT. Reni-cel used an AsCas12a nuclease to edit the distal CCAAT box (-118 to -113) of the promoter regions of the γ-globin genes (*HBG1/2*), mimicking naturally occurring variants of the *HBG1/2* promoters that lead to reactivation of γ-globin expression and persistence of HbF expression.

The Phase I/II Ruby study enrolled 40 patients, and treatment resulted in increases in HbF of higher than 40% of total Hb as early as 4 months and were sustained up to 24 months (exhibit 34). Of the 40 patients treated, 39 were vaso-occlusive crisis-free after reni-cel infusion. Treatment also resulted in early increases in mean corpuscular fetal hemoglobin (MCH-F)/F-cell, and levels were sustained above the anti-sickling threshold of 10 pg/F-cell through the last follow-up. In addition, treatment resulted in decreases in mean scores for pain intensity, pain interference, physical functions, and social roles. Patients also showed sustained levels at month 12 of allelic editing in peripheral blood nucleated cells and bone-marrow-derived CD34-positive cells with mean editing levels of 75.8% and 87.8%, respectively. Treatment was reasonably well tolerated, with 10% of patients experiencing an adverse event related to reni-cel, 7.5% of which were grade 3 or 4.

Exhibit 34
DECODER
Efficacy of Reni-cel in the Phase I/II Ruby Study



*Central laboratory reference range: 12.0-16.0 g/dL for females and 13.6–18.0 g/dL for males
†The last RBC transfusion in patients occurred a mean (SD) of 14.6 (5.6) days after reni-cel infusion
Source: Editas Medicine company presentation

Reni-cel was also studied in seven patients with TDT in the Phase I/II EdiThal study. Treatment with reni-cel resulted in mean Hb of 12.5 g/dL by month six, above the transfusion independence threshold of 9.0 g/dL. The safety profile of reni-cel was consistent with myeloablative conditioning with busulfan, and no serious adverse events related to reni-cel were reported. Both of EDIT-301's programs were discontinued in late 2024 and early 2025 because of challenges in finding a commercial partner and questions about the program's competitiveness with commercially available therapies.

iECURE (Private)
Location: Philadelphia

Technology
For its current gene editing assets, which serve to insert a functional gene of interest into the genome via nuclease-mediated repair, iECURE has licensed the ARCUS nuclease, a proprietary engineered meganuclease from Precision BioSciences described earlier in this report. Privately owned iECURE is delivering both an ARCUS editor and the repair template via viral vectors, which are co-administered to patients.

Key gene editing assets

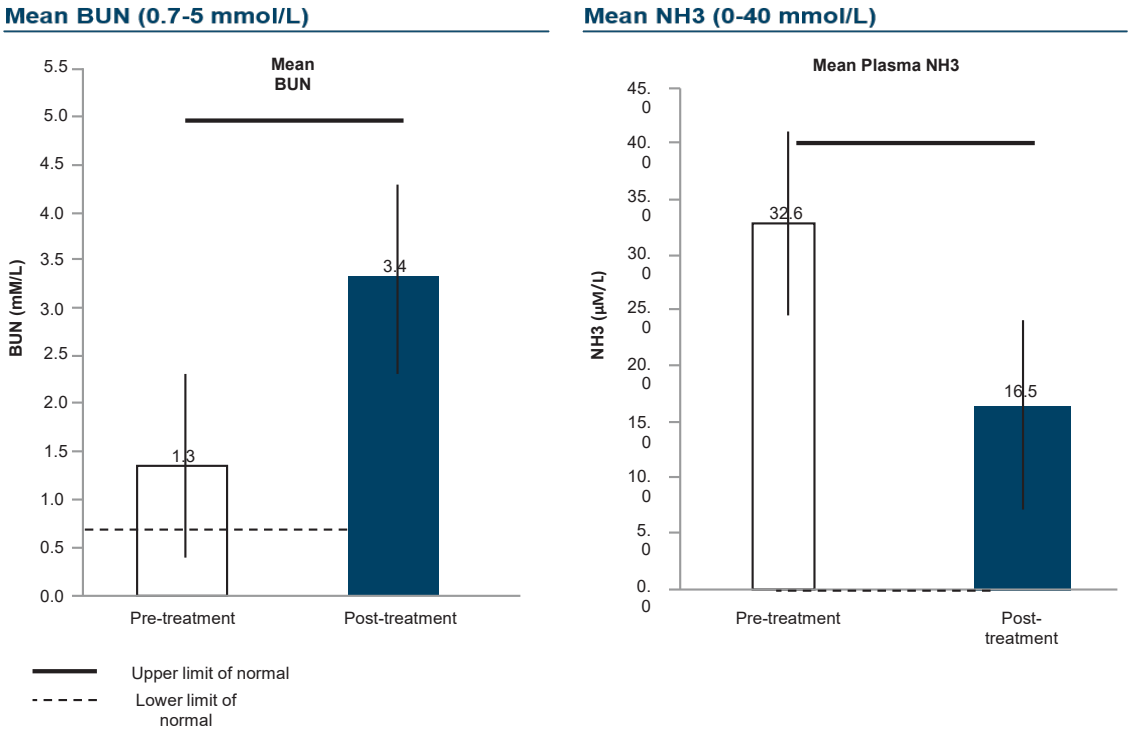
ECUR-506 is an ARCUS-driven gene addition therapy that triggers insertion of the *OTC* gene into the genome, providing the expression of functional ornithine transcarbamylase (OTC) protein in patients with OTC deficiency and urea cycle disorder. ECUR-506 has received fast-track designation from the FDA. The *OTC* transgene is inserted within the *PCSK9* gene via an engineered ARCUS

nuclease specific for the *PCSK9* locus. ECUR-506 uses a *PCSK9*-targeting ARCUS nuclease that targets exon 7 of the *PCSK9* gene, which has been previously described (Wang et al., 2018 Nature Biotechnology). Both the *PCSK9*-targeting ARCUS nuclease (ECUR-506A) and the OTC repair template (ECUR-506D) are being delivered via the AAVrh79 vector, which targets the liver and directs *OTC* gene insertion into the *PCKS9* locus in co-transduced hepatocytes.

The company is currently running the Phase I/II OTC-HOPE study (NCT06255782) in babies (younger than 9 months old) with genetically confirmed neonatal onset OTC deficiency. Given that the severe form of the disease arises almost exclusively in males, the trial is confined to male patients. OTC-HOPE aims to enroll about eight patients, and data from one patient was presented at the American Society of Gene and Cell Therapy (ASGCT) conference in 2025. This patient had a hyperammonemic crisis in the first week of life and was put on a protein-restrictive diet and nitrogen scavenger therapy (phenylbutyrate). The patient experienced a second hyperammonemic crisis at 5.5 months of age and was enrolled into the OTC-HOPE trial at 6.5 months of age. Data presented at ASGCT covered 9 months post-treatment (data cutoff of April 30, 2025). At week 24, serum PCSK9 levels were decreased by 37%, indirectly suggesting successful integration of the OTC transgene in the *PCSK9* locus.

After ECUR-506 treatment, the dosing of phenylbutyrate was consistently lowered from week 8 to week 12 and was eliminated by week 13. The patient maintained a protein-restricted diet through 12 weeks. The patient began increasing protein intake from weeks 13-15 but subsequently lowered protein intake to within urea cycle disorder guidelines until month 7, after which protein intake was increased through the data cutoff. On clinical endpoints, the patient showed stabilized glutamate levels below the upper limit of normal across the entire study. In addition, the patient showed a 2.1 mM/L increase in blood urea nitrogen and a 16.1 uM/L decrease in ammonia at the data cutoff, indicative of improvement in urea cycle function. Given the weaning of both nitrogen scavenger therapy and protein-restrictive diet, this patient was deemed to have achieved a complete clinical response (exhibit 35). ECUR-506 was generally well tolerated, though the patient experienced liver enzyme elevations beginning at week 4, at which point the patient was given steroid treatment; AST levels spiked at week 4 (about 3x the upper limit of normal) and returned to normal by week 10, while ALT levels spiked at week 4 and continued increasing up through a second spike at week 7 (about 5x the upper limit of normal) and subsequently diminished to normal by week 10. We note that the patient was asymptomatic and showed no increases in bilirubin.

Exhibit 35
DECODER
Blood Urea Nitrogen (Left) and Blood Ammonia (Right) in Patient Receiving ECUR-506



BUN: blood urea nitrogen
Source: Adapted from iECURE ASGCT 2025 presentation

Precision BioSciences, Inc. (DTIL)
Location: Durham, North Carolina

Technology

Precision has developed the ARCUS nuclease, a proprietary engineered I-creI meganuclease derived from an intron of the gene encoding the 23S ribosomal RNA of *Chlamydomonas reinhardtii.* The company uses iterative directed evolution on the domains/amino acids that provide sequence specificity to program ARCUS for different genomic sites depending on the therapeutic application. The ARCUS meganuclease cleaves its DNA substrate in a staggered manner, leaving a 3' overhang, which is thought to promote homology-directed DNA repair; this may increase the efficiency of predictable gene edits in the context of DNA insertion and excision applications. For delivery, the company is using both LNP and viral-based methods depending on the targeted tissue type.

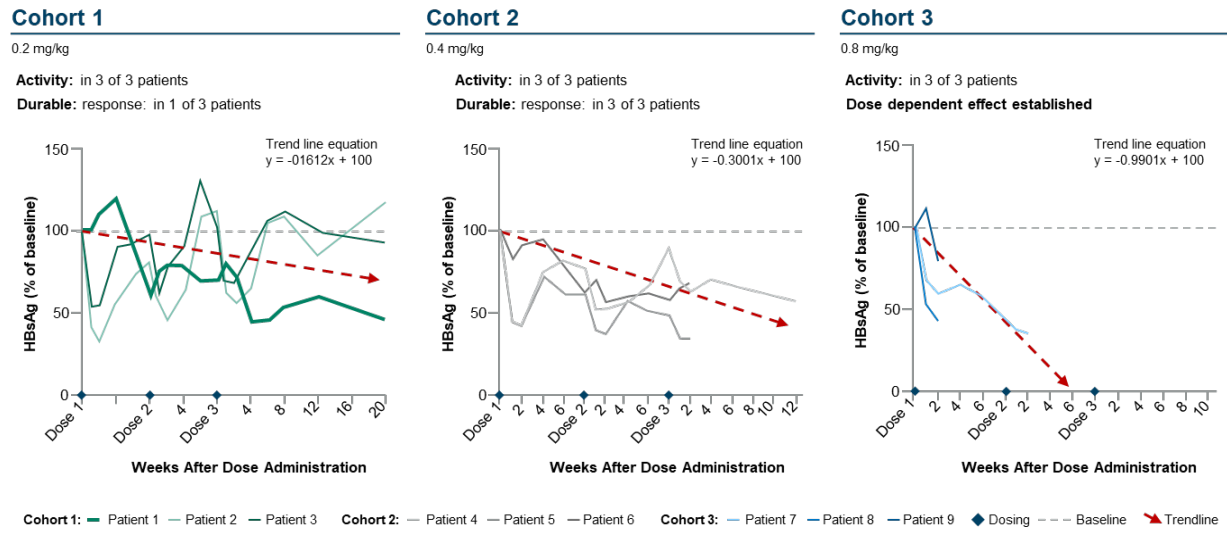
Key gene editing assets

PBGENE-HBV is an ARCUS nuclease-based asset targeting the hepatitis B virus (HBV) genome for the treatment of HBV infection. PBGENE-HBV has received fast-track designation from the FDA. PBGENE-HBV is an LNP-delivered payload (LNP licensed from Acuitas) containing an mRNA encoding an ARCUS nuclease specific for a conserved 22-base-pair sequence within the promoter region of the open reading frame of the HBV genome encoding the *POL* gene (ARCUS-POL). Expression of ARCUS-POL within hepatocytes transfected by PBGENE-HBV leads to dsDNA cleavage of the HBV genome at the *POL* locus, which may occur on instances of the HBV genome of infected cells in the form of covalently closed circular DNA (cccDNA) or instances of HBV genomic copies

integrated into the host's genome. Instances of dsDNA breaks aim to suppress viral replication by both promoting degradation of cccDNA through disruption of the covalently closed circle and introducing mutations in the *POL* gene that limit the virus's replicative capacity. For additional information on the development of ARCUS-POL and relevant preclinical data, see Precision's publication (Gorsuch et al., 2022 Molecular Therapy).

Precision is conducting the Phase I ELIMINATE-B study of PBGENE-HBV in adult patients with chronic HBV (NCT06680232). Part 1 of the trial enrolled three dose cohorts (0.2 mg/kg, 0.4 mg/kg, 0.8 mg/kg; n=3 per cohort) to receive three doses of PBGENE-HBV every eight weeks in combination with nucleoside analog treatment (current standard of care). As of the data cutoff of October 31, 2025, PBGENE-HBV has elicited a dose-dependent reduction in hepatitis B surface antigen (HBsAg) in patient serum. This included a durable reduction in HBV load in 100% (3/3) patients in the 0.4 mg/kg cohort. In addition, in a liver biopsy taken from a single patient in the study (0.4 mg/kg cohort, received 2 of 3 doses), 28% of HBV viral genomes were edited, suggesting that ARCUS-POL nuclease activity correlates with reductions in HBsAg abundance. While patients in the 0.8 mg/kg cohort have not advanced far enough for their response to treatment to be deemed durable, early data appears more dramatic than the 0.4 mg/kg cohort (exhibit 36). Additional dose cohorts may be added to part 1 of the study to reduce viral load sufficient to warrant cessation of nucleoside analog therapy.

Exhibit 36
DECODER
Hepatitis B Antigen Load After PBGENE-HBV Treatment



Source: Precision BioSciences company materials

PBGENE-HBV has been generally well tolerated with no dose-limiting toxicities observed to date, although one participant did not complete dosing because of a transient, reversible infusion reaction, which the study's data safety monitoring board did not deem to be dose-related or dose-limiting. PBGENE-HBV has elicited dose-dependent spikes in live enzymes (ALT, AST elevations), including one grade 3 incident of AST elevations, though such reactions resolved within three days. Given the timing of these liver enzyme elevations, we interpret these to be related to the LNP component. PBGENE-HBV also elicited grade 3 hypotensive events in 40% of patients, which all resolved in less than 24 hours after dosing.

Excision BioTherapeutics, Inc. (Private)
Location: Watertown, Massachusetts

Technology
Excision is developing CRISPR-based assets designed to excise DNA sequences from viral genomes for antiviral therapeutics. While activity from a single nuclease may introduce deleterious mutations that reduce viral fitness upon infection, combining activity of two nucleases at somewhat proximal sites adds a layer of potential efficacy by also potentiating genomic deletion of the intervening sequence, which may be more difficult for a virus to circumvent through iterative mutagenesis/selection events.

Key gene editing assets

EBT-101 is a *Staphylococcus aureus* Cas9 (saCas9) therapeutic targeting the genome of human immunodeficiency virus (HIV) for the treatment of HIV infection. EBT-101 has received fast-track designation from the FDA. EBT-101 packages a cassette encoding saCas9 as well as two sgRNAs into an AAV-DJ/8 vector, which allows systemic administration across multiple tissue types. The two sgRNAs guide saCas9 to the *GagD* and long terminal repeat (LTR) sequences of the HIV genome. Because the repeat sequences are similar (and identical at the specific protospacer sequence targeted), only one sgRNA is needed to hit both loci. It is important to note that the combination of *GagD* and LTRs yielded increased excision efficiency beyond other essential genes (*GagB, GagC, PolB*) in preclinical studies (Yin et al., 2017 Molecular Therapy).

A Phase I/IIa open-label trial of EBT-101 (NCT05144386) investigated excision-based therapy in HIV-positive patients on stable antiretroviral therapy with undetectable viral load for more than one year prior to dosing (n=6). Endpoints for the study were confined to safety and tolerability measures, where EBT-101 met all primary and secondary endpoints; no serious adverse events were observed and only one adverse event was associated with EBT-101 and was grade 1. No clinical or biomarker-based results were provided, and we note that enrollment of patients with undetectable levels of HIV viral load likely makes such measures difficult (see press release here).

Locus Biosciences (Private)
Location: Morrisville, North Carolina

Technology
Locus is using its CRISPR-Cas3-enhanced bacteriophage (crPhage) platform to develop CRISPR-based therapies targeted to microbiota. For its gene editing payload, the company is using CRISPR Cas3 to degrade the genome of target bacterial strains to prevent infectious disease. For delivery, the company is using engineered bacteriophages to deliver a CRISPR cassette to target bacteria.

Key gene editing assets

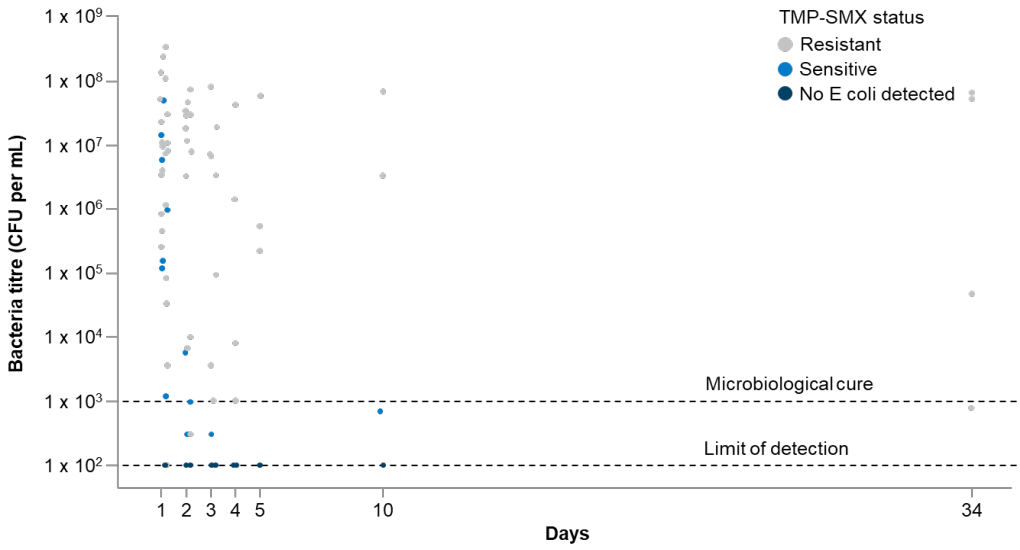
LBP-EC01 is a combination of engineered bacteriophages targeted at various strains of *E. coli* in humans for the purpose of warding off *E. coli* infections, with particular emphasis on *E. coli* strains that may be resistant to antibiotics. The approach is two-pronged, using intrinsic bacterial lytic activity of the phage to kill *E. coli* cells and the DNA degradation machinery to aid in *E. coli* cell death; upon synthesis of the Cascade machinery within the bacteriophage-transduced bacterium, the Cascade complex is guided to the *E. coli* genome via a gRNA and subsequently enacts its DNA degradation activity. In LBP-ECO1, six individual bacteriophages are used, three of which deliver the Cas3 Cascade complex machinery.

In the double-blind Phase Ib LBx-1001 trial of LBP-EC01 in patients with lower urinary tract infections (UTIs) caused by *E. coli* (NCT04191148), participants (n=36) were randomized 2:1 to receive LBP-ECO1 or a placebo control. Results indicate LBP-EC01 can decrease the level of infectious bacteria in the bladder.

The Phase II ELIMINATE trial in females with acute uncomplicated UTI (uUTI) caused by drug-resistant *E. coli* (NCT05488340) remains ongoing, but results from part 1 of the study have been published (Kim et al., 2024 The Lancet Infectious Disease). Patients were randomized 1:1:1 to receive LBP-EC01 via intraurethral and intravenous administration once daily. We note that the first eight patients on study were treated via a different protocol, which involved twice-daily intravenous dosing as well as oral dosing, but that protocol was discontinued after three patients discontinued treatment due to nonserious tachycardia and afebrile chills. We note that an additional tachycardia event spurring discontinuation arose in the high-dose group after protocol amendment, though no serious adverse events were reported in the study. On efficacy, LBP-EC01 elicited a rapid reduction of *E. coli* from patients' urine within four hours after initial treatment, microbiologic cure in 88% (14/16) of patients, and "clinical cure" in 100% (16/16) patients in the evaluable population by day 10 following treatment (exhibit 37).

Part 2 of the study will randomize about 288 female patients 1:1 to receive to receive LBP-EC01 or placebo for a three-day dosing regimen. The primary endpoint will be the resolution of uUTI symptoms and microbiologic response at day 10.

Exhibit 37
DECODER
***E. Coli* Load in Urine of Patients With Urinary Tract Infection After LBP-EC01 Treatment**



Source: Kim *et al*., 2024 *The Lancet Infectious Disease*

SNIPR Biome (Private)
Location: Copenhagen, Denmark

Technology
SNIPR is developing CRISPR-based therapies targeted to microbiota. The company may use multiple types of CRISPR systems (class 1 and class 2 Cas effectors) depending on the specific application (genome degradation, gene insertion, etc.). For delivery, the company is using both engineered bacteriophages and bacterial conjugation (the *E. coli* strain EC77 has been cited by the company) to deliver a CRISPR cassette to target bacteria.

Key gene editing assets

SNIPR001 is an orally administered combination of four engineered bacteriophages that deliver the Cas3 Cascade complex machinery to various strains of *E. coli* in humans for the purpose of warding off antibiotic-resistant *E. coli* infections. SNIPR001 has been granted fast-track designation by the FDA. The bacteriophages are engineered to not only contain the Cascade complex machinery within their genomes, but also specifically bind to *E. coli* via engineering of the bacteriophage's tail fiber protein. For additional information on the development of SNIPR001 and relevant preclinical data, see SNIPR's publication (Gencay et al., 2023 Nature Biotechnology).

In a Phase I trial in healthy volunteers (NCT05277350), individuals (n=36) across three dose cohorts were randomized 3:1 (cohorts 1-2) or 3:2 (cohort 3) to receive SNIPR001 or placebo twice daily for seven days. SNIPR001 was well tolerated, with mild/moderate adverse events and no withdrawals from the study. On pharmacodynamics, SNIPR001 was recovered in feces from treated individuals in a dose-dependent manner, and treatment with SNIPR001 numerically lowered gut *E. coli* levels (see press release here). SNIPR is running a Phase Ib/II trial for SNIPR001 in U.S. patients with hematologic malignancies receiving a hematopoietic stem cell transplant who are positive for fluoroquinolone-resistant *E. coli* pre-transplant (NCT06938867). The study will enroll about 24 patients who will be randomized 1:1 to receive BID SNIPR001 or placebo in combination with standard-of-care prophylactic treatment for *E. coli* infection for 30 days. The first patient was dosed in the trial in June 2025.

YolTech Therapeutics (Private)
Location: Shanghai, China

Technology
YolTech uses its proprietary, AI-powered High-Throughput Evolution Platform for Discovery and Optimization of Novel Editors (HEPDONE) platform as the technological basis for its gene editing platform. This includes mining and filtering of metagenomic data for discovery of CRISPR systems and other enzymes across species, followed by structure-function-based rational design strategies before testing of editing capabilities in cells and higher organisms. HEPDONE has identified about 1 million Cas proteins to date. The company's clinical-stage assets employ multiple Cas types including Cas9 as well as a high-fidelity Cas12 (YolCas12HF).

On delivery, YolTech also has a robust LNP delivery platform, which includes a lipid library of over 1,000 lipids from more than 10 lipid families with the goal of both hepatic and extra-hepatic delivery. YolTech's LNPs include a structural lipid, cholesterol, and an ionizable lipid, as well as a proprietary Stealth lipid, which we interpret to be a derivative of or alternative for a polyethylene glycol (PEG) lipid typically present in LNP formulations.

Key gene editing assets

YOLT-203 is a YolCas12HF-based therapy targeting the *HAO1* gene for the treatment of patients with primary hyperoxaluria type 1 (PH1). YOLT-203 serves to knock out the glycolate oxidase enzyme in target cells. The CRISPR payload of YOLT-203 is delivered via an LNP that targets the liver. YOLT-203 received RMAT designation from the FDA in early 2026.

YolTech is running multiple Phase I studies of YOLT-203 in patients with PH1 (NCT06892301 and NCT06511349). In February 2025, the company reported positive results from its Phase I trial (NCT06511349), which included data from seven patients dosed with YOLT-203 at either 0.3 mg/kg or 0.45 mg/kg. Data was presented at the ERA Congress in June 2025. For patients who received the 0.45 mg/kg dose, a nearly 70% reduction of 24-hour urinary oxalate levels was observed, which was sustained through the 16-week primary observation period. There have been no serious adverse events (SAEs), treatment discontinuations, or patient withdrawals (see the press release here).

In November 2025, YolTech received IND approval from the FDA for initiation of a double-blind, placebo-controlled pivotal study of YOLT-203 in PH1 patients. The company also holds FDA rare pediatric drug designation for YOLT-203, which would make the company potentially eligible for a priority review voucher if approved.

YOLT-201 is a Cas9-based therapy targeting the *TTR* gene for the treatment of patients with ATTR-PN and ATTR-CM. Similar to Intellia's nex-z, YOLT-201 serves to knock out TTR in target cells, though we are unaware of the specific genomic location where YOLT-201 is designed to induce a double-stranded DNA break. The CRISPR payload of YOLT-201 is delivered via an LNP that targets the liver.

YolTech is running a small-scale study of YOLT-201 specifically in ATTR-CM patients (NCT06082050). The study is fully enrolled and randomized ATTR-CM patients (n=7) across four dosing levels. In the high-dose group, subjects achieved about 90% reduction in serum TTR. For two patients in the low-dose group whose TTR reduction did not meet the target, a second administration was given, resulting in over 95% reduction in serum TTR. Fever and transient liver enzyme (ALT/AST) elevations were observed.

YolTech is also running the Phase I/IIa YT-YOLT-201-101 trial (NCT06539208) in patients with ATTR-PN and ATTR-CM. The YT-YOLT-201-101 trial is dosing YOLT-201 across two dosing cohorts. As of December 21, 2024, eight study participants (six ATTR-PN patients and two ATTR-CM patients) had been dosed in the Phase I portion. All ATTR-PN patients also completed study follow-up, and preliminary data from the high dose of YOLT-201 showed a decrease in serum TTR protein of over 90% with no grade 3 adverse events or dose-limiting toxicities observed. The study's safety review committee subsequently decided to suspend the Phase I dose-escalation portion for ATTR-PN patients and move straight to the Phase IIa portion of the study, allowing recruitment of additional ATTR-PN patients at the higher dose (see the press release here).

BRL Medicine (Private)
Location: Shanghai, China

Technology
BRL uses *Streptococcus pyogenes* Cas9 (spCas9) for its gene editing technologies, which span both ex vivo and in vivo applications; BRL has proprietary gene editing platforms in both HSCs (Modi-HSC platform) and T cells (Quikin CART and TyUCell platforms). For its ex vivo, applications, the company uses electroporation to deliver its gene editing payloads. For in vivo applications, which are focused on targeting T cells, the company has both viral and LNP-based methods for delivery.

Key gene editing assets

BRL-101 uses the company's ModiHSC platform for the treatment of patients with TDT. Similar to CRISPR/Vertex's exa-cel, BRL-101 spurs editing of the erythroid enhancer sequence of *BCL11A* to knock out BCL11A expression, which prevents BCL11A from suppressing fetal gamma-globin gene expression (*HBG1, HBG2*) and ultimately boosts HbF production. BRL is also developing the same therapy for patients with SCD (denoted in some instances as BRL-102).

BRL is conducting an open-label Phase I/II study of BRL-101 in TDT patients (NCT05577312). The company's latest presentation of results from BRL-101 was given at the European Hematology Association (EHA) conference in June 2025, which provided updates for 15 TDT patients ages 6-26 dosed with BRL-101. As of May 23, 2025, 100% (15/15) of patients had achieved transfusion independence for at least 12 months (minimum follow-up: 14.5 months; average follow-up 25.8 months). A subsequent press release highlighted that the first patient dosed in the study (7- to 8-year-old male) had achieved transfusion independence for five years after BRL-101 infusion. The company's EHA presentation also highlighted a single severe SCD patient dosed with BRL-101 (NCT06300723). One month after cell reinfusion, the SCD patient demonstrated significant preliminary efficacy: the HbF proportion increased from a baseline of 4.6% to 26.1%, and total hemoglobin increased from 52 g/L to 104 g/L without red blood cell transfusion for nearly three weeks. By the sixth month, total hemoglobin continued to increase to 134.1 g/L. The safety profile of BRL-101 was consistent with other autologous HSC transplant therapies.

