danaher

Scientific Exchange 2024: Advancing Gene Editing Platforms for Rare Diseases

November 20, 2024

2024 Scientific Exchange:
Objectives and approach

Objective

- Advance therapeutic development for rare conditions that are **scientifically feasible but commercially non-viable**

Approach

- Define repeatable gene editing platforms that **streamline development and regulatory requirements** across multiple therapies for **rare populations**[1]
- Understand **development and regulatory implications** from successful gene editing platforms

Benefits

- **Faster time to patient access**
- **Larger number of rare conditions with effective treatments**

Guardrails

- Maintain **favorable risk-benefit balance** for rare patients
- Ensure **appropriate regulatory rigor** maintained

1: Excluding oncology

Provisional Agenda (I)

Set up
9 – 9:30

- **Introductions, objectives, ground rules, logistics** (Galen/Atlantica)
- **Opening remarks and aspirations for the day** (ARM, ISCT, and Danaher)
- **Why is this topic important to the Agency?** (Peter Marks)

Problem definition & initial case study review
9:30-11:30

- **Gene editing platform components** (David Liu)
- **The need for sustainable gene editing models in rare indications** (Harry Malech and Vanessa Almendro Navarro)
- **Synthesis of opportunities from pre-meeting discussions:** Platform overview, case studies, common themes (G/A)
- **Case study #1: *ex vivo* Autologous T Cell Gene Editing** (Fyodor Urnov and Sadik Kasim)
  - Questions and reflection from attendees, including platform validation, needed evidence, and next steps
- **Case study #2: LNP *in vivo* Gene Editing Platform for Urea Cycle Disorder** (Kiran Musunuru)
  - Questions and reflection from attendees, including platform validation, needed evidence, and next steps

Breakout groups assignments, instructions, rapporteurs (Galen/Atlantica)

Working lunch

Consideration of gene editing platforms (case studies)
11:30 – 1:30

Technology-specific parallel breakout groups to explore gene editing platforms

- Assignment based on underlying gene editing technology
- Six additional case studies, three per breakout group
- Questions and reflection within each breakout group including platform validation, needed evidence, and next steps
- Rapporteur to summarize gene editing platform opportunities and challenges within each breakout group

Provisional Agenda (II)

Break

Case study outcomes, implications
2:00 – 3:30

Breakout group *rapporteurs* present findings

- Rapporteur presentations
- Additional observations from breakout group participants
- Questions from plenary

Galen / Atlantica: Test key opportunities across case studies

- Populate structured framework summarizing key opportunities across breakout groups
- Discuss and align on readiness, evidentiary needs, and next steps for promising opportunities

Break (if needed)

Next steps and closing
3:30 – 4:00

- **Closing reflections and key take-aways** (Nicole Verdun)
- **Roadmap for progress** (Galen/Atlantica)
- **Close**

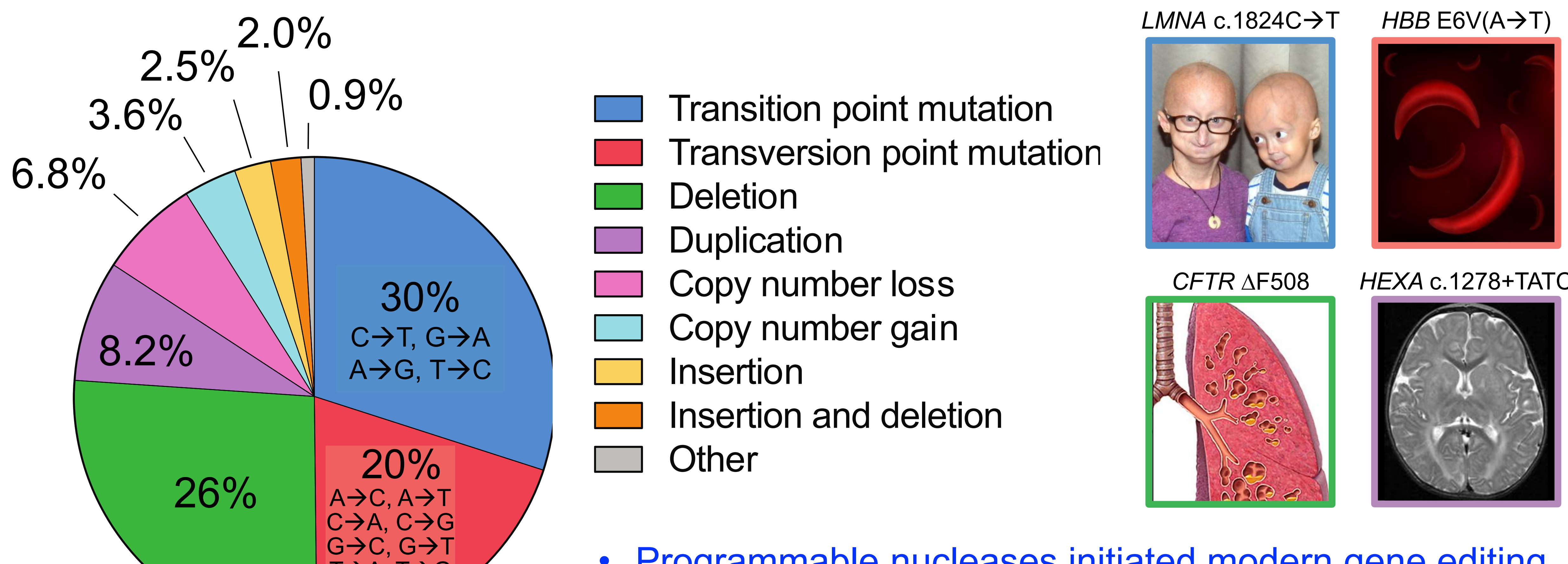
Current and Future Gene Editing Agents for Targeted Gene Correction and Integration in Cells, Animals, and Patients

David R. Liu Group
Merkin Institute, Broad Institute of MIT and Harvard
Department of Chemistry and Chemical Biology, Harvard University
Howard Hughes Medical Institute

Sammy Basso

December 1, 1995 –
October 5, 2024

Human Genetic Variants Associated with Disease



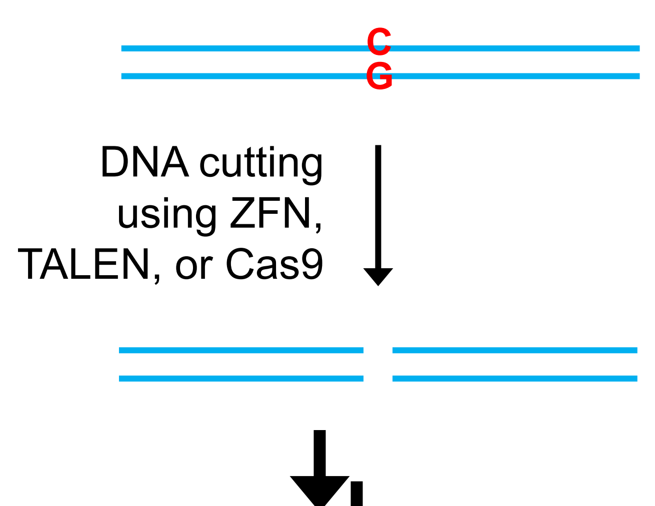
Total variants >100,000

- Programmable nucleases initiated modern gene editing and are well-suited for target gene disruption or deletion

ClinVar database: Landrum et al., *Nucl. Acids Res.* **44**, D862 (2016)

Programmable nucleases for editing: work of Klug, Pabo, Chandrasegaran, Bonas, Bogdanove, Charpentier, Church, Doudna, Jinek, Siksnys, Zhang *et al.*

Nucleases Are Well-Suited for Gene *Disruption*



End joining

..CACTGCGGCTGG--GTGGGGGTTAAAGC..
..CACTGCGGCTG----TGGGGGTTAAAGC..
..CACTGCGGCTGGAAGG---GGTTAAAGC..
..CACTGCGGCTG----TGGGGGTTAAAGC..
..CACTGCGGCTGG----GGGGGTTAAAGC..
..CACTGCGGCTGGG---GGGGGTTAAAGC..
..CACTGCGGCTGGA--TGGGGGTTAAAGC..

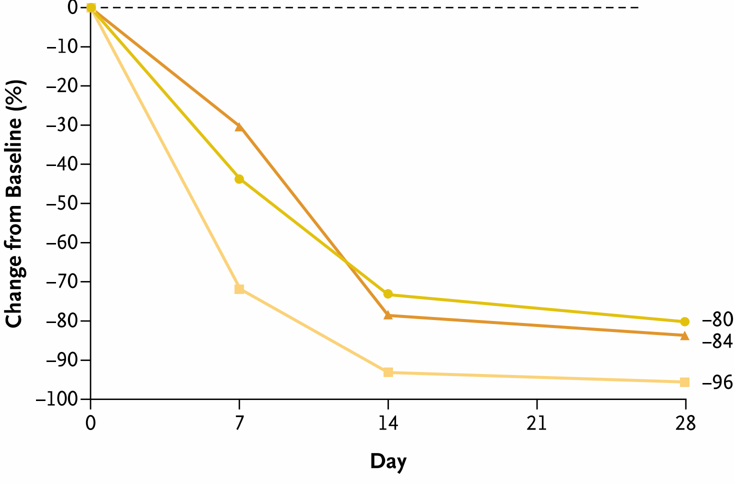
Mixture of insertions and deletions (indels)
Typically **disrupt** targeted genes

The NEW ENGLAND JOURNAL *of* MEDICINE

ESTABLISHED IN 1812 — AUGUST 5, 2021 — VOL. 385 NO. 6

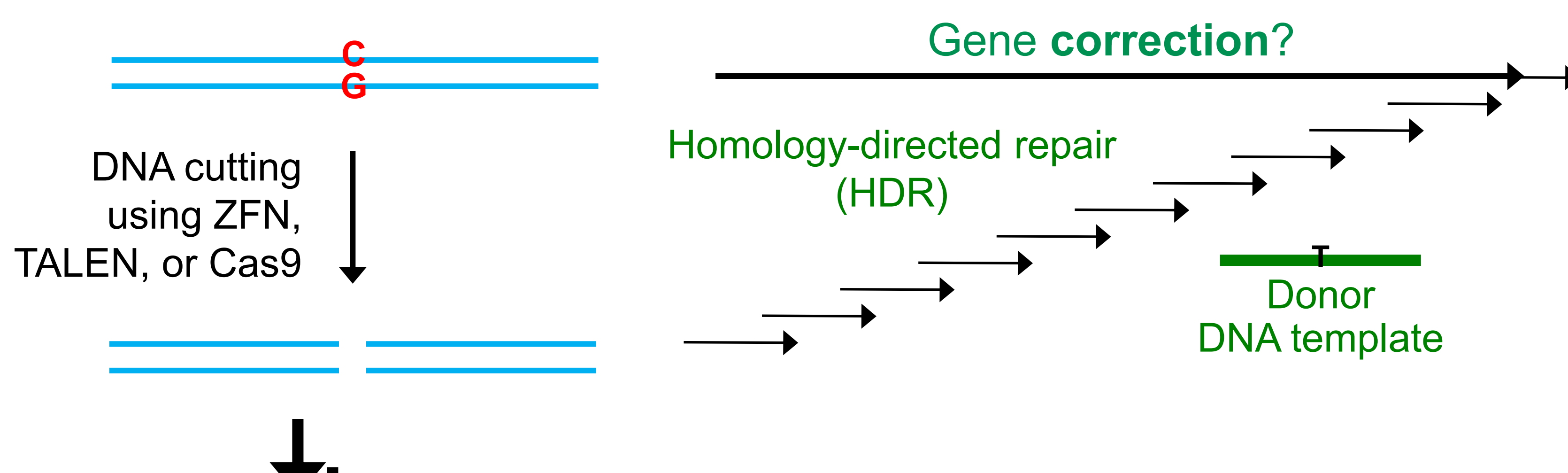
CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis

Julian D. Gillmore, M.D., Ph.D., Ed Gane, M.B., Ch.B., Jorg Taubel, M.D., Justin Kao, M.B., Ch.B., Marianna Fontana, M.D., Ph.D., Michael L. Maitland, M.D., Ph.D., Jessica Seitzer, B.S., Daniel O'Connell, Ph.D., Kathryn R. Walsh, Ph.D., Kristy Wood, Ph.D., Jonathan Phillips, Ph.D., Yuanxin Xu, M.D., Ph.D., Adam Amaral, B.A., Adam P. Boyd, Ph.D., Jeffrey E. Cehelsky, M.B.A., Mark D. McKee, M.D., Andrew Schiermeier, Ph.D., Olivier Harari, M.B., B.Chir., Ph.D., Andrew Murphy, Ph.D., Christos A. Kyratsous, Ph.D., Brian Zambrowicz, Ph.D., Randy Soltys, Ph.D., David E. Gutstein, M.D., John Leonard, M.D., Laura Sepp-Lorenzino, Ph.D., and David Lebwohl, M.D.



Frangoul *et al. N. Engl. J. Med.* **384**, 252–260 (2021); Gillmore *et al. N. Engl. J. Med* **385**, 493–502 (2021)
Cas9 editing: Jinek, Doudna, Charpentier *et al. Science* **337**, 816 (2012); Cong, Zhang *et al. Science* **339**, 819 (2013); Mali, Church *et al. Science* **339**, 823 (2013)

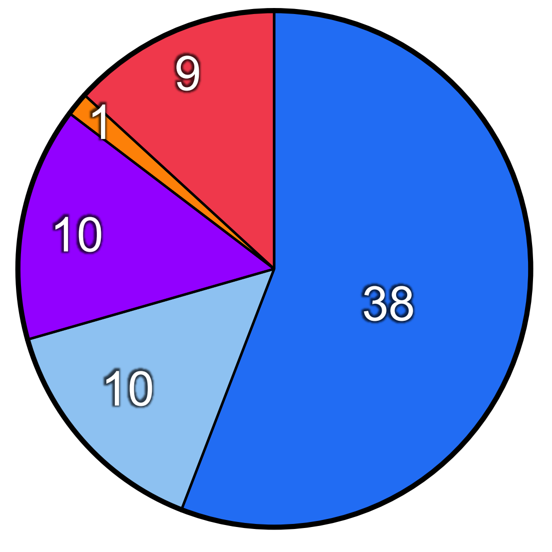
Nucleases Are Not Well-Suited For Gene *Correction*



Mixture of insertions and deletions (indels)
Typically **disrupt** targeted genes

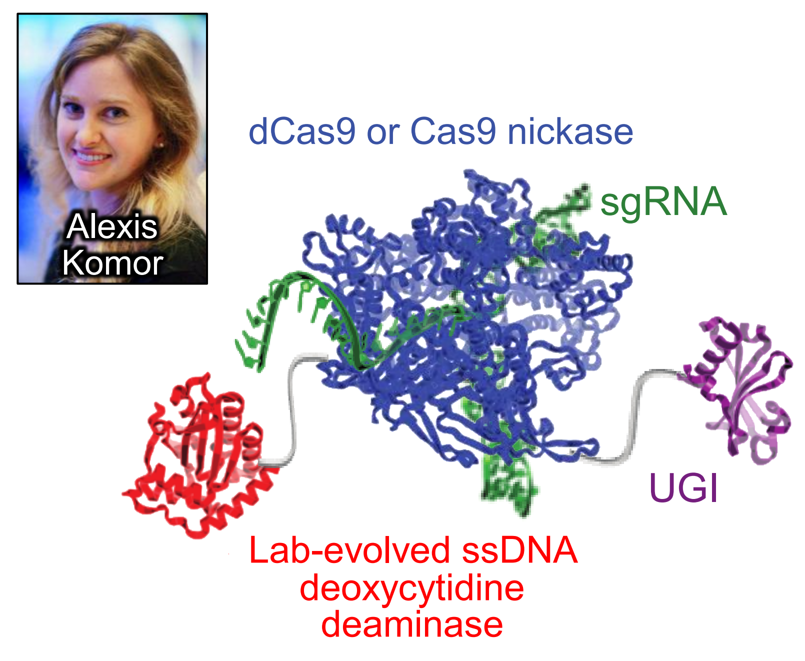
- HDR can correct genes, but is inefficient in most cell types
- Repair of double-strand breaks primarily results in gene disruption but also chromosomal abnormalities, allele loss, p53 activation, etc.

Therapeutic gene editing clinical trials (April 2024, n=68, clinicaltrials.gov)

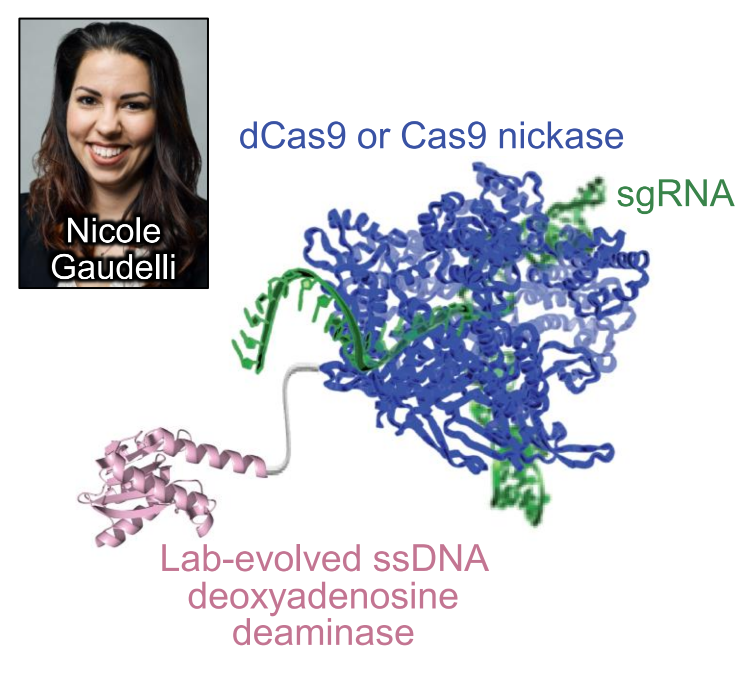


Rouet *et al. Mol. Cell. Biol.* **14**, 8096 (1994); Lukacsovich *et al. Nucl. Acids Res.* **22**, 5649 (1994); Choulika *et al. Mol. Cell. Biol.* **15**, 1968 (1995); Song *et al. Mol. Ther. Nucl. Acids* **21**, 523 (2020); Adikusuma *et al. Nature* **560**, E8 (2018); Kosicki *et al. Nat. Biotechnol.* **36**, 765 (2018); Stadtmauer *et al. Science* **367**, eaba7365 (2020); Turchiano *et al. Cell Stem Cell* **28**, 1136 (2021); Nahmad *et al. Nat. Biotechnol.* **40**, 1807 (2022)

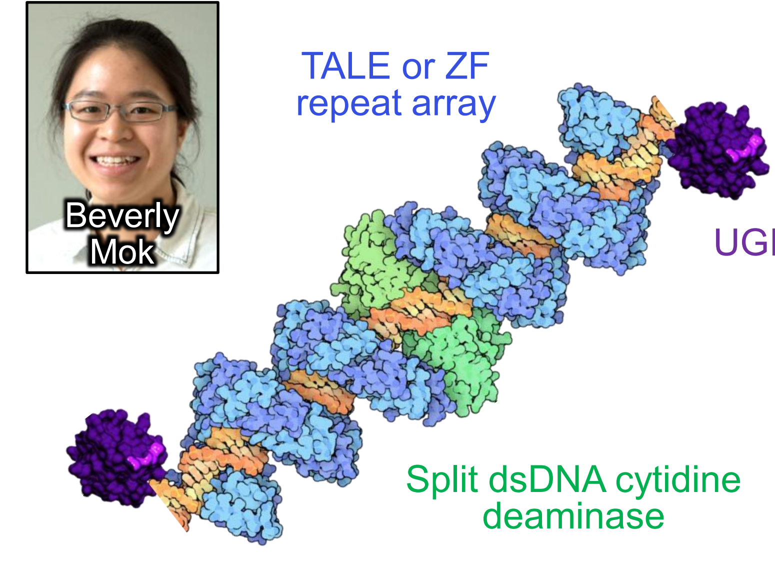
Base Editors Correct Point Mutations Without Requiring DSBs



Cytosine base editor (CBE)
C → T, G → A



Adenine base editor (ABE)
A → G, T → C



Mitochondrial base editors
C → T, G → A, A → G, T → C

- Base editors install or correct the four most common types of point mutations at targeted DNA sites without requiring DSBs or donor DNA templates

CBE: Komor, Kim, Packer, Zuris, Liu *Nature* **533**, 420 (2016); Nishida, Kondo *et al. Science* **353**, aaf8729 (2016); ABE: Gaudelli, Komor, Rees, Packer, Badran, Bryson, Liu *Nature* **551**, 464 (2017); TALE-DdCBE: Mok, de Moraes, Mootha, Mougous, Liu *et al. Nature* **583**, 631 (2020); TALE-ABE: Cho, Kim *et al. Cell* **185**, 1764 (2022); ZF-DdCBE: Willis, Liu *et al. Nat. Commun.* **13**, 7204 (2022); Lim, Kim *et al. Nat. Commun.* **13**, 366 (2022)

Ex Vivo and *In Vivo* Therapeutic Base Editing in Animal Models

nature

Base editing of haematopoietic stem cells rescues sickle cell disease in mice

https://doi.org/10.1038/s41586-021-03609-w

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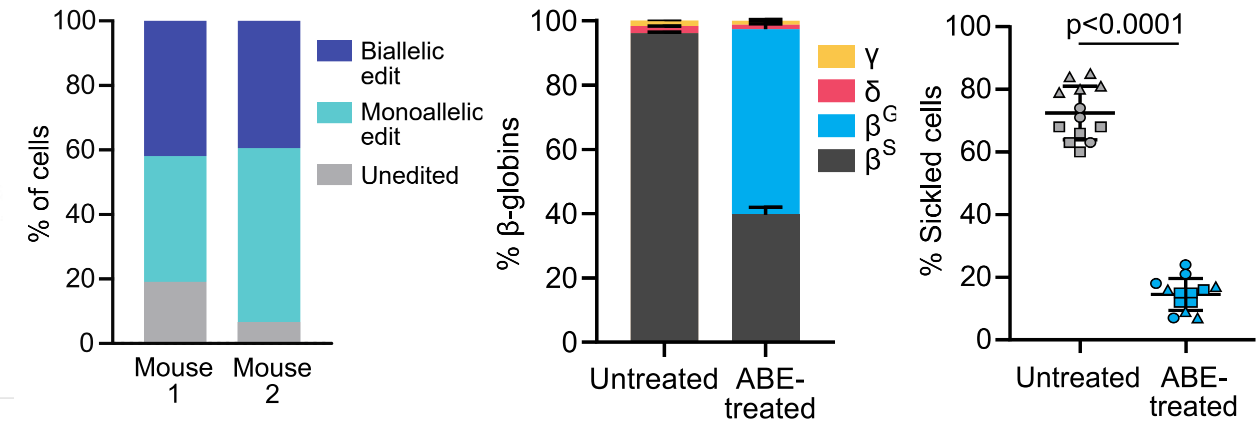
Published online: 2 June 2021