EdiGene (Private)
Location: Beijing, China

Technology
With regard to its nuclease-based gene editing efforts, EdiGene uses Cas9, which has been applied to its cell therapy platforms; these include HSC therapy and its monogenic knockout results in altered glycosylation and immune compatibility (MAGIC) platform for its allogeneic cell therapies (CAR-T cell and stem-cell-derived therapy).

Key gene editing assets

ET-01 is a Cas9-edited HSC therapy for the treatment of TDT. ET-01 edits the erythroid enhancer region of *BCL11A* to ablate its expression to upregulate HbF. ET-01 was investigated in the open-label Phase I EDI001 study (NCT04390971). Data from a single enrolled patient (12-year-old female) after infusion showed a significant increase in HbF production (3.35 g/L at baseline to 89.87 g/L) and total hemoglobin, which reached 110 g/L at month 18 after treatment. The patient received her last transfusion on day 87 and was transfusion-free for over 15 months. The patient experienced 62 cases of adverse events, which were mostly grades 1-2. Only 1 AE (grade 1) was attributed to ET-01, and grade 3 AEs were attributed to inappropriate diet after apheresis and the busulfan preconditioning regimen. Development of ET-01 was discontinued due to priority changes at EdiGene.

Reforgene Medicine (Private)
Location: Guangzhou, China

Technology
Reforgene discloses relatively little regarding specifics of its gene editing platform, though language from the company's website suggests that the platform is wide-ranging, including "a proprietary portfolio of gene editing technology tools, encompassing cutting-edge technologies such as CRISPR/Cas12, CRISPR/Cas13, base editing, and epigenetic editing" (reference [published in

Mandarin]). The company's clinical assets at the time of writing are CRISPR Cas9 based. On delivery, the company's in vivo gene editing candidates include both liver-targeting and extra-hepatic tissues, suggesting the company uses both LNP and viral-based delivery.

Key gene editing assets

RM-001 is a Cas9-based editor for ex vivo administration of CD34+ HSCs for the treatment of TDT. CRISPR/Vertex's exa-cel spurs editing of the erythroid enhancer sequence of *BCL11A* to knock out BCL11A expression, which prevents BCL11A from suppressing fetal gamma-globin gene expression (*HBG1, HBG2*), thereby boosting fetal hemoglobin production. RM-001 spurs editing of the BCL11A binding site within the promoter sequences of the gamma-globin genes (sequence is shared across *HBG1* and *HBG2*) to prevent BCL11A-mediated suppression of gamma-globin, which allows production of fetal hemoglobin. These are essentially the same therapeutic strategy, but RM-001's design also maintains BCL11A expression, which mitigates potential impairments of BCL11A suppression in HSCs (Jang & Feng, et al., 2025 Blood Advances). RM-001 is administered similarly to exa-cel treatment; patients' HSCs are collected and treated ex vivo with RM-001 before patient re-infusion.

Combined data from an investigator-initiated trial (IIT) and Phase I study of RM-001 in TDT patients (total n=19; data cut-off of July 15, 2025; median follow-up of 26.3 months) showed robust clinical improvement; all 19 patients were transfusion-free at data cutoff, with all patients experiencing at least 15 months of transfusion freedom after a 2-month washout period following the patient's last transfusion. The patients stopped transfusions at a median of 22 days post-RM-001 reinfusion. This was correlated with the level of HbF, which constituted 85% of total hemoglobin after 3 months of RM-001 treatment, 98% after 12 months of treatment, and 100% after 18 months of treatment (see abstract here).

AccurEdit Therapeutics (Private)
Location: Suzhou, China

Technology
AccurEdit's gene editing platform allows development of editors covering both DNA and RNA. The platform also enables the design, screening, optimization, and validation of gRNA aided by AccurEdit's independently developed algorithms. On delivery, the company is developing polymer-based technologies, including LNPs for targeting of the liver as well as extra-hepatic tissues.

Key gene editing assets

ART002 is an LNP-delivered CRISPR Cas9 therapy targeting the *PCSK9* gene for the treatment of familial hypercholesterolemia. ART002 induces a knockout of PCSK9, which triggers elevated LDL-receptor expression and subsequently lowers circulating LDL cholesterol. In an open-label investigator-initiated trial of ART002, a single administration of ART002 elicited an average of about 90% PCSK9 knockdown with a maximum observed knockdown of 93%. Twelve to 24 weeks after administration, LDL-C reductions exceeding 50% were achieved in most patients, with the highest reduction approaching 70% (see press release here). A Phase I study of ART002 in familial hypercholesterolemia (NCT07353398) is now recruiting patients (expected n=24, according to clinicaltrials.gov).

William Blair

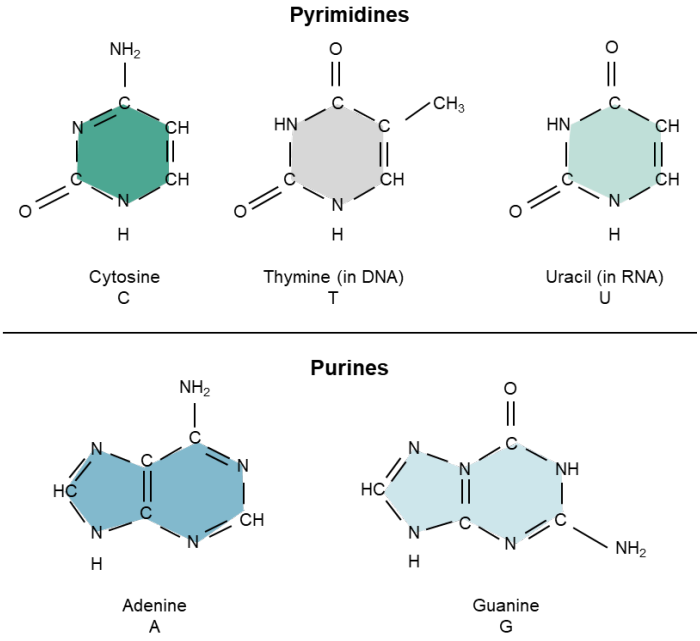
DNA Base Editing

DNA base editing strategies provide predictable, targeted gene edits in an efficient manner. Traditional non-templated gene editing strategies employed via nuclease-mediated DNA cleavage at a targeted genomic locus can kick-start error-prone DNA damage repair pathways, but the size (number of indels introduced) and type (specific bases added and/or deleted) are enacted by the cell and cannot be programmed by developers. While templated gene editing strategies via an exogenously delivered DNA donor sequence can channel the gene edit toward a specific sequence outcome, these methods are, as of now, inefficient and best purposed to insert large pieces of genetic material into the genome, such as entire gene sequences. Moreover, such nuclease-based gene editing methods require administration of double-stranded breaks to DNA, which may potentiate toxicities. However, many gene editing applications do not require large-scale genomic changes and are addressable by mutation of a single base. Such use-cases are well suited for base editors to enact targeted, efficient gene edits to single bases within the genome, without creating double-stranded DNA breaks, for therapeutic applications.

DNA Base Editing 101

Unlike other genome editing techniques that require insertion of a DNA base at a given position by the cell via a DNA polymerase enzyme, DNA base editing works by inducing transformation of an existing DNA base into another via chemical modification. Nucleic acid bases exist in two distinct families: purines and pyrimidines (exhibit 38). Purines [adenines (A) and guanines (G)] have a larger, double-ringed structure, while pyrimidines [cytosines (C), thymines (T), and uracils (U)] have a single ring. Note that T and U have very similar chemical structures, and of the five prototypical nucleic acids, only four are used in any given type of nucleic acid polymer: DNA exists as A, G, C, and T, whereas in RNA, U replaces T. Within the purine and pyrimidine families of DNA, the primary chemical characteristic that differentiates each base is the presence of either an amine group (NH2) or a carbonyl group (C=O) within the six-membered ring of the nucleic acid base; for purines, As have an amine group and Gs have a carbonyl group, while for pyrimidines, Cs have an amine group and Ts have a carbonyl group. This duality of amine-containing and carbonyl-containing purines and pyrimidines allows DNA base pairing to occur: each cognate DNA pair (As bond with Ts, and Gs bond with Cs) represents a matching of a purine base with a pyrimidine base as well as an amine-containing base with a carbonyl-containing base; a hydrogen bond between the amine group and carbonyl group is required for successful DNA pairing. DNA base editing works through de*amin*ation—the removal of the amine group from an amine-containing nucleic acid. In the end, DNA base editing uses deamination and the DNA repair machinery to recode a C into a T or an A into a G, creating a targeted edit to a single base in the genome.

Exhibit 38
DECODER
Molecular Structures of Nucleic Acids

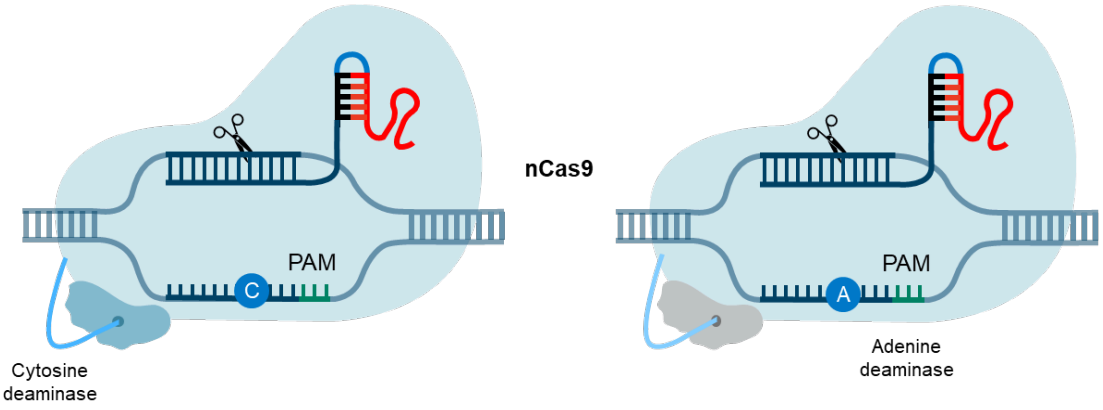


Source: SUNY Open Educational Resources

Guides and Deaminases Used for Base Editing

DNA deamination can be accomplished on either amine-containing DNA base, A or C, by adenine deaminases or cytosine deaminases, respectively. To accomplish base editing at a specific base within the genome, developers append a deaminase to a molecular guide. For most base editing constructs designed for genome editing therapeutics, the CRISPR-Cas system is used for the guide machinery, and the deaminase is added to the beginning (N-terminus) of the Cas protein (exhibit 39). Using the CRISPR system as a guide is advantageous for base editing not only due to its modular design and well-studied specificity, but also because it aids in the specificity of the deamination process itself: both adenine and cytosine deaminases used for base editing are specific for single-stranded (ss) nucleic acid polymers, ssDNA and ssRNA. When the CRISPR-Cas machinery finds its genomic target, the gRNA binds to the DNA of the target strand, which results in exclusion of the DNA from the opposite strand. This DNA-RNA structure, known as an R-loop, creates a stretch of ssDNA on the nontarget strand spanning the length of the protospacer sequence encoded in the gRNA. Therefore, deaminase activity by the base editor is limited to within this ssDNA, specifically on the nontarget strand. Further molecular characterization of CRISPR-guided deaminase events has demonstrated that the deaminases impart their own "deaminase window" within the ssDNA template, which is typically smaller than the ssDNA created by the CRISPR-mediated R-loop. Moreover, sequence motifs preferred by specific deaminases can further narrow deaminase activity to a targeted nucleotide.

Exhibit 39
DECODER
CRISPR Base Editing System



Source: Adapted from Gomez-Escribano et al., 2025 *eBioMedicine*

Choice of the specific deaminase used for a base editing therapeutic is chiefly dependent on the specific base needing to be corrected. For adenine deaminases, developers use an engineered isoform of *E. coli*–derived TadA (ecTadA), an enzyme naturally responsible for deaminating an adenosine within an arginine-encoding tRNA, which was subjected to multiple rounds of directed evolution to yield an ssDNA-specific adenine deaminase (ecTadA*) with broader sequence specificity than the wild-type enzyme. We note that TadA requires dimerization for its activity, and some adenine base editing constructs use two copies of the TadA enzyme appended to Cas9, one wild-type ecTadA copy and one ecTadA* copy. However, other iterations contain a single ecTadA* copy and may rely on dimerization between separate base editing molecules in cells to achieve deamination. The deaminase window for ecTadA*-containing adenine base editors has been reported to contain nucleotides 3-9 of the gRNA protospacer sequence, with optimal deamination efficiency at position 6.

Naturally occurring ssDNA cytosine deaminases in mammals provide developers with greater choice when designing a cytosine base editor. Canonical cytosine base editors use the APOBEC homolog from rat (rAPOBEC). APOBEC functions in mammalian cells to deaminate specific cytosines in certain mRNAs. Most typically, the rAPOBEC1 isoform is used, which has a preference for TC motifs when deaminating ssDNA. Characterization of rAPOBEC1 within the base editing context has revealed a deaminase window corresponding with nucleotides 4-8 of the gRNA protospacer. The size of the deaminase window depends on the sequence context of the targeted nucleotide, with sequence motifs preferred by the deaminase typically enlarging the potential window. Other APOBEC homologs have also been used for base editing, including APOBEC2, and multiple APOBEC3 isoforms (3A, 3B, 3C, 3D, 3F, 3G, and 3H). In addition, the APOBEC ortholog from sea lamprey (PmCDA1) has also been used for base editing efforts. Each isoform has a slightly different sequence motif preference and editing window. Such motifs are often defined by nucleotide sequence, but may also involve other factors, including DNA methylation status. We highlight APOBEC3A, which is more efficient at deaminating cytosines that are not methylated.

In addition to APOBEC, the deaminase AID has also been used. AID is particularly well known in immunology, as its base editing activity at the immunoglobulin locus initiates the DNA damage pathway that stimulates antibody isotype switching in B cells. While AID's relative specificity for ssDNA over ssRNA compared to APOBEC may be attractive to developers of base editors, its hyperactivity and large deaminase window (50-100 nucleotides) make specific DNA edits difficult

to manage, in our view. We note that GenAssist Therapeutics is using an AID-based base editor as part of its targeted AID-mediated mutagenesis (TAM) platform for development of potential therapies for Duchenne muscular dystrophy (DMD).

In addition to single adenine and cytosine deaminases, base editing constructs with the capacity for dual adenine and cytosine base editing have also been engineered. However, due to the increased potential for off-target deamination of surrounding nucleotides (bystander base editing), we do not see these dual base editors as particularly viable for therapeutic-based efforts. For more information on deaminases used for base editing purposes, including dual adenine and cytosine deaminase constructs, see this article.

While CRISPR is the predominant guide used for base editing, specific base editing applications, such as editing of mtDNA, are not suitable for CRISPR-based guides. Therefore, base editing has also been designed for TALE-based guides. Because docking of TALEs at a genomic locus does not create an R-loop structure, deaminases tailored for ssDNA applications are not well suited. Therefore, designers of TALE-guided base editors must use a dsDNA deaminase, such as DddAtox from the bacterium *Burkholderia cenocepacia,* which enacts cytosine deaminase activity on dsDNA. However, deaminases on dsDNA possess increased proclivity for spurious off-target deamination given that genomic DNA is double stranded. Therefore, a split deaminase approach has been developed, which separates the N-terminal and C-terminal portions of DddAtox onto two independent TALE guides targeting proximal mtDNA sites. Only when both TALEs have bound their respective mitochondrial genomic targets will a fully assembled DddAtox protein form, allowing deamination of the targeted cytosine. This approach is being used by Primera Therapeutics to target the mitochondrial leucine tRNA gene (mt-TL1), which is mutated in multiple primary mitochondrial myopathies.

Deaminase and guide RNA engineering

Engineering of deaminases aims to narrow the scope of deamination by increasing the specificity for ssDNA over ssRNA and narrowing the deamination window while maintaining efficient deaminase activity. For adenine base editing, the evolution of ecTadA into a state-of-the-art base editor hinges on key domains within the deaminase protein; ecTadA*, mentioned above, contains 14 mutations compared to the wild-type copy, which are located in the substrate-binding loops and the C-terminal helix. Such mutations balance increased ssDNA binding while preventing promiscuity, including deamination of cytosines in dsDNA. Current iterations of adenine base editors (ABE9) boast deaminase windows as little as 1-2 nucleotides with little off-target activity (Chen et al., 2023 Nature Chemical Biology).

For cytosine deaminases, efforts to increase specificity of APOBEC's deaminase activity have focused on the substrate binding site. Mutations at this site have successfully decreased enzyme processivity, which narrows the deaminase window. Mutations in the enzyme at amino acids that contact the sugar phosphate backbone of ssDNA can also narrow the deaminase window of engineered APOBEC enzymes. Successful engineering of APOBEC has also altered motif specificity. Mutations in the enzyme's active site predicted to contact the nucleotides ahead (on the 5' end) of the targeted cytosine create APOBEC mutants with reduced motif specificity and increase the number of potential mutations targetable for base editing (Thuronyi et al., 2019 Nature Biotechnology). Perhaps most notably, advancements in APOBEC engineering have curbed its affinity for and deaminase activity on ssRNA, which has been reported to occur on 40%-60% of expressed genes in human cells, depending on the cell type. Mutations to limit APOBEC activity on ssRNA reduced off-target RNA editing by about 3,800-fold while showing more precise on-target DNA editing (Grünewald et al., 2019 Nature). Engineering efforts on APOBEC3A have also been performed to reduce off-target deaminase activity, including narrowing the deaminase window (Wang et al., 2018 Nature Biotechnology), and enhance specificity to TC motifs for base editing (Gehrke et al., 2018 Nature Biotechnology).

In addition to APOBEC engineering for cytosine base editing, cross-base deaminase activity discovered in TadA mutants revealed potential for a TadA-based cytosine-specific base editor (Neugebauer et al., 2023 Nature Biotechnology). As mentioned above, engineering efforts in the context of adenine base editing have already limited the deaminase window for TadA down to 1-2 nucleotides, and further improvements have increased TadA cytosine base editor efficiency and sequence motif expansion (Lam et al., 2023 Nature Biotechnology; Li et al., 2024 Nature Communications). Given the relative on-target specificity of TadA compared to APOBEC, we see TadA-based cytosine deaminases as the future of cytosine base editing.

Beyond engineering of the deaminase to minimize the deamination window, additional engineering efforts have been made on the gRNA to suppress bystander edits by leveraging deaminase bias toward ssDNA substrates; by adding bases to the 3' end of the gRNA (opposite side of the gRNA from the protospacer) that hybridize with the nontarget DNA strand, design of a RNA hairpin structure at the specific target base minimizes the exposed ssDNA substrate for deamination. This work was recently published in the laboratory of George Church (Perrotta, Vinke, & Ferreira et al., 2025 Nature Biotechnology).

Cellular Response to Base Edits—Biasing DNA Repair Toward a Change in Sequence

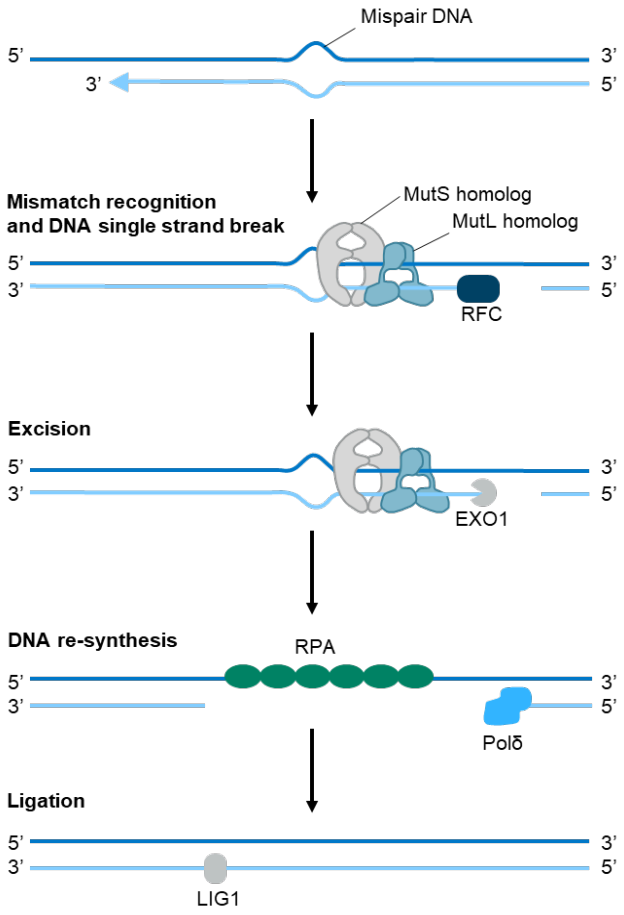
Deamination of adenine and cytosine DNA bases does not transform those bases into nucleic acids used in DNA. Deamination of adenine by an adenine base editor transforms adenine into an amineless purine called inosine (I), interpreted by cellular machinery as a G, and deamination of cytosine by a cytosine base editor transforms cytosine into uracil, interpreted by cellular machinery in the context of DNA as a T. Base editing efficiency, therefore, relies not only on the efficiency of deamination, but also on the ability of the base editor to bias cellular repair toward incorporating the edited base as its "new normal."

In the case of cytosine-to-uracil base edits, these switches are relatively commonplace; the high concentration of uracil required for RNA synthesis propagates mistaken incorporation of Us instead of Cs into the genome during DNA replication. The cell may fix an improper U in the genome through a process called base excision repair, which cleaves the uracil base, excises the sugar-phosphate backbone at that site, and fixes the gap with a T at that site. However, during base editing, base excision repair activation would negate the successful deamination event by the base editor, as base editing requires the uracil remain in the genome so that it may eventually be encoded as a T. As a result, cytosine base editing efficiencies rely on the suppression of base excision repair. To improve such efficiencies, recent generations of base editors incorporate a protein that inhibits uracil glycosylase, the enzyme in the uracil base excision pathway responsible for cleaving the base and triggering its removal. Uracil glycosylase inhibitors (UGIs) may be appended onto Cas9 in addition to the deaminase, and multiple UGIs may be appended in tandem to improve blockage of the base excision repair pathway and increase base editing efficiency. While inosine excision by glycosylases through base excision repair is possible, it is relatively inefficient, likely due to the low misincorporation of inosines into the genome. Therefore, no equivalent inosine glycosylase inhibitors are needed for efficient adenine base editing.

A successful deamination event paired with effective suppression of the base excision repair pathway will create an unpaired mismatch between DNA strands, as the deaminated base can no longer base pair with the base on the opposite DNA strand. This kink in the dsDNA helix will be recognized by the cell as an error in the genome that must be repaired through a process called mismatch repair. As with base excision repair, canonical mismatch repair is suited for the process of fixing errors derived from replication of the genome during cell division. Therefore, the enzymatic steps of this pathway are tailored for this process. In the context of base editing, the evolutionary design of this pathway is particularly pertinent to the issue of recognizing which DNA strand contains the required base at that position; it may seem plausible that if a cell encountered a position in the genome where two noncognate DNA bases were paired together, it would not know which

strand had the correct base at that position and which strand was the incorrect replicate. In the case of mismatch repair during DNA replication, this issue is resolved through relative methylation status of the two DNA strands; mismatch repair proteins identify the improperly replicated nascent daughter strand though its reduced methylation, triggering the endonuclease to nick the daughter strand and repair the incorrect base (exhibit 40; find more information about mismatch repair during DNA replication in this article).

Exhibit 40
DECODER
DNA Mismatch Repair



RFC: Replication Factor C; EXO1: Exonuclease 1; RPA: Replication Protein A
Polδ: DNA Polymerase delta; LIG1: DNA Ligase 1
Source: https://www.neophore.com/science/dna-mismatch-repair-inhibitors

However, in the case of base editing, a mismatch is created outside the context of DNA replication where both DNA strands are equally methylated. Therefore, to bias the mismatch repair pathway, the base editing system takes advantage of the CRISPR-Cas guide: base editors use the D10A nickase Cas mutation, creating an nCas, which introduces a nick to the target strand in tandem with base deamination on the nontarget strand. This nick biases the mismatch repair pathway to excise and recode the nonedited strand as cognate to the base-edited strand, solidifying the base edit within the genome. In support of the role of the nickase in facilitating base editing, an nCas mutant with hyper-nicking activity was shown to boost base editing efficiency (Gandadireja et al., 2024 ACS Synthetic Biology).

With base editing development expanding over recent years, there have been several efforts to improve efficiency, selectivity, and delivery packaging of this technology. Given the relative infancy of Cas12-based nickases in the gene editing field (we are unaware of any disclosed nCas12 enzymes that cleave the target strand), most currently used CRISPR-based base editors use Cas9. However, we believe this may change as Cas12 nickases become more developed and widely accepted. TALE-guided nickases have also been recently commandeered for strand-specific mtDNA base editing when combined with either an ssDNA adenine or cytosine base editor (Yi et al., 2024 Nature Biotechnology; Cho et al., 2024 Cell). Addition of an exonuclease to a similar scheme may improve base editing efficiencies and strand-specific bias through increased ssDNA creation. This method was recently published as CyDENT base editing (Hu et al., 2024 Nature Biotechnology).

Applications for DNA Base Editing

DNA base editing allows targeted mutations via switching base identities within a gene. In instances where the mutation is introduced within the gene's coding sequence—the sequence that encodes for the gene's protein product—the base edit will alter the gene's resultant mRNA and protein products. Because the mismatch repair process will synergize both DNA strands in line with the base edit introduced, the mutation can be made by editing a base on the template DNA strand, the DNA strand physically used as a template by the RNA polymerase to transcribe its RNA product, or the coding DNA strand, the partnered strand to the template strand. During transcription, RNA polymerase will use the template strand and add the RNA base that complements (pairs with) that DNA base to the growing RNA strand. Therefore, the template strand does not have the same sequence as the resultant RNA produced; instead, the template strand and RNA product are "complementary" in sequence. On the other hand, the coding strand is referred to as such because although it is not used directly by the RNA polymerase for transcription, its sequence encodes (is a match with) the resultant RNA product from that gene. Therefore, developers may introduce a base edit to the coding strand that directly matches the desired sequence of the gene's mRNA product, or, alternatively, introduce the complementary base on the template strand. This does allow for carbonyl-containing bases (Gs and Ts) within a gene's DNA sequence to be indirectly replaced by editing the amine-containing A or C on the opposite strand. However, base editing is inherently limited to purine-to-purine or pyrimidine-to-pyrimidine base switches (A ↔ G; C ↔ T).

Decisions by developers to target a base edit to the template strand or coding strand may be influenced by multiple factors. For example, preference for a specific type of base editor—adenine or cytosine—may influence this choice. In addition, parameters associated with the gRNA, such as the distinctiveness of the target sequence within the genome or the presence of a PAM site for CRISPR-guided base editing applications, must also be considered.

Gene knockout

A base editor may edit a nucleotide within an amino-acid-encoding codon, to create a Stop codon (UAA, UAG, or UGA) within the protein's reading frame, which will induce the ribosome to terminate the protein's production prematurely. The resultant mutant protein will either be degraded by the cell or exist in a truncated/dysfunctional state. This can only be accomplished with cytosine base editing, as A-to-G switches with adenine base editing capable of leading to Stop codon formation can only occur from the UAA codon, which is already a Stop codon. As with nuclease-based knockouts, developers attempting to knock out a gene by targeting the gene's protein-coding sequence typically design the introduction of the Stop codon near the beginning of the gene to maximize the likelihood of protein dysfunction and degradation.

Base editing may also be used to mutate an mRNA's Start codon (AUG), which will impact the ability of the ribosome to initiate translation at the proper site and/or frame. However, we note that translation from downstream START codons or noncanonical START codons (typically CUG, GUG, and UUG) may occur (an article with more information regarding non-AUG translation initiation is available here). These consequences may also create functional knockouts by reducing

translational output or enacting frame shifts that incite protein dysfunction. Abrogation of START codons can also be used as a potential strategy in cases where a gene contains multiple used START sites and/or reading frames to prevent translation from that alternative START site, which may alter cellular responses to stimuli.

In addition, as with nuclease-based gene editors, knockouts may also be administered via a base edit not within the protein-coding region, but in a genomic sequence that regulates the gene's transcription. Developers must take care that the specific mutation introduced is sufficient to influence the activity of the transcriptional machinery interacting with that genomic site. We note pioneering work is being done in this space through the use of artificial intelligence models to predict the effects of sequence variation in gene promoters on the expression of that gene (Jaganathan, Ersaro, & Novakovsky et al., 2025 Science).

Gene mutation

In diseases where a mutation is caused by a single amino acid switch in a protein leading to dysfunction, base editing may be used to edit the gene's protein sequence back to the wild-type sequence or mutate the pathogenic codon to a different codon encoding for an alternative amino acid that does not spur disease.

Alternatively, point mutations introduced from base editing may serve to modulate a protein's function in cells to nonphysiological levels. For example, a base edit could introduce a mutation that impedes certain functions of a protein while maintaining its expression in cells. While a knockout may be preferred in some cases, this application may be useful for curbing specific protein functionality in proteins with multiple functions, whether within one cell or from cell-to-cell or tissue-to-tissue. Mutations could also be introduced via base editing that boost a gene's expression or alter a protein's function to supraphysiological levels.

Gene mutations may also be introduced that are inconsequential to protein expression or function but endow the protein with resistance to a specific drug that is used adjunctively as part of a larger treatment regimen. This scheme has typically been applied to cell-based therapies; such cells are administered a modification (through gene editing or otherwise), which provides them with a characteristic that will make the cell therapeutically potent. Following that modification, base editing can be used to effect a point mutation that will make the base-edited protein resistant to a drug administered later in the treatment regimen. When that drug is administered as part of treatment, unmodified cells in the patient expressing the wild-type version of the drug's target protein will be affected, while modified cells receiving the base edit will be spared. We highlight Beam Therapeutics' Engineered Stem Cell Antibody Paired Evasion (ESCAPE) platform for bone marrow transplantation, which uses base editing to mutate CD117 in patient-derived HSCs, which are concomitantly modified to mitigate disease. To refresh a patient's existing hematopoietic cell compartment, an antibody specific for CD117 can then be used as a "preconditioning" regimen to bind to and eliminate all remaining disease-causing HSCs in the patient expressing CD117. When the modified HSCs are then reintroduced into the patient, their mutated CD117 will maintain its normal function but will not be bound by the CD117 antibody, allowing those edited therapeutic cells to survive and seed the patient's hematopoietic cellular compartment. In addition, IASO Biotherapeutics has developed a base editing strategy targeting the protein Lck to create CAR-T cells resistant to the tyrosine kinase inhibitor imatinib to reduce host rejection of the allogeneic CAR-T cell transplant.

Modulation of RNA splicing

Base edits can also be applied to modulate the splicing of a gene's RNA product, which may impact its protein's expression or makeup. Splicing occurs via sequence motifs called splicing donors and splicing acceptors, which fall near the barrier of a gene's introns and exons. Splicing occurs when splicing machinery forms a macro protein-RNA complex called the "spliceosome," which cleaves

the intron out of the pre-RNA, creating a seamless transition from exon to exon in the mature mRNA product (see a video animating the splicing process here). Mutation of a splicing donor site (GT motif) or a splicing acceptor site (AG motif) within a given intron via base editing will render the splice site dysfunctional, not allowing splicing to occur at that intron-exon junction.

Inhibiting splicing through a base edit could yield multiple effects, and designers may introduce a splicing mutation within a gene at a specific site depending on the intended application. For example, splicing inhibition may force inclusion of an intron within the mature mRNA sequence, which may introduce a knockout via either premature Stop codons, throwing off the protein's reading frame, or rendering the protein dysfunctional by introducing an extra array of amino acids into the protein's sequence. We highlight the design of Verve's (now Eli Lilly) base editing construct for the *PCSK9* gene, which edits the splicing donor site between the first exon and first intron of *PCSK9*, forcing the first intron to be included in the mRNA sequence. Inclusion of *PCSK9's* first intron creates a potent gene knockout due to an encoded Stop codon four codons into the intronic sequence.

In addition to inclusion of an intron, splicing inhibitors, when used beyond the first intron-exon junction, can induce a phenomenon called "exon skipping"; by blocking a given splicing donor or acceptor, the uninhibited splicing donor-and-acceptor pair from the exons sandwiching the targeted exon will join, leading to the targeted exon being spliced out. Exon skipping can cause multiple effects depending on the exon skipped. For example, the noncanonical joining of two distal exons may introduce a premature Stop codon in the mRNA sequence, leading to a protein knockout. Alternatively, as some codons are split between two exons (the triplet base sequence of the codon starts on one exon and finishes on the next exon), joining of two distal exons may create a frame shift that renders the protein dysfunctional. A knockout may also be created by skipping an exon that encodes amino acids necessary for the protein's function, though we are not aware of this application of exon skipping in any therapeutic assets to date.

While exon skipping may be used to create a protein knockout, the most common use-case for exon skipping for therapeutic purposes is to skip a pathogenic exon within the protein sequence. Such exons may be deleterious to function through mutations that introduce a premature Stop codon or contain expanded repetitive sequence elements. By inducing the cell to skip such exons via base editing, protein functionality may be restored. We highlight the preclinical therapeutic assets from GenAssist Therapeutics, which is designing base editors to enact exon skipping of pathogenic exons within the dystrophin gene to address various genetic forms of DMD.

In addition to pathogenic exons deriving from mutations, premature termination codons may also be encoded into exons as part of the wild-type mRNA sequence; while these exons are typically spliced out of the mRNA to create a functional protein product, the cell can regulate the abundance of that protein by enacting alternative splicing to selectively include that exon, thereby making those splicing events "nonproductive," since they will not encode a full-length protein. It has been estimated that up to 35% of human mRNA isoforms may contain such encoded premature Stop codons (Lewis et al., 2003 PNAS). Using exon skipping to prevent inclusion of these Stop codon-containing exons serves to incrementally increase the amount of protein expression by preventing nonproductive alternative splicing events. This concept is being implemented by Stoke Therapeutics, which is using antisense oligonucleotides (ASOs) as exon skippers. By targeting diseases in which only one of the two gene copies is mutated, Stoke aims to address the disease by boosting the expression of the wild-type allele through exon skipping. To date, we are unaware of any company using base editing for this specific application of exon skipping, but we see this as a potential application for base editing. Lastly, while exon skipping represents a powerful method for restoring or boosting protein function, the potential for the joining of two distal exons to create deleterious effects on protein expression or function highlighted above makes such exon skipping applications amenable only to specific genes and/or exons.

Mitigation of Off-Target Base Editing Activity

Compared with nuclease-based gene editing techniques and double-stranded breaks, the use of nickases in base editing reduces the potential toxicities associated with severe genetic lesions and malignant transformations. For example, both adenine and cytosine base editors spurred genomic translocations at a frequency of 0.19% and 0.22%, respectively, compared with 1.93% with a standard Cas9 nuclease (Yin et al., 2022 Nature Communications). These rates may vary depending on the cell type and genomic location targeted. Therefore, mitigation of off-target base editing primarily reflects the desire to limit deaminase activity in either 1) unwanted parts of the genome, which includes the proclivity of many deaminases used for base editing applications to target both ssDNA and ssRNA, or 2) unwanted nucleotides within the deaminase window.

In addition, as with nuclease-based applications, it is important to consider that not all off-target edits will lead to deleterious consequences; base editing in off-target noncoding or intronic regions of the genome will have a reduced impact on gene expression. In addition to those potential scenarios, the predictability of sequence correction by base editors allows an additional layer of tolerable off-target editing activity unavailable to nuclease-based applications via the creation of "silent mutations." Silent mutations are those in which the altered sequence creates a new codon, which encodes for the same amino acid as the original codon. While it is technically possible for these silent mutations to create downstream effects on the gene's mRNA molecule such as mRNA half-life, translation efficiency, and/or interaction of the mRNA with noncoding RNAs, these effects are unlikely. Regardless, the resultant protein sequence from silent mutations will be unaffected. Moreover, while off-target base editing changes may result in a change in amino acid at a given position, such a change may have no effect on protein function. However, given the unknown risks associated with undesired, permanent mutations to the DNA sequence, we still deem it paramount for therapeutic applications to limit off-target deaminase activity of DNA base editors.

Below we list several aspects of the base editing machinery design that can be tweaked to control for potential off-target deaminase activity and base editing.

Guide design

The primary driver of off-target base editing stems from shortcomings of the guide, which may bind to other regions of the genome if nonspecific. Given the shared guide designs for nuclease and base editing constructs, we refer to those considerations listed above, including selecting the targeted genomic sequence (when a choice can be made), engineering guides with increased fidelity, reducing the guide's affinity for DNA, tailoring the guide to bind only in certain chromatin structures, and/or shortening the editor's half-life in cells. In addition, as noted above, recent work has included modification of the guide RNA's 3' end to mitigate bystander edits by minimizing the amount of ssDNA substrate available to the deaminase.

Deaminase engineering

In addition to specificity considerations of the guide to target the deaminase to the proper genomic locus, reducing the ability of the deaminase to perform bystander base editing is crucial for enacting the specific gene editing outcome desired. At the DNA level, this can sometimes be addressed by guide design; selecting target sequences for which there is only one instance of the deaminase's cognate base within the deaminase window will ensure on-target editing. However, in instances where other potential deaminase targets cannot be avoided, engineering deaminases with a limited deaminase window, low processivity, or hyper-specific motif preferences tailored to the targeted nucleotide will aid in the elimination of potential off-targets. Also, reducing the affinity of the deaminase for ssRNA will reduce bystander base editing on RNA. We refer readers to the "Deaminase and guide RNA engineering" section above for specific advancements that have been made on this front.

NLS hyperactivity
Bystander deamination of RNA in the cytoplasm of cells is partly a problem of deaminase promiscuity and partly a problem of access to cytoplasmic RNAs. Many RNAs are transcribed in the nucleus of cells and are subsequently exported to the cell's cytoplasm, where they either are translated by ribosomes or, in the case of non-protein-coding RNAs, perform their cellular function. When the DNA base editing construct is introduced into the cell, the protein-based machinery (e.g., Cas-deaminase fusion protein) is translated in the cell's cytoplasm, but its NLS sequence will trigger it to translocate to the nucleus to perform its genome editing function. Therefore, the more efficient the NLS sequence is at forcing the deaminase to the nucleus, the less time the deaminase will spend in the cytoplasm with access to cytoplasmic RNAs that may spur bystander RNA deaminase activity. More efficient NLS sequences will not address bystander deaminase activity on DNA, nascent RNA that has not yet exported to the cytoplasm, or nuclear RNAs that remain there in association with their function.

Require multiple DNA binding events
As with nuclease-based editing, the requirement of multiple independent guides to bind to their cognate targets for base editing to occur lowers the potential for off-target genomic or RNA deaminase activity. Split, TALE-guided deaminase constructs have already shown utility in mtDNA base editing applications. Given the complementarity of an R-loop with ssDNA deaminases, it is quite difficult to create base editing constructs requiring the independent binding of two unique CRISPR-based guides. However, we note advancements in this area from CorrectSequence Therapeutics, which uses a unique deaminase inhibitor-activator construct to enable deaminase activity only in the context of two Cas docking events (see exhibit 47 on page 91).

Small-molecule-induced deamination
Split deaminase constructs can be applied not only to the tethering of independent guide molecules, but also to protein domains that require the administration of a small molecule to associate. For these designs, one part of a split deaminase is appended to a genomic guide along with a protein that can only bind its cognate protein binding partner in the presence of a small molecule. The other part of the split deaminase is attached to that cognate protein binding partner. Unlike other split deaminase approaches, we note that the specificity of this approach is achieved only if the two deaminase "halves" cannot enact deaminase activity through spontaneous assembly; deaminase activity must only be possible through association of the small-molecule-induced protein binding partners. To date, the FKBP-rapamycin binding (FRB) protein + FKBP3/12 system is the small-molecule-dependent protein binding system used for this approach, where the association of FRB with FKBP3/12, and therefore deaminase activity, can occur only in the presence of the small molecule rapamycin (Berrios et al., 2021 Nature Chemical Biology; Zeng et al., 2023 Nature Communications).

DNA Base Editing Business and Clinical Updates

Beam Therapeutics Inc. (BEAM)
Location: Cambridge, Massachusetts

Technology
Beam's gene editing platform consists of proprietary base editing technologies including cytosine or adenine base editors. The technology harbors a DNA targeting CRISPR protein that does not cause a DNA double-stranded break and is combined with a base editing enzyme that carries the desired based modification. The company uses electroporation and LNPs for ex vivo and in vivo delivery of its gene editing technologies, respectively.

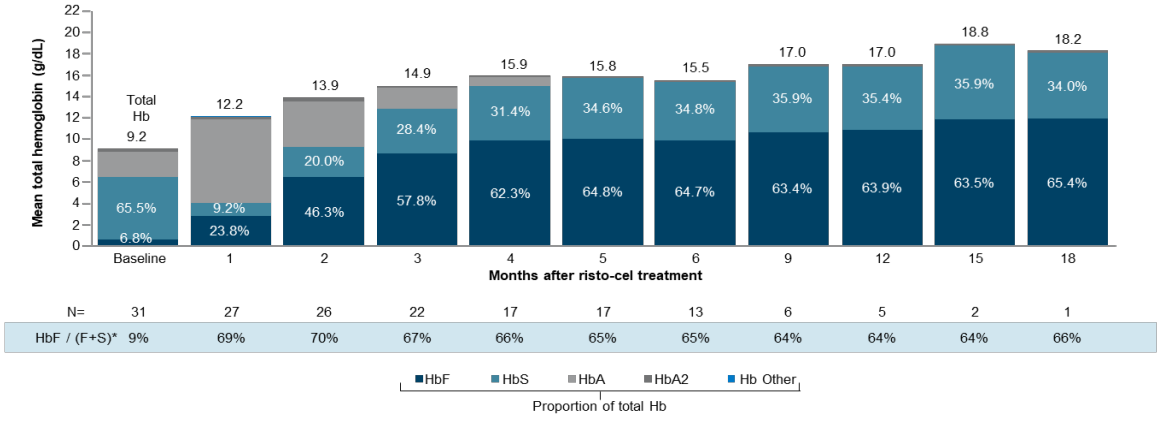
Lead assets

Risto-cel (BEAM-101) is a cell therapy in clinical development for the treatment of SCD. Risto-cel consists of ex vivo edited autologous CD34-positive hematopoietic stem and progenitor cells (HSPCs). CD34-positive cells are base edited to mimic naturally occurring A-to-G substitutions in the promoters of the HBG1/2 genes, which disrupts the BCL11A transcriptional repressor and leads to the upregulation of HbF.

The Phase I/II BEACON trial of risto-cel has enrolled 54 patients. As of August 6, 2025, 31 SCD patients age 16–34 years had been treated with a mean risto-cel dose of 11.9×106 viable CD34+ cells/kg, with a median follow-up of 6.6 months (range: 0.3-20.4 months). Patients treated with risto-cel achieved rapid and sustained increases in HbF, with mean levels exceeding 60% as early as four months post-treatment (exhibit 41). Increases in HbF were associated with a reduction in sickling hemoglobin (HbS) below 40%. Pancellular HbF expression was maintained throughout follow-up and was associated with the resolution of anemia and normalization or improvement in markers of hemolysis and red blood cell health (i.e., haptoglobin, lactate dehydrogenase, reticulocytes, erythropoietin). Editing efficiency was at an average of 67.4% at six months, which increased to 72.8% by 12 months, demonstrating durable engraftment of edited cells. Notably, patients required a median of one stem cell collection cycle (range: 1-5), with 65% of patients requiring only one mobilization cycle. Post-infusion, neutrophil engraftment occurred at a median of 17.5 days and platelet engraftment at 19 days, with 29% of patients avoiding platelet transfusions.

Exhibit 41
DECODER
Efficacy of Risto-cel in SDC Patients in Phase I/II BEACON Study

Patients achieved endogenous HbF >60% and HbS <40% by 1 month after risto-cel treatment that was sustained through follow up, suggesting resolution of anemia



Source: Beam Therapeutics company presentation

On safety (exhibit 42), the median duration of severe neutropenia was seven days, suggesting fewer hospital days compared with traditional transplant processes. No severe VOCs have been reported in any treated patients following engraftment. Other adverse events associated with risto-cel include stomatitis (77.4%), decreased appetite (32.3%), hypokalemia (32.3%), skin hyperpigmentation (32.3%), and anemia (22.6%).

Exhibit 42
DECODER
Safety of Risto-cel in SDC Patients in Phase I/II BEACON Study

Patients with, n (%)	N=31
Any TEAEs	31 (100)
Related to risto-cel	3 (9.7)*
Any TEAEs ≥ Grade 3	27 (87.1)
Related to risto-cel	1 (3.2)†
Serious TEAEs	12 (38.7)
Related to risto-cel	0
Death	1 (4)‡
Related to risto-cel	0

Most common TEAEs, n (%)	N=31
Stomatitis	24 (77.4)
Febrile neutropenia	22 (71.0)
Decreased appetite	10 (32.3)
Hypokalemia	10 (32.3)
Skin hyperpigmentation	10 (32.3)
Platelet count decreased	8 (25.8)
Anemia	7 (22.6)
Hypomagnesemia	7 (22.6)
Constipation	6 (19.4)
Hypertension	6 (19.4)
Nausea	6 (19.4)
Anxiety	5 (16.1)
Headache	5 (16.1)
Peripheral edema	5 (16.1)
Pruritus	5 (16.1)
Pyrexia	5 (16.1)

Source: Beam Therapeutics company presentation

BEAM-302 is an in vivo base editing therapy in development for alpha-1 antitrypsin deficiency (AATD). BEAM-302 is designed to correct the E342K point mutation in the *Serpina1* gene, leading to correct folding and functions of the AAT protein. BEAM-302 is encapsulated and delivered in LNPs that directly target the liver.

The ongoing Phase I/I study of BEAM-302 aims to enroll 106 AATD patients. Part A will study doses of 15, 30, 60, and 75 mg of BEAM-302 in patients with alpha-1-associated lung disease. Part B will consist of a dose exploration phase in patients with alpha-1-associated liver disease with or without lung disease.

As of the February 10, 2026, cutoff date, the Phase I/II study of BEAM302 has now treated 29 patients across parts A and B with followup extending up to 12-18 months. In the singledose 60 mg cohort, BEAM302 achieved a steadystate mean total AAT level of 16.1 µM, with all patients durably exceeding the 11 µM protective threshold, representing the highest mean serum AAT level clinically reported to date for a *Serpina1* correction approach. Corrected MAAT constituted about 94% of circulating AAT, while mutant ZAAT was reduced by about 84%, producing a biochemical profile meeting or exceeding that of natural MZ carriers (exhibit 43). Higher doses and repeat dosing did not improve efficacy, with the 75 mg and 2x60 mg cohorts achieving comparable AAT levels, supporting a saturation threshold at 60 mg and confirming this dose for pivotal development. It is important to note that BEAM302 preserved physiologic regulation of AAT expression, as demonstrated by one patient who mounted an acutephase response during infection, with total AAT rising to about 30 µM while maintaining roughly 95% MAAT, a mechanistic feature not achievable with augmentation therapy. Consistent efficacy was also observed in patients with underlying liver disease, supporting dose selection across phenotypes and reinforcing the 60 mg regimen for advancement into the pivotal part C cohort planned for the second half of 2026.

Exhibit 43
DECODER
Efficacy of BEAM-302 in AATD Patients

Dose Cohorts	15 mg (n=3) Part A	30 mg (n=3) Part A	60 mg (n=6) Part A	75 mg (n=9) Part A	2x60 mg (n=3) Part A	30 mg (n=3) Part B	60 mg (n=1) Part B
Mean total serum AAT (μM) at baseline†	5.2	5.6	6.6	6.3	5.5	6.8	5.5
Steady state total AAT (μM)†	8.4	9.9	16.1	14.4	16.5	12.5	17.2
Proportion M-AAT (steady state) mean	45%*	60%*	94%	91%	93%	75%	95%
% change in Z-AAT (steady state) mean	-15%	-40%	-84%	-79%	-80%	-51%	-86%

* as of 28 days post-treatment; values from March 2025 Update
† Baseline and steady state AAT values for the 15 mg and 30 mg cohorts were measured using turbidimetry in 2025; later cohorts used LC MS in 2026
Source: Beam Therapeutics company presentation

BEAM-302 has demonstrated a favorable safety profile across 26 single-dose patients treated through 75 mg (exhibit 44). In these cohorts, treatment-related adverse events have been limited to infusion-related reactions and transient grade 1-2 liver transaminase elevations, with no treatment-related serious adverse events and no bilirubin abnormalities reported. In a small re-dosing cohort (2×60 mg), one patient experienced asymptomatic grade 4 ALT and grade 3 AST elevations following the second dose, consistent with an inflammatory response to repeat LNP exposure; liver enzymes normalized without intervention. No similar events have been observed in any single-dose cohort, and future development is expected to focus on a single-dose regimen, substantially mitigating this risk. For more, see our note: BEAM-302 Shows Impressive AAT Dynamics Unique to SERPINA1 Correction; Pivotal Part C Commencing Second Half 2026.

Exhibit 44
DECODER
Safety of BEAM-302 in AATD Patients

Patients with	Single-Dose (up to 75 mg) N=26 (%)	Multi-Dose (2x60 mg) N=3 (%)
Any TEAEs ≥Grade 3	0 (0)	1 (33)*
Dose-limiting toxicities	0 (0)	0 (0)
Serious TEAEs	0 (0)	0 (0)
Death	0 (0)	0 (0)

*Grade 4 ALT and Grade 3 AST elevations in one patient following second 60 mg dose; asymptomatic and resolved without intervention
Source: Beam Therapeutics company presentation

Verve Therapeutics (formerly VERV, acquired by Eli Lilly)
Location: Boston

Technology
Verve is developing several base editing and broader gene editing assets for the treatment and prevention of cardiovascular disease via long-term lipid control. For base editing applications, adenine base editing is the focus, with an mRNA encoding the adenine base editor as well as an sgRNA packaged into an LNP for delivery to the liver. Verve's base editing technology was originally licensed from Beam Therapeutics. The company has used multiple LNP variants for delivery, but current clinical-stage assets use an LNP that contains a specialized ionizable amino lipid (licensed from Novartis) and is subsequently conjugated to a GalNAc moiety for enhanced liver targeting in instances of low LDL-receptor expression or LDL-receptor mutations. Verve has also listed a "novel editor" as part of its preclinical assets, although the nature/application(s) of that editor beyond Lp(a)-lowering have yet to be disclosed at the time of writing.

Key gene editing assets

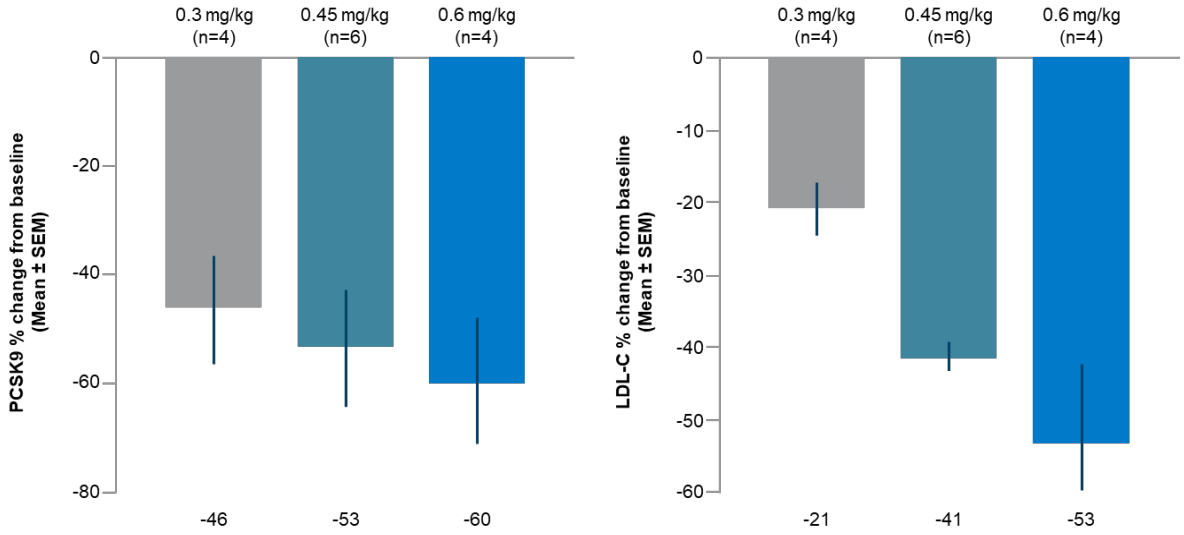
VERVE-101 is an ABE8.8 adenine base editor comprising an spCas9 fused to an adenine deaminase as well as an sgRNA for targeting to the *PCSK9* locus for the treatment of cardiovascular disease, including heterozygous familial hypercholesterolemia (HeFH) and atherosclerotic cardiovascular disease (ASCVD). VERVE-101's sgRNA targets the junction between the first exon and first intron of *PCSK9* and triggers a deamination event on the first adenine encoded on the template strand of the first intron of *PCSK9*. Of note, the editing window of ABE8.8 typically ranges from positions 3 to 9 in the protospacer DNA sequence, and peak editing is observed at position 6 of the protospacer, which is the location of the edited adenine within the protospacer sequence. In addition, the edited adenine is the only adenine within the 7-nucleotide deaminase window on the *PCSK9* locus, which strongly reduces the possibility of bystander edits. Editing of the first adenine within intron 1 of *PCSK9* precipitates a base switch on the coding strand from T to C, thereby mutating and deactivating the splice donor of intron 1 (Musunuru et al., 2021 Nature). By preventing successful mRNA splicing of intron 1, a functional knockout of *PCSK9* is created by translation into the intronic sequence, which contains a Stop codon four codons into the intron.

The Phase I Heart-1 study (NCT05398029) investigated increasing doses of a single VERVE-101 treatment in patients with HeFH (0.1 mg/kg, 0.3 mg/kg, 0.45 mg/kg, and 0.6 mg/kg), with 9 of 10 patients assessed for levels of serum PCSK9 and LDL-C, and average measurements assessed from day 28 to last available follow-up from baseline. VERVE-101 elicited PCSK9 reductions as much as 84% from baseline, which were coupled with dose-dependent reductions in LDL-C lowering (see our note: At the Heart of It: Trail-Blazing Heart-1 Data Shows In Vivo Base-Editing POC; Low Patient Number Variable but Hits Base Case). However, Heart-1 was paused after a grade 3 transient ALT elevation and thrombocytopenia was observed in the sixth patient enrolled in the 0.45 mg/kg cohort (see our note: Heart-1 Paused Following Safety Events; Prioritizing VERVE-102; Closer Look at Ionizable Lipid Safety Landscape). Longer-term follow-up data from the Heart-1 trial, which consisted of 6 patients treated with 0.45 mg/kg VERVE-101 and 1 patient treated with 0.6 mg/kg VERVE-101, showed sustained LDL-C reductions; patients treated at the 0.45 mg/kg showed average LDL-C reductions of about 51% from baseline at 18 months, while the patient treated with 0.6 mg/kg had LDL-C levels reduced by 57% from baseline after 18 months. The patient treated at the 0.6 mg/kg dose showed LDL-C reductions of 68% after 24 months.

VERVE-102 is a follow-on *PCSK9* base editor that contains the same base editing machinery as VERVE-101 but is delivered via an alternative formulation of an LNP, which contains a different PEG lipid, a different ionizable lipid (in-licensed from Novartis), and a GalNAc ligand. The target indication was also expanded into premature coronary artery disease as VERVE-102 moved into the clinic with VERVE-101 safety setbacks.