Gregory A. Newby[1,2,3,12], Jonathan S. Yen[4,12]✉, Kaitly J. Woodard[4,12], Thiyagaraj Mayuranathan[4,12], Cicera R. Lazzarotto[4], Yichao Li[4], Heather Sheppard-Tillman[5], Shaina N. Porter[6], Yu Yao[4], Kalin Mayberry[4], Kelcee A. Everette[1,2,3], Yoonjeong Jang[4], Christopher J. Podracky[1,2,3], Elizabeth Thaman[7], Christophe Lechauve[4], Akshay Sharma[8], Jordana M. Henderson[9], Michelle F. Richter[1,2,3], Kevin T. Zhao[1,2,3], Shannon M. Miller[1,2,3], Tina Wang[1,2,3], Luke W. Koblan[1,2,3], Anton P. McCaffrey[9], John F. Tisdale[10], Theodosia A. Kalfa[7,11], Shondra M. Pruett-Miller[6], Shengdar Q. Tsai[4], Mitchell J. Weiss[4]✉ & David R. Liu[1,2,3]✉



nature

In vivo base editing rescues Hutchinson–Gilford progeria syndrome in mice

https://doi.org/10.1038/s41586-020-03086-7

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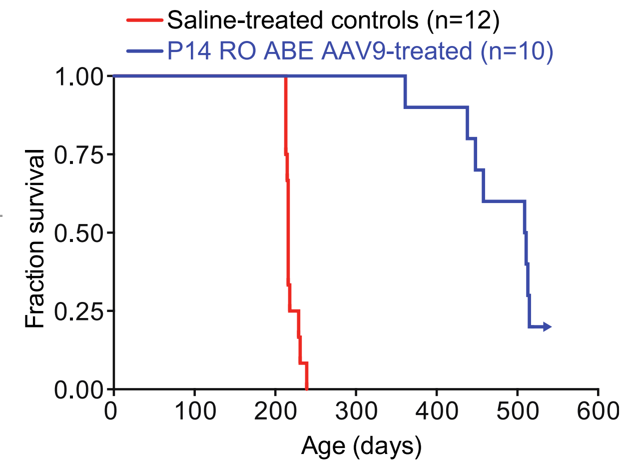
Published online: 06 January 2021

Luke W. Koblan[1,2,3,13], Michael R. Erdos[4,13], Christopher Wilson[1,2,3], Wayne A. Cabral[4], Jonathan M. Levy[1,2,3], Zheng-Mei Xiong[4], Urraca L. Tavarez[4], Lindsay M. Davison[5], Yantenew G. Gete[6], Xiaojing Mao[6], Gregory A. Newby[1,2,3], Sean P. Doherty[5], Narisu Narisu[4], Quanhu Sheng[7], Chad Krilow[4], Charles Y. Lin[8,9,12], Leslie B. Gordon[10,11], Kan Cao[6], Francis S. Collins[4]✉, Jonathan D. Brown[5]✉ & David R. Liu[1,2,3]✉



Untreated progeria mouse
7.5 months old

ABE-treated progeria mice
11 months old

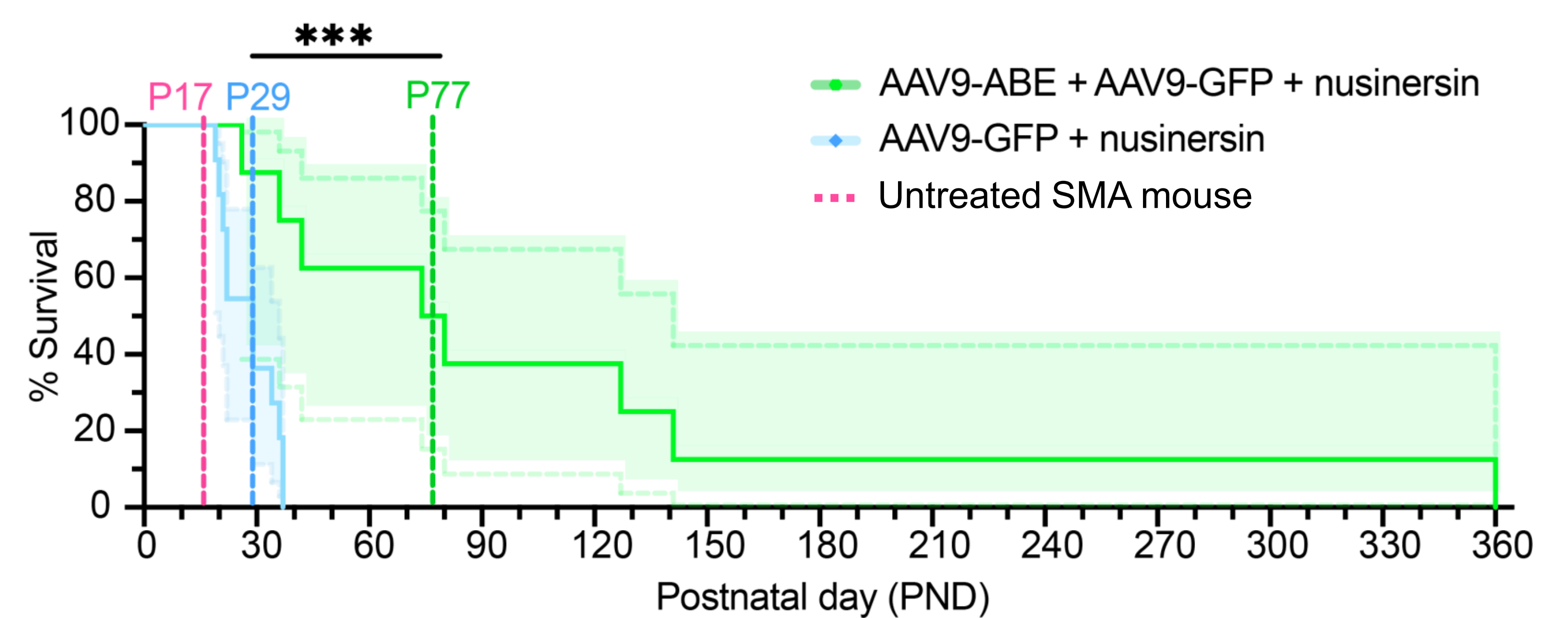
Newby, Yen, Woodard, Mayuranathan, Weiss, Liu *et al.* *Nature* **595**, 295 (2021)
See also Chu, Packer, Rees, Slaymaker *et al.* *CRISPR J.* **4**, 169 (2021)
Koblan, Erdos, Wilson, Cabral, Levy, Xiong, Tavaraz, Davison, Gete, Mao, Newby, Lin, Gordon, Cao, Collins, Brown, Liu *et al.* *Nature* **589**, 608 (2021)

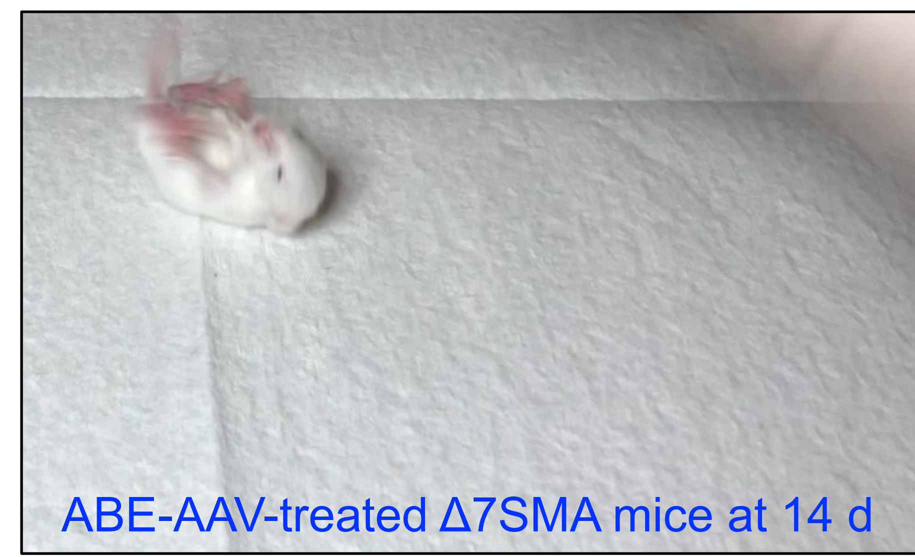
In Vivo Base Editing Rescues Spinal Muscular Atrophy in Mice

Science

Base editing rescue of spinal muscular atrophy in cells and in mice

Mandana Arbab[1,2,3,4]†, Zaneta Matuszek[3,4,5]†, Kaitlyn M. Kray[6], Ailing Du[7], Gregory A. Newby[3,4], Anton J. Blatnik[6], Aditya Raguram[3,4], Michelle F. Richter[3,4], Kevin T. Zhao[3,4], Jonathan M. Levy[3,4], Max W. Shen[3,4,8], W. David Arnold[9,10], Dan Wang[7,11], Jun Xie[7], Guangping Gao[7,12], Arthur H. M. Burghes[6], David R. Liu[3,4,13]*





Arbab, Matuszek, Kray, Du, Gao, Burghes, Liu *et al. Science* **380**, eadg6518 (2023)

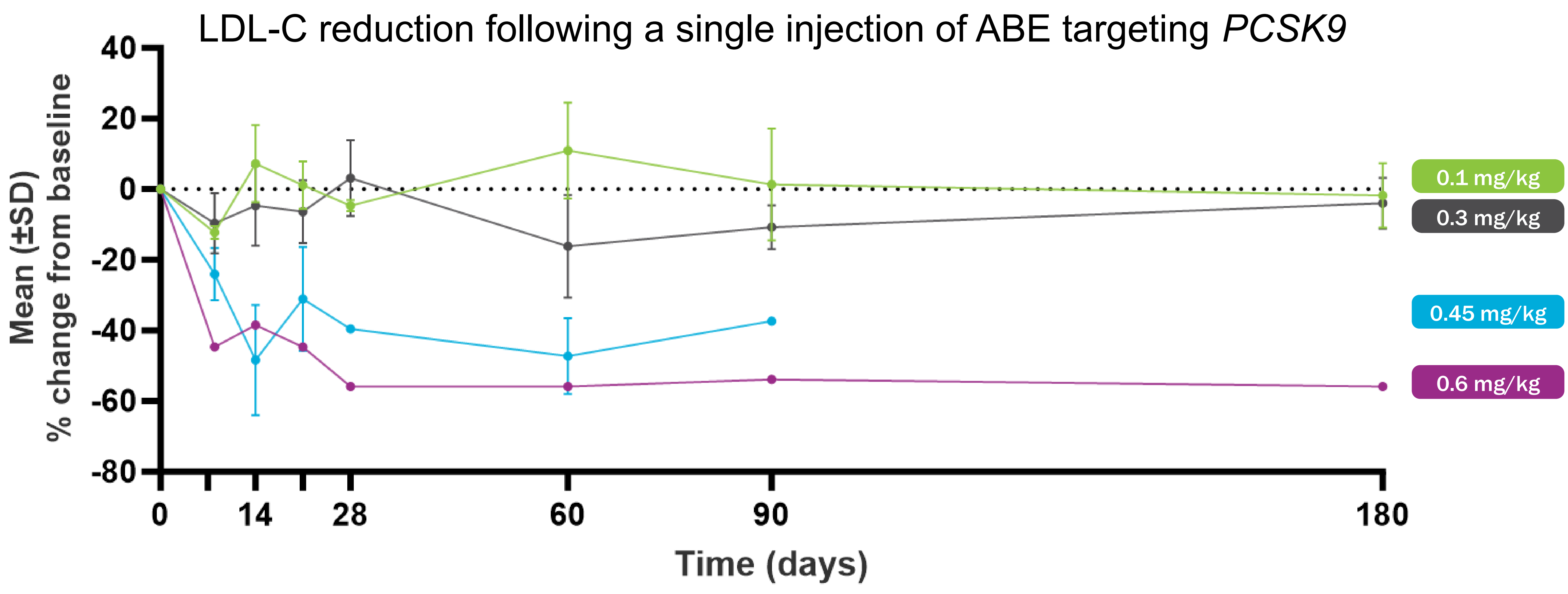
Base Editing Clinical Trials and First *Ex Vivo* Outcomes

Candidate	Target	Status
BEAM-101	*HBG* for SCD & β-thalassemia	Phase 1/2 US
TvT UCL/GOSH (Qasim lab)	Multiplex-edited CAR-T for T-cell leukemia	Phase 1 UK **readouts**
VERVE-101 VERVE-102	*PCSK9* for familial heart disease	Phase 1b NZ US UK CA **readouts**
BEAM-201	Multiplex-edited CAR-T ALL/AML	Phase 1/2 US
CARAML UCL/GOSH	Multiplex-edited CAR-T for AML	Phase 1 UK
BRL-103 (BioRay Labs)	*HBG* for β-thalassemia	Phase 1/2 CN
CS-101 (CorrectSeq)	*HBG* for β-thalassemia	Phase 1 CN **readouts**
BEAM-302	*AAT* for alpha-1 antitrypsin defic.	Phase 1/2 UK
U.S. NIAID	*CYBB* for chronic granulomatous dis.	Phase 1/2 US
BEAM-301	*G6Pase* for glycogen storage disease type 1a	Phase 1/2 US

- Alyssa, a 13-year-old T-cell acute lymphoblastic leukemia patient
- Chemotherapy and bone-marrow transplant failed
- Given T-cells triply base edited at *CD7*, *CD52*, *TCR* with αCD7-CAR
- Complete remission by 1 month, persisting >24 months

Chiesa, Qasim *et al.*, *Blood* **140**, 4579 (2022); Chiesa, Qasim *et al.*, *N. Engl. J. Med.* **389**, 899 (2023); photo: BBC

First *In Vivo* Base Editing Clinical Trial Outcomes



- ABE programmed to install a splice site mutation in *PCSK9* delivered as mRNA via LNP injection
- *In vivo* base editing in human patients results in durable reduction of LDL cholesterol levels

American Heart Association presentation by Verve Therapeutics November 12, 2023; Musunuru *et al. Nature* **593**, 429 (2021)

Prime Editing Uses Nicked Target DNA to Prime Reverse Transcription of Edited Sequence Encoded in a pegRNA

Anzalone, Liu, and co-workers *Nature* **576**, 149 (2019); U.S. patents 11,447,770, 11,643,652, 11,795,452, 11,912,985

Advancement of Prime Editing Systems

Stabilize pegRNAs with 3' motif (epegRNAs) or RNA-binding proteins (La)

Nelson, Randolph, Liu *et al.* *Nat. Biotechnol.* **40**, 402 (2022); Yan, Adamson *et al.* *Nature* **628**, 639 (2024)

Evolved reverse transcriptase and Cas domains (PE6)

Doman, Pandey, Osborn, Liu *et al.* *Cell* **186**, 3983 (2023)

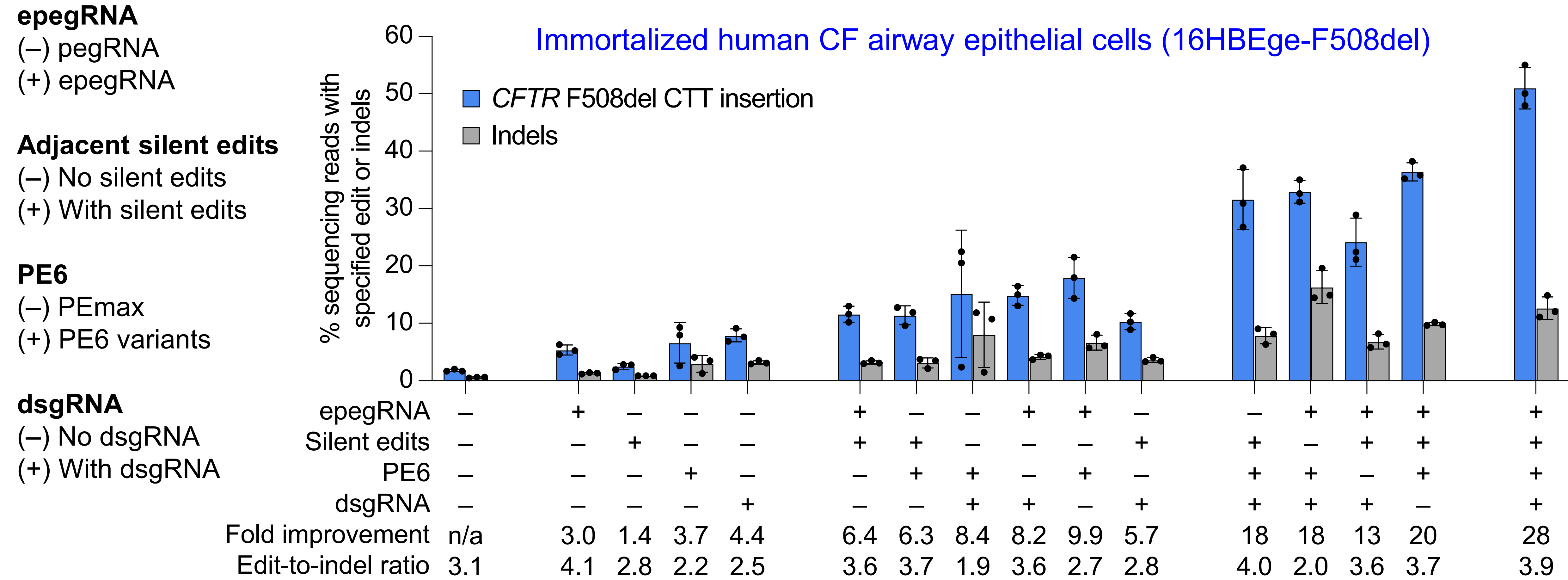
Mismatch repair evasion (silent edits, PE4/PE5)

Chen, Hussmann, Adamson, Liu *et al.* *Cell* **184**, 5635 (2021)

Protein architecture, nuclear localization signals, codon usage (PEmax)

Chen, Hussmann, Adamson, Liu *et al.* *Cell* **184**, 5635 (2021)

Prime Editing Improvements Enable Efficient, Precise Correction of *CFTR* Fdel508



- Insights into spacer, PBS, RTT, epegRNAs, PEmax architecture, mismatch repair evasion, strategic silent edits, evolved PE6 variants, and dsgRNAs enable efficient, direct correction of *CFTR* Fdel508

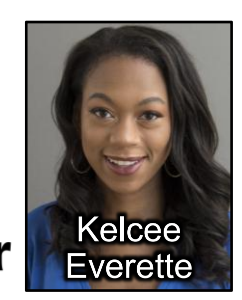
Sousa, Hemez, Lei, McCray, Liu *et al. Nat. Biomed. Eng.* **in press, online** (2024); dsgRNAs: Kiani *et al. Nat. Meth.* **12**, 1051 (2015) & Park *et al. Genome Biol.* **22**, 170 (2021)

Ex Vivo and *In Vivo* Therapeutic Prime Editing in Animals

nature biomedical engineering

Ex vivo prime editing of patient haematopoietic stem cells rescues sickle-cell disease phenotypes after engraftment in mice

Kelcee A. Everette[1,2,3], Gregory A. Newby[1,2,3], Rachel M. Levine[4], Kalin Mayberry[4], Yoonjeong Jang[4], Thiyagaraj Mayuranathan[4], Nikitha Nimmagadda[4], Erin Dempsey[4], Yichao Li[4], Senthil Velan Bhoopalan[4], Xiong Liu[5], Jessie R. Davis[1,2,3], Andrew T. Nelson[1,2,3], Peter J. Chen[1,2,3], Alexander A. Sousa[1,2,3], Yong Cheng[4], John F. Tisdale[5], Mitchell J. Weiss[4], Jonathan S. Yen[4] & David R. Liu[1,2,3]



HBB^S

Met Val His Leu Thr Pro Val Glu Lys

5′ ACC ATG GTG CAC CTG ACT CCT GTG GAG AAG 3′

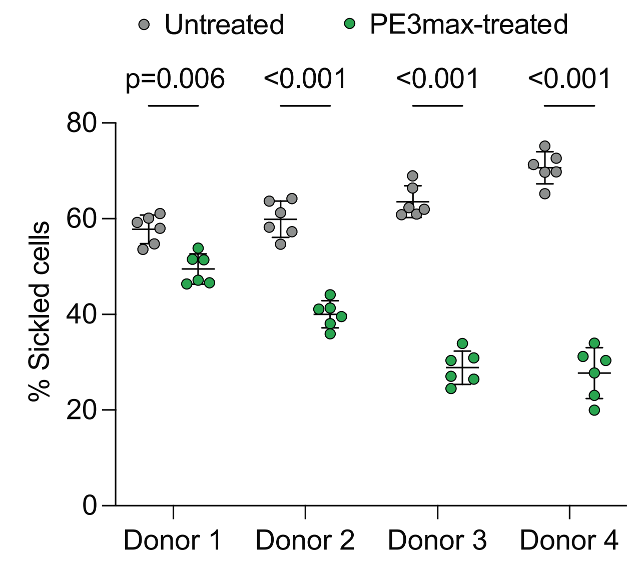
Protospacer — PAM

SCD reversion edit | Silent PAM edit

5′ ACC ATG GTG CAC CTG ACT CCT GAA GAG AAG 3′

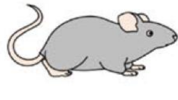
Met Val His Leu Thr Pro Glu Glu Lys

HBB^A



Vision rescue via unconstrained in vivo prime editing in degenerating neural retinas

Huan Qin[1*], Wenliang Zhang[1*], Shiyao Zhang[1*], Yuan Feng[1*], Weihui Xu[1], Jia Qi[1], Qian Zhang[1], Chunxiu Xu[1], Shanshan Liu[1], Jia Zhang[1], Yushuang Lei[1], Wanqin Liu[1], Shuyu Feng[1], Jingjing Wang[1], Xuefei Fu[1], Zifen Xu[1], Ping Li[1], and Kai Yao[1]



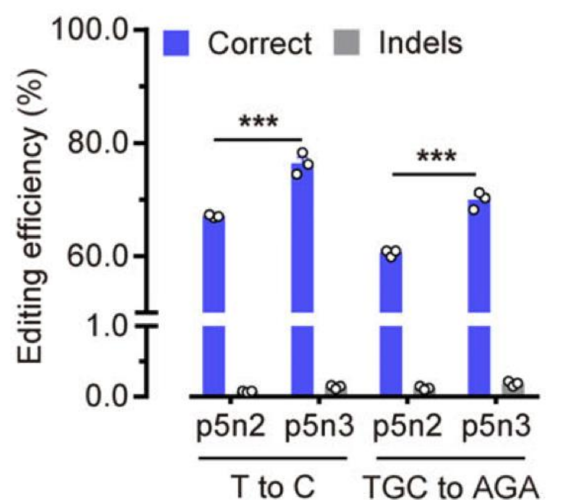
Pde6b^rd10

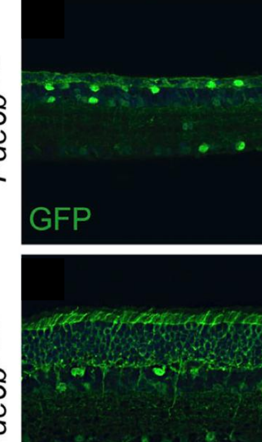
Control: PE^SpRY + gScrambled-GFP

Corrected: PE^SpRY + g*Pde6b*-GFP

subretinal injection → Analysis

P14 → P120





Everette, Newby, Weiss, Yen, Liu *et al. Nat. Biomed. Eng.* **7,** 616 (2023); Qin, Yao *et al. J. Exp. Med.* **220**, e20220776 (2023)

Prime Editing First U.S. IND Clinical Trial Clearance

- Chronic granulomatous disease (CGD): primary immunodeficiency caused by mutations in subunits of NADPH oxidase complex
- Hundreds of patients in the U.S.
- Median age of death (2017): 30-40 years, with progressive debilitation from infection, inflammation, autoimmunity
- Only cure currently: allogeneic bone-marrow transplant (GvH risk)

Prime editing preserves engraftment potential of long-term HSCs in mice

Bone marrow engraftment (% human CD45⁺)
100
80
60
40
20
0
Mock treatment
Prime edited

Long-term engrafted HSCs maintain correction of *NCF1* ΔGT

% precise correction
100
80
60
40
20
0
Therapeutic threshold
Mock treatment
Prime edited