Data from the Phase Ib HEART-2 study of VERVE-102 (NCT06164730), which dosed patients at 0.3 mg/kg, 0.45 mg/kg, and 0.6 mg/kg, showed impressive efficacy, eliciting dose-dependent reductions in LDL-C (0.3 mg/kg: 21%; 0.45 mg/kg: 41%; 0.6 mg/kg: 53%); see exhibit 45. In addition, the maximum LDL-C reduction observed was 69% from a patient in the 0.6 mg/kg dose cohort. VERVE-102 demonstrated a clean safety and tolerability profile, with no treatment-related SAEs, no dose-limiting toxicities, and no cardiovascular events observed. Across participants, there was one grade 2 infusion-related reaction that resolved with acetaminophen. There were no clinically significant changes in ALT, AST, bilirubin, or platelets observed at any dose level or any timepoint evaluated after dosing. In addition, at the time of data release, two participants had also been dosed in a fourth dosing cohort (0.7 mg/kg), and the early laboratory and clinical safety profile was in line with the first three dosing cohorts (see our notes: Heart-2 First Look: Reads as a Clear Win With Clean VERVE-102 Safety, Competitive LDL-C Lowering, Dose-Dependent Effects and Heart-2 Additional Takeaways: VERVE-102 Exceeds LDL-C Goals, Corrects Prior-Gen Safety Concerns; Best-Case Outcome for Verve).

Exhibit 45
DECODER
PCSK9 Protein and LDL-C Reductions Following VERVE-102 Treatment in the Heart-2 Trial



Source: Verve Therapeutics company materials

VERVE-201 is an adenine base editing asset targeting the *ANGPTL3* gene for the treatment of more extreme cardiovascular diseases: HoFH, refractory hypercholesterolemia, and dyslipidemia. VERVE-201 works under a similar base editing principle as VERVE-101/102; an adenine base edit within an intron on the template strand disrupts the intron's splicing donor, which prevents proper splicing, leaving an intronic sequence within the mature mRNA that contains a Stop codon. The splicing disruption caused by VERVE-201 occurs between exons 6 and 7 of *ANGPTL3*. For delivery, VERVE-201 uses a GalNAc-conjugated LNP, which we surmise is the same LNP used for VERVE-102. VERVE-201 is in a Phase Ib study (NCT06451770) in patients with refractory hyperlipidemia, with an aim to meaningfully lower both LDL-C and triglyceride levels.

On June 17, 2025, Eli Lilly announced its intent to acquire Verve for $10.50 per share in cash (about $1.0 billion aggregate value, roughly 67% premium to prior-day close), plus one nontradable contingent value right (CVR) for an additional $3.00 per share (about $1.3 billion total potential deal value, roughly 115% premium to prior-day close), payable upon the first patient being dosed with

VERVE-102 for ASCVD in a U.S. Phase III study within 10 years of the closing of the transaction. We note that before the deal, Eli Lilly acquired opt-in rights to co-develop and co-commercialize VERVE-101, VERVE-102, and an undisclosed liver target in October 2023. The deal provided parent company Beam $200 million cash and $50 million in equity purchasing up front and an additional $350 million in potential development milestones. The acquisition of Verve by Eli Lilly was completed on July 25, 2025. For additional information on the business development activity here, see our note: Agrees to Be Acquired by Lilly for $10.50 Per Share and $3 CVR for Up to $1.3 Billion; Downgrading to Market Perform.

YolTech Therapeutics (Private)
Location: Shanghai, China

Technology
For a full description of YolTech's technology, including its HEPDONE editor discovery platform and LNP delivery platforms, see our profile of YolTech's clinical updates in the section on nuclease-based editing. The company's base applications are included as part of the HEPDONE platform, including a YolTech adenine base editor (hpABE5), which consists of a Cas9 nickase fused to a newly discovered deaminase derived from the bacterium *Hafnia paralvei.*

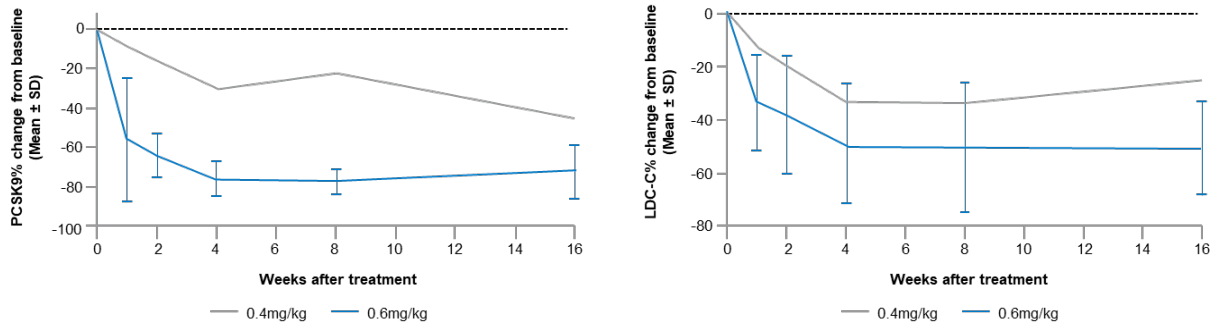
Key gene editing assets

YOLT-101 is an hpABE5-based editor targeting the *PCSK9* gene for the treatment of cardiovascular disease, including HeFH. The base editor payload of YOLT-101 is delivered via an LNP with conjugated GalNAc ligands for additional liver targeting in the context of underexpressed and/or mutated LDLR. YOLT-101 shares the same mechanistic rationale and sgRNA sequence as Verve's VERVE-101/102, albeit with different base editor machinery and a different LNP vehicle.

The company is conducting two Phase I clinical trials (NCT06461702 and NCT06458010), and data from NCT06458010 was recently published as non-peer-reviewed preprint (see Wan et al., 2025 MedRxiv). In the open-label study, 6 patients with HeFH were randomized into 3 dosing cohorts (0.2 mg/kg [n=1]; 0.4 mg/kg [n=2]; 0.6 mg/kg [n=3]) receiving a single dose of YOLT-101, and efficacy data from the 0.4 mg/kg and 0.6 mg/kg cohorts were provided. YOLT-101 elicited a dose-dependent reduction in PCKS9 levels, achieving maximal reductions of nearly 80% by four weeks after treatment in the high-dose cohort, which was largely maintained through the end of the reported follow-up period (16 weeks). This corresponded with a 50.4% reduction in LDL-C levels from baseline (exhibit 46). YOLT-101 elicited a dose-dependent increase in liver enzyme (AST and ALT) elevations, although no adverse events grade 3 or above were reported.

In June 2025, YolTech announced that the FDA had accepted the company's IND application for a trial of YOLT-101 in HeFH patients in the U.S.

Exhibit 46
DECODER
PCSK9 Protein and LDL-C Reductions Following YOLT-101 Treatment



Source: Wan et al., 2025 *medRxiv*

GenAssist Therapeutics (Private)
Location: Suzhou, China

Technology

GenAssist uses an AID-based cytosine deaminase as part of its targeted AID-mediated mutagenesis (TAM) platform. Based on preclinical work (Qiu et al., 2024 Cell Reports), the company's platform uses Cas9 nickases from multiple species (e.g., spCas9 and saCas9, among others) depending on the specific PAM sequence(s) surrounding the desired edit. The company's primary delivery focus is to muscle tissue, and the company uses an AAV9 vector for delivery of its gene editing payload into muscle.

Key gene editing assets

GEN6050X is a TAM-based dystrophin (*DMD* gene) exon 50 skipper for the treatment of DMD patients amenable to exon 50 skipping. GEN6050X received rare pediatric disease designation (RPDD) by the FDA in 2025. Similar to the designs of Verve/Lilly's VERVE-101/102 and VERVE-201, GEN6050X induces a base edit on the template strand of the *DMD* gene located at a splice donor for the purpose of inhibiting splicing at that location. However, in the case of perturbing *DMD* mRNA splicing at exon 50, perturbed splicing does not lead to a dystrophin knockout, but instead induces an alternative splicing event joining exons 49 and 52, creating a shorter but in-frame dystrophin mRNA that does not contain the deleterious mutation(s) encoded at the DNA level.

GEN6050X comprises two AAV9 vectors, which are co-administered. The first encodes the cytosine base editor (ss.AAV9.oTAM) and the second encodes copies of the base editors sgRNA along with a copy of the gamma-actin gene (*ACTG1*) to facilitate additional muscle reconstitution beyond boosting dystrophin functionality (ss.AAV9.hE50-sgRNA). We note that based on the design of this therapy, there may be more muscle cells that overexpress gamma-actin than those receiving both arms of the therapy (gamma-actin overexpression plus *DMD* editing).

GEN6050X is being dosed in DMD patients amenable to exon 50 skipping therapy as part of the Phase I GEN6050XIIT study (NCT06392724). As of November 10, 2025, three patients (ages 6.5-10 years old) had been dosed, with two such patients past 52 weeks of follow-up. All patients received a single dose of 5×1013 vg/kg. On the North Star Ambulatory Assessment (NSAA), the first patient obtained continuous gains in NSAA scores beginning at week 26, which was maintained through week 52. On the Performance of Upper Limb 2.0 (PUL2.0) scale, both patients reached the maximum PUL2.0 score by week 26 and sustained this level of performance throughout the study

period. On cardiac function, GEN6050X elicited an average 13.3% increase in left ventricular ejection fraction (LVEF) above baseline after 52 weeks. On pulmonary function, GEN6050X elicited an average 14.6% increase in predicted forced vital capacity (pFVC) above baseline after 52 weeks. None of the three enrolled patients have exhibited any clinically significant symptoms or laboratory abnormalities related to GEN6050X treatment (see press release here). Dystrophin expression data from muscle biopsy has not been made available, to our knowledge.

Reforgene Medicine (Private)
Location: Guangzhou, China

Technology
See our profile of Reforgene's clinical updates in the section on nuclease-based editing for a full description of its gene editing technology assets.

Key gene editing assets

RM-004 is a cytosine base editor for ex vivo administration to CD34+ HSCs and reinfusion for the treatment of alpha-thalassemia. Specifically, RM-004 corrects the mutation causing hemoglobin H–Constant Spring (HbH-CS) disease, a form of alpha-thalassemia that is a T-to-C switch in the gene encoding the hemoglobin subunit alpha 2 (*HBA2* c.427T>C). RM-004 is administered similarly to RM-001 treatment; the patient's HSCs are collected and treated with RM-004. Following myeloablative conditioning, RM-004-treated cells are reinfused into the patient.

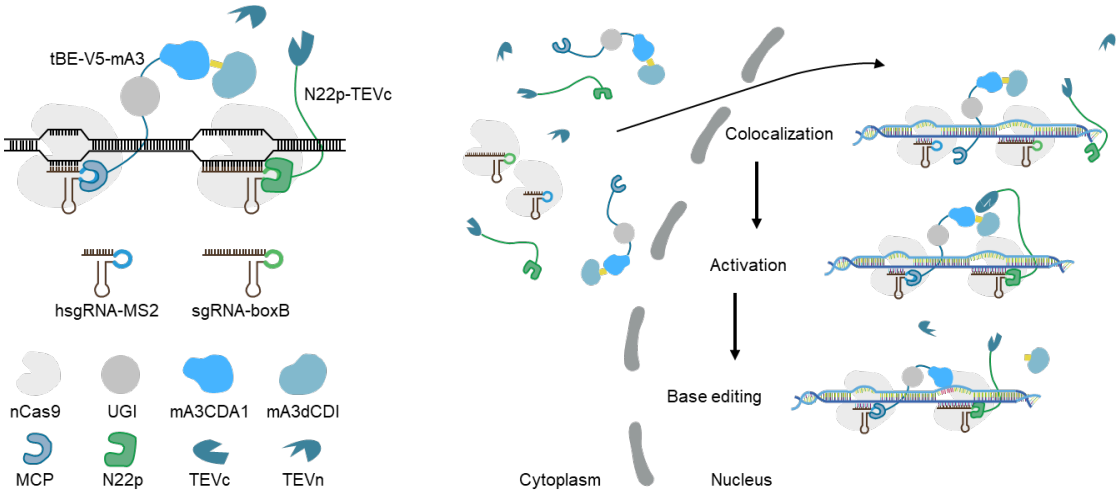
Reforgene is conducting a Phase I study of RM-004 in HbH-CS patients (NCT06107400). As of July 15, 2025, data from a single patient dosed with RM-004 (14-year-old female) included over 15 months of follow-up. After a single dose of RM-004, the patient achieved neutrophils engraftment and platelets engraftment on day 14 and day 20, respectively. The patient's last red blood cell (RBC) transfusion occurred on day 13 after RM-004 cell infusion, and the patient had remained transfusion free for 14.7 months. These clinical data were backed by biomarker data showing that the proportion of HbH-CS-positive RBCs decreased from 100% to approximately 10%, indicating that 90% of peripheral blood erythrocytes were derived from successfully edited HSPCs. RM-004-related adverse events were consistent with those observed from myeloablation and HSC transplantation, and no serious adverse events had occurred (see abstract here). We view these pharmacodynamic and safety data as encouraging, although we are tempering our conclusions given that only a single patient had been dosed. We note that the ongoing Phase I trial plans to enroll four additional patients, according to clinicaltrials.gov.

CorrectSequence Therapeutics (Private)
Location: Shanghai, China

Technology
CorrectSequence uses its transformer base editor (tBE) platform for base editing of target genes. The tBE technology is a cytosine base editor developed in 2021 as a means to limit off-target cytosine base editing by creating a base editing construct in which a single base editing event requires two separate Cas binding events (Wang, Xue, Zhang, Gao, & Qiu et al., 2021 Nature Cell Biology); see exhibit 47. Specifically, the tBE construct delivers four separate RNAs to cells: an mRNA encoding a Cas9 nickase; two separate sgRNAs, which bind at proximal sites, with one of the sgRNA protospacers binding directly at the target cytosine to create an adequate ssDNA substrate within the deaminase window (sgRNA 1) and the other binding at a nearby site (sgRNA 2); and an effector key RNA, which encodes the cytosine deaminase and auxiliary machinery to create an inducible cytosine deaminase system. Using P2A sites, the effector key construct encodes synthesis of three different proteins. It is important to note that the two sgRNAs not only are different in protospacer

sequence, but also contain different RNA sequence moieties that recruit different proteins encoded by the effector key; sgRNA 1 encodes a boxB sequence, which is a cognate for the N-peptide, and sgRNA2 encodes a MS2 sequence, which is a cognate for the MS2 coat protein (MCP).

Exhibit 47
DECODER
Scheme of CorrectSequence's Transformer Base Editor System



Source: Han, Gao, Zhu, & He et al., 2023 *Nature Protocols*

As mentioned, the effector key encodes three different proteins: 1) an MCP-tagged deactivated cytosine deaminase system (cytosine deaminase + uracil glycosylase inhibitor), which is constitutively deactivated via fusion with a deaminase inhibitor protein (the deaminase inhibitor is linked to the deaminase system via a peptide linker containing an amino acid sequence cognate for the TEV protease; 2) the C-terminal domain of TEV protease linked to an N-peptide; and 3) the N-terminal domain of TEV protease.

Upon cell entry, the MCP-tagged deactivated cytosine deaminase binds to sgRNA2 and is localized to its cognate site via the Cas9 nickase. Activation of the deactivated cytosine deaminase can occur only upon TEV protease cleavage of the domain between the deaminase and the deaminase inhibitor, which requires 1) the association of the N-terminal and C-terminal domains of TEV and 2) the localization of the now-functional TEV to the deaminase window via binding to sgRNA1/Cas9 nickase localization. In this way, both sgRNA binding events are necessary for a single cytosine deaminase event.

In preclinical experiments, a construct similar to tBE has also been applied to adenine base editing in mitochondria using an alternative effector key, which requires tandem, proximal binding of two TALE constructs to induce adenine deamination (Fan et al., 2025 Nature Biotechnology). We therefore infer that the company has the capacity to perform adenine deamination using nCas9 as well, although we are unaware of any such assets at the time of writing.

Key gene editing assets

CS-101 is a tBE for ex vivo administration of CD34+ HSCs for the treatment of TDT and SCD. Like Beam's risto-cel (the type of base editing enacted notwithstanding), CS-101 edits the BCL11A binding site within the promoter sequences of the gamma-globin genes to prevent BCL11A-mediated suppression of gamma-globin, which allows production of fetal hemoglobin (Han, Qiu, Sun, Fu, Wang, & Qian et al., 2023 Cell Stem Cell). CS-101 is delivered to a patient's collected HSCs via an LNP.

CorrectSequence is running multiple clinical trials of CS-101 in both TDT and SCD patients. As of August 26, 2025, CS-101 had been administered to nearly 20 patients, with the company reporting positive clinical outcomes in "several" TDT patients, including reported clinical cures (transfusion freedom for >6 months) in at least 4 TDT patients, with the longest period of transfusion freedom at 22 months. CS-101 has also been administered to at least one SCD patient (a 21-year-old female from Nigeria), who also achieved a clinical cure, which was backed by biomarker data showing an increase in HbF from 4.4% of total hemoglobin at baseline to 60% after three months. In addition, no VOCs or treatment-related adverse events have been reported (see the press release here).

CS-121 is an in vivo, LNP-delivered tBE targeting the *APOC3* gene for the treatment of familial chylomicronemia syndrome (FCS) and severe hypertriglyceridemia (sHTG). While the specific location of the intended edit has not been disclosed at the time of writing, the edit is intended to "mimic" naturally occurring loss-of-function genetic variants of *APOC3*, some of which have been demonstrated to reduce the risk of coronary heart disease in carriers (see the *NEJM* publication here).

On November 7, 2025, the company reported that the first patient had been dosed in a Phase I clinical trial of CS-121 (NCT07176923). This patient was a 63-year-old male with fasting triglyceride levels exceeding 1,100 mg/dL. Three days after receiving CS-121, the patient's fasting triglyceride levels dropped "significantly," with no reported adverse events.

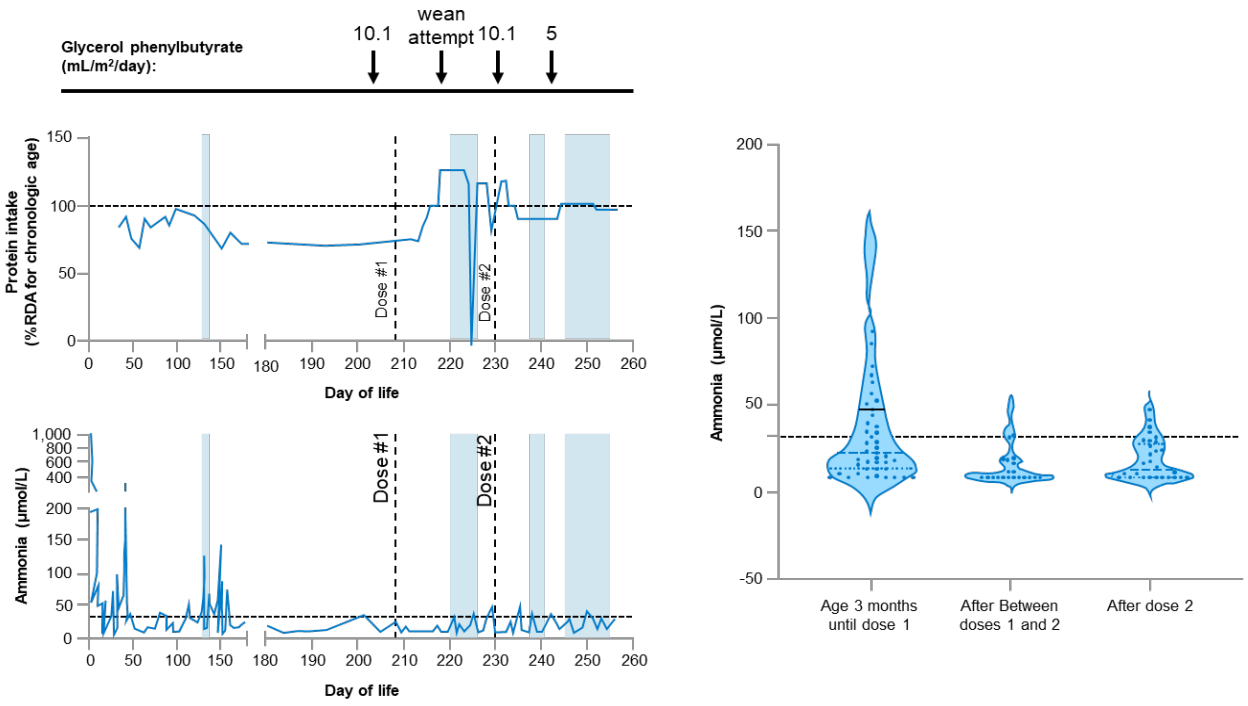
N of 1 Base Editing of the *CPS1* Gene in a Newborn Patient

In May 2025, Musunuru et al. published a case of using a customized LNP-delivered base editing asset for gene correction in a newborn patient (KJ Muldoon) with carbamoyl-phosphate synthetase 1 (CPS1) deficiency (Musunru et al., 2025 NEJM). This patient was placed on renal replacement therapy and scheduled for a liver transplant at five months of age. Through a screening process, researchers identified an adenine base editor with a preference for NGC protospacer-adjacent motifs (NGC-ABE8e-V106W), paired with a guide RNA targeting the Q335X adenine mutation in CPS1, as the most efficient base editing approach for this n=1 patient and termed the asset kayjayguran abengcemeran (k-abe). From conception to the first patient infusion, k-abe was developed over six months, including design, preclinical testing and potency evaluation, and eventual two-step infusion.

On days 205 and 209 post-birth, the patient underwent prophylactic immunosuppression with sirolimus and tacrolimus, respectively. On day 208 post-birth, the patient received an IV infusion of 0.1 mg/kg k-abe (total RNA dose). Because of several biochemical parameters and only a mild effect on dietary restriction, a higher 0.3 mg/kg IV infusion was performed 22 days after the receipt of the first 0.1 mg/kg infusion. As shown in exhibit 48, the median blood ammonia levels before the first k-abe dose (23 µmol/L [39 µg/dL]), between the first and second doses (9 µmol/L [15 µg/dL]), and after the second dose (13 µmol/L [22 µg/dL]) indicate a treatment-related reduction. CPS1-deficiency patients also have low urinary orotic acid levels, and treatment with k-abe resulted in levels at the high end or above the normal range (2.4 nmol/mol to 2.6 nmol/mol of creatinine). The patient's weight also increased from 7.14 kg at 207 days post-birth to 8.17 kg at 256 days under a normal protein intake diet and halving the glycerol phenylbutyrate background scavenger therapy dose (10.1 ml/m2/day to 5.0 ml/m2/day).

The patient had a viral infection after the first k-abe infusion (0.1 mg/kg) and two viral infections, with vomiting and diarrhea, after the second infusion (0.3 mg/kg IV). Off-target editing was assessed in preclinical assays using RNAseq. Cells exposed to super-saturating doses of k-abe showed low-level synonymous bystander editing at the endogenous wild-type CPS1 locus. Low levels of off-target base editing at an intronic site in *ATP7B* (encoding a copper transporter) were observed but not replicated in primary human hepatocyte cell preparations.

Exhibit 48
DECODER
Protein Intake and Ammonia Reductions Following Personalized Cps1 Base Editor in Newborn Patient



Source: Musunuru et al., 2025 *NEJM*

DNA Writing and Prime Editing

Genome editing using homology-based repair is employed by developers looking to make specific gene corrections or insertions. Such techniques are inefficient, however, and require the administration of double-stranded breaks. Moreover, while breakthroughs in ssDNA donor manufacturing may allow more seamless and efficient homology-based DNA insertion in the future, delivering DNA-based homologous donors concomitantly with nucleases is cumbersome and potentiates additional safety risks beyond off-target nuclease activity. While additional tools for transgene insertions, such as molecular integrases, are also at the disposal of developers, such tools are ideally suited for large-scale gene insertions and may not be ideal for dexterous gene edits. Therefore, site-specific writing of new DNA sequences (DNA polymerization) into the genome from an RNA-based template across multiple scales of insertion represents an additional, potentially powerful template-based DNA editing technique amenable to therapeutic applications.

DNA Writing 101

Flexibility in location-specific gene editing outcomes in both type (insertions, deletions, or base changes [including purine ↔ pyrimidine base switches not possible with base editing]) and scale (minor indels, medium-size indels, large-scale indels) can best be holistically captured through site-specific, templated DNA polymerization (DNA writing). While other genome editing applications allow either the cell to polymerize semi-random changes into the genome or developers to externally manufacture the physical piece of DNA being inserted at a specific site, gene writing allows the cell to polymerize the DNA from a customized RNA template provided to the cell. This not only reduces the potential for random genomic integrations inherent in delivery of DNA-based templates, but also potentiates a range of enactable sequence changes.

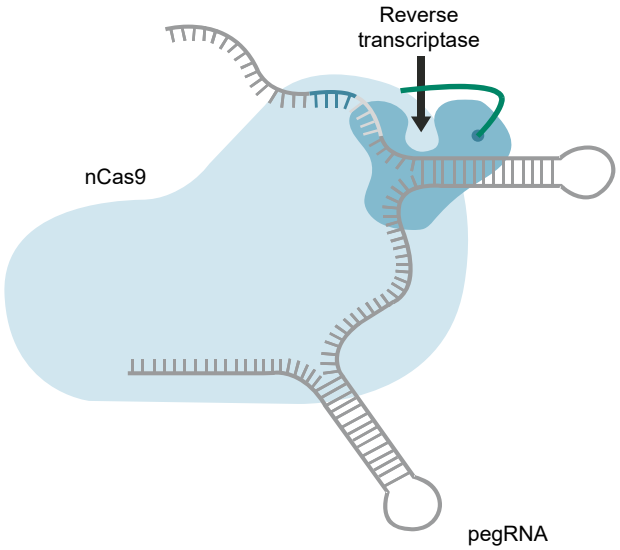
The central dogma from DNA to RNA to protein uses DNA-templated nucleic acid polymerases: DNA-templated DNA polymerases create a complementary piece of DNA from a DNA template, which are used to replicate the cellular genome during cell division, and DNA-templated RNA polymerases create a complementary piece of RNA from a DNA template (this process is also called transcription). Conversely, RNA-templated DNA polymerases exist and are the founding basis for DNA writing technologies. Such polymerases are termed "reverse transcriptases" as they perform the opposite function of traditional transcription (RNA to DNA).

Reverse transcriptases (RTs) create complementary DNA (cDNA) from an RNA template. RTs were originally discovered in retroviruses (e.g., HIV), viruses with RNA genomes where an RT encoded within the viral genome creates the viral genome's cDNA, which allows a DNA copy of the viral genome to be integrated into the genome of the infected mammalian cell. RT activity was also found in retrotransposons, which synthesize their DNA sequence into a distal genomic location via an RNA-based template from a transcribed copy. Both CRISPR-guided and retrotransposon-guided reverse transcription are being applied to gene editing therapeutics. By using the cDNA polymerization activity of these enzymes in combination with an RNA template, novel DNA sequences may be written into the genome for therapeutic applications.

Prime Editing 101

Prime editing is a CRISPR-guided and -templated reverse transcription approach capable of introducing site-specific designed indels. Prime editing was originally developed in the lab of Dr. David Liu in 2019 (Anzalone et al., 2019 Nature). Like other gene editing applications discussed in this report, prime editing derives from a Cas fusion protein, where an RT domain is appended to the end (C-terminus) of the Cas protein. In addition, a modified gRNA is used (prime editing gRNA [pegRNA]); the pegRNA not only binds to and guides the Cas to the proper genomic location, but also encodes the specific indel(s) to be incorporated as the gene edit. The 3' end of the pegRNA is the RNA template used by the reverse transcriptase to write new DNA into the genome (exhibit 49).

Exhibit 49
DECODER
Prime Editing System



Source: Adapted from Gomez-Escribano et al., 2025 *eBioMedicine*

Like other genome editing applications, prime editing initiates upon binding of the pegRNA's guide portion (protospacer) to its cognate sequence in the genome. Prime editing uses an H840A nCas9, which nicks the genome on the nontarget strand. This frees the nontarget strand to interact with the template portion of the pegRNA; inherent in the initiation of polymerase activity by reverse transcriptases is the need for priming: the 3' end of the RNA template must be hybridized to a complementary piece of DNA onto which the remaining cDNA will be synthesized. Therefore, the 3' of the pegRNA, termed the primer binding site (PBS), is designed to be complementary to the 5' end of the spacer sequence on the nontarget strand. While the PBS has typically been designed up to 15 nucleotides in length to ensure proper RT initiation, this can be problematic; the PBS and 5' end of the protospacer pegRNA sequence will be complementary to each other, and if that stretch of complementarity is long enough, the pegRNA will form an end-to-end duplex with itself, which may prevent Cas9 association or proper guide functionality by the pegRNA. Subsequent experimentation suggests that a 7-nucleotide PBS may be optimal to minimize this complementarity while maintaining primer function (Ponnienselvan et al., 2023 Nucleic Acids Research).