NADPH oxidase rescued in neutrophils from edited HSCs

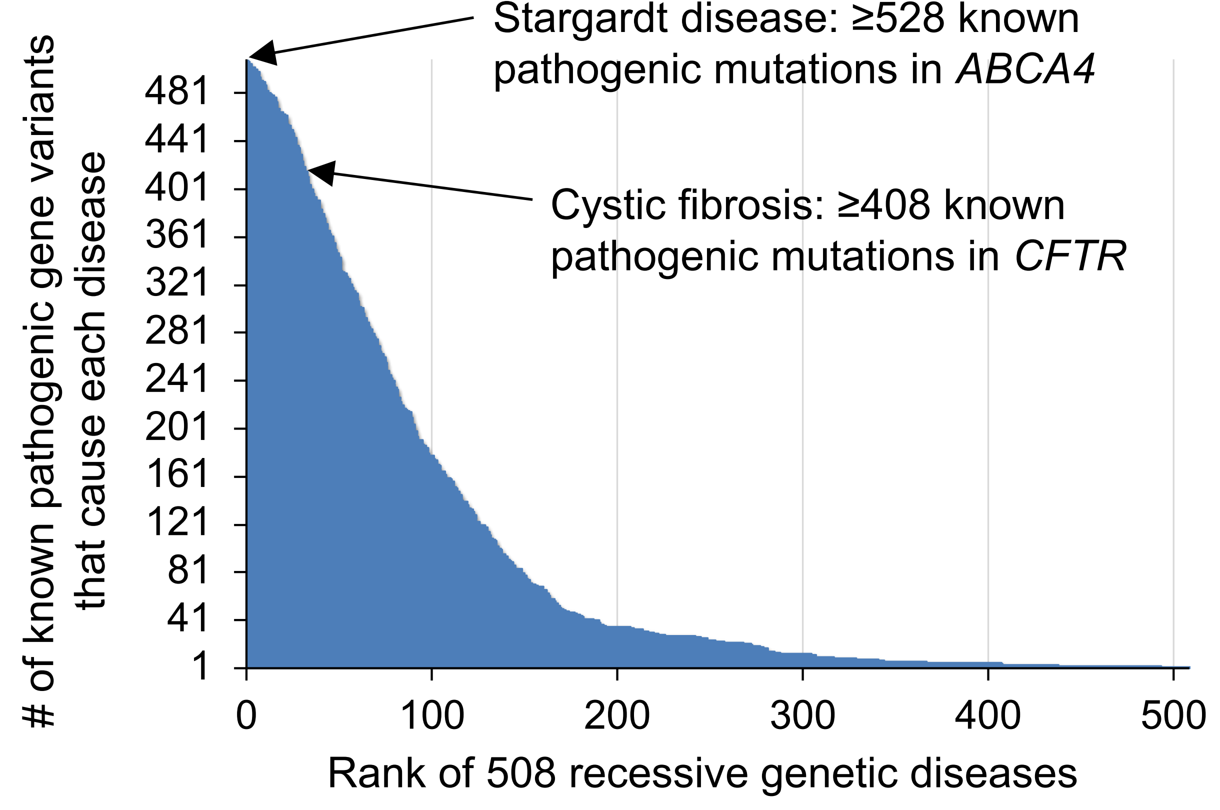
% DHR-positive cells
100
80
60
40
20
0
Mock treatment
Prime edited

No detected off-target edits in human HSCs

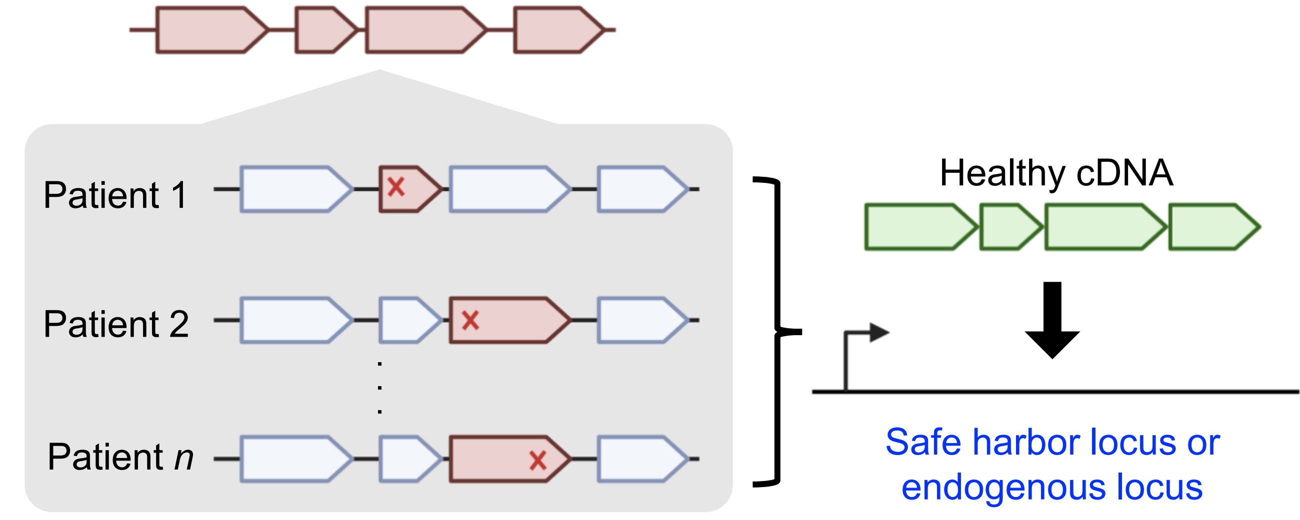
% indels
40
30
20
10
2.0
1.5
1.0
0.5
0.0
• Target (*NCF1*)
chr1 chr2 chr3 chr4 chr5 chr6 chr7 chr8 chr9 chr10 chr11 chr12 chr13 chr14 chr15 chr16 chr17 chr18 chr19 chr20 chr21 chr22 chrX

Prime Medicine (Apr 29, 2024); CGD review: Arnold *et al. Adv. Ther.* **34**, 2543 (2017); therapeutic threshold: Marciano *et al. J Allergy Clin Immunol.* **141**, 365 (2018)

Some Genetic Diseases Are Caused by Many Mutations



- A major drug development, manufacturing, and regulatory challenge that limits patient benefit

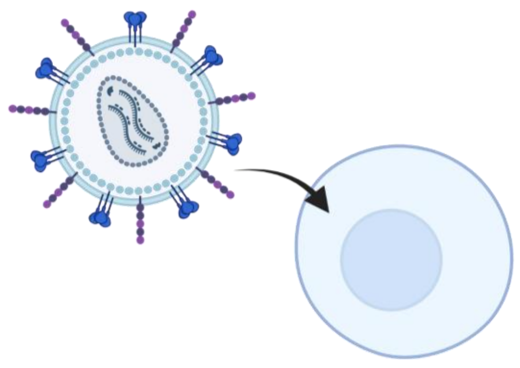


- Patients suffering from a loss-of-function genetic disease *regardless of their specific mutation* could benefit from a single treatment that introduces a healthy gene copy into their cells

Data from Xiao and Lauschke *NPJ Genom. Med.* **6**, 41 (2021)

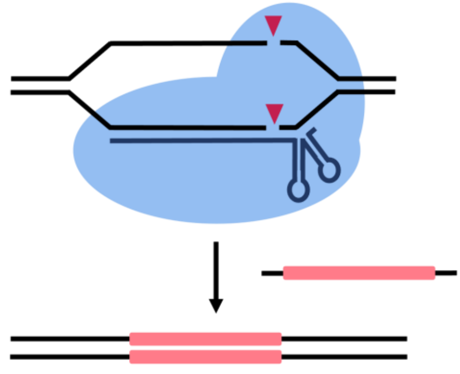
An Old and New Opportunity

Viral introduction of healthy gene copies



- Not targeted to native locus
- Toxicity from viral vector
- Risk of oncogenic integration
- If non-integrating, how to redose?

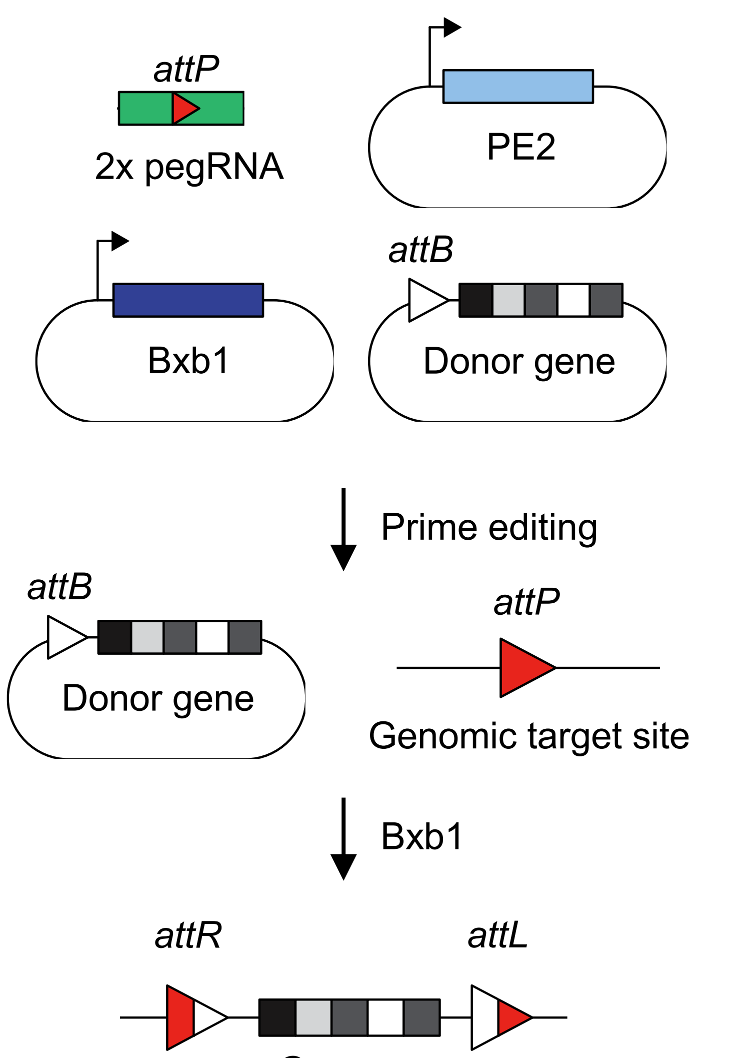
Nuclease-mediated HDR or end-joining



- Requires DSBs and associated risks
- Low HDR efficiency in most cell types
- Generates mixtures of indels, insert orientations, and insert copy numbers

- Ideally: efficient integration of gene-sized DNA at any genomic target site without requiring DSBs

Targeted Gene Integration in Human Cells by PASSIGE (Prime Editing + Recombinase)



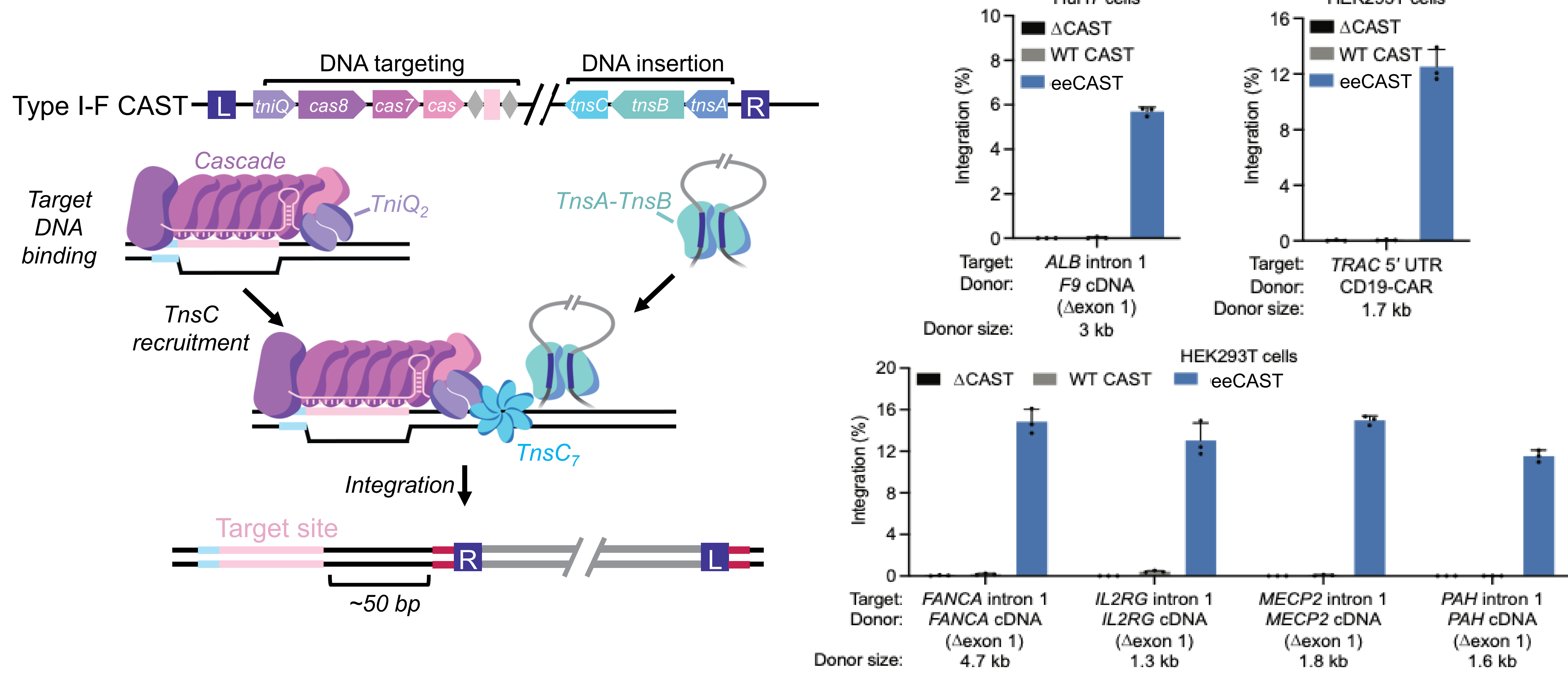
PASSIGE   eePASSIGE   PASTE   no prime editor (+eeBxb1)

% Integration

	HEK293T cells					N2a cells
Locus:	*FANCA*	*GBA1*	*ALB*	*B2M*	*TRAC*	*Smn1*
Donor:	*FANCA* cDNA (Δ Exon 1)	*GBA1* cDNA (Δ Exon 1)	*Factor IX* cDNA (Δ Exon 1)	CD19-CAR	CD19-CAR	*Smn1* cDNA (Δ Exon 1)
Donor size:	8.8 kB	6.1 kB	7.1 kB	5.9 kB	5.9 kB	5.3 kB
Landing site:	*attP* in intron 1	*attP* in intron 1	*attP* in intron 1	*attP* in 5'UTR	*attP* in 5'UTR	*attP* in intron 1

Pandey, Gao, Osborn, and coworkers *Nat. Biomed. Eng.* **in press, online** (2024)

Targeted Gene Integration in Human Cells by Evolved CASTs



With Isaac Witte, George Lampe, Simon Eitzinger, Shannon Miller, Sam Sternberg, and coworkers (2024); Type I CAST in bacteria: Klompe, Sternberg *et al. Nature* **571**, 219

Summary: Mammalian Cell Gene Editing Technologies

"We the People of the United States, in Order to form g more perfeat Union…"

Nuclease editing	"We the People of the United States, in Orderimperfeat Union..."	• Robust for gene disruption/deletion • On-target edits are indel mixtures of many sequences • Cannot *correct* mutations w/o HDR & donor DNA • Induces undesired consequences of DSBs
Base editing	"We the People **of the United States, in Order to form** a more perfeat Union…"	• Robust for transition mutation correction or disruption • On-target edits are primarily one product • Does not require DSBs or donor DNA • Bystander editing stymies editing of some targets • Cas-independent off-target deamination for older BEs
Prime editing	"We the People of the United States, in Order to form a more perfect Union…" ***or*** "We the People of the United States, in Order to form a ~~more~~ perfect and resilient Union…"	• Highly versatile: substitutions, insertions, deletions • On-target edits are primarily one product • Does not require DSBs or donor DNA • Mechanism inherently resists off-target editing • Larger size, pegRNA optimization often required

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http://liugroup.us, @davidrliu

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Prime editing animation
by David Bolinsky
and Prime Medicine

Harry Malech

2024 Scientific Exchange: Gene editing platforms

Facilitating Gene Therapy for Rare Genetic Diseases

Harry L. Malech, MD
National Institute of Allergy and Infectious Diseases
National Institutes of Health

My comments and opinions are my own and I do not represent NIAID nor NIH policy during my participation in the 2024 Scientific Exchange.

My area of expertise and experience is with the development and application of lentivector and gene editing technology, including the conduct of clinical trials of gene therapy for patients with inborn errors of immunity.

Rare diseases often share a genetic component or origin

Estimated range in frequency and absolute number of various disease classes[3]

Lifetime prevalence	Disease class
1	Flu virus
~10^{-2}	Autoimmune
~10^{-4}	Leukemia
10^{-3}–10^{-5}	Monogenic X-linked
10^{-4}–10^{-10}	Monogenic Autosomal

Lifetime prevalence axis: 1, 10^{-2}, 10^{-4}, 10^{-6}, 10^{-8}, 10^{-10}; line labelled Rare[2]

# of diseases axis: 10^2, 10^4, 10^8

>10,000+ rare diseases, with 250 novel diseases added annually

EURORDIS estimates 3.5-5.9% of the world's population affected by rare diseases.

>500 Inborn Errors of Immunity[4]
(My area of clinical expertise.)

80%

of rare diseases have a genetic component[1]

1: Rare Genomics Institute; and Editorial, The landscape for rare diseases in 2024. The Lancet Global Health. 2024 March, 12(3):e341.
2: Rare disease definition vary by geography but generally definitions ranging from 1/1,000 to 1/2,000 people, with 200K patient in the US; Ultrarare lacks a standard definition but is sometimes defined as 1/50,000 or 1/100,000 people. These materials focus on rare conditions that are scientifically feasible but not commercially viable – a threshold of 10,000 affected US patients is sometimes used as a threshold for viability
3: Adapted from Estimating the number of diseases – the concept of rare, ultra-rare, and hyper-rare - PMC (nih.gov); A call to arms against ultra-rare diseases | Nature Biotechnology
4: Tangye SG, et al. Human Inborn Errors of Immunity: 2022 Update on the Classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol. 2022 Jun 24;42(7):1473–1507.

The state of play for rare disease therapeutic progress

Gene therapies, particularly gene editing are among the most promising therapeutic modalities to treat rare diseases[3], with high technical feasibility for targeted interventions of monogenic diseases

Despite this, <5% of rare disease patients have effective treatments

Medicines with fewer than 10,000 patients in the U.S. struggle to be commercialized

The NIH, academic labs, and hospital centers play an important role in advancing rare disease treatments, but operate at a smaller R&D scale and may stop short of full licensure for a rare disease therapy,

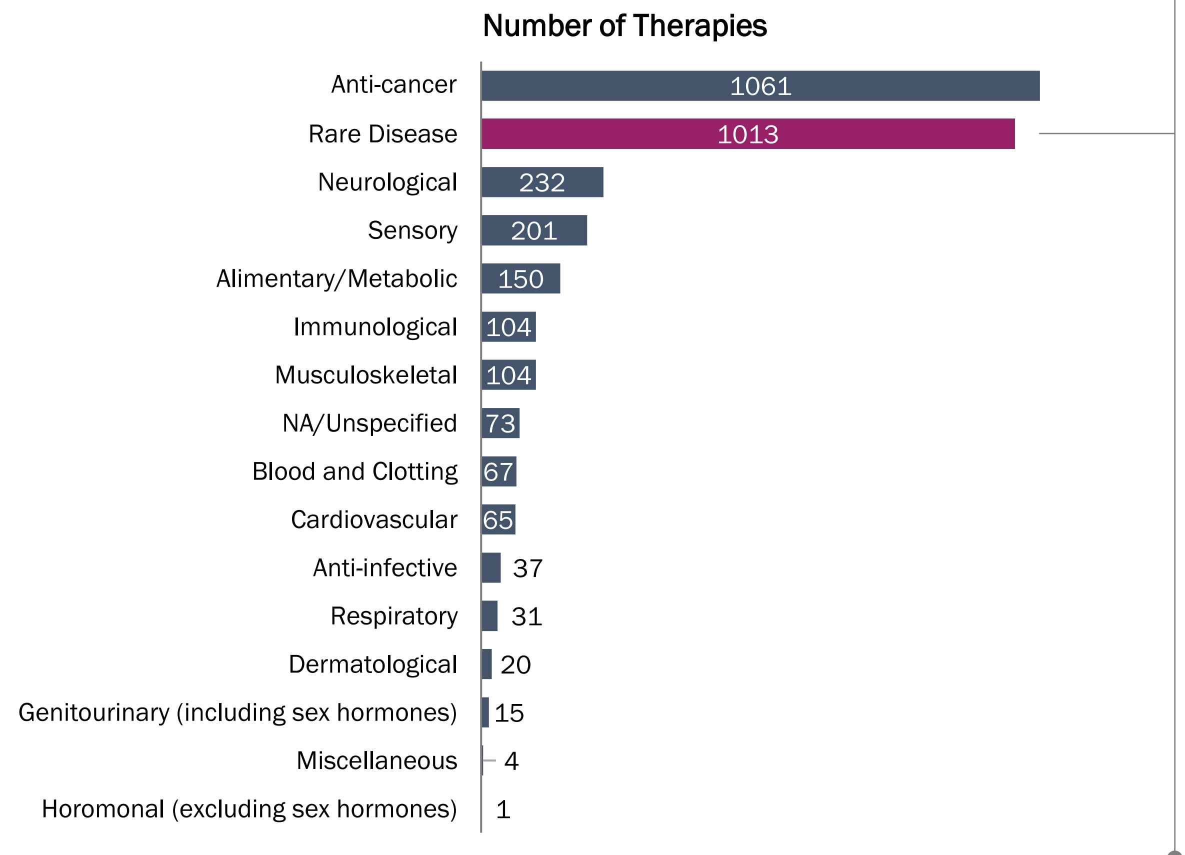
Many experts believe that very small disease areas may never be commercially viable targets for pharmaceutical and biotech developers without new, significant incentives or development approaches

Source: Patent-to-Launch Time for Orphan Drugs is 2.3 Years Longer (globenewswire.com); NORD; Can you hear us now? The impact of health-care utilization by rare disease patients in the United States | Genetics in Medicine (nature.com); Realizing the Potential of Gene Therapies for Rare and Ultra-Rare Inherited Diseases | Human Gene Therapy (liebertpub.com); Microsoft Word - adapations of value framework for URD 111317

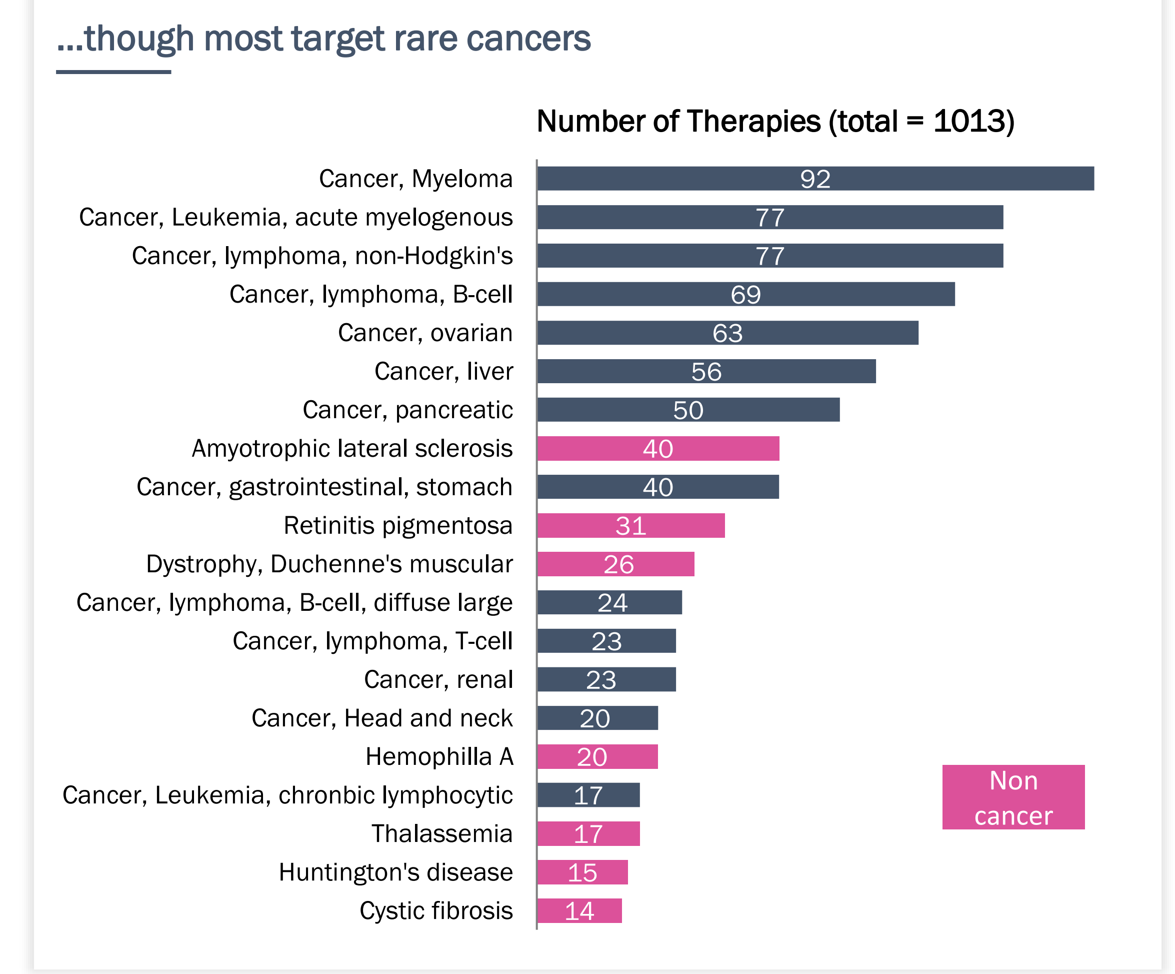
danaher

Gene therapy initiatives disproportionately target cancers and more ‘common’ rare conditions

1013 gene therapy trials target rare disease indications…



…though most target rare cancers



Note: Figures based on indications in pipeline development only for each therapy
Source: Pharmaprojects| Citeline, October 2024

The Goal and Promise of Today's 2024 Scientific Exchange:

Innovative platform approaches to gene editing integrated with innovative approaches to regulatory review will facilitate development and approvals of treatments for rare diseases.