In addition, given the smaller influence of gRNA-DNA pairs distal to the PAM site, designed mismatches at positions 18-20 (5' end) of the protospacer can alleviate such secondary structures with limited effect on its guide function and have a noted benefit on prime editing efficiency and error rates (Fei et al., 2025 Nature Communications). Upon priming via pegRNA-nontarget DNA strand interaction, the RT domain of the prime editor polymerizes DNA onto the nontarget DNA strand using the pegRNA's reverse transcription template (RTT). We note that computational-based methods for optimal pegRNA design suggest that the median lengths of PBS, RTT, and edit-to-nick distance should approximate 9 nt, 12 nt, and 5 nt, respectively (Liu et al., 2023 Nature Machine Intelligence). Further rules of thumb for pegRNA design can be found in an article here. We acknowledge that specific permutations to pegRNA design for a given genomic site and/or application likely require tailoring on such parameters through extensive testing.

RT Domains Used in Prime Editors

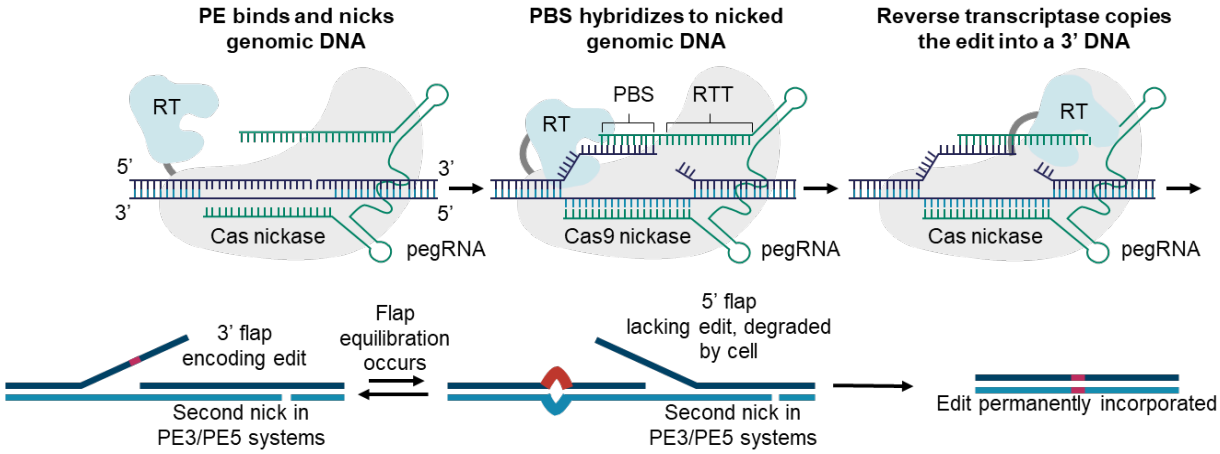
It is important to note that characteristics of a prime editor's RT may influence successful gene writing efficiency. Such characteristics include the RT's error rate (inserting the wrong DNA base corresponding to a given RNA base within the pegRNA's RTT template). Current prime editing methods primarily use RTs derived from oncoretroviruses, most commonly Moloney murine leukemia virus (M-MLV). Oncoretroviruses are 10-15 times more faithful than the prototypic HIV-1 RTs, which are evolutionarily programmed to have boosted error rates compared to other polymerases in order to introduce natural genetic diversity into iterations of the replicating virus to find advantageous mutations (Barrioluengo et al., 2012 FEBS; Abram et al., 2014 Journal of Virology); the estimated error rate of M-MLV is 1 in about every 86,000 bases polymerized (Barrioluengo et al., 2011 Biochemical Journal). Additional complexity relating to RT error rates arises from the reverse transcription of RNA templates with chemically modified RNAs, which can increase RT error rates. Given the known advantages of certain RNA chemical modifications for therapeutic applications considering such modifications can improve RNA half-life or other pharmacodynamic factors in target cells, engineering of RTs that exude improved fidelity on chemically modified RNA templates may be advantageous. To this end, we highlight CRISPR Therapeutics' synthetic nucleotide template polymerase (SyNTase) editing platform, which features an RT capable of reading chemically modified RNA templates. In addition to fidelity, characteristics of RTs, including RT protein stability and processivity (length of polymerase activity before failure), influence prime editing efficiency.

Attempts to reduce the size of the RT domain identified smaller M-MLV variants that performed just as well as the full length M-MLV, suggesting that there may be multiple routes of RT domain optimization (Gao et al., 2022 Molecular Therapy). We note, however, that such assays were performed testing relatively small base insertions (1-3 bp in length), and full-length M-MLV variants may still be required for additional fidelity and/or processivity in the context of larger genome edits. We anticipate that development of RT variants with superior design and activity will continue to be investigated from multiple angles; in addition to prime editing, other biotechnological assays, including standard RNA sequencing practices, rely on stable, faithful, processive RTs for proper output. Therefore, any future development on RT functionality from those arenas could be applied to prime editors to improve the RT component.

In addition to rational-design-based evolution of the M-MLV RT, metagenomic analyses are also being employed to discover additional RTs from other organisms that may be used in prime editing contexts. As with discovery of novel Cas nucleases for gene editing applications, biotechnology companies such as Arbor, Mammoth, and Metagenomi are applying their metagenomic discovery frameworks to identify and improve RTs for potential prime-editing-based therapeutics.

Cellular Response to Prime Editing—Biasing Toward a Desired Change in Sequence

Exhibit 50
DECODER
Prime Editing Mechanism and Edit Incorporation



Source: Adapted from Doman & Sousa et al., 2022 *Nature Protocols*

DNA flap excision

DNA insertions created with prime editing may require instances of DNA flap excision: recall that the nCas of the prime editor will nick the nontarget strand three bases from the PAM site, which leaves two single-stranded DNA pieces complementary to the 20-nucleotide protospacer—17 nucleotides on the 5' piece and 3 nucleotides on the 3' piece. The RT domain will write DNA onto the 17-nucleotide 5' piece, and in instances where prime editing is used to create a DNA insertion, any insertions to that strand will leave more than 20 nucleotides across both single-stranded DNA pieces to hybridize with the 20 nucleotides on the protospacer-binding portion of the target strand. This will require one piece to displace the other, leaving a DNA flap that may be excised by DNA repair machinery. While few studies have fully investigated this process, we reason that the 5' piece, which will contain the templated edit(s), will most often displace the 3' piece for hybridization because of bias for the piece with the larger "toehold" to win out due to its higher local association rate (for a more detailed review of DNA strand displacement, see the article here). However, in instances in which the edited DNA 5' piece is displaced, noncomplementarity of the edited portion with the target strand may cause the displaced DNA flap of the 5' piece to include the edit, which would then result in the edit being excised by DNA repair machinery. We note that in this context, increasing expression of the enzyme Fen1, which facilitates excision of the 3' piece, increases prime editing insertion efficiencies, while increased expression of the enzymes TREX1 and TREX2, which facilitate the excision of the 5' piece, reduces prime editing insertion efficiencies (Koeppel & Weller et al., 2023 Nature Biotechnology).

Base excision and mismatch repair

As with other DNA editing applications, cellular mechanisms for DNA base excision repair and DNA mismatch repair may cause the cell to remove indels written via prime editing. For DNA base editing applications, bias toward use of the edited DNA strand as the template for DNA mismatch repair is enforced via nicking of the unedited strand by a D10A nCas nickase. However, prime editing requires nicking of the edited DNA strand with a H840A nCas9 nickase, which should bias DNA mismatch repair against incorporation of written indels into the genome. We note that for certain iterations on prime editing technology, a second gRNA is added for the purpose of nicking the unedited DNA strand, resulting in staggered nicks, which may relieve some of bias against indel incorporation. Additional expression of a dominant-negative variant of MLH1 that reduces

mismatch repair can also improve prime editing efficiency (Chen & Hussmann et al., 2021 Cell). We note that anti-MLH1 siRNAs have also been used. We discuss those and further iterations of prime editing below.

Versions of Prime Editors

- ***PE1*** (Anzalone et al., 2019 Nature): The first-generation prime editing construct contains a Cas9 nickase (H840A) with the wild-type version of the M-MLV RT domain.
- ***PE2*** (Anzalone et al., 2019 Nature): A Cas9 nickase (H840A) with an engineered M-MLV RT domain to improve DNA writing efficiency.
- ***PE3*** (Anzalone et al., 2019 Nature): The prime editor used in PE2 and adds a gRNA targeted to the edited DNA strand. This triggers the expressed prime editing nickase to create an additional nick on the nonedited DNA strand (40-100 bp downstream), which removes bias toward the nonedited strand as the template for DNA mismatch repair.
- ***PE4*** (Chen & Hussmann et al., 2021 Cell): The prime editor used in PE2 and adds expression of dominant-negative variant of MLH1, which inhibits cellular mismatch repair to increase the likelihood the DNA edit will be preserved.
- ***PE5*** (Chen & Hussmann et al., 2021 Cell): The prime editor plus gRNA construct used in PE3 and adds expression of dominant-negative variant of MLH1, which inhibits cellular mismatch repair to increase the likelihood the DNA edit will be preserved.
- ***PEmax*** (Chen & Hussmann et al., 2021 Cell): A Cas9 nickase (H840A) with additional mutations previously shown to enhance nuclease activity with a codon-optimized version of the engineered M-MLV RT from PE2, which likely improves PE expression. The Cas and RT domain are linked with a linker of optimized length (34 amino acids). Additional NLS sequences are also added to improve nuclear localization.
- ***PE6*** (Doman & Pandey et al., 2023 Cell): A prime editor with an engineered Cas nickase (H840A) and RT domain optimized for prime editing via phage assisted continuous evolution (PACE).
- ***PE7*** (Yan et al., 2024 Nature): The prime editor used for PEmax with the N-terminal RNA-binding domain of the La protein appended to the C-terminus of the prime editor. While the exact mechanism for how La enhances prime editing efficiency has not been elucidated, its potential mechanism is thought to be mediated through direct binding with the pegRNA.

Passing the Character Limit—Getting the RT to Stop

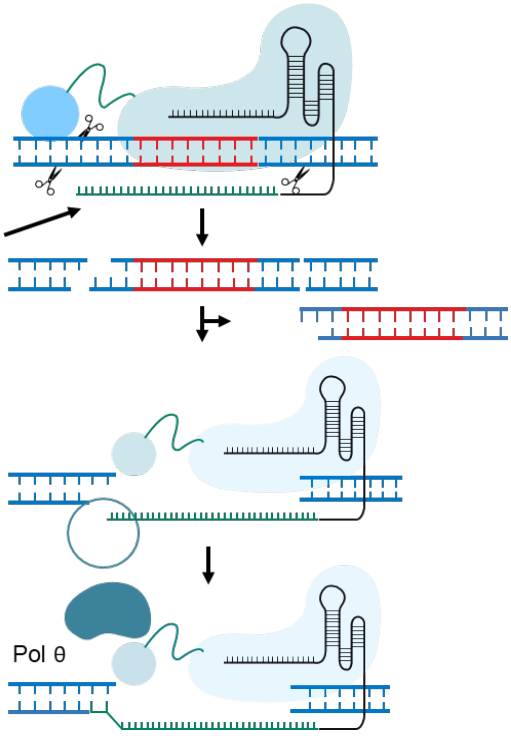
Prime editing guide RNAs must account for not only RT priming and initiation of reverse transcription, but also the termination of reverse transcription at the RTT. After the PBS and RTT at the pegRNA's 3' end, the pegRNA contains a constant, structured Cas-interacting sequence, which is not complementary to the genomic target. If the RT transcribes beyond the RTT and into Cas-interacting sequence, additional unwanted DNA nucleotides will be added, which could throw off the gene's reading frame or add amino acid(s) to the protein product that could alter its function; "run-on" RT activity into the Cas-interacting sequence has been observed in multiple studies, with typically 1-3 DNA nucleotides from the Cas-interacting sequence added in addition to the desired prime edit. Notably, structural studies suggest that steric hinderance from the Cas, not the 3-dimensional structured RNA stem loops of the pegRNA's Cas-interacting sequence, is the cause of RT termination (Shuto & Nakagawa et al., 2024 Nature), though we note that a recent study demonstrates reduced Cas-interacting sequence transcription via pegRNA base modifications (Antoniou et al., 2025 Nature Communications).

Rates of desired prime edits in the RTT typically outpace rates of undesired insertions from the Cas-interacting domain. This suggests that genome repair machinery (DNA flap excision and/or DNA mismatch repair pathways mentioned above) can remove these mismatched bases from the Cas-interacting sequence, which presents a counterargument to eliminating the activation of such repair pathways during prime editing altogether. Ultimately, we view RT run-on into the Cas-interacting domain as a meaningful risk to faithful prime editing for therapeutic applications, and optimization of pegRNA design to eliminate or account for such run-on, or engineering of Cas and/or RT mutants to enforce steric hinderance-based termination of reverse transcription at a desired base, may be required.

Endogenous Polymerase-Based Templated Reverse Transcription

While not strictly prime editing, CRISPR gRNA-templated DNA writing can also be accomplished via utilization of endogenous DNA polymerases in mammalian cells, which may exert reverse transcription activity (capable of using an RNA template for DNA polymerization). Such polymerases are predominantly used during double-stranded DNA repair to fill in missing DNA nucleotides and can begin polymerizing via priming from sticky DNA ends derived from staggered breaks or the creation of sticky ends during the DNA damage response. We highlight DNA polymerase theta, which is known to act in this process and is capable of RT activity (for a review of DNA polymerase theta, see the article here). Specific Biologics is using the RT activity of DNA polymerase theta by enacting a prime-editing-adjacent setup: a Cas9-restriction enzyme fusion protein uses a pegRNA-like molecule (repair template guide RNA [rep-gRNA]) to localize nuclease activity to a specific site. Upon creation of a staggered DNA break from the restriction enzyme and a blunt DNA break from the Cas9, the DNA damage repair response will use cellular DNA polymerase theta to mend the excision using the template sequence from the rep-gRNA (exhibit 51). This setup requires the formation of double-stranded breaks, which differs from the single-stranded nick required for prime editing, but we view this as an alternative, prime-editing-like DNA writing technology that may assuage potential delivery and/or fidelity limitations of exogenous RT activity from prime editors by using endogenous polymerase machinery for editing.

Exhibit 51
DECODER
Specific Biologics' Polθ-Reliant Dualase Prime Editing-Like System



Source: Adapted from Specific Biologics company materials

Retrotransposon-Based Target-Primed Reverse Transcription 101

In addition to standard transposons, which are mobile DNA sequences that integrate throughout the genome via physical "cutting and pasting" of the transposon's sequence out of one genomic locale and into another, retrotransposons integrate into new genetic sites via reverse transcription, following more of a "copy and paste" method; in this process, the DNA from a retrotransposon element is transcribed by the cell into RNA, which is then reverse transcribed as DNA into the genome at a distal site via a RT-containing retrotransposase encoded by the retrotransposon element. For non-long-terminal repeat retrotransposons (non-LTR retrotransposons), reverse transcription of the transposon element into the genome typically requires sequence recognition by the retrotransposase as well as some level of homology between the 3' end of the transposon RNA and the genome for proper reverse transcription priming (typically about 4 nt); therefore, the process of retrotransposon-based reverse transcription is often referred to as target-primed reverse transcription (TPRT).

For TPRT-mediated therapeutic applications, two copackaged RNAs are delivered: one encoding the retrotransposase and the other encoding the RNA template for DNA writing. Retrotransposases possess multiple protein domains that facilitate TPRT:

1. ***An RNA-binding domain*** that facilitates complex formation between the retrotransposase and template RNA.

2. ***A DNA binding domain*** that allows the retrotransposase to dock onto DNA and help facilitate the interaction between the genome and the template RNA. In naturally occurring retrotransposons, these DNA binding domains are often zinc finger domains. The contribution of the zinc finger domains to the sequence specificity of the retrotransposase is not fully elucidated and may vary among different retrotransposons, though we reason that developers of retrotransposases for TPRT could design a zinc finger domain to endow a sequence-specific guiding mechanism.

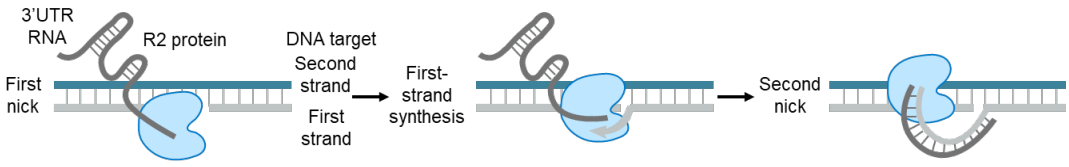
3. ***An endonuclease domain*** that nicks the DNA at the retrotransposase's cognate site to allow RNA template-DNA interaction and RT priming. We note that the RT priming occurs via interaction of DNA with the template RNA's 3' UTR, and we reason that the 3' UTR sequence also confers a layer of sequence specificity for the targeted insertion site.

4. ***An RT domain*** that transcribes the template RNA into cDNA.

There are multiple classes of site-specific non-LTR retrotransposons, and a TPRT-capable therapeutic retrotransposase may be a combination of domains from multiple classes assembled together (for more information on site-specific non-LTR retrotransposons, see chapter 50 of *Mobile DNA III* here); Tessera Therapeutics has screened over 100,000 combinations of such domains in cells for optimal gene writing activity. We note that the R2 class of retrotransposons has been engineered to write DNA into human cells at the retrotransposase's cognate locus (the 28S ribosomal RNA gene) by the lab of Kathleen Collins, a method dubbed precise RNA-mediated insertion of transgenes, or PRINT (Zhang & Van Treeck et al., 2025 Nature Biotechnology), which is being developed by Addition Therapeutics. However, the design of retrotransposases used by companies employing TRPT for therapeutic aims, such as Tessera Therapeutics, Averna Therapeutics, Addition Therapeutics, and Typewriter Therapeutics, has not been disclosed at the time of writing. We note that limited comparisons of error rates of RTs derived from retroviruses versus retrotransposons showed comparable fidelity (Jamburuthugoda & Eickbush, 2011 Journal of Molecular Biology), suggesting that further mutations in the RT domain of retrotransposases may be used to achieve desired fidelity.

Upon cDNA transcription, the end (3' end) of the cDNA anneals to the unedited DNA strand via homology, and presumably DNA repair machinery is used to excise the 5' flap from the original genomic strand. To fill in the complementary DNA on the unedited DNA strand, a second nick by the endonuclease domain on the unedited strand must take place, and cellular DNA polymerase is then used to fill in the missing complementary strand (exhibit 52).

Exhibit 52
DECODER
Target-Primed Reverse Transcription (TPRT) Mechanism



Source: Adapted from Thawani & Rodriguez-Vargas et al., 2025 *Science Advances*

Integrating the 3' end of the cDNA into the genome is the largest bottleneck for TPRT efficiency, in our view. Multiple potential errors may occur during integration of the 3' end of the cDNA into the genome and are more common than errors related to off-target insertion of the cDNA's 5' end upon reverse transcription initiation. Most commonly, the cDNA is directly ligated (joined) to the other piece of DNA derived from the DNA nick, which will retain extra genomic bases that should

have been excised on the 5' flap. This spurious, non-homology-based end joining can occur with both truncated and full-length cDNA products. Alternatively, though rare, additional spurious RT priming events can occur through hybridization of other genetic material with the transcribed cDNA strand, which essentially adds another template used for reverse transcription onto the cDNA product. Such genetic material could derive from either DNA (e.g., the template folded back on itself) or a strand of RNA located in the nucleus. This unintended template priming would cause the incorporation of unwanted sequences into the genome.

DNA Nucleotide Access—Improving Processivity and Fidelity

In the context of polymerases used for gene editing, processivity can be defined as the ability of the polymerase to complete full synthesis of its product polymer. In the case of RTs, processivity refers to creating a complete cDNA copy from its RNA template. To enact efficient DNA writing activity, RTs require access to DNA nucleotides to create cDNA, and it has recently been demonstrated in the context of prime editing that increased access to DNA nucleotides improves prime editing efficiency through M-MLV RT processivity (Liu & Ponnienselvan et al., 2025 Nature Biotechnology). Cellular concentrations of DNA nucleotides are controlled by cells and are chiefly regulated as part of the cell cycle; cells in synthesis phase (S-phase), which are copying their genome in preparation for cell division, must upregulate intracellular concentrations of DNA nucleotides to enact this process. Original findings from investigations of prime editing in vivo revealed that cell replication status (dividing versus nondividing cells) contributes to prime editing efficiency, with dividing cells enacting higher levels of prime editing than nondividing cells. Moreover, prime editing efficiencies are correlated with cell cycle speed, suggesting that reaching a specific cell cycle stage while the prime editor machinery is expressed may be important to achieving higher DNA writing efficiencies (Wang et al., 2021 Nucleic Acids Research). Such data in tandem suggest that prime editing may be most efficient in S-phase when DNA nucleotide concentrations are at their peak, which maximizes M-MLV RT processivity.

RT domains of retrotransposons are significantly more processive than the RT from M-MLV. Therefore, considerations surrounding nucleotide abundance from a *processivity* perspective are less relevant for TPRT than they are for prime editing, which allows TPRT to be applied to nondividing cells. However, access to DNA nucleotides also increases RT *fidelity,* which will influence the activity of all RTs across gene writing applications. As a result, we see maximizing nucleotide abundance in targeted cells as paramount for executing faithful gene writing.

Multiple lines of inquiry may allow faithful gene writing in nondividing cell types in a therapeutic setting. First, mutations in RTs at the enzyme's active site that heighten its affinity for DNA nucleotides have been shown to improve processivity. In other words, though cellular concentrations of DNA nucleotides are low in nondividing cells, engineered RTs with specific mutations may become more processive in this context by maximizing their ability to capture and incorporate DNA nucleotides while reverse transcribing cDNA. Alternatively, addition of exogenous factors to boost cellular concentrations of DNA nucleotides may aid in prime editing efficiencies. For example, the VPX protein, encoded by HIV-2 virus, blocks activity of the human cellular protein SAMHD1, which cleaves DNA nucleotides and helps regulate low nucleotide levels during the non-DNA-replicative phases of the cell cycle. In this way, the HIV-2 protein induces DNA nucleotide accumulation to maximize reverse-transcription-based viral replication. Recent work demonstrated that adding VPX protein in tandem with a prime editor boosts prime editing efficiency in vivo through increases in DNA nucleotides (Liu & Ponnienselvan et al., 2025 Nature Biotechnology).

Applications of Gene Writing

DNA writing's greatest advantage over other gene editing tools is its seamless flexibility to make different types of genomic edits; multiple types of edits (DNA insertions, deletions, or base switches) may be encoded by the DNA writer's template RNA and reverse transcribed into the genome. As with other genome editors, modifications may be made to either the coding or template strand,

with the DNA repair machinery responsible for synergizing both DNA strands in line with the edit introduced. However, unlike DNA base editing applications, where the targeted nucleotide must be within the protospacer binding site due to restrictions on deaminase activity for ssDNA, RT processivity allows targeted edits to be made downstream of the protospacer, adding flexibility for the genome sequence selection for the guide.

Prime editing boasts well-documented site specificity, although the scale of its editing potential may be limited. For DNA insertions, classic prime editing methods have been limited to insertions of about 44 base pairs in length. It has recently been postulated, however, that those limitations stem not from the biophysics of prime editing itself, but from limits on the length of pegRNAs; typical prime editing applications in cells use a U6 promoter, which recruits RNA polymerase III to transcribe the pegRNA. However, U6 promoters are known to be optimal for smaller transcripts, and therefore pegRNAs synthesized via U6-driven transcription may run into issues of length. Recent workarounds with Pol II-driven promoters for pegRNA synthesis, which do not have transcriptional length restrictions, have demonstrated DNA insertions of 700-800 base pairs (He, Xue, & Zhou et al., 2025 Trends in Biotechnology), and may be more in line with biotechnology companies with robust in vitro RNA synthesis platforms in place. A method for synthesis of long pegRNAs, created via ligation of two distinct RNAs, was recently developed (Lei et al., 2024 Nature Biotechnology). For deletions, prime editing constructs using a single pegRNA have achieved deletions of about 80 base pairs. However, we note that dual pegRNA constructs may achieve much larger genomic deletions (roughly 10,000 base pairs).

For TPRT-based gene writing, similar types of edits can be achieved based on the design of the template RNA. We note that the largest differentiator compared with prime editing is the capability of longer DNA insertions. While the maximum-length DNA insertions attainable by specific gene writers may vary, the R2 retrotransposon-based method (PRINT) achieved DNA insertions up to 4,000 bases. This assay was performed in highly replicative cell lines, which may have positively influenced the retrotransposase's processivity due to abundant availability of DNA nucleotides.

Indel-based editing outcomes

Because of the flexibility of DNA writing to create small-scale DNA insertions, deletions, and base changes, a number of different gene editing outcomes are available to developers. These include gene knockout, gene correction/mutation, and RNA splicing modulation applications, as previously detailed in this report. We highlight genetic knockouts, as gene writing represents a combination of flexibility and precision; unlike nuclease-based genetic knockouts, gene writing can dictate a specific Stop codon or frame-shift mutation to ablate target gene production and is not reliant on random DNA repair processes for mutagenesis. On gene correction, we note that DNA writing provides more efficient capabilities for small-scale DNA insertions and deletions than do other gene editing methods, and therefore are particularly useful for fixing gene mutations in which a gene's sequence has deleted or inserted base(s), which may throw off the gene's reading frame or preclude an important amino acid for protein functionality. Moreover, gene editing may correct or incorporate any combination of base switches, unlike base editing applications, which are limited to A-to-G or C-to-T switches for adenine and cytosine base editing applications, respectively.

DNA writing events at a target gene's locus may be applied to append elements that regulate the protein product's production via novel UTR sequences or additional amino acids that control various functions such as the protein's half-life (degron), subcellular localization (zip code), secretion (signal peptide), or even trigger cell death upon that gene's expression (suicide peptide). Outside a gene's protein coding sequence, DNA writing could be used to adjust a gene's regulatory elements to either attract or repel a transcription factor to modulate transcription levels. As stated earlier, developers would need to be aware of the gene's underlying transcriptional circuitry for such an edit to be effective.

Large DNA insertions

TPRT-based gene writing allows large DNA insertions at a targeted genomic locus. Such applications allow developers to insert a functional copy of a mutated gene to supplement protein activity or to insert a novel gene that endows the cell with a new function/phenotype. Recall that developers may elect to write DNA with a gene's intron or exon, including the use of safe-harbor loci, to facilitate transgene expression.

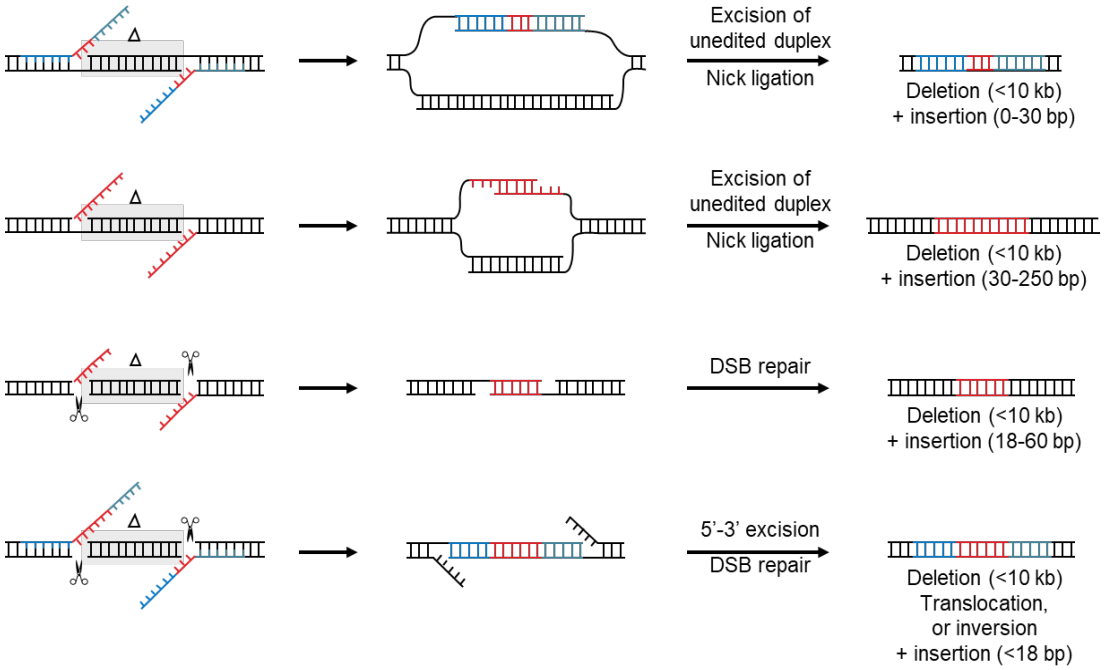
While we are unaware of any applications in a therapeutic setting, we also see potential for writing specific gene regulatory elements into the genome to control the expression of genes. This could manifest as the insertion of known transcriptional promoters or enhancers, which may modulate a gene's transcription. Alternatively, known transcriptional regulatory elements could be introduced that link the expression of a target gene to the expression of another gene. As an example, upon inserting a transcriptional promoter and/or enhancer endogenous for gene A into the gene regulatory locus of gene B, expression of genes A and B may then occur in similar contexts and/or at similar amounts. Alternatively, sequences governing DNA methylation (e.g., CpG binding sites, which are known for being highly methylated) or sequences enforcing specific 3-dimensional chromatin structures (e.g., CTCF binding sites, which serve to insulate specific DNA regions from one another via DNA looping) could be written into the genome site-specifically to influence gene expression.

While limits to the size of insertions mediated by prime editing itself prevent writing of entire genes (average cDNA length of eukaryotic gene is 1,000-2,000 base pairs), large DNA insertions into the genome may be facilitated by prime editing in a two-step methodology using the sequence specificity of integrase-mediated gene insertion. In this process, a prime editor writes an integrase's attB insertion site into the genome, which allows an integrase to insert a transgene at that written site. This technology is being advanced by Prime Medicine with its proprietary PASSIGE platform.

Large genomic deletions

In addition to single-site template RNA-mediated DNA writing to facilitate small-scale indels, two DNA writing events can be engineered in tandem to achieve additional genetic manipulations. We view the primary utility of this strategy as a method to achieve targeted large-scale genomic deletions. For this application, writers are designed at the border of the desired genetic sequence aiming to be deleted, with one template RNA targeted to each DNA strand. DNA writing at each site is then used to write a sequence that is complementary to the genetic sequence at the other site, such that the two free strands may hybridize from complementarity. As with standard single-site DNA writing, efficiency relies on probabilities of strand displacement and excision of the unedited DNA strands. In addition, small DNA insertions may be included in each edit to replace the deletion, which can aid in the maintenance of sequence and/or reading frame in instances where docking sites do not perfectly border the desired deletion (exhibit 53). We note that such applications have reduced efficiency because of the need for two separate DNA writing events to take place, and DNA writing activity from one of the editors but not the other may leave unintended insertions in the genome.

Exhibit 53
DECODER
Large-Scale Genomic Deletions Accomplishable With Prime Editing



Source: Adapted from Chen & Liu, 2023 *Nature Reviews Genetics*

Mitigation of Off-Target DNA Writing Activity

DNA nickase activity, which is used in both prime editing and TPRT-based gene writing, significantly reduces the potential for unintended cellular toxicities, although we note that additional nicks on the target DNA strand in certain prime editing versions (PE3, PE5) may increase potential genomic toxicities through creation of staggered DNA breaks. We view DNA writing methods as having a two-step process for DNA insertion, which reduces the risk for DNA writing at off-target sites: 1) a guide-based navigation of the genome to the proper DNA sequence and 2) proper RT priming with the RNA template, which should match the genomic insertion site in a sequence-specific manner. Nonetheless, strategies to mitigate off-target DNA writing activity are paramount to success for these applications.

For prime editing, similar off-target mitigation strategies related to the CRISPR-based guides, including guide design and Cas engineering discussed earlier in this report, make up the basis for mitigation of off-target activity. While we are unaware of any approaches requiring separate Cas/prime editor binding events to enact a single prime edit, we note that split prime editors have been designed for ease of AAV-based delivery (She et al., 2023 Signal Transduction and Targeted Therapy). This also includes constructs with an untethered RT and separated gRNA and RT template (Liu et al., 2022 Nature Biotechnology), which may be particularly amenable to methods to limit prime editor activity to specific contexts.

For retrotransposon-based methods, variability in the sequence homing strategies of specific retrotransposons makes engineering for target specificity more case-by-case. We also view limiting retrotransposon half-life as a potential way to limit unwanted DNA writing activity and

note that newly discovered small-molecule inhibitors of retrotransposon endonuclease activity may pave the way for small-molecule-mediated timing of TPRT activity (D'Ordine et al., 2024 Nature Communications).

Ultimately, while off-targeting activity of DNA writing should be monitored, we view unintended pitfalls in RT activity mentioned above at desired DNA writing sites as the chief concern for developers. For prime editing, these include failure of RT processivity in nondividing cells, which could leave "unfinished" DNA products in the genome that could be harmful to cells. In addition, run-on RT transcription during prime editing into the pegRNA's Cas-interacting sequence may potentially thwart the intended DNA sequence and needs to be mitigated. For TPRT, potential variability regarding integration of the cDNA's 3' end into the genome may leave unpredictable indels that could throw off the frame or function of inserted gene(s), though RNA base modifications have been demonstrated to improve faithful cDNA integration (Palm et al., 2024 RNA). Lastly, pertaining to all gene-writing-based methods, we see intrinsic RT fidelity as a potential bottleneck to faithfully corrected/inserted genes. Strategies to maximize nucleotide abundance in edited cells could be integrated to address RT fidelity-related limitations, particularly for the large DNA insertions.

DNA Writing Business and Clinical Updates

Prime Medicine (PRME)
Location: Cambridge, Massachusetts

Technology
Prime Medicine uses prime editing as the centerpiece of its therapeutic modality, and we note that the company uses the technology to enact multiple types of gene editing outcomes, including gene mutations, genomic deletions, and genomic insertions. Specifically, on genomic insertions, the company's proprietary PASSIGE platform pairs prime editing with the delivery of a recombinase, which inserts a DNA payload into the site of prime editing (the prime editor writes the cognate recombination site for recombinase activity; see a further explanation of integrases in "Non-homology-based gene correction and insertion" portion of the nuclease-based editing section above).

On prime editing technologies, Prime Medicine has reported use of multiple prime editing systems, which include PE4, PE5, and PEmax systems, along with additional novel prime editing proteins (variants of both Cas guide and RT domains). Beyond protein engineering, the company has developed novel pegRNA designs (presumably sequence and chemical modifications), which may contribute to higher prime editing efficiencies. On delivery, the company is using both LNP and AAV technologies for hepatic and extra-hepatic modalities, respectively.

Key gene editing assets

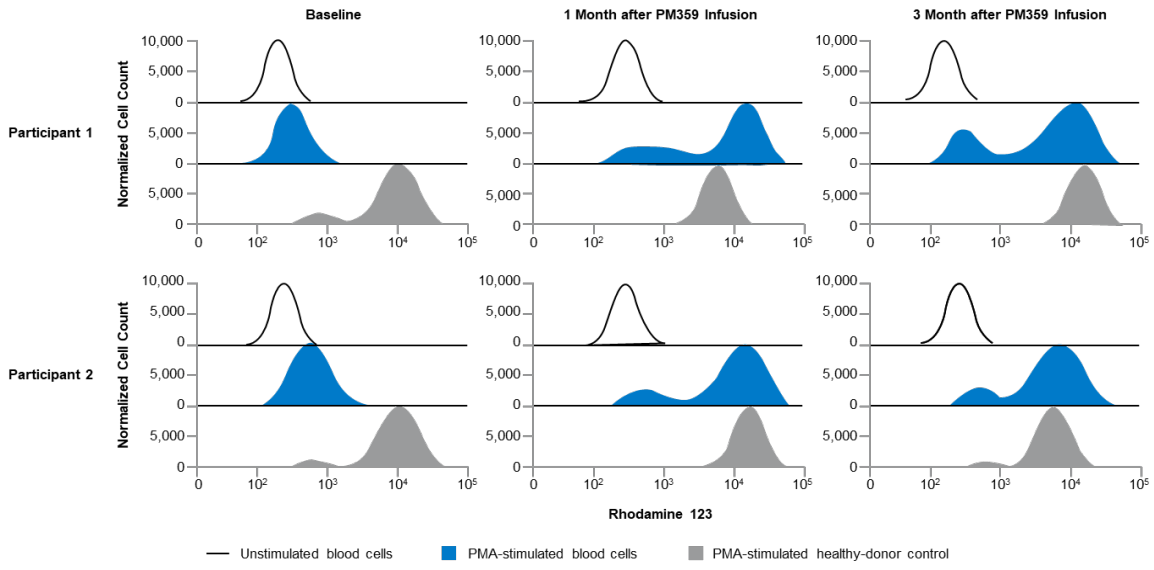
PM359 is a prime-editing-based therapy designed to correct the ΔGT mutation in exon 2 of the *NCF1* gene in patients with chronic granulomatous disease (CGD) amenable to ΔGT mutation correction. *NCF1* encodes the $p47^{phox}$ protein, which aids in the active NADPH oxidase complex. PM359 is an autologous cell therapy; after CD34+ HSCs are collected from a patient, PM359 is electroporated ex vivo into cells for editing. After myeloablative conditioning, edited cells are returned to the patient via bone marrow transplantation.

The two pseudogenes of *NCF1* (pseudogenes are nonfunctional copies of specific genes in the genome that may accrue over evolutionary time due to gene duplication events), *NCF1B* and *NCF1C,* naturally harbor the ΔGT mutation, and in CGD patients with ΔGT mutations, correction of any of the six ΔGT alleles (either copy of *NCF1, NCF1B,* or *NCF1C*) is disease-modifying, which increases the likelihood for PM359's therapeutic effect. We note that all six alleles contain the same sequence context surrounding the site of the ΔGT mutation, meaning the guide RNA sequences used are

applicable to all gene copies. Regarding the specific nature of the editor used, PM359 uses a PE3-based prime editing design. This includes delivery of three distinct RNAs: 1) an mRNA encoding the prime editor construct (Cas9 nickase-RT fusion protein), 2) a pegRNA for guiding the prime editor and providing a template for reverse transcription, and 3) a second sgRNA that triggers a second DNA nick on the nonedited strand (target strand) to bias inclusion of the edit during mismatch repair. On nicking of the nonedited DNA strand, we note that the design of the sgRNA appears to be distal to the site of prime editing (about 80 bp) and located in an intronic site before the beginning of exon 2, based on our review of the company's reports and patents.

PM359 is being investigated in a Phase I/II study in CGD patients amenable to ΔGT mutation correction (NCT06559176); initial data were recently published in *The New England Journal of Medicine* (Gori et al., 2025 NEJM). As of the last clinical update provided by Prime, two evaluable participants received PM359 infusions (participant 1: 18-year-old male; participant 2: 57-year-old male). After infusion, neutrophil engraftment occurred on day 16, while platelet engraftment was identified on day 19. Analysis of peripheral blood cells drawn from patients before and after infusion revealed significant increases in the number of p47phox-expressing and NADPH oxidase activity-competent cells at one month after PM359 infusion (83% of neutrophils enacted NADPH oxidase activity similar to wild-type samples), which was also sustained at three months post-infusion (exhibit 54). A sequencing-based analysis from the clinical course of participant 1 of both the PM359-treated HSCs pre-infusion as well as the patient's bone marrow aspirate six months after cell infusion revealed that most successful editing events occurred on a single allele of *NCF1, NCF1B,* or *NCF1C* loci, though successful gene writing events on 2 and 3 alleles within the same cell were also common. Prime has decided to pause clinical advancement of PM359 beyond the Phase I/II study with the hopes of finding an external development partner.

Exhibit 54
DECODER
NADPH Oxidase Activity From Patient-Derived PBMCs Dosed With PM359



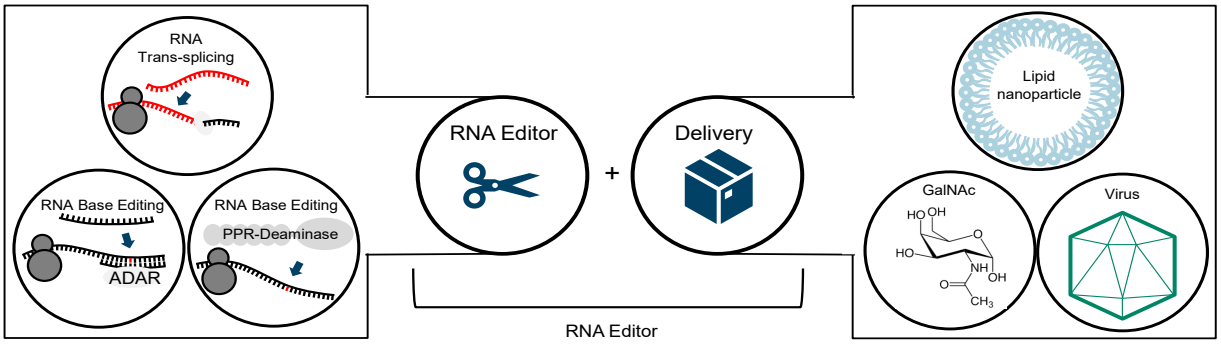
Source: Gori et al., 2025 *NEJM*

RNA Editing

Gene editing therapies that edit the genome make permanent edits for the most part; once a genomic correction, insertion, or deletion has been made at the DNA level, genetic information from that cell (and any daughter cells derived from that cell) is permanently changed, and the resulting RNA and protein products from that gene will incorporate the edit(s) made. This allows genome editing therapies to have one-and-done potential—with disease-mitigating impacts even in dividing cell types. However, such permanence also applies to any spurious genomic alterations that are enacted by the editor, which has raised concerns about off-target activity and creates additional regulatory risk for genome editing assets progressing through clinical trials. There have been recently-issued FDA draft guidance documents requiring post-marketing monitoring of genome-edited patients for 15 years or more, which we believe highlights regulatory concern but also a path forward, given the immense therapeutic potential of these modalities for devastating diseases (see here for a list of FDA-issued cell and gene therapy development guidance documents).

However, recall that we have defined gene editing as the gene-specific sequence alteration of any material along the trajectory of the "central dogma" of molecular biology (DNA → RNA → protein). Within the central dogma, RNA mediates the conveyance of genetic information encoded by DNA into functional enzymes that perform a vast array of cellular tasks. Cellular RNAs include messenger RNAs (mRNAs), which are translated by ribosomes into functional proteins, and noncoding RNAs, a diverse category of RNAs that are not translated but that carry out various cellular functions. Most of the current RNA editing applications used for therapeutic applications focus on protein-coding mRNAs. As with DNA, building of a modular RNA can be accomplished via combination of an editor component and delivery component to enact activity in specific tissue(s) (exhibit 55).

Exhibit 55
DECODER
Build Your Own RNA Editor



Source: William Blair Equity Research

Unlike DNA, RNA molecules have finite lifetimes in cells before they are degraded; endogenous RNA half-lives in mammalian cells vary widely and are dependent on several factors but are thought to be in the range of minutes to hours (Friedel et al., 2009 Nucleic Acids Research; Wang & Liu 2022 Scientific Reports). Therefore, RNA editing therapeutics allow direct targeting of the genetic material underlying disease without the risk of permanent, heritable off-target edits that may spur unwanted, unpredictable side effects. However, the limited lifetime of successfully editing RNA in cells also means that RNA editing therapies require sequential dosing regimens for sustained effects and there are high editing efficiency requirements for each dose of a given therapeutic modality.

In addition, in contrast with genome editing, because RNA is single stranded (whereas DNA is double stranded), there are no base excision repair or mismatch repair pathways to overcome. This means that compared with genome editing, the rate of incorporated RNA sequence modification more closely aligns to the rate of RNA editing. This does not (necessarily) mean that RNA editing is more efficient than genome editing; the rate of RNA editing is tied in with inherent regulatory and enzymatic processes of the respective DNA and RNA editing machinery. Also, while there are, for most DNA base editing applications, one or two genomic copies that must be edited (each diploid cell has two copies of a gene in its genome), dozens or hundreds of copies of a gene's RNA may exist in the cell at a given time, which requires significantly more successful base changes to occur during RNA base editing to correct the same percentage of RNA/protein products. While considerations related to copy number may reduce potential RNA editing efficiencies compared to genome editors, the increased scale of target substrates, combined with a sequential dosing paradigm, allows tunable dosing of RNA editors to elicit specific editing efficiencies, particularly for use-cases where maximal RNA editing is not needed/wanted.

RNA Base Editing 101

In addition to genomic base editing, which stems from guided deamination events on DNA bases, RNA bases may be similarly chemically modified to alter an RNA's sequence. Such RNA base editing may have similar applications as DNA base editing, but because of the transience of RNA lifetimes in cells, does not pose risks associated with permanent off-target base editing events that could yield unpredictable consequences.

RNA-guided RNA base editing via ADAR

While DNA base editing strategies have mostly spurned mammalian DNA deaminases for more effective counterparts from bacteria (e.g., Cas9), opportunities to leverage endogenous RNA deaminases represent the most effective and exciting RNA base editing strategies in the field to date. Such work uses the adenosine deaminase acting on RNA (ADAR) family of enzymes, which are capable of deaminating adenines (As) within RNA sequences. There are three distinct ADAR enzymes—ADAR1, ADAR2, and ADAR3—which each have unique activities and expression patterns. ADAR1 is ubiquitously expressed in all tissues; ADAR2 is expressed at low levels in most tissues and higher in neuronal tissue, including the brain, as well as vascular cells and the heart; and ADAR3 is expressed in neuronal tissues. ADAR3 is not catalytically active and may serve to negatively regulate the activity of ADAR1 and 2 by binding potential editable RNA sequences and obstructing editing activity.

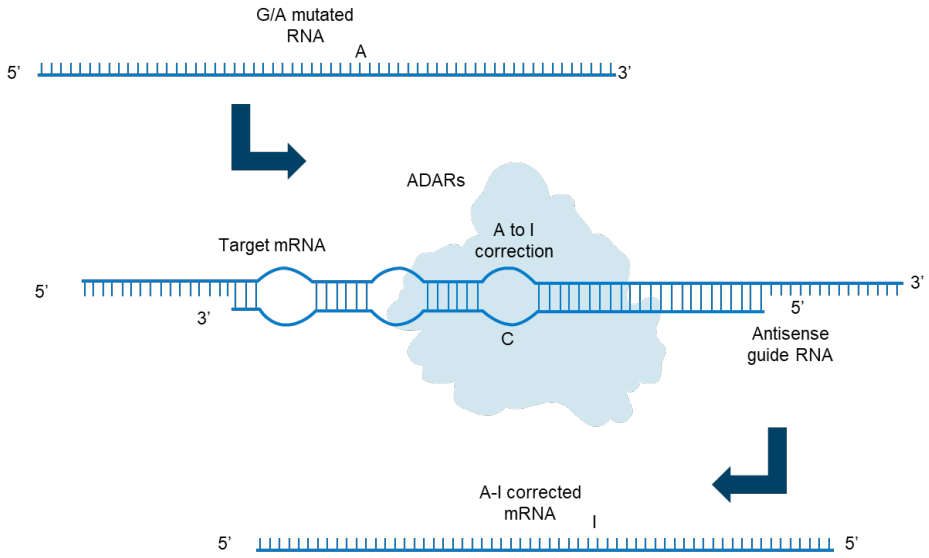
Endogenous activity by ADAR1 and 2 occurs within double-stranded RNA (dsRNA) structures of RNA sequences; though RNAs are synthesized as single-stranded polymers, local areas of homology within an RNA sequence can hybridize into intra-RNA dsRNA structures. In the context of cell physiology, deamination of adenine (A) into inosine (I) within dsRNA reduces the potential for autoimmune reactivity to endogenously transcribed RNAs. Specific immune sentinels ubiquitously expressed in tissues are responsible for recognizing foreign RNAs, typically derived from RNA viruses. One such mechanism by which viral RNA is recognized is via double-stranded RNA structures, a feature of double-stranded RNA viruses. RNA editing activity by ADAR converts adenines into inosines within dsRNA structures on self-derived RNAs, which breaks adenine-uracil base pairing and destabilizes the RNA structure; recall that inosines are interpreted as guanines in the context of nucleic acid hybridization. This A-to-I switch is enough to ward off immune recognition. In a seminal paper, ADAR editing of dsRNA was shown to prevent activation of the dsRNA sentinel MDA5, limiting production of the type I interferon signaling and immune system activation (Liddicoat et al., 2015 Science).

Given the importance of RNA editing in the context of viral infection to prevent an exacerbated immune response, ADAR1, which is typically expressed as a 110 kilodalton protein (ADAR1 p110), is transcribed from an alternative transcription start site to create a 150 kilodalton isoform (ADAR1

p150) in response to type I interferon (IFN) signaling. While ADAR1 p110 is expressed in the nucleus of cells and is thought to enact RNA editing activity on intronic regions of RNA co-transcriptionally, ADAR1 p150 is expressed predominantly in the cytoplasm and is thought to prefer sequences in the 3' UTR of transcripts after RNA processing (Kleinova et al., 2024 Nucleic Acids Research). This has raised a key question in the ADAR-editing field about whether type-I IFN is required to permit editing of cytoplasmic RNA prior to ribosomal translation, which could have important ramifications for therapeutic development leveraging this endogenous mechanism.

Though endogenous ADAR activity is heavily concentrated in regions of RNAs not encoding for proteins such as introns, UTRs, and transcribed retrotransposon elements (Alu elements) to avoid creating changes to the proteome (Wang et al., 2023 BMC Biology; Eggington et al., 2011 Nature Communications), developers may spur ADAR activity at a specific adenine in the transcriptome by leveraging ADAR's affinity for dsRNA structures; in this modality, a guide RNA (mostly) complementary to the desired site of editing is delivered, whereby hybridization of the guide RNA to the target RNA creates a dsRNA substrate for ADAR editing at the target adenine (exhibit 56). A seminal methods paper in 2019 from the lab of Prashant Mali demonstrated the ability to stimulate RNA base editing with an exogenous guide RNA without requiring the exogenous overexpression of ADAR in cells (Katrekar et al., 2019 Nature Methods), opening the possibility that endogenous cellular ADAR could be employed for RNA base editing at sufficient levels for therapeutic applications.

Exhibit 56
DECODER
ADAR-Mediated RNA Base Editing



Source: Bertoli et al., 2025 *Genes*

Given that ADAR-mediated RNA editing largely leverages endogenous cellular machinery, its safety profile may be less immunogenic than other techniques that require delivery and/or expression of a foreign protein (often bacterial derived), which can cause immune reactivity. However, RNA editing via cellular ADAR is subject to variables, such as cellular modulations of ADAR expression, that may be difficult to control or predict. Post-translational modifications of ADAR may also influence global activity or alter ADAR's interaction with the guide RNA, altering on- or off-target activity rates. Even the cellular environment, such as pH, has been shown to influence ADAR activity (Malik et al., 2021 Nucleic Acids Research). In addition, chemical modifications to the guide RNA made by the cell in certain contexts could influence its activity. Alternatively, post-transcriptional

modifications made by the cell to the target RNA may complicate ADAR editing efficiencies; for example, the post-translational adenosine modification N6-methyladenosine (m6A) has been demonstrated to reduce ADAR editing activity of m6A-modified adenosines by blocking ADAR recruitment (Xiang et al., 2018 Molecular Cell). Such variables may be difficult to predict or measure, but should be considered by developers, particularly if such factors are relevant to cells within targeted tissue(s).

As with any gene editing technique, RNA editing for therapeutic purposes aims to maximize on-target editing efficiency to reduce dosing requirements while minimizing off-target editing to prevent unwanted edits to the transcriptome, no matter how transient. Like DNA base editing, off-target editing may be an unwanted edit on a separate RNA transcript, or a bystander edit—editing of a neighboring adenine within the "window" of dsRNA hybridization. While RNA editing via endogenous ADAR restricts developers from altering/mutating the deaminase itself, biochemical characterization of ADAR editing activity has precipitated advancements in guide RNA design, in both sequence and chemical modification, which have advanced targeted ADAR editing while minimizing off-target activity. We highlight some notable advancements below.

Insights into ADAR-mediated RNA editing have allowed the optimization of guide RNA sequences. Such insights include:

- *Sequence context on the target RNA strand to promote ADAR activity:* ADAR has a hierarchy of preference for the base 5' of the target A (U>A>C>G) as well as the base 3' of the target A (prefers a G, with C being the second most preferred). This aids in choosing which RNA sequences to target (when flexibility in sequence in a therapeutic context is allowed) as well as predicting potential hot spots for off-target editing—UAN, AAG, CAG (the targeted A in each sequence is underlined) are RNA sequence motifs known to potentiate off-target ADAR activity.

- *Sequence context on guide RNA strand to promote ADAR activity:* For example, it is well established that ADAR prefers an A-C mismatch at the target adenine, and therefore most (if not all) guide RNAs engineer a C aligned with the target A in their guide.

- *Sequence context to avoid bystander ADAR editing:* Multiple strategies have been employed to avoid bystander editing, including placing Gs in line with bystander As (Qu et al., 2019 Nature Biotechnology) as well as placing abasic gaps (no base instead of a U) in the guide RNA in line with bystander As, which seems to suppress bystander editing more efficiently (Yi et al., 2022 Nature Biotechnology). Both such strategies are being employed under the LEAPER platform from Wensheng Wei's lab, which is being furthered by EdiGene Biotechnology. As opposed to guide RNAs employing contiguous hybridization, the CLUSTER approach for guide RNA designs the guide RNA to hybridize with multiple noncontiguous portions of the target RNA to create a dsRNA substrate for ADAR. As part of this approach, developers can pick sections of the target RNA particularly void of adenines, which lowers the likelihood of bystander edits (Reautschnig et al., 2022 Nature Biotechnology). This strategy has been enhanced via designing of G-U mismatches between the guide and target RNA, which has recently been shown to mitigate off-target editing of UAN motifs (Reautschnig et al., 2024 Nature Biotechnology).

In addition to sequence designs developed due to intrinsic biochemical properties between ADAR and the nucleotides themselves, guide sequences can also be designed to create RNA secondary structures, either within the guide RNA itself or as created by the guide RNA-target RNA interface, which select for on-target ADAR activity. For example, an RNA sequence in the GluR2 mRNA forms a well-characterized dsRNA structure that is known to facilitate ADAR binding and editing, and therefore multiple guide RNA constructs have appended the GluR2 sequence to an RNA sequence homologous with a target RNA to facilitate ADAR recruitment. In addition, circular guide RNA constructs have been shown to facilitate better stability and editing efficiencies (Yi et al., 2022 Nature

Biotechnology; Katrekar et al., 2022 Nature Biotechnology). Lastly, placement of gaps and bulges between the guide RNA and target RNA may facilitate specific secondary structures amenable for preferred ADAR activity. For example, inclusion of 8 bp loops positioned 5 bp upstream and 30 bp downstream and every 15 bp after that along the guide RNA substantially reduced bystander editing while retaining on-target efficiency (Katrekar et al., 2022 Nature Biotechnology). More recent work used high-throughput studies of ADAR editing activity on ALU retrotransposon elements to design effective guide RNAs that, when paired with a target RNA, mimic the secondary structures of such edited Alu elements (Sun & Cao et al., 2025 Nature Biotechnology). This MIRROR platform is being used by RecoRNA Biotechnology. As a complement to rational guide RNA design, screening-based methods have been employed to select for guide RNA sequences that maximize on-target ADAR editing in an unbiased manner (Quiroz et al., 2023 Nucleic Acids Research), as Korro Bio is developing.