While the focus of today's meeting is on industry, non-profit academic approaches to achieve gene therapy licensure such as the European **AGORA** initiative, the **Global Gene Therapy Initiative, the Bespoke Gene Therapy Consortium**, and the new **ARPA-H DECIDE-ET** initiative will also benefit from consensus outcomes of today's meeting.

https://www.rarediseaseadvisor.com/features/agora-ggti-consortia-strive-improve-global-access-expensive-gene-therapies/

https://ncats.nih.gov/research/research-activities/BGTC

https://arpa-h.gov/news-and-events/arpa-h-launches-exploration-topic-improve-access-life-changing-cellular-therapies

Alliance for Regenerative Medicine

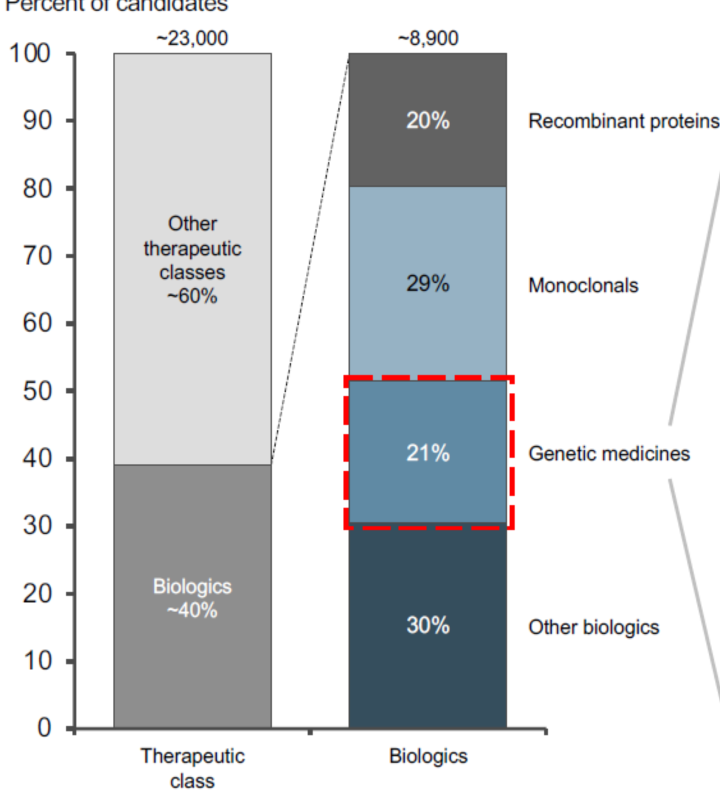
Vanessa Almendro Navarro

Platforming gene editing technologies

Innovation at the speed of life.

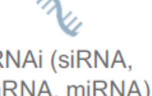
Genomic Therapies Hold Potential to Target Disease-Causing Mechanisms…

WW drug candidate pipeline by therapeutic class (2020)
Percent of candidates



Note: *Other biologics include non-gene edited cell therapy, oncolytic viruses, etc.
Source: L.E.K. research and analysis, PharmaProjects, BIS Research

Antisense / aptamer oligos
- Short DNA or RNA that bind and inhibit or alter gene expression via steric hindrance, splicing alterations, or initiation of target degradation



RNAi (siRNA, shRNA, miRNA)
- Short RNA molecules (siRNA or miRNA) that degrade mRNA transcripts through the Dicer / RISC complex

In vivo gene editing
- DNA / protein complexes (CRISPR-cas9, ZFN) that enable direct editing of the genome to address faulty proteins in the patient

Gene therapy (mRNA)
- Longer RNA strands which directly induce protein expression in diseases with non-functional proteins

DNA / RNA vaccines
- DNA / RNA administered to patients for expression of disease specific antigens to initiate an immune response

Gene therapy (DNA)
- Exogenous DNA delivered into cell nuclei to express a gene, typically to correct an error in production of a specific protein

Cell therapies (*ex vivo* gene editing)
- Whole cells with engineered DNA for infusion into patients to target a specific disease state

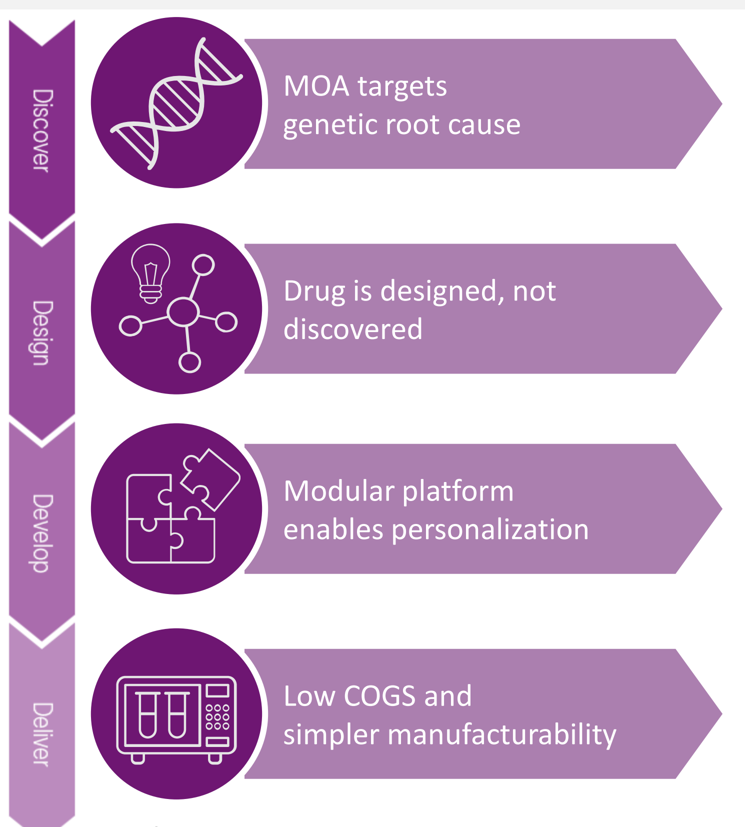
… however:

The curative potential of genomic medicines for rare disorders is not compatible with our current drug development models, biomanufacturing technologies, and health care system.

We need **alternative development frameworks** combined with **advanced biomanufacturing innovation** to advance genomic medicines for rare disorders from discovery to patients in a sustainable manner.

What is the Ideal State? Realizing Economies of Scale and Scope of Genomic Medicines for Rare Disorders

The ideal genomic therapy for rare disorders would be designed to directly target a genetic mutation, will be modular, with independent well-characterized components, and would benefit from simpler regulatory process and low-cost manufacturing



An ideal genomic therapy MOA directly addresses an **upstream cause of disease**, as proximal to the causative **genetic mutation** as possible, increasing the likelihood of efficacy and reducing pharmacokinetic unpredictability

Genomic therapies for rare disorders must be **rationally designed** based on a known molecular target suspected to cause disease; identifying hits through large screens and subsequent optimization is too costly and slow

Genomic therapies for rare disorders should be modularized to provide speed and regulatory advantages; changes to one component of the therapy result in **predictable effects that are independent of changes in other components** such that relevant payloads can be tweaked for new patient groups

Genomic therapies for rare disorders could potentially benefit from **economies of scale** from well-characterized platform components and **economies of scope** from platform designations

Source: L.E.K. interviews, research, and analysis

How Can we Achieve this Ideal State? And is it Truly Possible?

Key barriers to realize economies of scale and scope for genomic medicines for rare disorders

Stage	*Key barriers*	Technical	Financial	Regulatory	Structural
Discover	Patient sourcing & identification	• Lack of comprehensive genetic testing • Lack of data analytics capability / capacity	• Limited reimbursement for WGS	• Lack of universal screening guidelines	• Limited disease awareness • Under-established referral system • Lack of centralized databases • WGS data sharing / privacy concerns
Discover	Target identification & validation	• Prolonged research cycle due • Lack of relevant disease models			• Fragmented, uncoordinated research efforts
Design	Modality selection & drug design	• Lack of modality diversification		• Lack of modular design regulatory guidelines	• Restricted IP access
Design	Process development & manufacturing	• Lack of modular and cost-effective mfg. • Lack of phase and stage appropriate mfg.		• Lack of modular mfg. regulatory guidelines	• Concentrated expertise in select stakeholders
Develop	Preclinical testing	• Lack of relevant disease models		• Lack of tox study regulatory guidelines • Limited pre-approved non-Tx components	
Develop	Patient dosing, monitoring, and follow-up	• Lack of disease natural history • Lack of prior knowledge to inform dosing		• Unclear safety and efficacy expectations	• Lack of care delivery infrastructure • Minimal precedence for single-patient studies • Lack of centralized patient databases
Deliver	Regulation and institutional oversight			• No existing regulatory approval framework • Fragmented global regulatory bodies	
Deliver	Funding / reimbursement		• No sustainable funding / reimbursement model • Underinvestment in N-of-1 therapies		

Barriers vary by modality

Non exhaustive

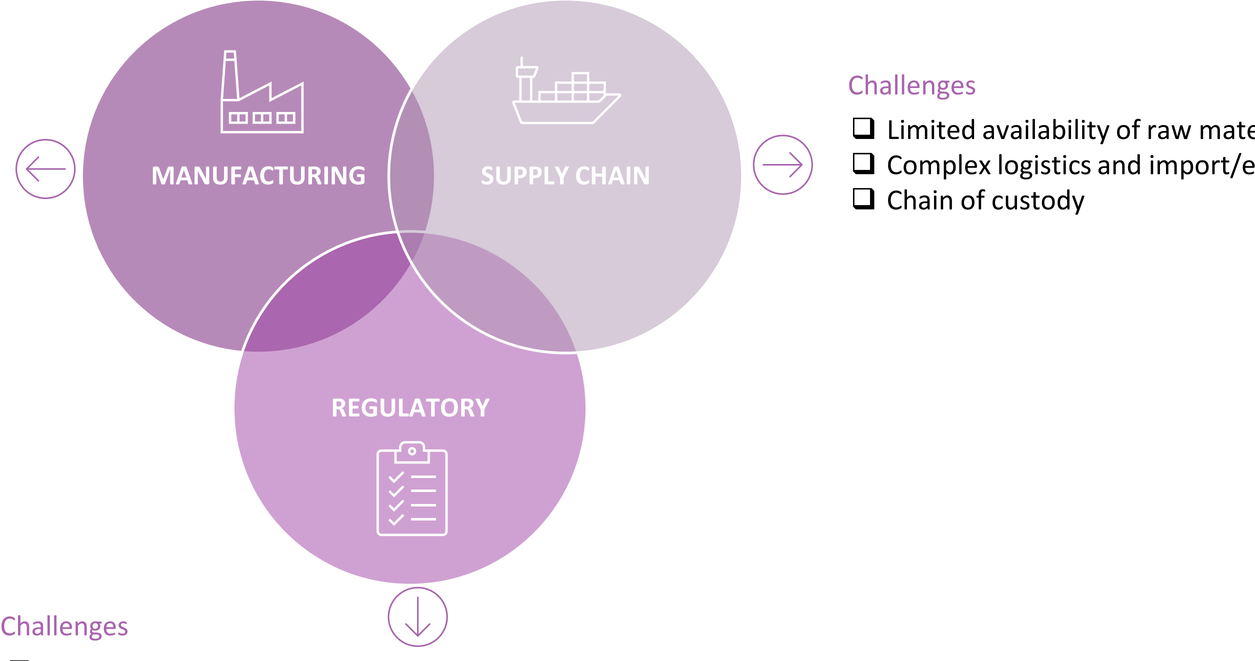
Source: L.E.K. interviews, research, and analysis

There is an Evolving Interconnectivity in Advanced Therapeutics Biomanufacturing

Increased complexity in the manufacturing of a broad portfolio of multicomponent modalities

Challenges

- ❑ Yield and quality of individual components
- ❑ Formulation in biological and synthetic material
- ❑ Consistent potency, purity and potency of final product



Challenges

- ❑ Limited availability of raw materials
- ❑ Complex logistics and import/export laws
- ❑ Chain of custody

Challenges

- ❑ Need for timely evolution of regulatory guidelines in a dynamic industry
- ❑ Need for updated regulatory frameworks that build on economies of scope
- ❑ Need for early interactions and co-development with drug developers

danaher.

Biotechnology

Life Sciences

Diagnostics

We are a leading global life sciences and diagnostics innovator, committed to accelerating the power of science and technology to improve human health.

Relentless Pursuit of Breakthrough Workflow Solutions to Reduce Complexity

Diagnose the disease	Write the code	Make the drug	Deliver to the right tissue	Measure efficacy & safety

~3 misdiagnoses and **5 doctors** before receiving accurate Dx

Unknown function of 98% of our DNA

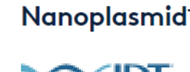
Manufacturing failure ~5% for mRNA; up to 10% for CAR-T cell therapies

>90% of IV-administered treatments end up in the liver

Patients may experience **diagnostic delays** that can averages **4-5 years**

~10% of patients may not receive a potentially curative therapy and may die because of **failed manufacturing** batch

Danaher is Uniquely Positioned to Address These Challenges



CRISPR nuclease with **lowest off-target effects**

Most reliable industrial-scale plasmid production

1st widely adopted bioreactor tech for production of Viral Gene Therapies

Gold standard in lipid nanoparticle delivery

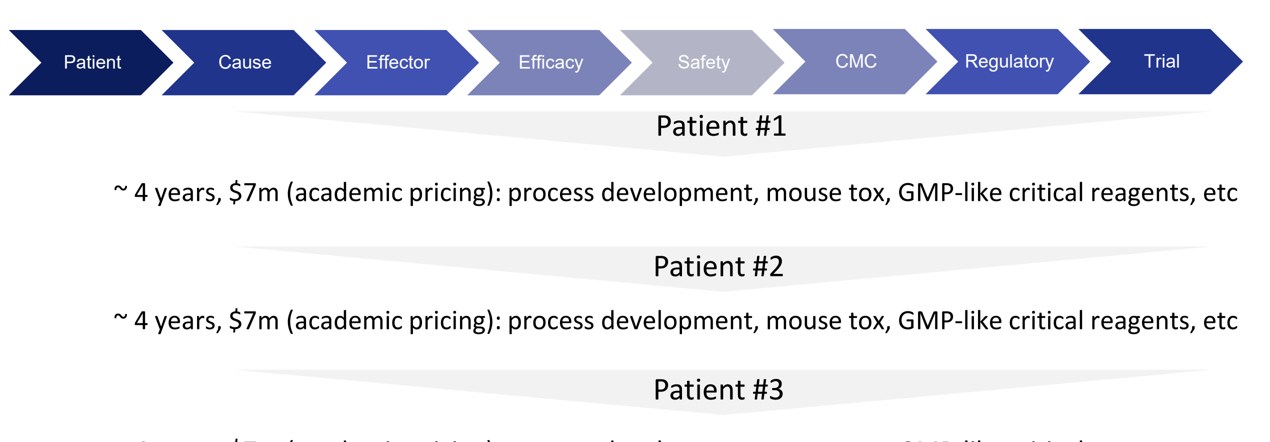
Best measurement of cell viability

Addressing major gaps and inefficiencies in genomic medicine biomanufacturing

What if we could “platform” the development of gene editing drugs?

From traditional, inefficient, and costly linear step-wise process…

…to consistently reproducible standardized process.



…

Patient

Causative Variant

Editing approach

Delivery

Efficacy Safety

Clinical Trial

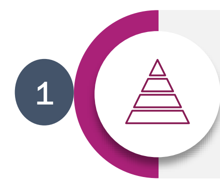
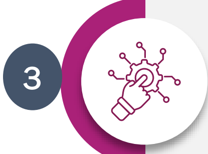
Engineer and deploy platform processes

Critical **success factors**:

1. Right development plan
2. Rigth technologies
3. Right regulatory path

Gene editing platform case studies…

Platforms can be described along six characteristics

1 	***Definition:*** **What is included in the platform?**	What components are fixed within a platform? Consider delivery method, editing technology, target cell/tissue/ disease area, or other key components. What is the rationale for fixing these components within the platform?
2	***Medical benefit:*** **What diseases could the platform address?**	What specific conditions could the platform target? What unmet need does the platform address? How does the platform potentially benefit the rare disease community?
3 	***Performance assessment:*** **How is the platform characterized?**	What measurable parameters ensure the platform is performing as expected? What body of evidence indicates a predictable, safe and efficacious platform?
4	***Efficiency:*** **What steps does the platform streamline?**	What steps in R&D and manufacturing can be omitted or done more efficiently due to platform predictability? How is a manufacturing process linked to the platform? How do these efficiencies manifest in early or late-stage development, or manufacturing?
5	***Value:*** **What is the value from platform efficiency?**	What are the efficiency gains associated with each 'streamlined' development and manufacturing step? Are there regulatory review savings from the platform? Please quantify if possible.
6	***Risk mitigation:*** **How viable is the proposed platform?**	What uncertainty is associated with skipped or streamlined steps? How can this uncertainty be addressed? What regulatory flexibility (if any) is required to proceed with this platform? Do regulators have the necessary tools to oversee the platform?

…Reflect modular components

Technical elements of a gene editing platform

		Fixed	Variable
Therapeutic target	Disease / syndrome	☐	☐
	Specific indication / target gene	☐	☐
	Target organ / cell	☐	☐
Therapeutic platform	Delivery	☐	☐
	Gene editing method	☐	☐
	Gene editor	☐	☐
	Guide RNA	☐	☐
Quality	Manufacturing (APIs / DP)	☐	☐
	QA / QC / analytical validation	☐	☐
	CQAs	☐	☐
Trial	Route of administration	☐	☐
	Endpoints	☐	☐
	Trial protocol	☐	☐

Individual developers to identify fixed versus variable components of a gene editing platform

Fixed components remain equivalent across more than one development program

Fixed components offer the potential for more efficient development and regulatory approach

Gene editing platforms can rely on multiple approaches to sharing information

Gene editing platforms…

…can rely on multiple vehicles to use or share information to validate performance

Vehicle	Who develops	Requires
Create / codify: Sector-wide alignment or investment to develop building blocks that can be shared	▪ Coalition; Public-private partnership	▪ Review decision but prior
Share: Sharing of existing best practice / prior knowledge amongst developers	▪ Coalition of developers/ Association	▪ Review decision ▪ Regulatory alignment (informal or guidance)
Use: Developer's prior knowledge, expertise	▪ Medicine sponsor, CDMO	▪ Meet guidance ▪ Review decision
Platform Technology Designation	▪ Medicine sponsor	▪ Approved drug ▪ FDA-designated platform

Overview of case studies

Fyodor Urnov

Ex Vivo Autologous T Cell Gene Editing For Inherited Hemophagocytic Lymphohistiocytosis (HLH)

Kiran Musunuru

LNP *in vivo* Gene Editing Platform for Urea Cycle Disorder

AAV ALS Gene Editing Platform

Base Editing Opportunities for Platform Development in Glycogen Storage Disease

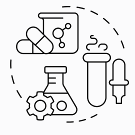
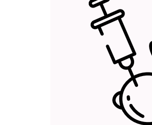
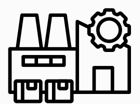
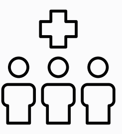
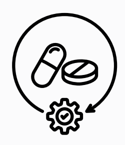
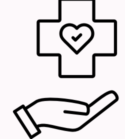
Modular platform for *in vivo* LNP Formulated Prime Editors Targeting Rare Genetic Diseases of the Liver

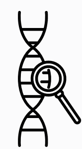
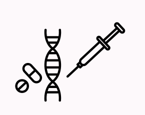
In Vivo Gene Editing for Sickle Cell Disease and other HSC targets

Streamlining CMC in gene editing

In vivo Gene Editing Platforms

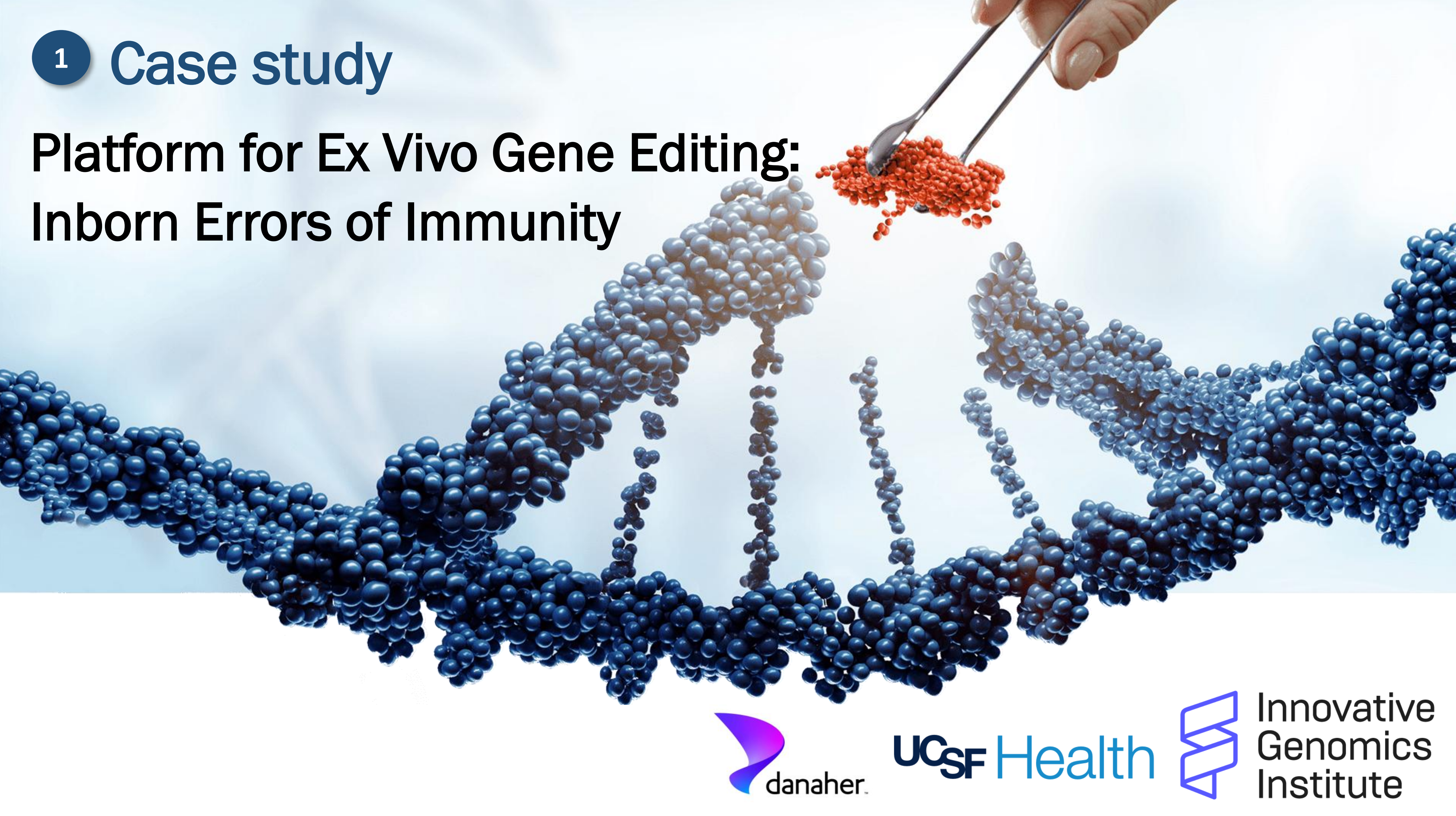
Case studies identified similar opportunities for progress