In addition to channeling ADAR editing via RNA sequence and/or structure, chemical modifications to nucleotides within the guide RNA may be designed to further improve ADAR activity. Advancement of RNA-based therapeutics into the clinic has been fueled by the ability to synthesize RNA sequences that possess chemical modifications to the RNA structure. These modifications are typically placed on the RNA molecule's sugar-phosphate backbone; every nucleotide of both DNA and RNA contains a nucleic acid (A, C, G, T, or U) along with a ribose sugar molecule and a phosphate (PO4) group, which links to its neighboring nucleotides via a phosphodiester bond. All three components can be modified, with biotechnology companies leveraging these to prevent nuclease degradation of oligonucleotide-based therapeutics systemically. For additional information on RNA nucleotide modifications for therapeutic applications, see our previous white paper: DECODER – Drugging RNA: Key Chemistry Advancements in the New Era of Titratable Genetic Medicines.

RNA modifications exert effectiveness across therapeutic modalities, in part by elongating the therapy's pharmacokinetics, in both targeted tissue(s) and targeted cells. In addition, RNA modifications can work to suppress immune reactivity to foreign RNA molecules, which improves their safety profile. Such attributes apply to guide RNAs for RNA base editing, and we note that 2'-OMe ribose modifications on all RNA bases, as well as utilization of DNA bases in the three most central bases surrounding the targeted adenine for ADAR editing, showed roughly 15-fold improvements in RNA base editing efficiency, which was attributed to improved RNA stability (Quiroz et al., 2023 Nucleic Acids Research). However, RNA chemical modifications have also shown utility in RNA base editing by influencing ADAR activity, and targeted placement of chemical modifications of guide RNA molecules has been demonstrated to both promote on-target RNA base editing and deter off-target bystander activity. Initial experiments demonstrated that chemical modifications to either the ribose or the phosphodiester of the GluR2 ADAR-capturing sequence and a 20-40 antisense domain, dubbed the RESTORE platform, could improve ADAR-mediated RNA base editing with endogenous ADAR but likely requires type-I IFN signaling (Merkle et al., 2019 Nature Biotechnology).

Subsequent work from Wave Life Sciences using the company's AIMer platform showed that stereopure (pure for only a single molecular conformation, not its "mirror image" orientation—other chemically modified RNAs are typically "stereorandom" molecules) chemically modified RNA bases enabled short RNAs (about 30 nucleotides) to be used as guides for ADAR editing. Stereopure guide RNA isomers not only improved ADAR editing efficiencies, but also reduced dependence on type-I IFN signaling, suggesting more efficient shuttling to and activity in the nucleus; further data suggests that these stereopure AIMers predominantly recruit ADAR1 p110 for activity. However, different chemical modifications and stereochemistries may differentially impact editing efficiencies with specific ADAR enzymes (Monian et al., 2022 Nature Biotechnology). Additional improvements to AIMer-driven editing were observed via insertion of targeted stereorandom nucleotides within the guide RNA, along with specific chemical modifications proximal to the targeted editing site, which suggests that impacts on editing efficiencies via chemically modified guide RNAs

extend into position-specific effects through direct biochemical/biophysical impacts on ADAR. This model has been supported by additional work. For example, in a subsequent publication from Wave, the company observed that AIMer-mediated editing was enhanced by replacing the cytosine in line with the targeted adenine with an N-3-uridine (N3U; isouridine). Isouridine is a modification to the nucleic acid itself, but is compatible with additional chemical modifications to the ribose and/or phosphate for additional tailoring (Lu, Shivalila, & Monian et al., 2024 Nucleic Acids Research). A separate group observed that insertion of a 4'-C-methyladenosine (4'-C-MeA) ribose modification inhibited bystander editing of UAA motifs while maintaining on-target ADAR activity when aligned with the -1 position of the target adenine (1 base to the 5' end). Of note, placement of 4'-C-MeA aligned with the -2 position of the target adenine ablated on-target ADAR activity, underscoring the location-specific effects of chemical modifications to guide RNA-mediated editing (Jauregui-Matos et al., 2024 Nucleic Acids Research).

Tailoring a guide RNA for ADAR editing—delivery matters

Insights into the biochemistry of ADAR base editing of specific adenines within a dsRNA sequence have paved development of guide RNAs that aim to maximize on-target ADAR activity through optimizations of sequence and chemical modification. However, considerations surrounding chemical modifications of guide RNAs limit the potential delivery vehicles able to be used to bring the guide RNA to its target tissue(s). Chemically modified guide RNAs can be administered only via nonviral delivery. This is because viral delivery requires the guide RNA to be encoded within the virus's genome, which is then transcribed by the transduced cell. Targeted incorporation of such chemical modifications in the context of viral delivery would require 1) the specific chemically modified nucleotide(s) to be delivered to the targeted tissue(s) and 2) instructions for where the targeted nucleotides were to be assembled into the guide RNA sequence by the cell. Neither of these two processes are currently possible. Therefore, while advancements to ADAR editing mediated by chemically modified RNA nucleotides introduced by Wave and others appear to improve ADAR activity and specificity, their use-case is limited to tissues where developers have the means to efficiently deliver such guide RNAs in a nonviral manner. What is more, such delivery methods must be decently tolerated given the necessity for RNA editors to be redosable.

By contrast, viral delivery diversifies the options for targetable tissues and potentiates durable RNA editing after a single-dose administration due to constitutive expression of the guide RNA from the viral genome, particularly in nondividing cells, but chemical modifications cannot be used, so the developers must rely on the sequence and/or 3-dimensional RNA structure of the encoded guide RNA to enact efficient on-target ADAR activity. In this area, we highlight Shape Therapeutics, which is using machine learning to gain insights into sequence motifs and 3-dimensional RNA shapes to develop highly efficient ADAR guide RNAs that are virally delivered to the brain to address CNS-related disorders; it has collaborations with both Roche and Otsuka.

Additional RNA Base Editing Modalities

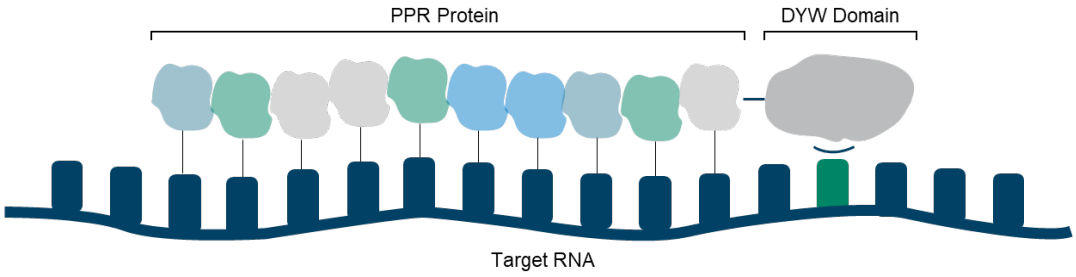
In addition to delivery of guide RNAs in isolation to create tailored dsRNA structures to mediate endogenous ADAR-driven RNA base editing, delivery of exogenous RNA editing enzymes can spur targeted RNA base editing. One such construct appends the deaminase domain of ADAR to a CRISPR guide for targeted deamination of adenines. Instead of using DNA-cognate Cas systems as detailed in the genome editing sections of this report, such applications require use of Cas13, a type VI Cas that uses a gRNA to identify specific RNA species via sequence. As a bacterial immune effector, hybridization of the Cas13's gRNA with the target RNA sequence spurs Cas13-mediated degradation of the target RNA molecule via successive RNA cleavage events (Abudayyeh et al., 2017 Nature). We note that the type I Cas, Cas7-11, is an additional Cas cognate for RNA that cleaves at a specific location with no spurious RNA cuts and RNA degradation (Özcan et al., 2021 Nature). Unlike DNA-cognate Cas proteins, RNA-cognate Cas proteins do not use a PAM to link protospacer hybridization with RNA cleavage; PAMs are located on the nontarget DNA strand and therefore cannot be present on ssRNA. However, certain Cas13 proteins show preference for protospacer

flanking sequences (PFSs), though the mechanism of how PFSs contribute to Cas13 function is not well understood (for a review of Cas13 biology and therapeutic applications, see Yang & Patel, 2024 Nature Chemical Biology). We note that Cas13-mediated RNA cleavage activity can be used for gene-selective RNA degradation as a form of genetic knockdown, although we do not consider this application to fall under the scope of gene editing.

For RNA base editing applications, CRISPR-guided (dCas13) ADAR deaminase domain fusions have been explored to deaminate specific adenines in the transcriptome, with the Cas13's gRNA hybridizing with the target RNA to create the dsRNA structure needed for ADAR activity (Cox, Gootenberg, & Abudayyeh et al., 2017 Science). While such applications may offer extra potential compared to RNA-based RNA base editors due to utilization of specific ADAR deaminase domain variants with supraphysiological activity, complexities such as off-target editing and delivery-related considerations make dCas13-ADAR fusion-based editors difficult. We are unaware of any such dCas13-ADAR assets in development for therapeutic applications to date.

Instead, we see DYW-type pentatricopeptide repeat (PPR) proteins (PPR-DYW proteins) as the more likely protein-guided RNA base editing platform to be used for therapeutic applications due to their complementarity to ADAR-mediated adenine base editing. PPR proteins were discovered in the mitochondrial genomes of plants and found to naturally induce RNA base editing of mitochondrial transcripts. The sequence-guide function relationship of PPR protein design is analogous to TALEs: each PPR protein is constructed as a repetitive sequence of PPR motifs, where each PPR motif binds a single RNA base, and RNA base binding of a given PPR motif is defined by the identity of three specific amino acids within the PPR motif. For PPR-DYW proteins, the PPR domain serves as the protein-based molecular guide, while the DYW domain can edit a targeted RNA nucleotide (exhibit 57). We note that PPR proteins confer specificity for RNA editing of the fourth base downstream of the PPR domain (for a review of PPR proteins, see Small et al., 2020 The Plant Journal). It is important to note that the DYW domain of the PPR-DYW:PG subfamily of PPR-DYW proteins catalyzes the deamination of RNA cytosine (C) into uracil (U). Unlike in DNA, uracil is an endogenous nucleic acid used in RNA species, and therefore PPR-mediated C-to-U edits may exude native functionality (changes to the RNA sequence notwithstanding) and expand the A-to-I ADAR editing capabilities of more traditional RNA base editing approaches. PPR-based RNA cytosine base editing is currently being developed by EditForce. We note that EditForce has also discovered that the PPR-DYW:KP subfamily of PPR-DYW proteins catalyzes a U-to-C conversion in RNA (Ichinose et al., 2025 Scientific Reports). However, as currently understood, this chemical reaction requires chemical crosslinking of the PPR protein to the RNA, so it is not suitable for RNA editing for therapeutic applications. We do not rule out the possibility, however, that advancements may be made to allow U-to-C RNA base editing to also become a viable option within the RNA editing landscape.

Exhibit 57
DECODER
PPR-DYW Proteins for RNA Base Editing



Source: Adapted from EditForce company materials

RNA Trans-Splicing 101

In addition to correction of individual RNA bases, RNAs may also be edited by replacing an entire end of a gene's RNA sequence with an alternative copy. This is done by promoting the joining of two separate RNAs at a specific location via a process called RNA trans-splicing. Trans-splicing is an alternative and rare splicing process used in mammalian cells.

Recall that protein-coding genes contain stretches of sequence that do not encode for the protein's sequence, and that such regions (introns) are included in the RNA sequence after transcription but are subsequently removed during RNA processing via RNA splicing. By cutting out the intronic regions and conjoining the previously noncontiguous protein-coding sections of the RNA's sequence (exons), a mature mRNA contains a contiguous protein-coding sequence able to be translated by ribosomes into a functional protein. It is important to note that most splicing in cells is performed in cis; exons from the same RNA strand are joined together by the spliceosome (see a video animating the splicing process here). RNA trans-splicing uses cellular mechanisms of cis splicing to mediate the conjoining of two separate RNAs at a specific location, which allows developers to swap in introns into a RNA's sequence. After initial evidence demonstrated that trans-splicing occurred in mammalian cells (Eul, Graessmann, & Graessmann 1995 EMBO), seminal work from the biotechnology company Intronn demonstrated programmed trans-splicing of a donor RNA in 1999 (Puttaraju et al., 1999 Nature Biotechnology).

For therapeutic applications of RNA trans-splicing, a donor RNA is delivered that encodes for the piece of RNA developers want to replace within the target gene's RNA sequence. For 5' RNA trans-splicing, the donor RNA is added to the 5' end of the targeted splice site within the target RNA. For protein-coding mRNA, this alters the 5' UTR and/or N-terminal portion (beginning) of the target gene's protein product. For 3' RNA trans-splicing, the donor RNA is added to the 3' end of the targeted splice site. For protein-coding mRNA, this alters the 3' UTR and/or C-terminal portion (end) of the target gene's protein product. In this way, an end of the targeted mRNA is replaced, which allows developers to create single RNA trans-splicing therapies that may alter a gene's sequence in the context of any number of mutation types within that gene.

Spliceosome-mediated RNA trans-splicing (SMaRT)

The cell's spliceosome is an RNA-protein complex that catalyzes the process of splicing. RNA trans-splicing therapies may be designed by harnessing the cell's spliceosome machinery to catalyze trans-splicing of a donor RNA with a target RNA.

Multiple motifs within introns facilitate intron removal and the joining of neighboring exons. Notable intronic motifs needed for proper RNA splicing include (listed in order from 5' to 3' within a given intron):

1. A splicing donor sequence located at the 5' end of the intron. This sequence contains a GU.

2. A branch point sequence located somewhere in the middle of the intron. While branch point sequences can vary, an A located within it is the catalytic nucleotide of interest.

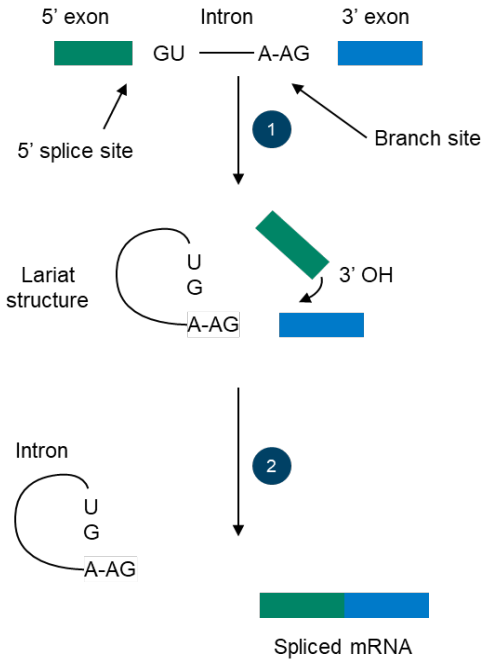
3. A polypyrimidine tract located 3' of the branch point. Recall that Cs and Us are the pyrimidines found in RNA. The polypyrimidine tract aids in the assembly of the spliceosome within the intron.

4. A splicing acceptor sequence located at the 3' end of the intron. This sequence contains an AG.

Briefly, during RNA splicing in cis, the spliceosome facilitates the GU sequence within the splice donor to chemically attack the A within the intron's branch point, which also facilitates cleavage of the splice donor from the 5' exon. This partly looped intermediate formed by the intron is called a

lariat. Following lariat formation, the spliceosome facilitates the chemical attack of the 5' exon by the 3' exon via the splice acceptor, which joins the exons together and cleaves off the intron lariat (exhibit 58). For an in-depth review of the biochemical and -physical steps of RNA splicing, see Wilkinson, Charenton, & Nagai 2020 Annual Review of Biochemistry.

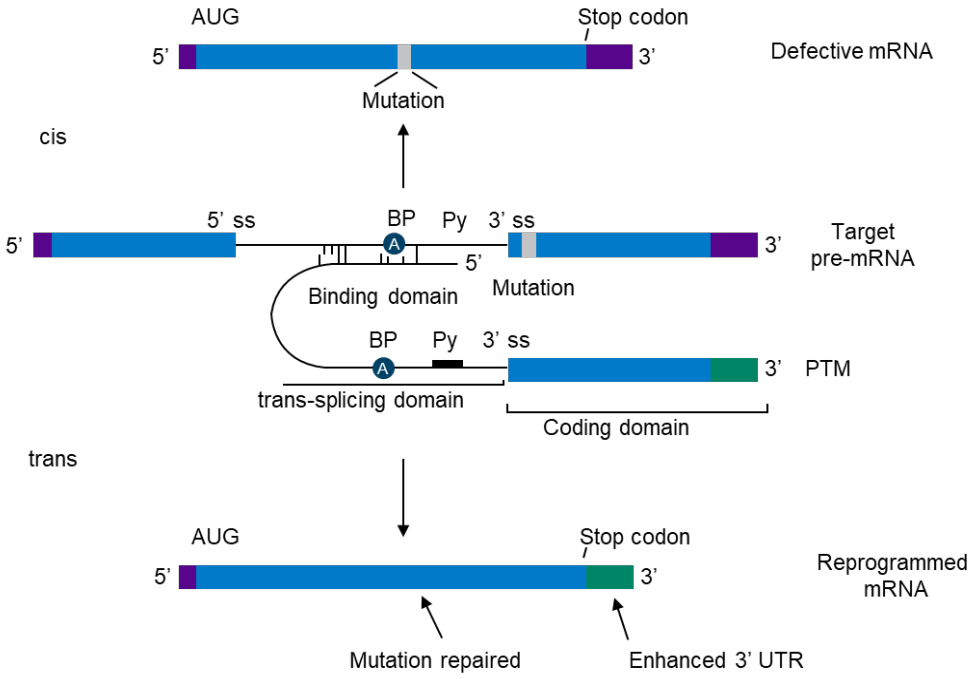
Exhibit 58
DECODER
Canonical RNA Cis-splicing Mechanism



Source: Newman, 1998 *Current Biology*

To facilitate trans-splicing into the target RNA by the spliceosome, donor RNAs contain the requisite intron motifs to facilitate splicing. For 5' RNA trans-splicing, the donor RNA contains the desired RNA sequence to be spliced in, followed by an intronic sequence containing a splice donor. Upon localization of the RNA to the correct intron within the target RNA, the branch point, polypyrimidine tract, and splice acceptor within the target RNA's intron help facilitate trans-splicing using the donor RNA's splice donor. For 3' RNA splicing, the donor RNA contains an intronic sequence containing branch point, polypyrimidine tract, and splice acceptor sequences followed by the desired RNA sequence to be spliced in. The spliceosome is then able to use the donor RNA in combination with the target RNA's intronic splice donor to facilitate trans-splicing (exhibit 59).

Exhibit 59
DECODER
Splicesome-Mediated RNA Trans-splicing (SMaRT) Mechanism



Source: Garcia-Blanco, 2003 *The Journal of Clinical Investigation*

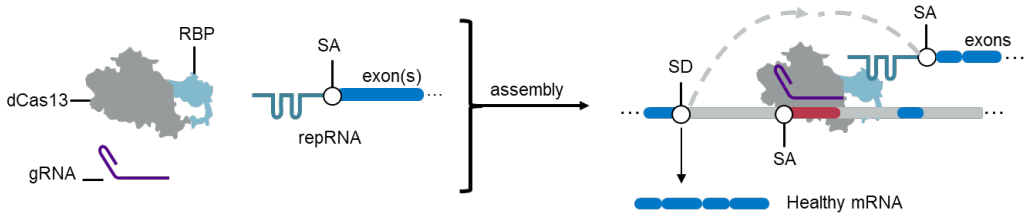
Strategies to facilitate SMaRT primarily differ in the ways the donor RNA is localized to the targeted intron within the targeted RNA to facilitate trans-splicing at that location. The primary method of localization is via an RNA sequence, which is appended to the donor RNA and hybridizes with the targeted intron; for 5' RNA trans-splicing, the guide sequence is located at the 3' end of the donor RNA, while for 3' RNA trans-splicing, the guide sequence is located at the 5' end of the donor RNA. For therapeutic applications, this modality is being developed by Ascidian Therapeutics. The company's lead asset, ACDN-01, is being evaluated in the Phase I/II STELLAR trial in patients with ABCA4-related Stargardt disease (see its review paper here: Doi et al., 2024 Molecular Therapy: Nucleic Acids; NCT06467344).

Because RNA trans-splicing is a rare event in cells, and successful trans-splicing must divert spliceosome machinery away from entrenched cis-splicing events, development of RNA trans-splicing strategies capable of generating RNA editing events at a therapeutically relevant scale is the largest bottleneck in the field and requires optimization of the donor payload. Ascidian is using in silico–based strategies to optimize the sequence of donor RNAs that may be more likely to induce trans-splicing. However, other strategies have also been used, which primarily aim to promote trans-splicing by thwarting cis-splicing. For example, targeted RNA oligonucleotides that block cis-splicing may be used to promote trans-splicing of a donor RNA (Liemberger et al., 2018 International Journal of Molecular Sciences). In addition, development of the Programmable RNA Editing & Cleavage for Insertion, Substitution, and Erasure (PRECISE) method, which directs targeted RNA cleavage at the site of trans-splicing to limit cis-splicing and promote trans-splicing, was also recently published as a preprint (Schmitt-Ulms et al., 2024 BioRxiv). We note that RNA cleavage of the RNA containing the splice donor (the 5' component of the spliced RNAs) mediates the best trans-splicing efficiencies in this context: for 5' trans-splicing, RNA cleavage is directed to cut the

donor RNA, and for 3' RNA splicing, RNA cleavage is directed to cut the target RNA. This method was adapted for multiple types of RNA-cleaving enzymes, including a guided Cas7-11 as well as targeted ribozymes (RNA that enact enzymatic activity).

In addition to versions of SMaRT where the donor RNA encodes its own localization via sequence hybridization with the target intron, which can lead to instances of off-target splicing activity, a donor RNA may be guided to the target RNA/intron via an RNA-cognate Cas. Unlike PRECISE, this scheme does not use RNA cleavage activity; a dCas13 is used for localization of the donor RNA. The dCas13 construct also contains an RNA binding domain, which allows the donor RNA to bind to the dCas13 via an RNA sequence cognate to that RNA binding domain. Though this strategy may raise additional immunogenicity concerns compared with an RNA-based SMaRT system due to the expression of a bacterial protein, using the dCas13 for localization may improve trans-splicing specificity beyond RNA-based strategies. Also, we note that targeted binding of the dCas13 via gRNA hybridization may serve to promote trans-splicing by both localizing the donor RNA to the site of trans-splicing and deterring cis-splicing. The dCas13 binding at the target intron's splice donor or acceptor sequence for 5' and 3' trans-splicing, respectively, can inhibit cis-splicing of the target RNA, allowing more efficient induction of trans-splicing (exhibit 60). This technology is being developed by Amber Bio and has been published in an initial preprint (Borrajo & Javanmardi et al., 2023 BioRxiv), while another group has since published a dCas13-mediated trans-splicing method, dubbing it CRISPR-assisted mRNA fragment trans-splicing, or CRAFT (Fiflis et al., 2024 Nature Communications).

Exhibit 60
DECODER
CRISPR Cas13-Based Splicesome-Mediated RNA Trans-splicing Mechanism



Source: Borrajo & Javanmardi et al., 2023 *BioRxiv*

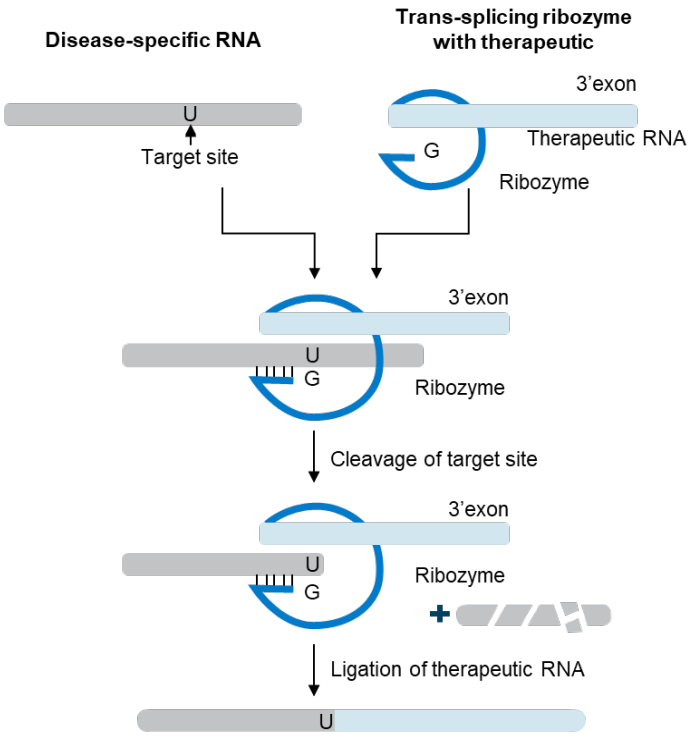
Ribozyme-mediated RNA trans-splicing

In addition to recruitment of the cell's endogenous spliceosome to carry out trans-splicing, additional strategies use intron sequences that can carry out the catalytic activity to splice themselves out of an RNA sequence. Such ribozymes are found in a number of different physiological contexts and are subdivided into group I introns and group II introns (for an overview of the comparison between group I and group II intron splicing, see Saldanha et al., 1993 FASEB). Group I introns are the primary modality being developed for trans-splicing therapeutics. This is because the structure-function relationship of group I introns is more thoroughly understood and because their ability to self-catalyze splicing is more effective at physiological conditions than group II introns; self-splicing of group II introns is most efficient at nonphysiological concentrations of salt and magnesium, and exogenous factors are required for group II intron splicing in physiological conditions. By contrast, group I introns need only an exogenous guanosine to catalyze self-splicing and perform such reactions in physiological conditions.

The large rRNA intron encoded in the genome of the *Tetrahymena* genus of unicellular eukaryotes is the group I intron that has been primarily investigated for therapeutic use of ribozyme-mediated trans-splicing. The *Tetrahymena* group I intron ribozyme donor RNAs have been redesigned for therapeutic applications to act in 3' RNA trans-splicing by converting the cis-acting

splice site recognition sequence at the ribozyme's 5′ end to a guide sequence. The donor RNA to be spliced into the target RNA is encoded at the 3' end of the ribozyme (exhibit 61). Seminal work in the lab of Bruce Sullenger demonstrated that a *Tetrahymena* group I intron could be used for RNA trans-splicing in mammalian cells (Jones, Lee & Sullenger 1996 Nature Medicine). Subsequent work has demonstrated that trans-splicing efficiency can be improved by extending the length of homology between the 5' guide sequence on the donor RNA and the target RNA (Kwon et al., 2005 Molecular Therapy). Design of group I introns for RNA trans-splicing applications continues to evolve (see a review article here). More recently, a split group I intron approach for trans-splicing of two exogenously delivered RNAs was created and improved the ribozyme's trans-splicing activity via in silico–driven sequence modifications, which may lend insight into applications for donor RNA-target RNA interactions for therapeutic purposes (Gao et al., 2025 Nature Chemical Biology). Rznomics is using a *Tetrahymena* group I intron ribozyme-based platform to create RNA trans-splicing therapeutics.

Exhibit 61
DECODER
Ribozyme-Mediated RNA Trans-splicing Mechanism



Source: Rznomics company materials

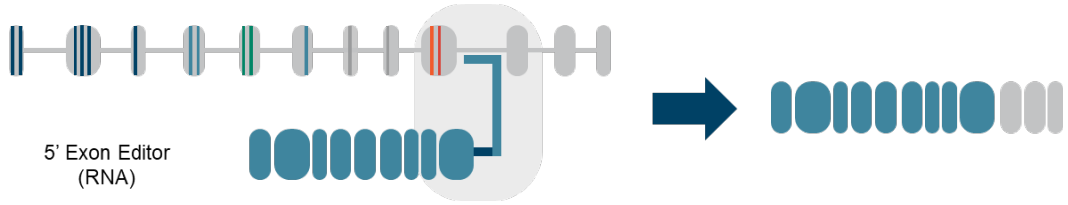
Choosing a target intron for RNA trans-splicing

Trans-splicing technology allows the replacement of an entire end of an RNA sequence, which, when applied to correcting mutations in a given gene, can splice in a corrective RNA sequence regardless of the specific mutation causing disease. Therefore, for gene correction applications, trans-splicing technology is best used for genes that cause disease but for which multiple types/locations of mutations are common or similarly prevalent. For such instances, using an RNA base

editing approach, which corrects only a single mutation (a single mutation type at a single location), would require iterative development of multiple RNA base editing therapies, one for each individual mutation, to address the same patient population.