Common themes across case studies		Potential opportunity
 The **delivery vehicle primarily** drives biodistribution including germline, and toxicology		 **Streamline animal studies** - and retain focus on *in silica* on- and off-target editing studies
 Fixing manufacturing (same location, equipment, process, starting materials, etc.) yields **consistent process characterization**		Consistent process characterization enables **streamlining of process validation steps**
 Enrolling patients in a single trial (same **phenotypical disease but differing genotypes)** will yield efficiencies…		…in enrollment, clinical trial protocols, dosing, sites, and long-term follow up – and is **patient-friendly for fragmented rare disease populations**
 **GMP requirements for guide RNA** should be more precisely tailored for stage of development and benefit-risk of target population		 **Limiting # of batches of gRNA required** within and across patients would substantially increase drug development efficiency

 If validated, streamlining opportunities from gene editing platforms could translate into **material efficiency gains** (2x – 5x)	 These gains present opportunity to address additional rare disease therapeutic targets that are **currently not viable**

Evaluation matrix:

Potential opportunity	Size of opportunity (efficiency gains)	Biggest challenge(s)	Evidentiary needs (validate performance)	Next steps / comments
Consistent delivery vehicle primarily allows streamlining of animal studies	▪ […]	▪ […]	▪ […]	▪ […]
Consistent process characterization enables streamlining of PV	▪ […]	▪ […]	▪ […]	▪ […]
One trial for multiple genotypes/same phenotype patient-friendly and efficient	▪ […]	▪ […]	▪ […]	▪ […]
Benefit-risk GMP for gRNA substantially increases development efficiency	▪ […]	▪ […]	▪ […]	▪ […]


1
Case study
Platform for Ex Vivo Gene Editing:
Inborn Errors of Immunity
danaher
UCSF Health
Innovative
Genomics
Institute

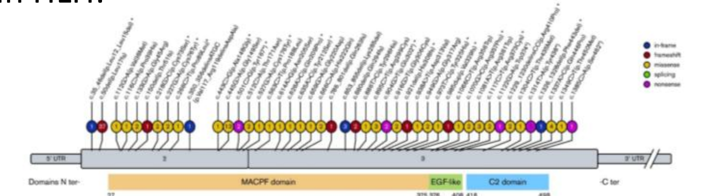
Case Study: Ex Vivo Autologous T Cell Gene Editing For Inherited Hemophagocytic Lymphohistiocytosis (HLH)

- HLH is an appropriately triggered (infection, malignancy) immune response that leads to excessive and self damaging inflammation due to the inability to terminate the response secondary to mutations causing defective cytolytic function
- *Standard of care:* suppressing the immune response with steroids, chemotherapy, and/or anti-cytokine directed therapy followed by allogenic hematopoietic stem cell transplant.
- 40% of patients do not survive on SOC
- Deaths largely due to inability to control hyperinflammation and get patients to transplant
- **Only need 30% chimerism (normal donor T cells) to control disease** = corrected T cell therapy could be life saving

Mutations in a number of genes are associated with HLH:

Known defect	Gene	Function
Primary ("genetic") Hemophagocytic Lymphohistocytosis (HLH)		
FHL2	Perforin (PFR 1)	Cytolysis of target cells
FHL3	MUNC13-4 (UNC13D)	Vesicle priming and secretion of lysosomes
FHL4	Syntaxin-11 (STX 11)	Vesicle intracellular trafficking/membrane fusion
FHL5	STXBP2(MUNC 18-2) syntaxin binding protein 2)	Vesicle trafficking and membrane fusion
HLH-associated immunodeficiencies		
Immunodeficiency	Gene	Associated features
Chediak Higashi syndrome	LYST	Oculocutaenous albinism
Griscelli syndrome type 2	Rab27A	Hypopigmentation
X linked lymphoproliferative disorder 1	SH2D1A	Hypogammaglobulinemia
X linked lymphoproliferative disorder 2	XIAP /BIRC	Lymphoproliferative disease
IL-2 Inducible T-Cell Kinase (ITK) Deficiency	ITK	Lymphoproliferative disease

Patients typically present with unique mutations. Mutational landscape of PRF1 in HLH:



Rationale for a CRISPR-Cas-based approach:

- Mutation correction in T cells
- Lenti GT in mouse model
- AlloVST ameliorates HLH
- T cell editing has a 15 yr clinical track record

Patients have the T cells they need

- Polyclonal TCRs target infection / malignancy
- Just need to fix the killing defect

Hypothesis

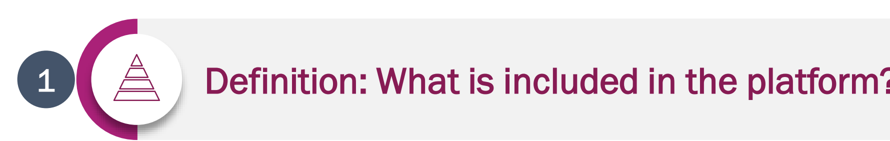
Gene correction of mutations in (auto) T cells will control HLH

- How long will control last?
- Corrected T cells as a bridge to gene edited HSCs?

Goal

Develop a platform where a patient-specific genome editor can be rapidly developed, derisked, and cell product generated and administered.

Case Study: IGI platform for IEI – fHLH use case



Legend

Fixed component of platform	Detail	Variable components	Platform evolution

	Platform 1.0 (same gene, same editor)		Platform 2.0 (same gene, many editors)		Platform 3.0 (many gene, many editors)	
Therapeutic target	Disease / syndrome	Inborn Errors of Immunity (IEI)	Disease / syndrome	IEI	Disease / syndrome	IEI
	Specific indication / target gene	PRF1	Specific indication / target gene	PRF1	Specific indication / target gene	HLH (Multiple genes)
	Target organ / cell	T cell	Target organ / cell	T cell	Target organ / cell	T cell & HSPC
Therapeutic platform	Delivery	Ex vivo: Electroporation	Ex vivo delivery	Ex vivo: Electroporation + LNP	Ex vivo delivery	Ex vivo: Electroporation + LNP
	Gene editing method	Cas nuclease	Gene editing method	Cas nuclease + base editor	Gene editing method	Cas nuclease + base editor + prime editor + ?
	Guide RNA	Multiple guides (for one gene)	Guide RNA	Multiple guides (for one gene)	Guide RNA	Multiple guides for multiple genes
Quality	Manufacturing (APIs / DP)	Fixed location, equipment, and labor – with surrogate CQAs for lot release	Manufacturing (APIs / DP)	Fixed location, equipment, and labor – with surrogate CQAs for lot release	Manufacturing (APIs / DP)	Fixed location, equipment, and labor – with surrogate CQAs for lot release
	QA / QC / analytical validation	Standardized validation (*e.g.,* minimize lots for PD / PV)	QA / QC / analytical validation	Standardized validation (*e.g.,* minimize lots for PD / PV)	QA / QC / analytical validation	Standardized validation (*e.g.,* minimize lots for PD / PV)
	CQAs	Purity, identity and potency	CQAs	Purity, identity and potency	CQAs	Purity, identity and potency
Trial	Route of administration	IV (adoptive transfer)	Route of administration	IV (adoptive transfer)	Route of administration	IV (adoptive transfer)
	Surrogate endpoint	Recovery of cytopenias Improvement in inflammatory markers	Surrogate endpoint	Recovery of cytopenias Improvement in inflammatory markers	Surrogate endpoint	Recovery of cytopenias Improvement in inflammatory markers
	Trial protocol	Common trial protocol (basket / inclusion) and dosing	Trial protocol	Common trial protocol (basket/inclusion) and dosing	Trial protocol	Common trial protocol (basket/inclusion) and dosing

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Global patients impacted by inborn errors of immunity (IEI)

2

Medical benefit: What diseases could the platform address?

Patient prevalence (2020)

106,373 patients

Category	Patients with IEI
Phenocopies of Inborn Errors of Immunity	
Bone Marrow Failure	
Complement Deficiencies	5,017
Autoinflammatory Disorders	6,946
Defects in Intrinsic and Innate Immunity	2,253
Congenital Defects of Phagocyte # or Function	7,576
Diseases of Immune Dysregulation	4,841
Predominantly Antibody Deficiencies	55,494
Combined Immunodeficiencies with Associated or Syndromic Features	16,045
Immunodeficiencies Affecting Cellular and Humoral Immunity	7,528

Axis: 0, 20,000, 40,000, 60,000, 80,000, 100,000, 120,000

Patients with IEI

Addressable patients

Platform 1.0

HLH (PRF1 deficiency): ~**30% of 161 patients**

Platform 2.0

HLH (PRF1 deficiency): ~**70-90% of 161 patients**

Platform 3.0

- *HLH (PRF1 deficiency):* **161 patients**
- *HLH (UNC13D deficiency):* **191 patients**
- *HLH (STX11 deficiency):* **15 patients**
- *HLH (STXBP2 deficiency):* **67 patients**
- *HLH (LYST):* **197 patients**
- *HLH (RAB27A):* **164 patients**
- *HLH (SH2D1A):* **242 patients**
- *HLH (XIAP deficiency):* **256 patients**
- *HLH (ITK deficiency):* **34 patients**

1300+ patients

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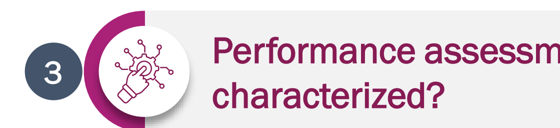
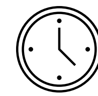
Source: "2020-2021 Global Survey on Primary Immunodeficiencies", Jeffrey Modell Foundation



Platform performance



3 **Performance assessment: How is the platform characterized?**

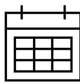
What measurable parameters ensure the platform is performing as expected?
What body of evidence indicates a predictable, safe and efficacious platform?

At time of therapeutic use

- Certificate of analysis from manufacturer on critical reagents and APIs with CQAs consistently met or exceeded
- Certificate of analysis from manufacturer on critical autologous cell product (viability, efficacy) with CQAs met or exceeded

Key target product profile metrics achieved – **efficacy, potency, safety, purity, identity**

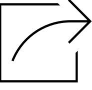
- All input technologies and individual steps are at a level of maturity to appropriately 'platform'
- Patient is dosed with the cell product before severe disability / death



Over treatment duration & follow-up

Endpoints:

- Primary (safety): Treatment related AEs compatible with benefit-risk considerations
- Exploratory endpoints (efficacy): Clinically meaningful benefit relative to SOC
  - No hyperinflammatory flares at 1 yr
  - Overall survival



To evolve platform

- What unlocks transition from platform 1.0 to 2.0 to 3.0?
  - Consistent platform performance, as measured by CQAs
  - Safety profile justifies expanding treatment population
  - Durability of clinical benefit relative to SOC
  - Urgency to treat necessitates timely delivery of new platform therapeutics

Can we Optimize Manufacturing for sgRNA at the Right Time, Quantity, and Quality for the Right Patient?

Downstream purification impacts sgRNA quality but not specificity

Quality	Manufacturing technology	Time to manufacture	Purification method	Purity (%)	Specificity
GMP	AKTA	6 to 18 months	HPLC	92.75	99.9
RUO	IDT	2 days	HPLC	84.8	99.9
RUO	IDT	2 days	Std Desalt	44.5	99.9

Cell-Based Editing Activity:
Cas9 RNP Electroporation, K562 cells

Rampseq data

***In vitro* Activity:**
Cas9 RNP cleavage of dsDNA

Functional performance of 100 nt Cas9 sgRNAs targeting *HPRT1* when used in K562 CRISPR editing and *in vitro* DNA cleavage experiments. CRISPR editing outcomes were assessed via NGS using the rhAmpSeq CRISPR Analysis System following electroporation (Lonza) into K562 cells. Cas9-mediated DNA cleavage *in vitro* was assessed using the Fragment Analyzer system.
(*n* = 3, error bars represent standard deviation. RUO controls were purified using either HPLC or standard desalt methods.)

QC assessment of 100 nt Cas9 sgRNAs purity was executed on PA-800 Plus (Sciex) to determine purity-by-length of guide RNAs on a developed RUO method for this instrument and for this class of oligonucleotide.

rhAmpSeq as an Analytical Method for Measuring Purity

rhAmpSeq assessment of sgRNA grade comparability

100 nt sgRNAs targeting *HPRT1* (chrX:134498409) were complexed with WT *S.p.* Cas9 (4 µM) and delivered via electroporation in K562 cells.

rhAmpSeq panels were designed for targeted amplification at 11 off-target loci and the on-target locus. Panel included 2 sites nominated by cell-based empirical methods and 10 via *in silico* nomination.

% Specificity is calculated by the following:

$$100 \times \frac{\text{(On-target editing)}}{\text{(Sum On + Off-target editing)}}$$

sgRNA Grade	sgRNA Purification	Mean % Specificity (n=3)
cGMP - vial 1	HPLC	99.9
cGMP - vial 2	HPLC	99.9
Pilot Run	HPLC	99.9
RUO	Std Desalt	99.9

On-Target Editing Locus

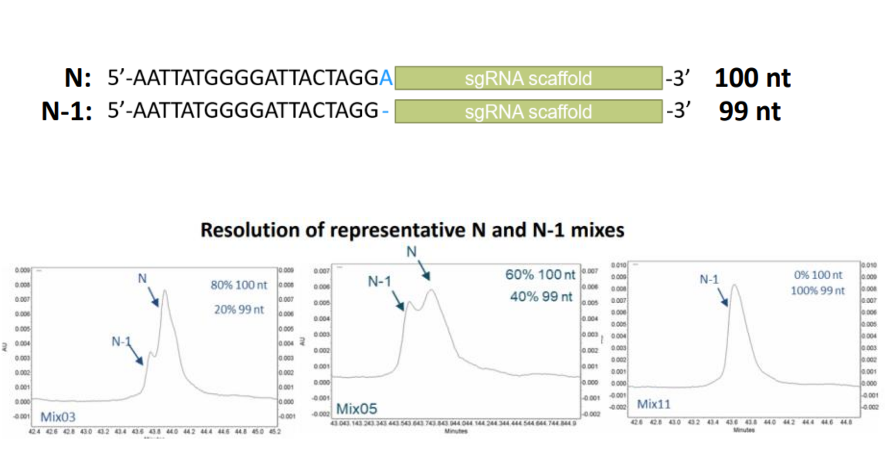
chr	start	end	strand	hg38_seq	Levenshtein_distance
chrX	134498409	134498429	-	CTTATATCCAACACTTCGTG	0

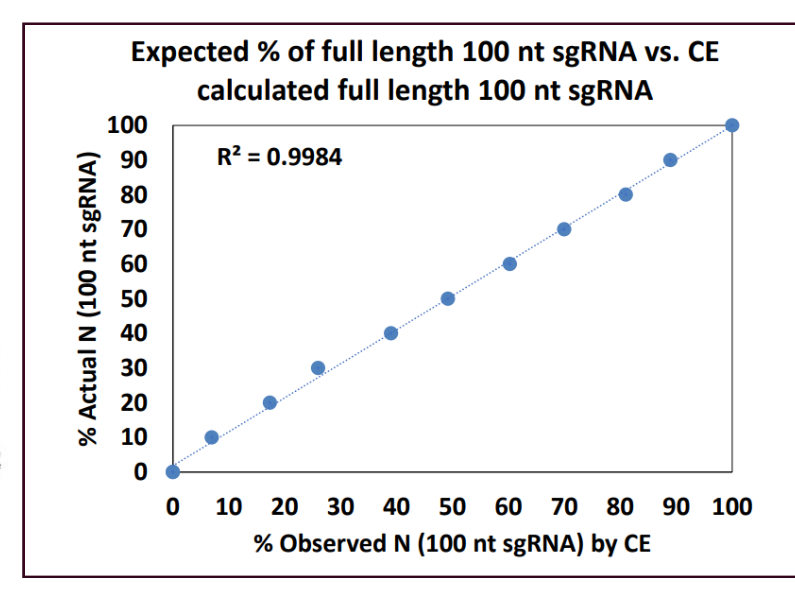
Putative Off-Target Loci

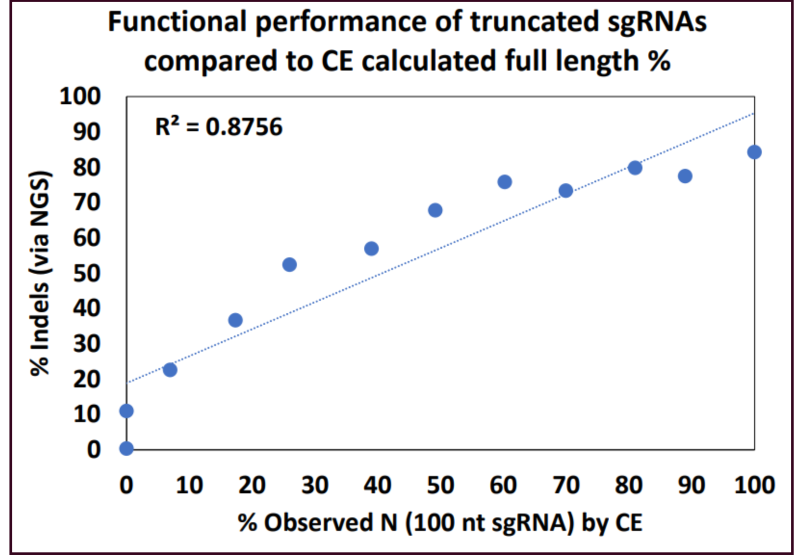
chr	start	end	strand	hg38_seq	Levenshtein_distance
chr8	42379946	42379966	+	CTTGTACCCAGCACTTCGTG	3
chr3	100929120	100929140	+	CTTATTTCCAACACTTAGTT	3
chr11	84719391	84719411	-	TTTATATAAAACACTTCATG	4
chr14	74725529	74725549	+	ATAATTCCCAACACTTCGTG	4
chr7	133980572	133980592	-	CTTACATACAACACTTCTTT	4
chr5	167982469	167982489	+	CATATATTAAACACTTCCTG	4
chr13	92678145	92678165	+	TTTATATCCAACACTTTTTA	4
chr12	83944674	83944694	-	ATTATATCAAACACTTTATG	4
chr4	95366406	95366426	+	CTTTTATCCTAAACTTCTTG	4
chr4	10634263	10634283	-	TTTATATCCAAAACTTCCTT	4
chr10	35291832	35291852	+	CTTGTGTATGACTTTTTGTG	8

A C T G = mismatched NT

What is the Relationship Between sgRNA Purity and Activity?







An optimized CE method can detect expected abundance of 1 nt base changes in *RUO standard desalt* sgRNAs that simultaneously result in loss of sgRNA function. To assess the ability of the analytical QC method to detect single nt differences in RNA oligo length, a standard 100 nt single guide RNA (sgRNA) targeting *HPRT1* (N) was mixed at varying ratios with the same sgRNA lacking a single nucleotide at the 3'- end of the protospacer sequence (N-1). Sequences are depicted above, left; representative CE traces are also shown. Observed vs actual FLP abundance is plotted across 11 concentrations with an R2 value of 0.9984. For functional comparison, the N:N-1 mixes (11 total) were electroporated into K562 cells (ATCC) as RNP complexes with *S.p.* Cas9 WT V3 (IDT) at a final concentration of 1 µM, containing 4 µM Cas9 electroporation enhancer (IDT). Genomic DNA was extracted after 48 hrs (Quick Extract) and total editing was assessed via NGS using the rhAmpSeq CRISPR library prep and analysis system. Total CRISPR editing (% indels) for each mix is plotted against the detected presence of the full length sgRNA via CE. As expected, % editing decreases as the presence of truncated sgRNA is increased in the mix. The R2 of the % editing compared to the analytical assessment of % full length material is 0.8756 (E).

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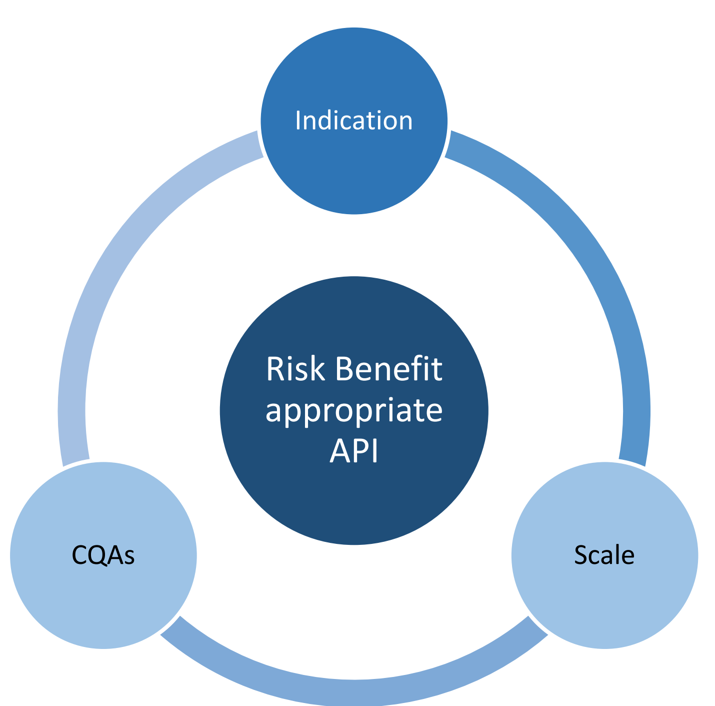
Opportunity for the Future: Risk-Benefit Appropriate Products

Today: cGMP

- cGMP manufacture is a warranted standard for large batch/large indication risk/benefit management
- Timelines and cost associated with this risk mitigation is not practical for n=1/few
- Many of the n=1/few diseases are indications with limited treatment timeframes to mitigate substantial harm

Future: Risk-Benefit Appropriate guideRNAs

- Create manufacturing solutions that solve for scale, cost and timelines
- Establish data for risk-based determination of MVP CQAs for guideRNA using pre-clinical comparability and clinical outcome data



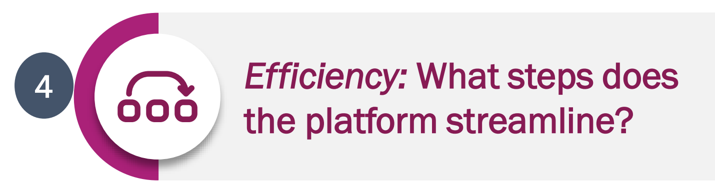
Cost & Manufacture timeline

Grade	RUO	Risk-Benefit Appropriate	cGMP
Equipment certification	XXX	TBD	XXX
Manufacturing Standard	ISO 9001:2015	TBD	ICH Q7
Clean room	no	clean room certified	ISO8 clean room certified
Minimum Scale	N/A	TBD	100 mg
Purification	De-salt	HPLC	HPLC
Stability	No	No	Yes (customer)
CQAs			
Sterility, bioburden, endotoxin	no	Criteria established (equal to cGMP)	Criteria established
Purity	no	Criteria TBD	Criteria established
Identity, molecular weight	no	Criteria TBD	Criteria established
Yield	yes	Criteria TBD	Criteria established
Appearance	yes	Criteria TBD	Criteria established

Pre-clinical, functional comparability

- Efficacy
- Safety
- Potency

Efficiency from platform



Therapeutic platform	Platform 1.0 (same variant) Same editor, same target gene, different gRNAs		Platform 1.5 (multiple variants) Different editors, different gRNAs, same gene		Platform 2.0 (multiple genes) Different editors, different gRNAs, different genes, same disease	
	Delivery	Ex vivo: Electroporation	Ex vivo delivery	Ex vivo: Electroporation + LNP	Ex vivo delivery	Ex vivo: Electroporation + LNP
	Gene editing method	Cas nuclease (HDR)	Gene editing method	Cas nuclease (HDR) + base editor	Gene editing method	Cas nuclease + base editor + prime editor + ?
	Guide RNA	Multiple guides (for one gene) + ssODN	Guide RNA	Multiple guides (for one gene) + ssODNs (for HDR)	Guide RNA	Multiple guides for multiple genes

For each amended IND…

Preclinical	Rodent disease model	Streamline/rely on initial IND	*Ex vivo* studies providing robust correlation with safety and efficacy in humans
	Ex vivo on target	Proceed in full, limited ability to reference first IND	Delivery + Gene Editor + gRNA yields unique on- and off-target profile. Potency/specificity assessment pipeline used for all
	Ex vivo off target	Proceed in full, limited ability to reference first IND	
Clinical	…		
CMC	Benefit-risk appropriate GMP	Streamline/rely on initial IND	Benefit-risk appropriate GMP and comparability studies demonstrating consistent GE reagent safety and quality across class
	Benefit-risk appropriate GMP	Streamline/rely on initial IND	Cell product manufacturing process used across all patient cell lots in the platform

Legend Streamline/rely on initial IND; Proceed in full, limited ability to reference first IND

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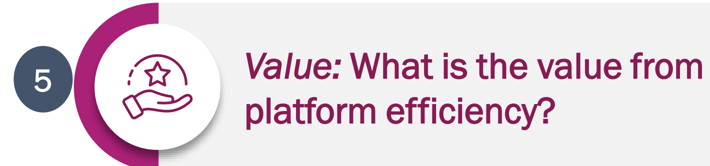
Value from IGI case study



What are the savings ($, FTEs, time) associated with each ‘streamlined’ development and manufacturing step? Are there regulatory review savings from the platform? Please quantify.

	Platform 1.0	Platform 1.0+
Development time for each therapy[1]	Initial (to first IND) – 2.5 years. Subsequent: 4 months.	4 months
Patient benefit	Decreased toxicity (shortened exposure to immunosuppressive agents and chemotherapy leading to decreased organ toxicity and infection risk). Less inflamed entering transplant (pre-transplant inflammation is a strong predictor of poor outcome); likely associated with better overall outcome.	Gene-edit within 4 months of presentation. Prevent future life-limiting HLH episodes
Therapeutic access implications	Ultrarare populations of n of 1 (unique guides) or n of ~100 (unique editor) insufficient for sustainable development	1K US patients sufficient to develop sustainable development model, attract commercial developer interest

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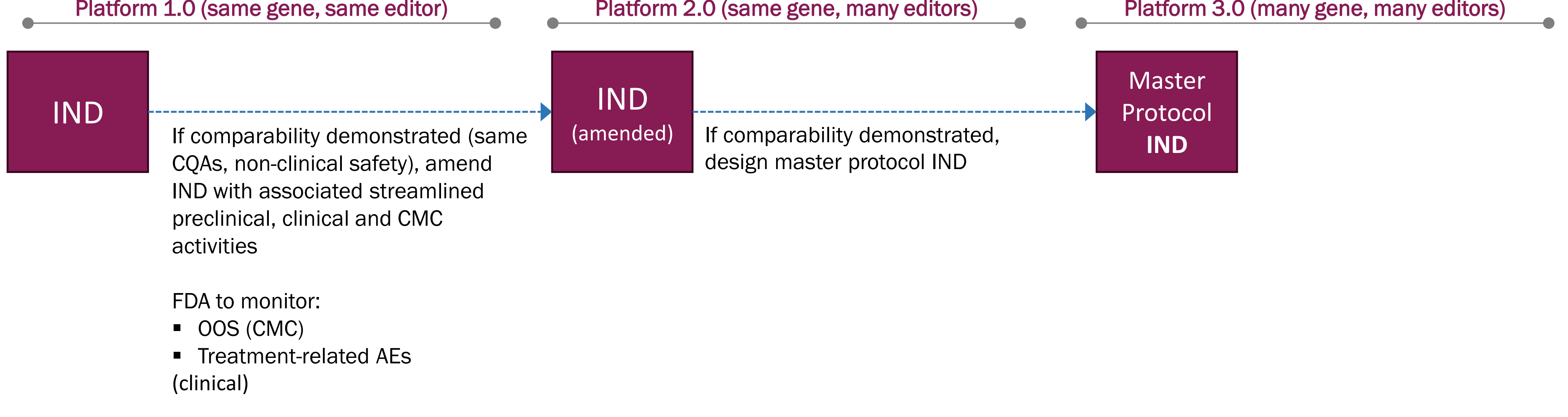
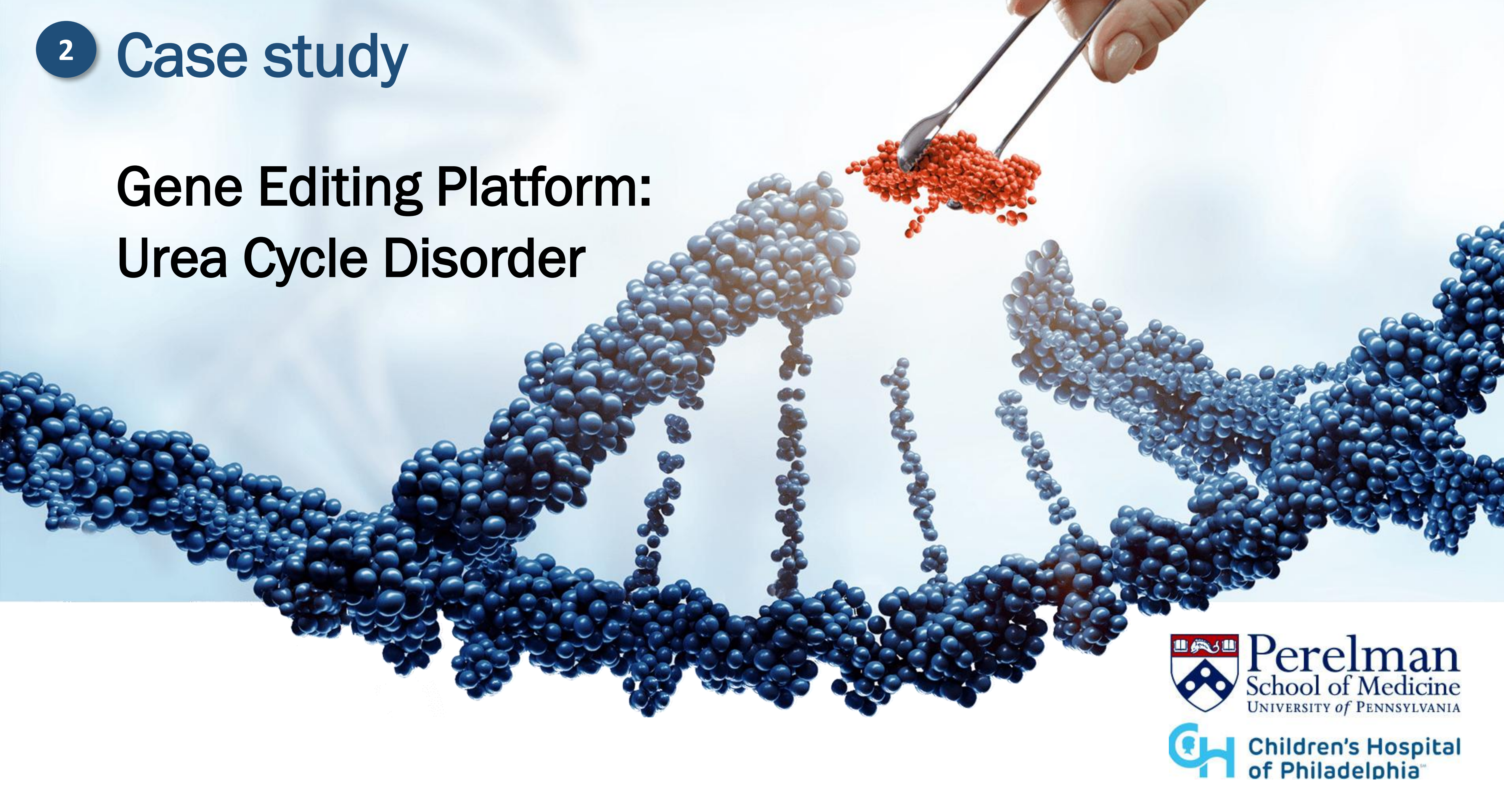
Perceived risks, and mitigation

What uncertainty is associated with skipped or streamlined steps? How can this uncertainty be addressed? What regulatory flexibility (if any) is required to proceed with this platform? Do regulators have the necessary tools to oversee the platform?

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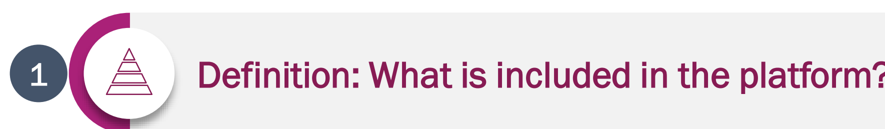
Case study
Gene Editing Platform:
Urea Cycle Disorder
Perelman
School of Medicine
University of Pennsylvania
Children's Hospital
of Philadelphia

Evolving platform targets UCD-associated genes



Legend: Fixed component of platform | Detail | Variable components | Platform evolution

Category	Component	Platform 1.0 (multiple guides)	Platform 1.5 (multiple editors)	Platform 2.0 (multiple genes)
Therapeutic target	Disease / syndrome	UCD	UCD	UCD
	Specific indication / target gene	CTLN1[1] (defective ASS1 gene)	CTLN1 (defective ASS1 gene)	Six different genes along UCD pathway
	Target organ / cell	Hepatocytes (liver)	Hepatocytes (liver)	Hepatocytes (liver)
Therapeutic platform	Delivery	LNP *in vivo*	LNP (same formulation)	LNP (same formulation)
	Gene editing method	Base editor	Base editor	Base editor
	Gene editor	Same editor	Different editors (same gene)	Different editors (different genes)
	Guide RNA	Multiple guides (for one gene)	Multiple guides (for one gene)	Multiple guides (for multiple genes)
Quality	Manufacturing (APIs / DP)	Fixed location, equipment, and labor – with surrogate CQAs for lot release	Fixed location, equipment, and labor – with surrogate CQAs for lot release	Fixed location, equipment, and labor – with surrogate CQAs for lot release
	QA / QC / analytical validation	Standardized validation (e.g., minimize lots for PD / PV)	Standardized validation (e.g., minimize lots for PD / PV)	Standardized validation (e.g., minimize lots for PD / PV)
	CQAs	Purity, identity and potency	Purity, identity and potency	Purity, identity and potency
Trial	Route of administration	IV	IV	IV
	Surrogate endpoint	Blood ammonia / glutamine levels	Blood ammonia / glutamine levels	Blood ammonia / glutamine levels
	Trial protocol	Lead IND with single variant, amendments for additional variants	Lead IND with multiple variants / editors, umbrella clinical trial	Master protocol, common control arm, 'umbrella of umbrellas' trial

1: Citrullinemia type I (CTLN1), also known as arginosuccinate synthetase deficiency

Must define trigger for *new* editor

Definition: What is included in the platform?

Platform 1.5 (multiple editors)

Therapeutic target	Disease / syndrome	UCD
	Specific indication / target gene	CTLN1 (defective *ASS1* gene)
	Target organ / cell	Hepatocytes (liver)
Therapeutic platform	Delivery	LNP *in vivo*
	Gene editing method	Base editor
	Gene editor	Different editors (same gene)
	Guide RNA	Multiple guides (for one gene)
Quality	Manufacturing (APIs / DP)	Fixed location, equipment, and labor – with surrogate CQAs for lot release
	QA / QC / analytical validation	Standardized validation (*e.g.*, minimize lots for PD / PV)
	CQAs	Purity, identity and potency
Trial	Route of administration	IV
	Surrogate endpoint	Blood ammonia / glutamine levels
	Trial protocol	Lead IND with multiple variants / editors, umbrella clinical trial

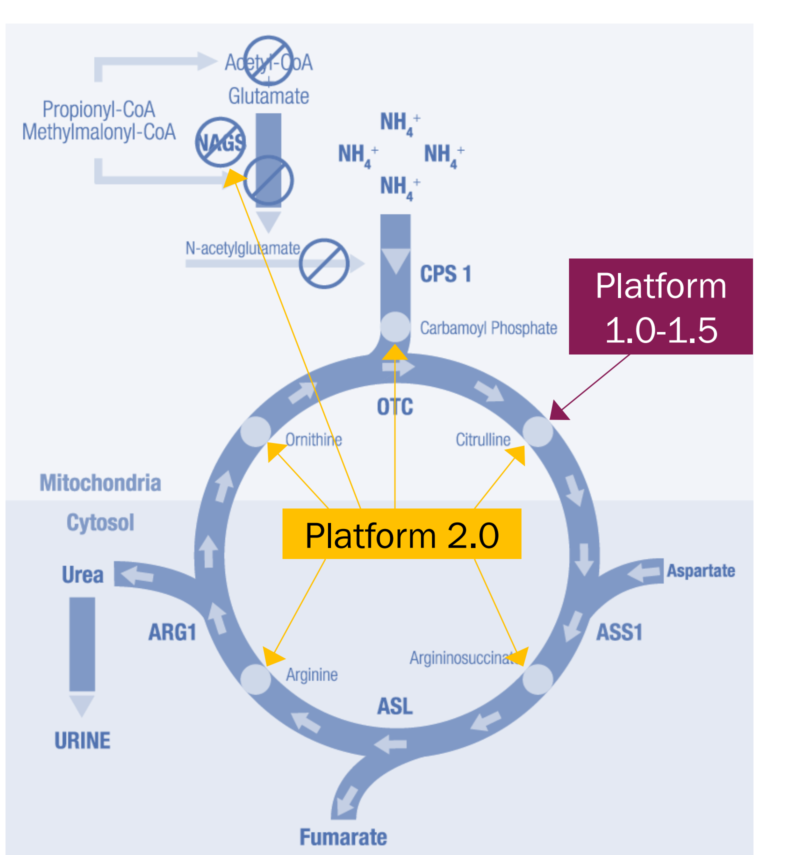
What exactly constitutes a *different* editor?

- Would the use of two mRNAs encoding editor proteins that differ only with respect to PAM preference be considered the use of different editors?
- Would the use of two mRNAs encoding editor proteins that differ only with respect to a deaminase domain be considered the use of different editors?
- Would the use of two mRNAs encoding editor proteins that differ only with respect to a nuclear localization signal be considered the use of different editors?
- Would the use of two mRNAs encoding editor proteins that differ only with respect to conservative amino acid substitutions that do not affect the editing activity be considered the use of different editors?
- Would the use of two mRNAs encoding the same editor protein but with different 5'UTR and/or 3'UTR elements be considered the use of different editors?

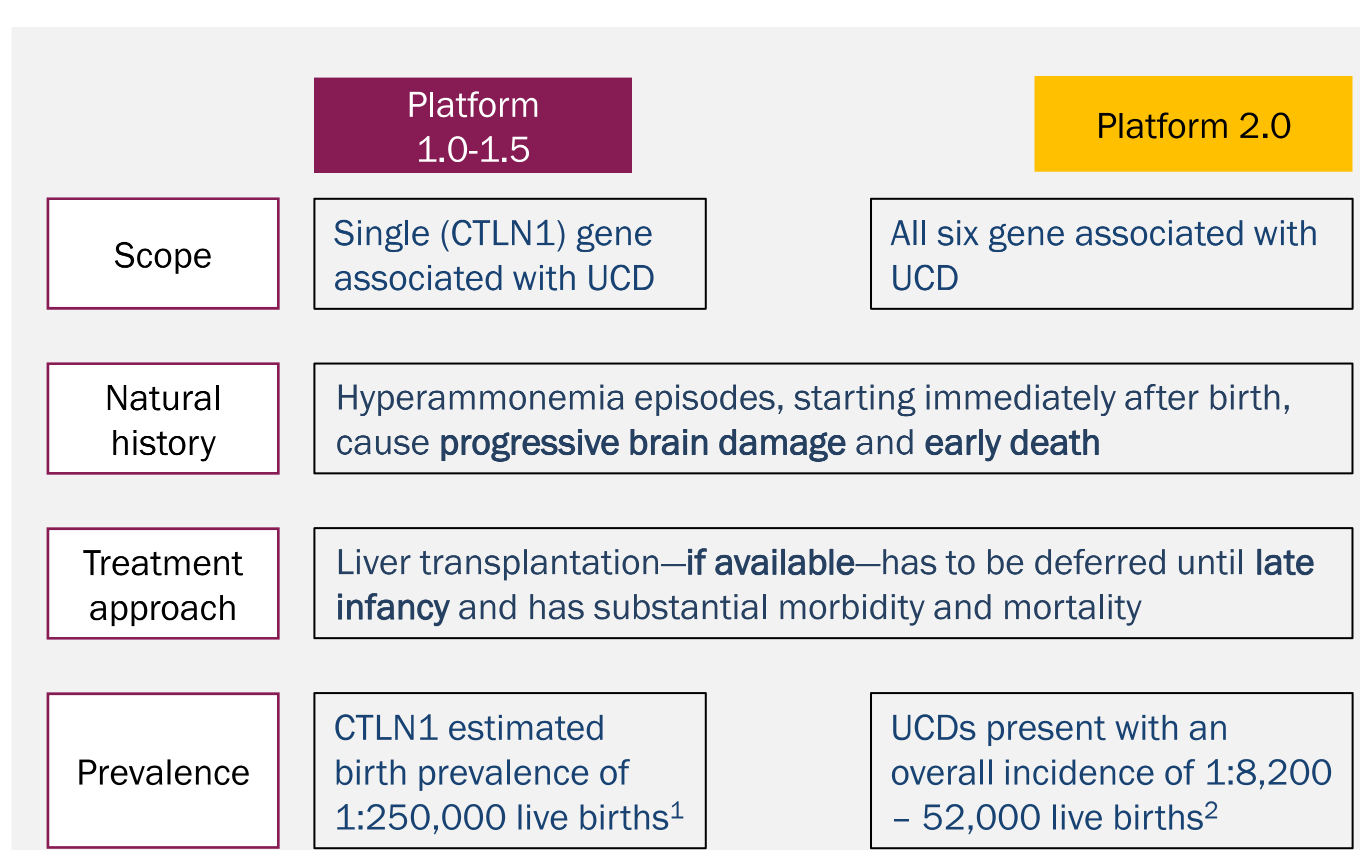
Initial platform addresses small fraction of UCD patients, with potential for broader application

Medical benefit: What diseases could the platform address?

Overview of UCD and platform applicability



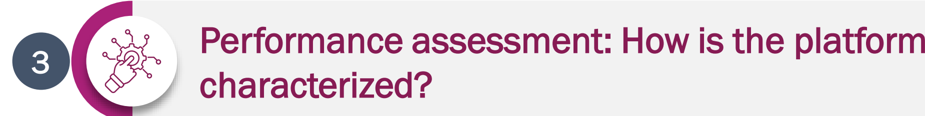
Platforms address significant unmet need

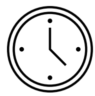


	Platform 1.0-1.5	Platform 2.0
Scope	Single (CTLN1) gene associated with UCD	All six gene associated with UCD
Natural history	Hyperammonemia episodes, starting immediately after birth, cause **progressive brain damage** and **early death**	
Treatment approach	Liver transplantation—**if available**—has to be deferred until **late infancy** and has substantial morbidity and mortality	
Prevalence	CTLN1 estimated birth prevalence of 1:250,000 live births[1]	UCDs present with an overall incidence of 1:8,200 – 52,000 live births[2]

1: Summar et al. 2013 – Approximately 300 CTLN1 patients in the US
2: See https://doi.org/10.1002/jimd.12609 and https://my.clevelandclinic.org/health/diseases/23470-urea-cycle-disorder

Performance



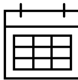


At time of therapeutic use

- Certificate of analysis from manufacturer on critical reagents and DSs with CQAs consistently met or exceeded
- Certificate of analysis from manufacturer on LNP DP with CQAs met or exceeded

Key target product profile metrics achieved – efficacy, potency, safety, purity, identity

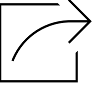
- All input technologies and individual steps are at a level of maturity to appropriately 'platform'



Over treatment duration & follow-up

Endpoints:

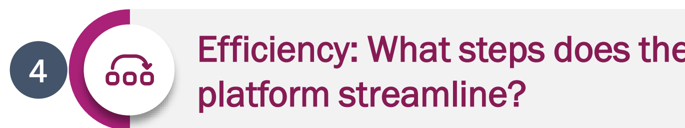
- Primary (safety): Treatment related AEs compatible with benefit-risk considerations
- Secondary endpoints (efficacy): Reductions in blood ammonia and glutamine levels
- Exploratory endpoints (efficacy): Clinically meaningful benefit relative to SOC



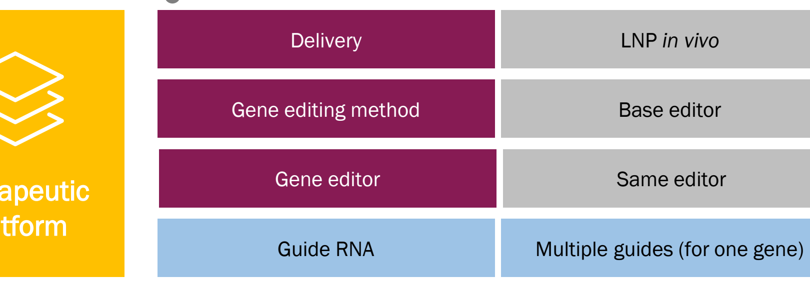
To evolve platform

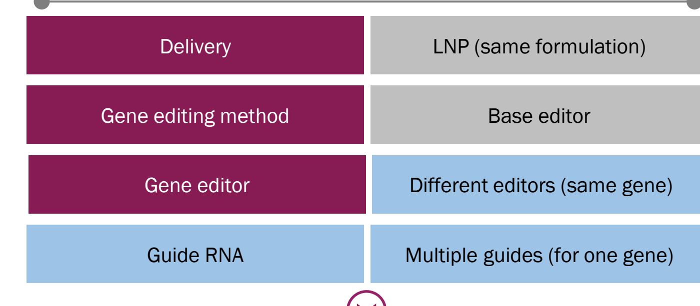
- What unlocks platform evolution?
  - Consistent platform performance, as measured by CQAs
  - Safety profile justifies expanding treatment population
  - Durability of clinical benefit relative to SOC
  - Urgency to treat necessitates timely delivery of new platform therapeutics

Platform approach to streamline R&D



Efficiency: What steps does the platform streamline?