Given that trans-splicing is a more unbiased approach to RNA editing, the decision of where within the target RNA to perform the trans-splicing event (said another way, how much of the target RNA to replace with the donor RNA) is an important design-related decision for developers. We surmise that this choice is primarily predicated on the location of the common mutations in the target gene that lead to disease, such that RNA trans-splicing can pave over such potential mutations. Given that specific domains within a protein are responsible for different functional properties of the protein, disease-causing mutations within a gene often occur in clustered "hot spots." For example, if inactivating mutations in the protein-coding gene Gene X causes disease, and Gene X's mRNA contains 12 exons interspersed by 11 introns, disease-causing mutations may be largely sequestered to exons 1-9. Therefore, targeting 5' RNA trans-splicing at intron 9 of Gene X would allow the replacement of exons 1-9 and correction of any such mutations (exhibit 62).

Exhibit 62
DECODER
RNA Trans-splicing Corrects RNA Sequences Across Multiple Mutation Hotspots



Source: Doi et al., 2024 *Molecular Therapy*

Choice of the specific intron may also be influenced by relative trans-splicing efficiencies of different introns within the target RNA. Such efficiencies could be affected by several variables, including the 3-dimensional structure(s) and/or post-transcriptional modifications of/within the target intron, which may either inhibit or promote donor RNA binding and splicing. Alternatively, the intrinsic dynamics of cis-splicing of the target RNA may also be at play. For example, biochemical processivity of cis-splicing may vary across introns within an mRNA, making certain introns more amenable to rerouting toward trans-splicing outcomes. In addition, the order of intron splicing may influence relative trans-splicing efficiencies at specific introns. Evidence suggests that the order at which introns are spliced out of a given gene's RNA is a relatively fixed process (consistent across mRNA molecules from a given gene), but the specific order is not necessarily based on the timing of their transcription (introns are not necessarily spliced out in order from 5' to 3'; Choquet et al., 2023 Nature Structural & Molecular Biology). We surmise that introns spliced out later in the splicing process would be more amenable to trans-splicing given that it would allow additional time for the donor RNA to bind after gene transcription, but such timing may be difficult to predict, and we note that alternative splicing may further complicate such dynamics.

Applications for RNA Editing

Compared with genome editing technologies discussed above, RNA editing allows similar genetic manipulations to occur without the risk of instances of off-target editing persisting indefinitely given the limited lifetime of RNA species in cells. Broadly, we view the scope of applications possible with RNA base editing and RNA trans-splicing as similar to those of DNA base editing and DNA writing, respectively. Therefore, our commentary on applications of RNA editing below is focused on those use-cases that we deem most common and/or RNA editing-specific.

Gene mutation

The most common application of RNA editing currently is gene-correcting mutations in the context of disease, where RNA editing can be used to fix mutant transcripts at the RNA level, leading to functional, wild-type protein production. With RNA base editing, specific point mutations may be corrected; however, limits exist. RNA base editing can be used for base substitution mutations (one nucleotide exists instead of another), specifically in cases where a deaminating event (A-to-I switch or C-to-U switch) in the coding sequence fixes the amino acid sequence of the protein product back to wild-type, and unlike DNA base editing, RNA base editing cannot back into base corrections to an A or C by editing a complementary strand. In other instances, such as for alternative mutation types (e.g., base insertions or deletions) or base substitutions from carbonyl-containing nucleic acids (Gs or Us), RNA trans-splicing can be used to splice in a donor RNA with a wild-type sequence. As mentioned above, this may be amenable for multiple genotypes within a gene, allowing developers to develop a single disease-correcting therapy that covers multiple mutation hot spots.

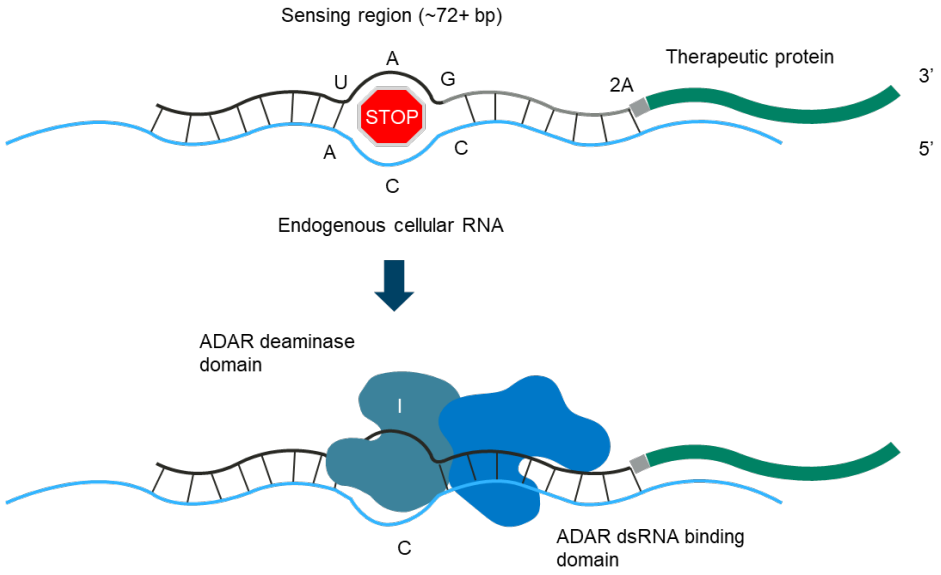
RNA edits can also induce mutations that alter a gene's sequence to a non-wild-type outcome. This could be performed to alter protein function to sub- or supraphysiological levels. Alternatively, levels of protein production could be modulated by RNA edits that do not impact a protein's sequence and/or function by inducing edits in UTRs. While genome editing allows tunable protein expression by editing regulatory sequences that affect gene transcription (e.g., gene promoters or enhancers), such methods are not possible with RNA editing methods. RNA edits that alter mRNA splicing—to exclude deleterious sequences (e.g., premature Stop codons, toxic repetitive sequences), prevent splicing to introduce a premature Stop codon from an unspliced intron, or alter the rates of nonproductive splicing—may also be enacted, though we note that care must be taken to ensure that such edits are enacted before RNA splicing can occur.

Such edits can be made to protein-coding mRNAs, as is most common in practice, but can also be made to noncoding RNAs to alter their function by, for example, endowing alternative sequence recognition, changing their cellular interacting partners via sequence-intrinsic or 3-dimensional structure changes, or altering their enzymatic capacity. However, we note that large-scale changes to noncoding RNA sequences via RNA trans-splicing may be compatible only with ribozyme-mediated RNA trans-splicing, as most (if not all) noncoding RNAs are not spliced and therefore do not have the requisite sequence motifs to mediate spliceosome-mediated RNA trans-splicing.

RADAR

In addition to ADAR-mediated RNA editing designs in which a guide RNA is introduced to cells to induce RNA editing of an endogenous cellular transcript via dsRNA formation, formation of a dsRNA structure may also allow inducible RNA editing of the exogenous therapeutic RNA introduced to cells, with a specific endogenous RNA serving as the unedited "guide" transcript. This methodology has been termed RNA sensing using adenosine deaminases acting on RNA (RADAR). RADAR can be used for inducible, condition-specific mRNA transgene expression: an exogenous protein-coding mRNA is introduced that harbors a premature Stop codon, such that the encoded protein product is not translated constitutively. The exogenous RNA also contains a sequence surrounding the premature Stop codon, which is complementary to a prespecified cellular transcript such that hybridization of the exogenous RNA with the endogenous cellular RNA creates a dsRNA substrate for ADAR to edit the adenine within the Stop codon (UAG or UGA) to UGG (a codon that encodes the amino acid tryptophan), which then permits production of the encoded protein product (exhibit 63). Multiple seminal papers describing this technology from Stanford and MIT were published in quick succession in 2022 (Kaseniit et al., 2022 Nature Biotechnology; Jiang et al., 2022 Nature Biotechnology). Unlike traditional guide RNA-driven ADAR editing in which tailored sequence and chemistry designs are placed on the guide RNA to promote editing of an unmodified cellular transcript, ADAR editing in RADAR occurs on the manufactured exogenous RNA. This allows developers to tailor specific sequence motifs and/or chemical modifications to the actual ADAR substrate, which may increase ADAR editing efficiency above other ADAR-based methods.

Exhibit 63
DECODER
RNA Sensing Using Adenosine Deaminases Acting on RNA (RADAR) Mechanism



Source: Adapted from Radar Therapeutics company materials

RADAR allows an exogenous mRNA transgene to be expressed only when a specific endogenous transcript is expressed in a cell. This allows developers to tailor the choice of the endogenous "guide" transcript depending on the type of condition the developer wants the transgene to be expressed in. For example, RADAR allows the exogenous mRNA transgene to be delivered to a heterogenous cell population while tailoring transgene expression to only the specific cell type(s) that expresses the endogenous guide transcript. Alternatively, the guide transcript may be one that is expressed in a multitude of cell types but is expressed only under certain cellular signaling conditions, therefore tailoring the expression of the transgene to those contexts (we note that these two scenarios are not mutually exclusive; an endogenous target RNA may be restrictive in both cell type and signaling context). This technology is being developed for therapeutic applications by Radar Therapeutics; on the mRNA side, although outside the scope of this report, Strand Therapeutics is developing cell-specific mRNA-based expression cassettes.

Mitigation of Off-Target RNA Editing Activity

The finite lifetime of RNAs in cells slightly eases the pressure for curbing all off-target RNA editing activity. However, we still view restriction of off-target activity as paramount to regulatory approval of RNA editors, as off-target RNA activity may still spur serious adverse consequences. For example, evidence that ADAR1 overexpression in cells may be oncogenic (see a review here: Baker & Slack 2023 Trends in Genetics) supports the argument that indiscriminate RNA base editing activity transcriptome-wide may cause deleterious effects. In addition, fusion proteins are a known driver of oncogenesis, and while such proteins are typically thought of as the product of genetic rearrangements at the DNA level, we view potential off-target RNA trans-splicing activity as a possible source of such proteins in certain contexts (see review of fusion proteins here: Tang et al., 2025 Genes & Diseases).

The requirement for RNA editors to perform activity at large scale (editing a significant number of target transcripts over time) means that any affinity for off-target activity will detract from an RNA editor's efficiency. On this point, we note that the relative scales of RNA editing and genome editing dictate the ability to tailor editor half-life in cells to limit off-target editing activity. As mentioned

above, genome editing requires (in most cases) a maximum of only two editing events, one DNA editing event on each chromosomal copy of a gene, to be successful and permanent. This means that shortening a genome editor's half-life in cells can be used to prevent unwanted editing events over time. To analogize this process, genome editing is designed as a covert military operation—the goal is to perform a finite task while getting in and out quickly and quietly to prevent additional harm. In contrast, RNA editing requires multiple successive events, one on each RNA molecule, and edits are impermanent by nature (additional gene transcription adds RNA molecules to be edited), which motivates developers to elongate the half-life of the editor in cells to maximize RNA editing efficiency and reduce dosing burden. To extend the analogy, if genome editing is designed as a covert military operation, RNA editing is designed as an occupation, and methods to reduce editor half-life cannot be used to curb off-target editing activity as they are more deleterious to therapeutic efficacy.

Guide design

As most RNA editing technology is enacted via RNA-based modalities, we view design of the guide molecule as the chief means of controlling off-target activity. We view both sequence-based and chemistry-based designs as the means by which developers can chiefly mitigate off-target editing by RNA editors. As with other methods using a guide RNA, selection of the appropriate target sequence within a gene is of utmost importance, namely ensuring the uniqueness of that sequence compared with the rest of the transcriptome, which should mitigate off-target editing for both RNA base editing and RNA trans-splicing applications. In addition to considerations related to the target RNA itself, the guide RNAs used for RNA editing applications should be assessed for their ability to hybridize with DNA given that the targeted RNA sequence could also be found in the genome on the coding strand of the target gene with thymidine residues replacing the uridine only found in RNA. Given that RNA-DNA interactions (often referred to as R-loops) are capable of influencing gene regulation, particularly via transcription (see a review of RNA-mediated genome regulation here: Statello & Guo et al., 2020 Nature Reviews Molecular Cell Biology), we surmise that delivery of RNA editing guide RNAs to a cell at high concentrations to participate in RNA editing may have unintended consequences on the target gene's transcription. While these consequences may be difficult to address, certain target regions within a gene's sequence, perhaps further downstream of the gene's promoter, may be less prone to unintended effects on transcription.

We note that like DNA base editing, RNA base editing raises additional off-target considerations related to bystander editing of proximal adenines within the deaminase's editing window. Sequence considerations for the guide RNA related to avoiding target sequences rich in adenines (when possible), targeted guide RNA-target RNA mismatches, 3-dimensional RNA structure design, and placement of abasic sites, as well as chemical modifications to the guide RNA, are used to mitigate bystander editing activity.

Reducing RNA affinity

While we are unaware of discrete studies demonstrating how reducing RNA affinity restricts RNA off-target editing, we envision that like genome editing techniques, the ability to reduce RNA affinity could be used to improve stringency of an RNA editor. In the case of a guide RNA, specific chemical modifications and/or integration of DNA nucleotides into the guide RNA sequence may aid in achieving this property. Alternatively, for protein-based RNA editors such as PPR proteins, specific protein engineering to lower the protein's affinity to RNA could be employed.

Require multiple RNA binding events

While we are unaware of such constructs to date, we envision that split RNA binding systems could be tested for RNA editing applications. We see particular applications for RNA base editing modalities using delivered protein factors, such as a split ADAR approach or split PPR approaches, as possible. In addition, while spliceosome-mediated RNA trans-splicing applications may be difficult to enact using a split RNA system, we view recent engineering of a split group I intron approach

for trans-splicing of two exogenously delivered RNAs as potentially promising for opening this application (Gao et al., 2025 Nature Chemical Biology). We offer the caveat that time constraints relevant to RNA processing may significantly hinder RNA editing techniques requiring multiple RNA binding events; while DNA is a comparatively static molecule within the cell, allowing time for multiple DNA binding events to occur and/or assemble for editing to occur via genome editors that require more than one DNA binding event, RNA editing techniques commandeer RNA processing events (RNA deamination and RNA splicing) that occur co-transcriptionally or, at the latest, during final stages of mRNA processing before RNA export into the cell's cytoplasm. Because the necessary factors for RNA editing (e.g., ADAR, spliceosome factors) reside in the cell's nucleus to perform such duties, RNA editors must catalyze the desired reaction within a specific time window before the RNA is exported from the nucleus. While we are not aware of the comparative time frames of RNA editing reactions versus total RNA processing times, we envision that such time constraints could limit the ability of multistep reactions to take place while maintaining robust RNA editing efficiencies.

RNA Editing Business and Clinical Updates

Wave Life Sciences Ltd. (WVE)
Location: Cambridge, Massachusetts

Technology
Wave uses its AIMer platform to develop novel guide RNAs, which induce ADAR-mediated RNA base editing at targeted adenosines in the transcriptome. As highlighted earlier in the report, the AIMer platform is based on utilization of novel RNA chemistries, including phosphoryl guanidine-containing backbone linkages (known as PN linkages), which may enable greater ADAR deaminase activity or facilitate increased ADAR specificity toward the target adenosine. Such chemical modifications may be related to the nucleotide's ribose sugar or the phosphodiester linkage between nucleotides (Monian et al., 2022 Nature Biotechnology) or to the nucleic acid base itself (Lu, Shivalila, & Monian et al., 2024 Nucleic Acids Research). We note that such individual chemical modifications are not mutually exclusive and can be combined to produce highly customized, diverse nucleic acids. In addition to the development of RNA editors, Wave is also generating RNA interference-based therapeutics, which we mention given the company's presentation at its 2025 investor day of preclinical data of novel RNA molecules that enact both RNA editing and RNA interference (see the presentation slides here). We note that the RNA editing and RNA interference functions are targeted to different genes.

For delivery, the company's liver-targeted assets have used GalNAc conjugation. However, the company is also developing extra-hepatic delivery techniques, with preclinical RNA editing data of both NHPs using subcutaneous administration and mice using intravenous administration. The nature of the lung delivery molecule(s) used in these experiments is unknown.

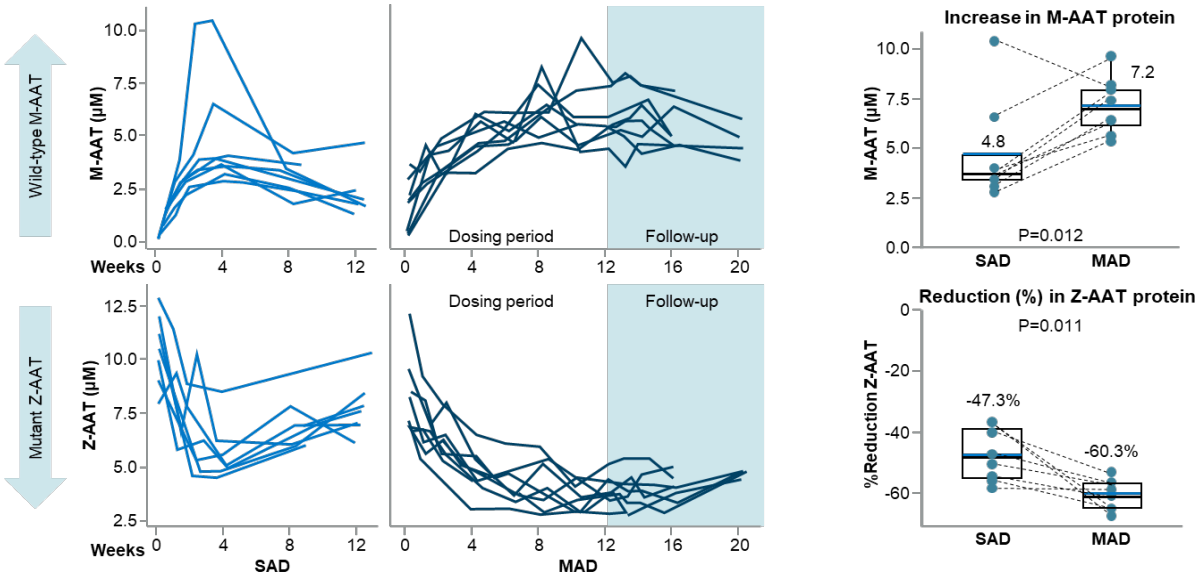
Key gene editing assets

WVE-006 is an RNA base editing asset targeting the *Serpina1* gene for the treatment of alpha-1 antitrypsin deficiency (AATD). Specifically, it corrects the E342K mutation (Z-AAT allele) of *Serpina1* back to the wild-type sequence (M-AAT) by guiding ADAR-mediated deamination of *Serpina1* mRNA. WVE-006 is an RNA-editing guide RNA conjugated to GalNAc and is delivered subcutaneously with GalNAc-conjugation for hepatic targeting. Based on Wave's preclinical and patent literature, we believe WVE-006 is a 30 base pair guide RNA with a base sequence of CCCAGCAGCTUCAGUCCCUUTCTUIUCGAU, where the underlined uracil (presumably N3U) is aligned with the targeted adenine in the *Serpina1* transcript. Specifics regarding the nature of chemical modifications to the raw RNA sequence used for WVE-006 have not been disclosed.

Wave is conducting the Phase I/II RestorAATion-2 study of WVE-006 in AATD patients harboring the Z-AAT allele (NCT06405633). Data presented to date includes single ascending dose (SAD) and multiple ascending dose (MAD) data from the 200 mg dose cohort and SAD data from the 400 mg dose cohort (n=8 per cohort). Efficacy data presented included two key metrics: 1) total circulating AAT levels and 2) levels of M-AAT, which indirectly correlate with RNA editing efficiencies. For total AAT levels, 11 uM total serum AAT (Z-AAT or M-AAT) is used as a lower threshold predictive of efficacy given that is the lowest AAT expression observed in unaffected heterozygotes and is the serum trough level of AAT augmentation therapy that was used for regulatory approval.

For the 200 mg dose, the SAD portion showed average total AAT levels peaked at 12.9 µM, which included 4.8 µM (37.2%) of the corrected M allele (M-AAT). This included one patient in the 200 mg cohort who had higher levels of circulating AAT expression due to an incident of a kidney stone (20.6 µM total AAT), which included 10.3 µM M-AAT (50% editing). M-AAT levels sharply declined four weeks after dosing, consistent with a return to normal AAT levels after resolution of the kidney stone. Although only from a single patient, these data are emblematic of the RNA editing capacity at a low dose of 200 mg, in our view. In the multidose portion of the 200 mg dose (every two weeks) cohort, total AAT levels peaked at 11.9 µM with 7.2 µM M-AAT. In total, M-AAT reached 64.4% of total AAT levels, and total AAT levels were slightly above the 11 µM trough concentration associated with AAT augmentation therapy. We also note that Serpina1 editing in the multidose setting proved durable, with M-AAT levels maintained above 50% of total AAT for 2 months after the final WVE-006 dose (exhibit 64).

Exhibit 64
DECODER
***Serpina1* mRNA Editing Elicited by WVE-006 in Phase I/II RestorAATion-2 Trial**



Source: Wave Life Sciences company materials

SAD data from the 400 mg dose cohort showed 12.8 µM total AAT with 5.3 µM (41.4%) of M-AAT. Based on the data stemming from the outlier patient whose AAT levels spiked, we do not view total AAT levels as a proper proxy for editing capacity or efficiency. However, circulating M-AAT levels showed an incremental dose response, which we view as positive, though again, not reflective of the capacity of RNA editing, which is likely dictated by levels of transcription of the Serpina1 gene after dosing initiation as much as by ADAR editing efficiency. WVE-006 was well tolerated; all AEs

were mild to moderate in intensity, and there were no SAEs or discontinuations (see our note: WVE-006 Shows Dose-Dependent RNA Editing in RestorAATion-2; Room for Korro to Exert Additional Serpina1 Editing Efficiency).

Korro Bio, Inc. (KRRO)
Location: Cambridge, Massachusetts

Technology
Korro uses its proprietary oligonucleotide promoted editing of RNA (OPERA) platform to generate guide RNAs for ADAR-based RNA editing referred to as Customized High-fidelity Oligonucleotides for RNA Deamination (CHORD). This platform incorporates both computational and rational design to generate guide RNAs that induce highly specific ADAR activity. We note that Korro's identification and optimization of ADAR guide RNAs uses a novel screening platform (Quiroz et al., 2023 Nucleic Acids Research). For delivery of its CHORDs, Korro has used both GalNAc and LNPs (licensed from Genevant; GVT-1, GVT-2).

Key gene editing assets

KRRO-110 is an RNA base editing asset targeting the *Serpina1* gene for the treatment of AATD. Specifically, it corrects the E342K mutation (Z-AAT allele) of *Serpina1* back to the wild-type sequence (M-AAT) by guiding ADAR-mediated deamination of *Serpina1* mRNA. The guide RNA payload of KRRO-110 is encapsulated in an LNP, which differentiates the asset from WVE-006. Although Korro demonstrated greater than 50% editing in preclinical studies in mice using ADAR-editing oligos delivered to hepatocytes via GalNAc-conjugation or LNP encapsulation, pharmacokinetics/dynamics observed with LNP delivery showed faster onset of editing as well as higher peak editing rates, which would require multiple doses to achieve from GalNAc-conjugated molecules.

Korro ran the Phase I/II REWRITE study of KRRO-110 in AATD patients harboring the Z-AAT allele (NCT06677307). Data presented pertained to both the SAD portion of the trial (data cutoff: November 6, 2025) in healthy volunteers dosed at 0.04, 0.1, 0.2, 0.4, 0.8, and 1.2 mg/kg (n=4 active dose, n=2 placebo per cohort) and to an open-label portion in AATD patients in two dose cohorts (0.6 mg/kg [n=3] and 0.8 mg/kg [n=4]). KRRO-110 demonstrated evidence of *Serpina1* RNA editing; all AATD patients with AAT levels above the turbidimetric assay's level of detection demonstrated an increase in AAT levels (n=7), and for every LC-MS sample measured to date (n=3 from the 0.8 mg/kg dose cohort), evidence of corrected M-AAT was present. However, robustness of editing was modest; the largest increase in total AAT observed was 3 μM, which led to a total of 10 μM AAT in that patient, below the 11 μM threshold considered to be within the asymptomatic carrier range of AAT levels and below that of peer clinical therapeutic development programs. LC-MS analysis revealed a maximum of 2 μM M-AAT, which was observed after days 2-4 post-dosing. These data, combined with functional neutralization data, suggest that *Serpina1* editing occurred but was insufficient to produce clinically meaningful amounts of M-AAT protein.

The company noted pitfalls related to KRRO-110's pharmacokinetics—specifically in AATD patients—that may have resulted in the disappointing RNA editing levels observed. In our follow-up, management specified that it observed a 40% reduction in the pharmacokinetic profile (a gross measurement based on multiple pharmacokinetic measurements of individual components of KRRO-110) and a roughly threefold faster rate of LNP dissipation in the AATD patient cohorts compared to the dosing in healthy volunteers, which showed PK profiles similar to those anticipated by the company based on preclinical experiments in mice and NHPs. The drug batches of KRRO-110 were the same across all cohorts, and management believes this could be an AATD human patient phenomenon. No treatment-emergent adverse events occurred, though mild to moderate infusion-related reactions (IRRs) were observed in two healthy volunteers at the highest dose cohort (1.2 mg/kg), as well as in two AATD patients at 0.6 mg/kg and two AATD

patients at 0.8 mg/kg. Management noted that the moderate IRRs, which occurred in the two healthy volunteers in the 1.2 mg/kg cohort, were resolved within 12-14 hours (see the press release here as well as our note: Third-Quarter Earnings; Further ADAR Editing Validation but KRRO-110 Disappointing on Profile; Downgrading to Market Perform).

Conclusion

The gene editing field is experiencing renewed momentum, evidenced by the approval of Casgevy, a robust pipeline of over 480 assets, and a plethora of near-term late-stage clinical readouts expected. We see the recent resurgence in financing and significant large-scale business development deals not just as a recovery, but as a strategic shift toward new technologies that prioritize safety but enable broader edit types with the ability to address more clinical applications.

We contend that the long-term value of gene editing lies beyond simple knockouts, which face increasing competition from safer, titratable RNA interference therapies. Instead, we see gene correction, precise DNA insertion, and durable gene upregulation as the differentiated applications for next-gen gene editors that justify the technology's risk profile. We continue to believe nonviral delivery will remain important for safety, potential redosing, and overcoming packaging constraints of AAV. Overall, our analysis suggests the gene editing field is at a significant inflection point after several years of market underperformance. Sponsors are now more focused not only on innovation but directing that innovation to tangible end-markets for their products. This enables logical value accretion and return on investment that we argue is relatively new to the gene editing field, which was historically ascribed a "platform valuation," and we are bullish on the space.

The prices (as of 3/30) of the common stock of other public companies mentioned in this report follow:

Alnylam Pharmaceuticals (Outperform)	$306.66
Bayer AG	$44.89
Biogen (Outperform)	$187.57
Bristol-Myers Squibb (Market Perform)	$59.73
CAMP4 Therapeutics (Outperform)	$3.97
Caribou Biosciences	$1.72
Cellectis SA	$3.21
CRISPR Therapeutics (Outperform)	$44.34
Editas Medicine	$2.23
Eli Lilly	$886.63
GSK	$27.17
Intellia Therapeutics (Outperform)	$11.66
Ionis Pharmaceuticals (Outperform)	$72.52
Krystal Biotech (Outperform)	$246.79
MeiraGTx Holdings	$7.65
Metagenomi Therapeutics	$1.26
Neurogene	$19.29
Novartis AG	$149.78
Novo Nordisk A/S	$35.85
Otsuka Corp.	$18.39
Pfizer	$28.32
Precision BioSciences	$8.12
Prime Medicine	$3.44
Regeneron Pharmaceuticals	$823.11
Regenxbio	$11.24
Roche Holding AG	$284.15
Sangamo Therapeutics	$0.92
Sanofi SA	$104.22
Stoke Therapeutics	$34.12
Vertex Pharmaceuticals (Outperform)	$412.56
Wave Life Sciences	$12.04

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