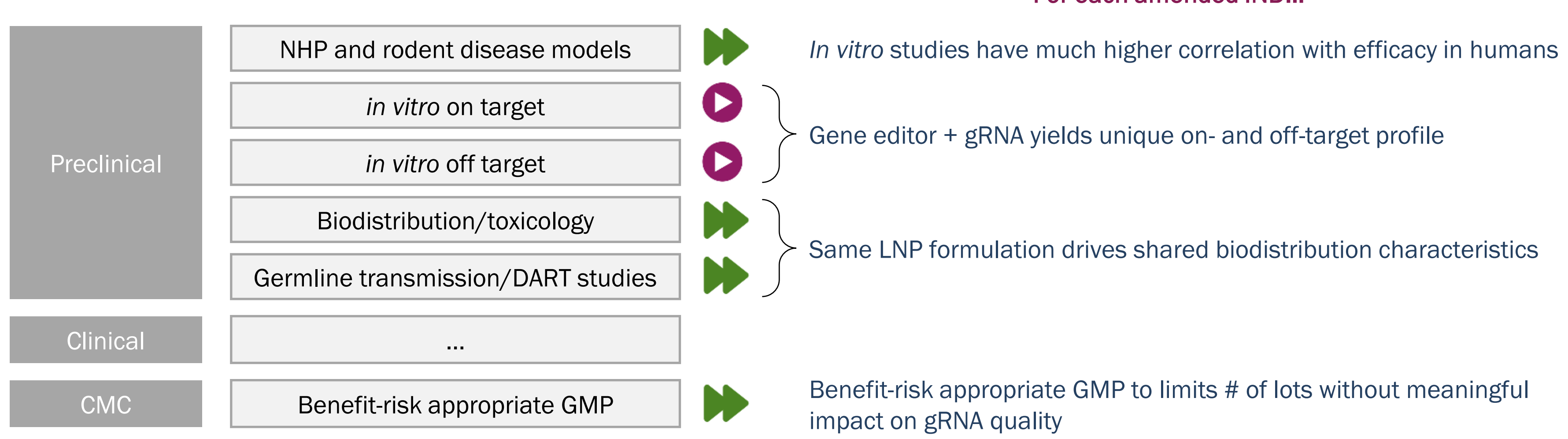




Platform 2.0 (multiple genes)
Different editors, different gRNAs,
different genes, same disease endpoints

Delivery	LNP (same formulation)
Gene editing method	Base editor
Gene editor	Different editors (different genes)
Guide RNA	Multiple guides (for one gene)

For each amended IND...



In vitro studies have much higher correlation with efficacy in humans

Gene editor + gRNA yields unique on- and off-target profile

Same LNP formulation drives shared biodistribution characteristics

Benefit-risk appropriate GMP to limits # of lots without meaningful impact on gRNA quality

Legend Streamline/rely on initial IND ▶ Proceed in full, limited ability to reference first IND

Value from platform

Value: What is the value from platform efficiency?

	Pre-platform 1.0	Platform 1.0+
Development time for each therapy[1]	36 months	6 months[2]
Patient benefit	Liver transplantation (if available) at 1 year of age, significant accumulated neurological disability	Gene edit at 6 months of age, less invasive than transplant and lower accumulated disability
Therapeutic access implications	Ultrarare populations of n of 1 (unique guides) or n of 300 (unique editor) insufficient for sustainable development	5-10K US patients sufficient to develop sustainable development model, attract commercial developer interest

1: Assumes new guide only
2: Assumes no BD, Tox, animal studies required – *in vitro* only for on- and off-target editing

Perceived risks, and mitigation

Risks	Acute adverse effects from the LNP formulation, including effects on the germline	Long-term genotoxicity in the form of off-target editing (specific to the editor/gRNA)	Underestimating on-target editing efficiency
Mitigation approach / theoretical regulatory agency perspective[1]	**Platform 1.0** (Δ in gRNA) FDA requests safety data from a Phase 1 clinical trial with the lead editor/gRNA before approving amendments (Platform 1.0) **Platform 1.5** (Δ in gRNA and editor) Agency may require separate INDs[2], but mitigate uncertainty about different acute AEs by evaluating all editors in a singe set of NHP/rodent tox studies and showing equivalency **Platform 2.0** (Δ in gRNA, editor, target gene) Agency would need different set of POC studies in a mouse model for each disease before adding disease to the master protocol	**Platform 1.0 – 2.0** Off-target editing would be evaluated for each individual editor/gRNA combination in a platform approach, so platform does not introduce risk relative to standard IND approach	**Platform 1.0 – 2.0** Agency may require preclinical evidence of strong *in vitro* activity, with in vivo correlation of on-target editing efficiency, - *i.e.*, results in cells are highly predictive of results in the target organ in mouse models (such data could be readily generated)

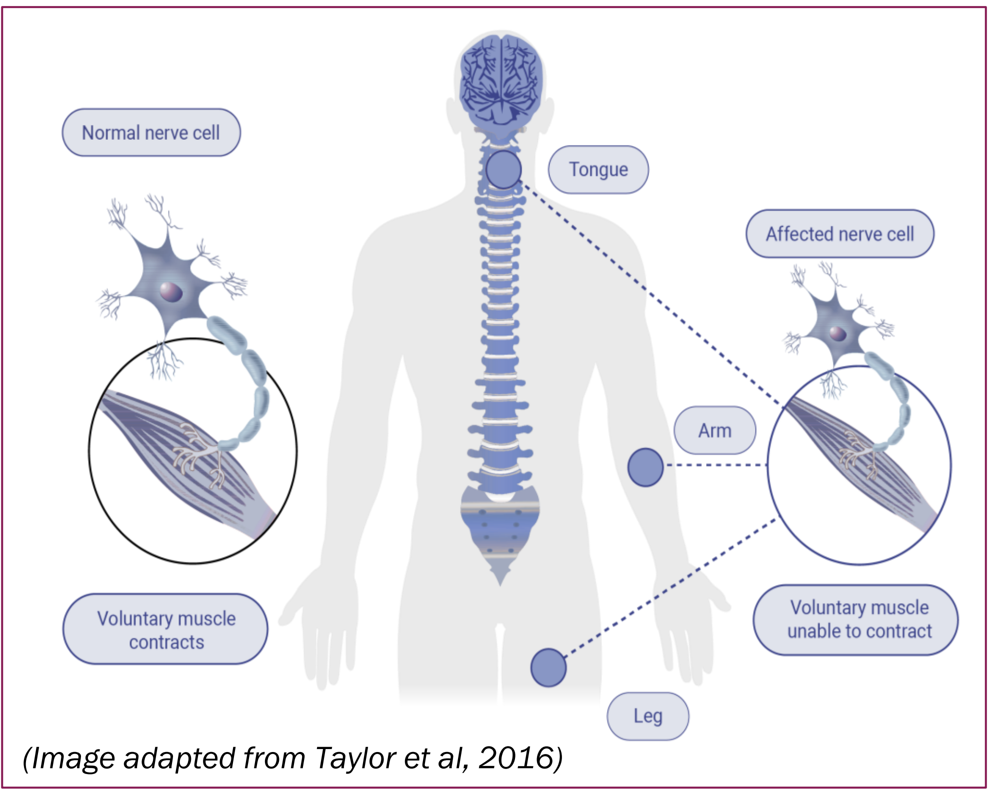
1: Summary perspective offered by the author of the case study
2: Though not necessarily

3
Case study
AAV ALS Gene
Editing Platform
arbor
biotechnologies

Amyotrophic Lateral Sclerosis Unmet Need & Medical Benefit

Medical benefit: What diseases could the platform address?

What specific conditions could the platform target? What unmet need does the platform address? How does the platform potentially benefit the rare disease community?



(Image adapted from Taylor et al, 2016)

Amyotrophic Lateral Sclerosis

- Progressive neurodegenerative disease that affects upper and lower motor neurons, leading to muscle wasting and paralysis
- Often progresses to death from respiratory failure within 3 to 5 years from symptom onset (~50-66 years)
- ~80K prevalent with ~17K incident cases in US & EU[2-5].
- Most common form of ALS (about 90%) is sporadic and associated with TDP-43 proteinopathy.
- Genetic drivers of familial ALS[6-9]:
  - C9ORF72: ~5% of all ALS (4,000 prevalent patients)
  - SOD1: ~2% of all ALS (1,500 prevalent patients)
- Current treatments slow disease progression and control symptoms but fail to address underlying cause

AAV Gene Editing platform could enable increased and faster drug development to bring novel therapies to ALS patients

1. Claims data of physician diagnosis (ICD-9 code 335.2 or ICD-10 code G12.21) and further classification based on El Escorial criteria. **2.** Golby R, 2016. **3.** Freer C, 2015 **4.** Chio A, 2017. **5.** Scialo C, 2016. **6.** Brown et al 2021. **7.** Ducharme S et al 2017. **8.** Rohrer JD et al 2015. **9.** Majounie E et al 2012.

AAV ALS Gene Editing Platform Modular Components

Platform AAV gene editing approach enables more efficient development of therapies and benefits more ALS patients.

Category	Component	Fixed	Variable
Therapeutic target	Disease / syndrome	X	
	Specific indication / target gene		X
	Target organ / cell	X	
Therapeutic platform	Delivery	X	
	Gene editing method	X	
	Gene editor	X	
	Guide RNA		X
Quality	Manufacturing (APIs / DP)*	X	
	QA / QC / analytical validation	X	
	CQAs	X	
Trial	Route of administration	X	
	Endpoints	X	
	Trial protocol	X	

Specific gRNA sequence for target GOI.

Targeting the same disease and organ/cell type allow for fixed vector, nuclease and plasmid configuration.

Fixed components allow for efficiency across manufacturing, quality, and clinical execution.

**Plasmid gene of interest (GOI) manufacturing would be program-specific.*

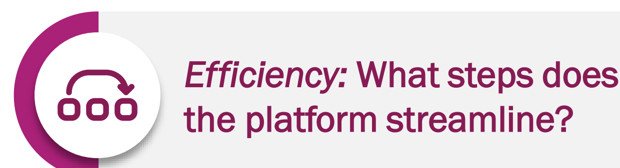
AAV ALS Gene Editing Platform Attributes

Definition: What is included in the platform?

What components are fixed within a platform? Consider delivery method, editing technology, target cell/tissue/ disease area, or other key components. What is the rationale for fixing these components within the platform?

Platform Attributes		Fixed vs. Variable
Disease / Indication	Amyotrophic Lateral Sclerosis (ALS)	Fixed
Target Gene	Various target genes (*i.e.*, STMN2, SOD1)	Variable
Target Organ / Cell	Whole brain, brain stem, cervical and thoracic spinal cord	Fixed
Patient Prevalence	> 80,000 patients US & EU	Fixed
Delivery	Adeno-associated viral vector (AAV)	Fixed
Gene Editing Method	CRISPR-Cas mediated knockdown	Fixed
Gene Editor	Small CRISPR-Cas nuclease	Fixed
gRNA	Standard nucleotide gRNA lengths	Variable
Promoters & Vector	Promoter selection and vector configuration optimized once for whole platform	Fixed
Manufacturing / CMC	AAV vector system; Rep2/Cap9 and pHELP plasmid Master Cell Banks (MCB) and Engineering of MCBs; CDMO process feasibility and optimization; Engineering runs; CMC sections of IND. *Specific GOI plasmid production, MCB, and ENG; GMP manufacturing & engineering runs for DS/DP required.*	Fixed
QA / QC / Analytics	Overlap in CDMO, required QA/QC, shared vector system, and analytic processes. *Specific activities for final DS/DP will be required.*	Fixed
Route of Administration	Intra-CSF (cerebrospinal fluid)	Fixed
Clinical Endpoints	ALS Functional Rating Scale – Revised (ALSFRS-R), change in slow vital capacity from baseline	Fixed
Trial Protocol	Ability to leverage standard trial protocol across platform. *Potential variations would be inclusion criteria (for specific populations i.e., SOD1) and gene specific biomarkers.*	Fixed
Clinical Execution	Shared centers of excellence, HCP and patient advocacy community	Fixed

Platform Synergies Drive Impact and Value Creation



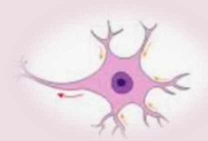
What steps in R&D and manufacturing can be omitted or done more efficiently due to platform predictability? How is a manufacturing process linked to the platform? How do these efficiencies manifest in early or late-stage development, or manufacturing?

Platform Synergies

R&D
Nonclinical

Nuclease, AAV Vector & Plasmid Configuration

In vitro pharmacology & off-targets



Biodistribution & GLP-Tox

ROA Optimization

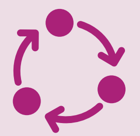
Efficiencies & Impact

- Reduced cost & time to IND
- Reduced *in vivo* animal use burden
- Streamlined data package for IND & ability to reference initial IND reports
- **Reduced FDA IND review burden**

Manufacturing
CMC, QA/QC

CDMO & Vector Platform

Process Optimization



QA / QC

Analytics

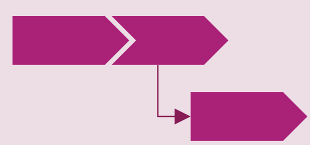
Efficiencies & Impact

- Reduced cost & time to IND
- Streamlined manufacturing process
- Shared analytics and scalable process
- Platform QA & QC synergies
- **Reduced FDA inspection burden**

Clinical
Clinical trial execution

Patient Population & Community

Clinical Plan



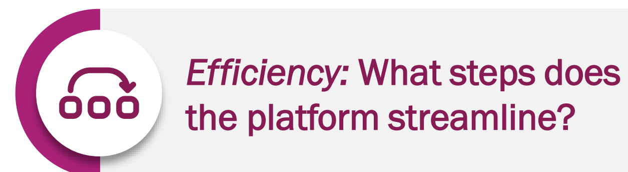
Endpoints

CRO & Clinical Sites

Efficiencies & Impact

- Increased value to patients
- Shared analytics processes
- Efficiencies in execution and decreased time to market
- **Reduced FDA review burden**

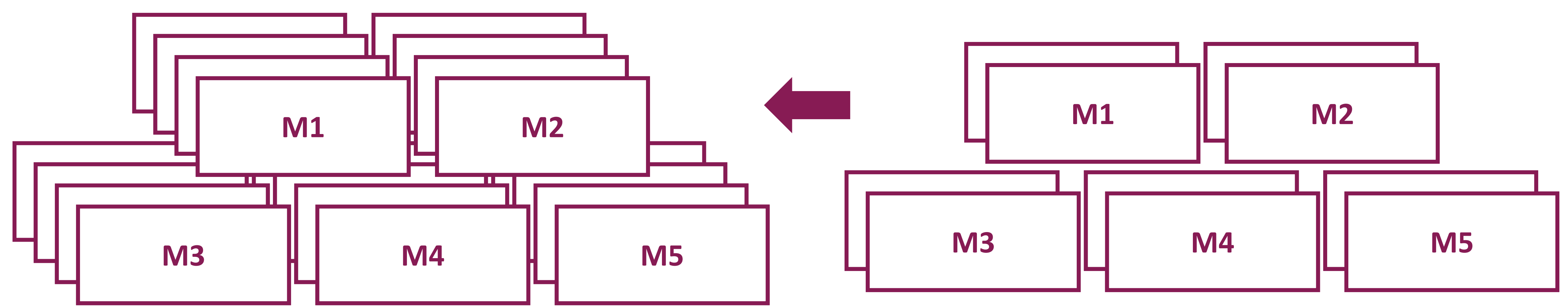
Reference IND Platform Approach



What steps in R&D and manufacturing can be omitted or done more efficiently due to platform predictability? How is a manufacturing process linked to the platform? How do these efficiencies manifest in early or late-stage development, or manufacturing?

Initial "Platform Reference" IND

Subsequent "Subsidiary" Streamlined INDs

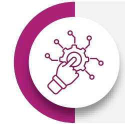


Initial program IND serves as general reference IND for subsequent platform programs

Subsidiary INDs only include specific module sections of difference with reference back to initial IND

Reduced time-to-file and review burden enabled by reference IND approach

Evidence generation will support platform validity and rigor



Performance assessment: How is the platform characterized?

What measurable parameters ensure the platform is performing as expected?
What body of evidence indicates a predictable, safe and efficacious platform?

Nonclinical Development

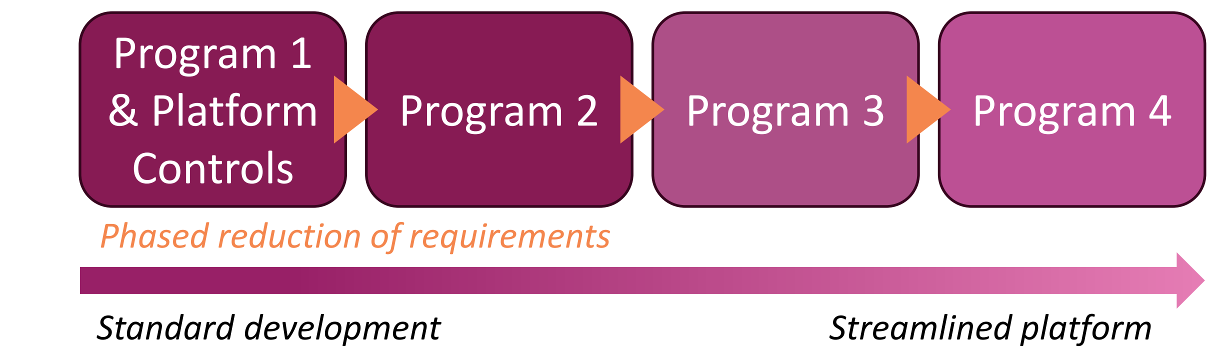
- Biodistribution parameters
- PK / PD parameters
- General and vector-specific toxicity (include scrambled vector)

Manufacturing / CMC / QA / QC

- Engineering runs
- Process development
- Analytical assays
- Stability panels

Clinical

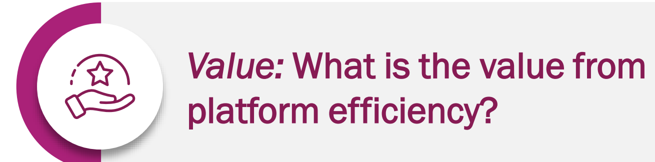
- PK / PD measures
- Safety and tox outcomes



Evidence generation to support platform

- Reference data standards from platform controls
- Reproducibility of data across programs
  - Nonclinical data packages
  - Manufacturing & quality processes
  - Clinical pharmacology measures

Estimated value to sponsor, FDA & community



Value: What is the value from platform efficiency?

What are the efficiency gains (time. FTEs, $) associated with each 'streamlined' development and manufacturing step? Are there regulatory review savings from the platform? Please quantify.

Value to Sponsor*	Value to FDA*	Value to Community*
≥25% reduction in cost to IND (FTE and program spend**)	**15-20%** synergies in IND module reports & time saved in IND review***	**2+ years** acceleration of more gene editing drugs to market (BLA)
6-12 months time savings to IND	**2+ fold reduction** in inspection requirements	**≥30%** reduced *in vivo* experiments (fewer NHP & murine studies)

Opportunity to apply platform to additional CNS diseases and/or different target tissues

*Gains do not apply to initial program in platform but would be assumed for each subsequent program within platform.
**Assumes synergies in R&D and CMC FTE to support program in research, development and CMC together with reduced spend for NHPs, analytics, process development, and CDMO costs.
***Assumes synergies in modules 2, 3, 4 and 5 from first IND to subsequent platform filings to be leveraged as reference.

What uncertainty is associated with skipped or streamlined steps? How can this uncertainty be addressed? What regulatory flexibility (if any) is required to proceed with this platform? Do regulators have the necessary tools to oversee the platform?

Risk	Mitigation	Regulatory Requirements
Cross-program impact for manufacturing site deviations.	Appropriate QA/QC at CDMOs to quickly identify and address deviations/CAPAs.	Appropriate initial FDA oversight of CDMO and manufacturers.
Cross-program impact on upstream supply components.	Increase QA/QC and supply chain monitoring for upstream components to quickly identify and address deviations/CAPAs.	Appropriate initial FDA oversight of CDMO and manufacturers.
Target specific toxicity and off-target effects.	Required nonclinical assessment of guide-specific toxicity and off-targets for all programs. Potential to run platform toxicity study to support future programs.	Early guidance or alignment between sponsor and agency on guide-specific requirements.
Inconsistent pharmacology upon gene KO across different targets.	Ensure robust assessment of PK/PD and safety parameters in research with multiple gRNAs including scramble control to build confidence.	Early guidance or alignment between sponsor and agency on guide-specific requirements.

6
Case study
In Vivo Gene Editing
for Sickle Cell Disease &
other HSC targets
CRISPR
THERAPEUTICS

Case Study | In Vivo Gene Editing for Sickle Cell Disease

Definition: What is included in the platform?

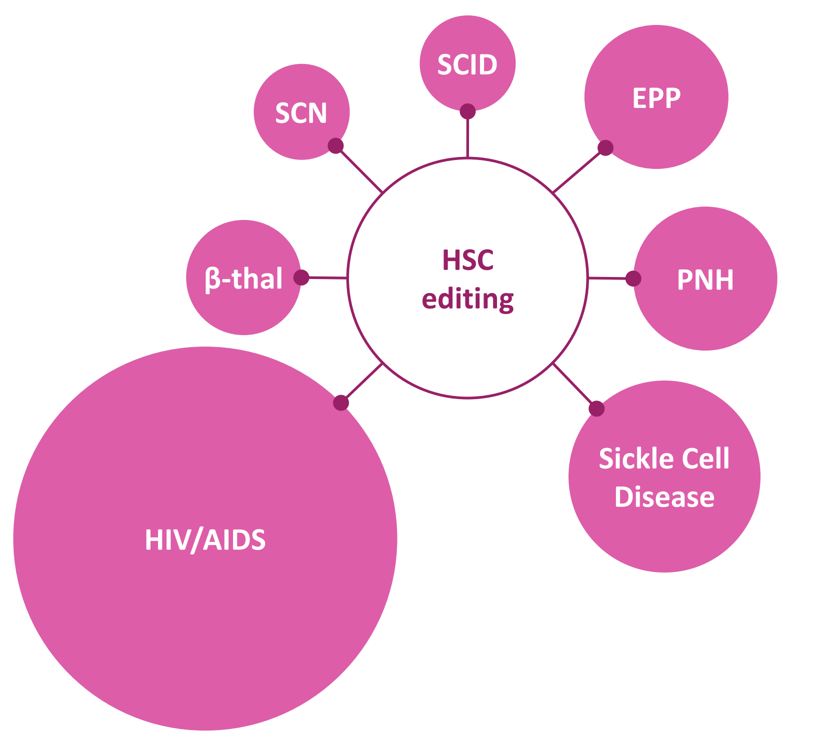
Category	Element	Platform 1: same gene, same editor	Platform 2: many genes, same editor	Platform 3: many genes, many editors
Therapeutic target	Disease / syndrome	Severe sickle cell disease, TDT	Potential other HSC KO targets	Potential other HSC correction targets
	Specific indication / target gene	BCL11a	Multiple	Multiple
	Target organ / cell	Hematopoietic stem cells	Hematopoietic stem cells	Hematopoietic stem cells
Therapeutic platform	Delivery	In vivo: LNP	In vivo: LNP	In vivo: LNP
	Gene editing method	CRISPR/Cas9	CRISPR/Cas9	Next gen editing
	Guide RNA	Single guide	Target specific guide	Target specific guide
Quality	Manufacturing (APIs / DP)	mRNA, sgRNA, LNP	mRNA, sgRNA, LNP	mRNA, sgRNA, LNP
	QA / QC / analytical validation	Standard phase appropriate qualification / validation	Standard phase appropriate qualification / validation	Standard phase appropriate qualification / validation
	CQAs	Potency, Integrity, Identity	Potency, Integrity, Identity	Potency, Integrity, Identity
Trial	Route of administration	IV	IV	IV
	Endpoints	VOC free	Specific to indication	Specific to indication
	Trial protocol	Common trial protocol & dosing	Common trial protocol & dosing	Common trial protocol & dosing

Dozens of diseases unlocked by effective targeting of HSCs

Medical benefit: What diseases could the platform address?

What specific conditions could the platform target?
What unmet need does the platform address? How does the platform potentially benefit the rare disease community?

Illustrative, abbreviated list of diseases potentially addressable with HSC editing



Example rare diseases	US burden	Unmet Need
X-linked Severe Combined Immune Deficiency (SCID)	SCID affects 1:58,000 births in US / yr and X-linked SCID is ~40%	▪ Allogeneic transplant is only potentially curative treatment with significantly better outcomes for patients transplanted younger than 3.5mos, but majority (>66%) of patients don't have access to matched sibling donor and time to transplant is longer for unrelated donors
Erythropoietic Protoporphyria (EPP)	~4000 people (prevalence)	▪ SCENESSE, afamelanotide, only approved therapy; subcutaneous implant every other month that totals ~$100k/yr for treatment with limited uptake and questions about efficacy
Paroxysmal Nocturnal Hemoglobinuria (PNH)	12-13:1,000,000 incident rate	▪ ULTOMIRIS, ravulizumab, and SOLIRIS, eculizumab, are approved C5 inhibitor mAbs with high discontinuation rates (~30%), as well as incomplete disease control as characterized by breakthrough hemolysis (~15-30%) and ongoing needs for transfusions (~30-50%)

Scriver, C. *Human Mutation.* 2007; 28(9): 831-845. Cazzorla, C. *Molecular Genetics & Metabolism Reports.* 2018; 16: 39-45. Lah, M. *Molecular Genetics & Metabolism Reports.* 2022; 33. Buckley, R. *NEJM.* 1999; 340(7): 508-516. afamelanotide Prescribing Information. EMA. Jalbert, J. *Blood.* 2019; 134(1): 3407. Broderick, K. *J Manag Care Spec Pharm*. 2023 Aug; 29(8): 941 – 951.

Platform performance

Performance assessment: How is the platform characterized?

What measurable parameters ensure the platform is performing as expected?
What body of evidence indicates a predictable, safe and efficacious platform?

Nonclinical studies

- Biodistribution studies
- Dose range finding studies, in specific disease models
- GLP toxicology studies
- DART
- Off target assessments, including variant aware searches
- Validated infusion study
- Predefined QTPP that is phase appropriate: defined release and characterization assays

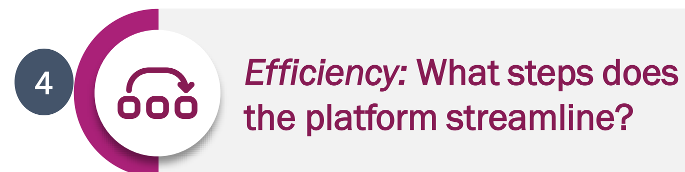
Dosing & Follow up

- GMP CoA on all Drug Substances and Drug Product
- Endpoints:
  - Primary (safety): treatment related AEs compatible with benefit-risk considerations
  - Exploratory (efficacy): clinically meaningful benefit relative to SOC
- Key TPP metrics achieved to advance to next phase

Platform evolution

- Tolerable safety, particularly as can be attributed to consistent editing cargo or delivery vehicle
- Consistent platform performance, as measured by CQAs
- Consistent manufacturing process & analytical methods to build database of cross-product CMC controls

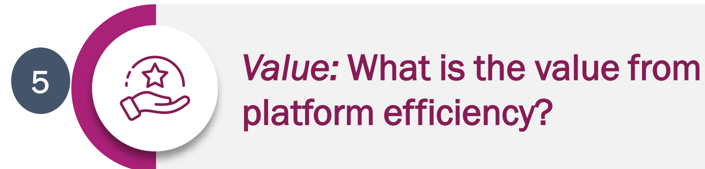
Efficiency from platform



What steps in R&D and manufacturing can be omitted or done more efficiently due to platform predictability? How is a manufacturing process linked to the platform? How do these efficiencies manifest in early or late-stage development, or manufacturing?

	Platform 1: same gene, same editor	Platform 2: many genes, same editor	Platform 3: many genes, many editors
IND enabling	GLP tox	Dependent on findings from 1.0: potential to shorten or not repeat	
	DART	DART	DART
	Off target	Off target	Off target
	Biodistribution		
Clinical trial	Individual clinical strategy	Individual clinical strategy	Individual clinical strategy
	CMC: Release & characterization	Dependent on findings from 1.0: PSQ on only particular assays	
BLA enabling	Analytical validation		
	mRNA process control strategy		
	sgRNA process control strategy	Selected PSQ	
	PPQ	Dependent on 1.0	

Value from efficiencies



What are the efficiency gains (time. FTEs, $) associated with each 'streamlined' development and manufacturing step? Are there regulatory review savings from the platform? Please quantify.

	Platform 1.0	Platform 2.0+
Development time for each therapy	De novo for each step, standard development times	Ability to accelerate if certain steps aren't repeated or can be shortened
Patient benefit	Allogeneic transplant (only 10% of patients have available donor) or recently approved ex vivo therapies	Rapid, streamlined iteration enables trials in rare diseases, particularly those without predominant mutations
Therapeutic access implications	Broader patient population than if myeloablative conditioning is required	Broad and deep: more diseases covered and ultrarare mutations addressed
Accessibility & patient access	Initial targeted footprint of centers, until experience level is sufficient	Infusion clinics with storage & thaw capabilities

Risks

Risk mitigation: How viable is the proposed platform?

What uncertainty is associated with skipped or streamlined steps? How can this uncertainty be addressed? What regulatory flexibility (if any) is required to proceed with this platform? Do regulators have the necessary tools to oversee the platform?

Potential risk	Description
On target, off tissue editing	• Delivery vehicle distribution is potentially modified due to even small changes in cargo
sgRNA specific potency	• Disease specific sgRNAs have potency that are dramatically different from one another

Guidelines with enough flexibility to evolve as the science changes, which might require external experts (whether platform or disease specific)

7
Case study
Streamlining CMC in
Gene Editing
charles river

Gene Editing Overview

Setting the stage for the CMC Discussion

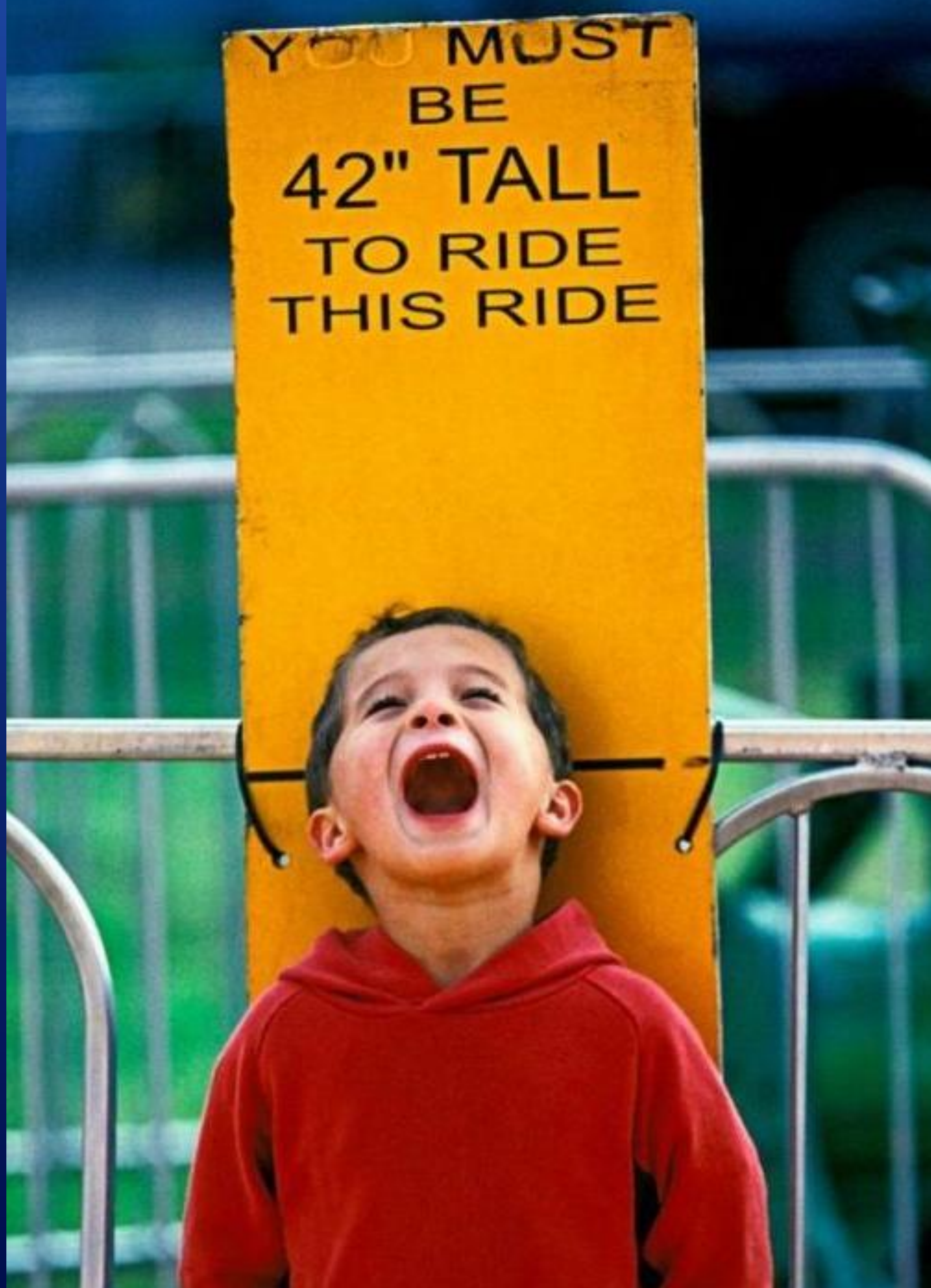
Technology	DSB inducing?	What is an off-target edit?	What is an on-target, undesired edit?	Sources of potential off-target/unintended edits
CRISPR/Cas-9	Yes	When DNA other than the target site is cut by the nuclease and modifies	When DNA repair fixes the gene in a way that does not result in the desired biological change	+ Cas9•gRNA binds and cuts off-target site + NHEJ generates a mixture of many deletions and insertions at the target site and at each off-target site + Large deletions at on-target DNA site can occur + HDR can fail to recombine full template sequence + Simultaneous DSBs at distinct loci rejoin to cause chromosomal aberrations like translocations
Base editing	No	When a site other than the target site is edited	When the target base is edited to an unintended base, or when bystander editing of a base close to the target base occurs	+ Cas9•gRNA binds off-target site + Bystander edits caused when the activity window of the base editor includes bases other than the intended base, leading to unintended base pair substitutions at the on-target site
Prime editing	No	When a site other than the target site is edited	When the target DNA sequence is edited to an unintended sequence	+ Cas9•gRNA binds off-target site + Prime editor (nickase or reverse transcriptase) makes unintended edit, such as an indel

Adapted from ARM Gene-Editing L&L October 2024

Central Tenets

Readily achievable, standard (as possible) CQAs

- Intent is to establish a minimum set of CQAs which will provide certainty to therapeutic developers employing gene editing
- Overarching goal is to speed innovation and lower the barrier of entry for programs, especially in the rare/ultra-rare space to enter the clinic
- While the goal is to streamline innovation and the path to clinic, the overarching goal of working to ensure patient safety must be at the forefront



Topics for Streamlining CMC in Gene Editing

Streamline CMC while maintaining Drug Product Quality and Safety

- Data used in the context of mitigating risk while maintaining drug product quality and safety
- Leverage existing cell and gene critical quality attribute (CQA) experience
  - Current knowledge from ex vivo (i.e. CAR-T) vs in vivo (i.e. in vivo AAV)
  - Phase-appropriate quality standards for critical raw materials/drug substance
  - Devise CQA framework based on Operation Warpspeed, establish minimum CQA requirements to be met
- Bifurcate CMC regulatory pathways for rare/ultra-rare vs non-rare to increase therapy accessibility for patients with high unmet need
- Open pathway for academic centers to manufacture for rare/ultra-rare indications; follow other licensure paths already established

Trade Secrets versus Common Know-How

A path to share common CQAs while protecting proprietary methods

Attribute	Test parameter	Test method	Acceptance criteria
General	Appearance color	Visual examination	(b) (4)
	Appearance of primary container	Visual examination	Each bag is without visible defects or leaks
Safety	Sterility	(b) (4)	No growth
	Endotoxin	(b) (4)	(b) (4)
	Mycoplasma	(b) (4)	Not detected
	Replication-Competent Lentivirus	(b) (4)	(b) (4)
	(b) (4)	Calculation [A]	(b) (4)
Purity	(b) (4) Viability	(b) (4)	(b) (4)
	Phenotype (b) (4)	(b) (4)	(b) (4)
	Phenotype (% NK)	(b) (4)	(b) (4)
	Phenotype (b) (4) purity)	(b) (4)	(b) (4)
Identity	CAR identity	(b) (4)	Positive for detection of CAR transgene
Quantity	Viable cell concentration	(b) (4)	(b) (4)
Dose	Number of CAR$^+$ viable T cells per kg of patient weight of total CAR$^+$ viable T cells in the final container	Calculation [B]	Patient 100.0 kg or below: 0.5 - 1.0 x 10^6 CAR$^+$ viable T cells/kg
			Patient above 100.0 kg: 0.5 -1.0 x 10^8 CAR$^+$ viable T cells
Potency/Identity	CAR expression from viable T cells	(b) (4)	(b) (4)
Potency	(b) (4)	(b) (4)	(b) (4)

[A] The calculation is based on results from (b) (4)
[B] The calculation is based on results from (b) (4)

Attribute	Test	Method	Acceptance criteria
General	Appearance	Visual examination	Translucent cell suspension, essentially free of visible foreign particles
Identity	CD34 expression	Flow Cytometry	Positive
	On-Target Editing Frequency	TIDE	Positive
Purity	CD34 Purity	Flow Cytometry	(b) (4)
Potency	On-Target Editing Frequency	TIDE	(b) (4)
	(b) (4)	(b) (4)	(b) (4)
	(b) (4)	(b) (4)	(b) (4)
Quantity and Content	Viable Cell Count	(b) (4)	(b) (4)
	Cell Viability	(b) (4)	(b) (4)
Safety	Sterility	(b) (4)	Drug Product: No growth (b) (4)

Adapted from Carvykti and Casgevy BLA documents

Leverage existing cell and gene CQA experience

Prior experience should inform future use-cases to streamline CMC

Operation Warpspeed CQA framework, establish minimum CQA requirements

+ Minimum Drug-Product CQAs may generally apply or be product specific

Attribute	Test	Method	Proposed Criteria
General	Appearance	Visual Inspection	Translucent, essentially free of visible foreign particles
Identity	Product Specific Purity (Surface and/or Intracellular)	Flow Cytometry	Positive (Program Specific)
Potency	On-Target Editing Frequency	TIDE (Sanger/NGS)	>30%
Potency	Product Specific Potency	Product Specific	Product/Cell Type Specific
Cell Quantity	Viable Cell Number/Viability	Automated Cell Counter	Product Specific/>70%
Safety	Residual RNP	ELISA	Negative
Safety	Sterility	BacT Alert/Celsis or equivalent	No Growth @ 14 days
Safety	Mycoplasma	qPCR - ABL7500 or QuantStudio5	Negative
Safety	Endotoxin	EndoSafe PTS/MCS	<1.5 EU/kg

Leverage existing cell and gene CQA experience

Prior experience should inform future use-cases to streamline CMC

Operation Warpspeed CQA framework, establish minimum CQA requirements

+ Minimum requirements for RNP and sgRNAs as critical raw materials and/or drug substance
+ Note: Many times RNP and sgRNAs come separately and need to be complexed on-site

Attribute	Test	Method	Proposed Criteria
sgRNA Identity	Guide Purity	Capillary Gel Electrophoresis	>90%
sgRNA Identity	Sequence Identity	TIDE (Sanger/NGS)	>80%
RNP Potency	Cleavage Assay	in vitro cell culture	Product Specific
RNP Stability	Cleavage Assay	In vitro cell culture	1+ year
sgRNA Stability	Cleavage Assay	In vitro cell culture	1+ year

Case Study to Assess Gene Editing Performance

Assess performance of different platforms to enable editing

CASE STUDY PROCESS FLOW DIAGRAM

Day -1

Thaw T-cells: Donor 1 (250e6 cells)
+ 50e6 cells/GREX in 50 mL media
+ 5 GREX100M

Day 0

Activate with Transact (RUO)
+ Sample (cell count/viability)

Day 2

Electroporate T-cells
+ 50e6 per electroporator
+ 4 electroporators + 1 control
+ Cas9/TRAC gRNAs
+ Sample pre/post electroporation (1-2 hrs)
+ Sample cells
+ Feed GREX (1L)

Day 4

Seed cells back into GREX
+ 5 GREX100M
+ 50 mL media/GREX

Day 7

Sample cells

Day 10

Sample cells

Case Study to Assess Gene Editing Performance

Assess performance of different platforms to enable editing

4 Electroporation systems

- Transfection System #1 (TFS1)
- Transfection System #2 (TFS2)
- Transfection System #3 (TFS3)
- Transfection System #4 (TFS4)

4 systems x 3 donors per system

- Cryopreserved T-cells, 50M per system (300M per donor)
- T-cell cost per donor:
  - Source purified T-cells directly: $13,000/run (1 run = 1 donor)
- Reagent cost per run (exc. Cas9/gRNAs) (4 systems): $15,000/run

Gene modification: TRAC (TCR) KO

- Cost w. Cas9/gRNAs from ThermoFisher: $1,500/donor
- Aldevron Cas9, Synthego sgRNAs

Case Study to Assess Gene Editing Performance

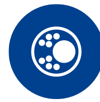
Assess performance of different platforms to enable editing

Media

- XVivo15, 5% Human AB serum (Sigma), 200 IU/mL IL-2

Gene Editing Reagents

- X um Cas9 and Y um TRAC gRNAs (Aldevron) per 25e6 cells (per electroporation)
- Client to follow up with gRNA sequences and protocols



Sampling Points

- Cell count/viability: D-1, D2 (pre & post electroporation), D4, D7, and D10

Flow cytometry (phenotype/KO efficiency): D4, D7 and D10

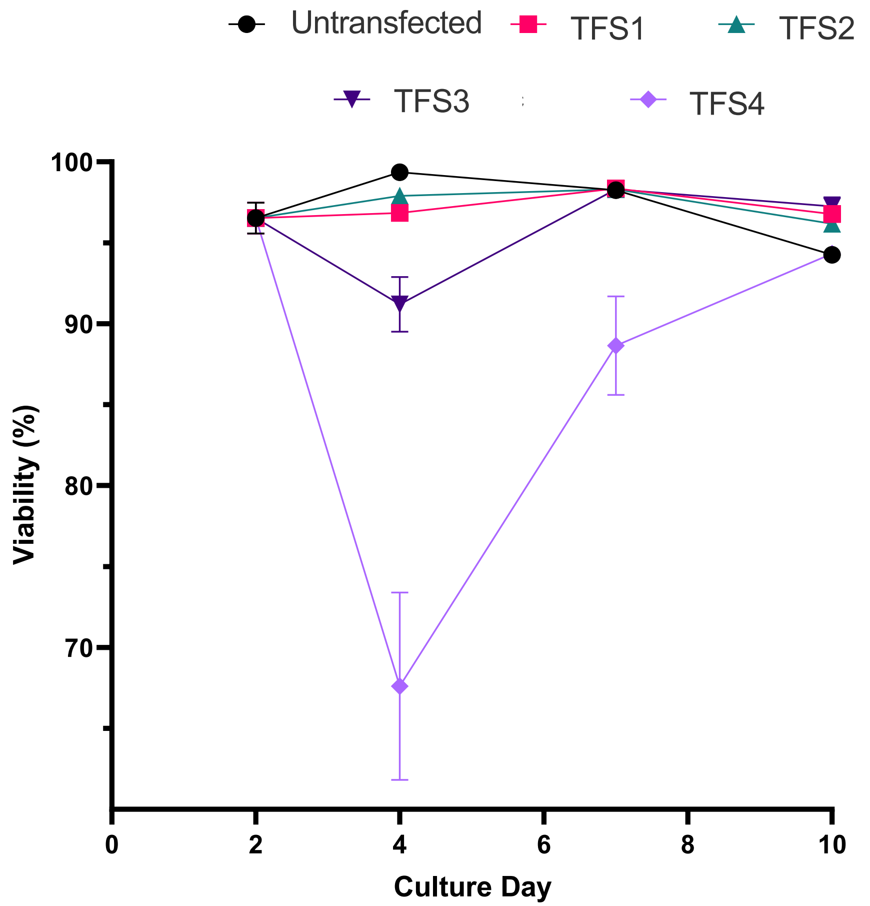
- Cells for assay: run fresh (preferred) or frozen

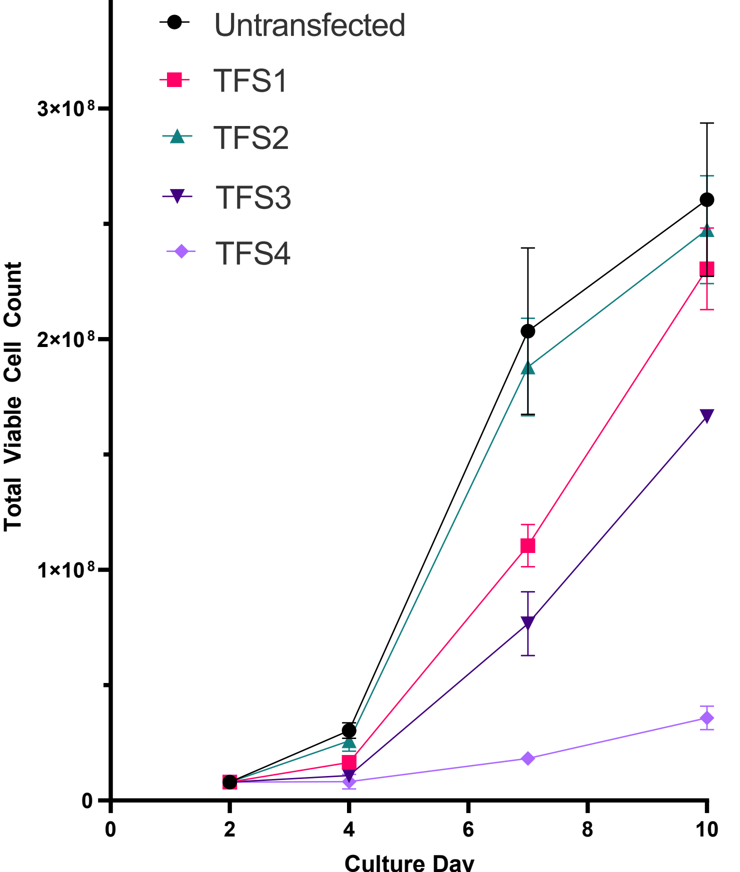
Final harvest

- Cells will be washed, formulated in CS10, and vialed at 20e6 cells/mL in 1 mL cryovials (5-10 vials per condition)

Case Study to Assess Gene Editing Performance

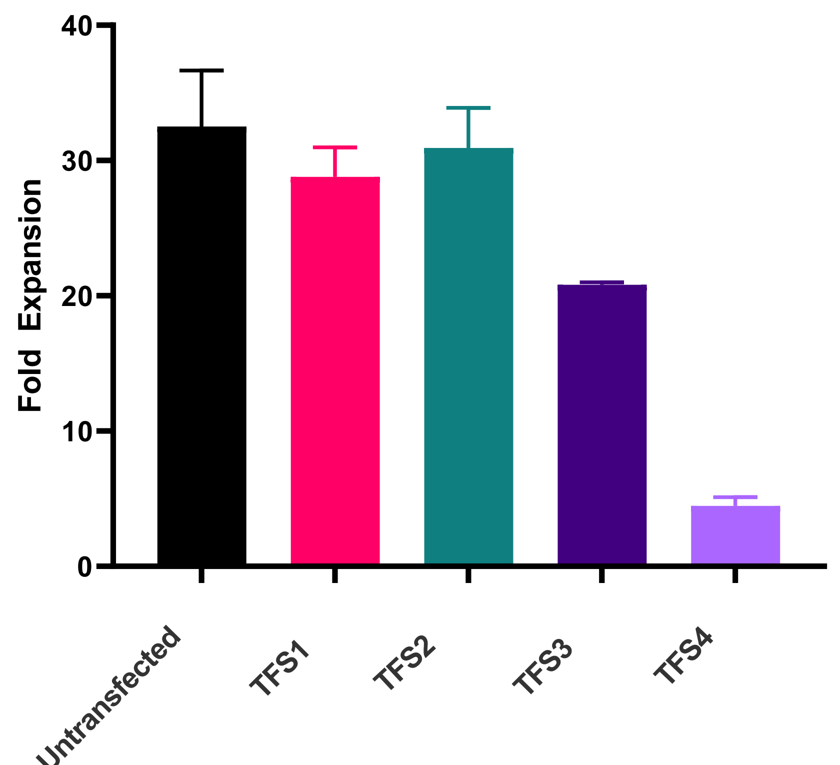
Assess performance of different platforms to enable editing

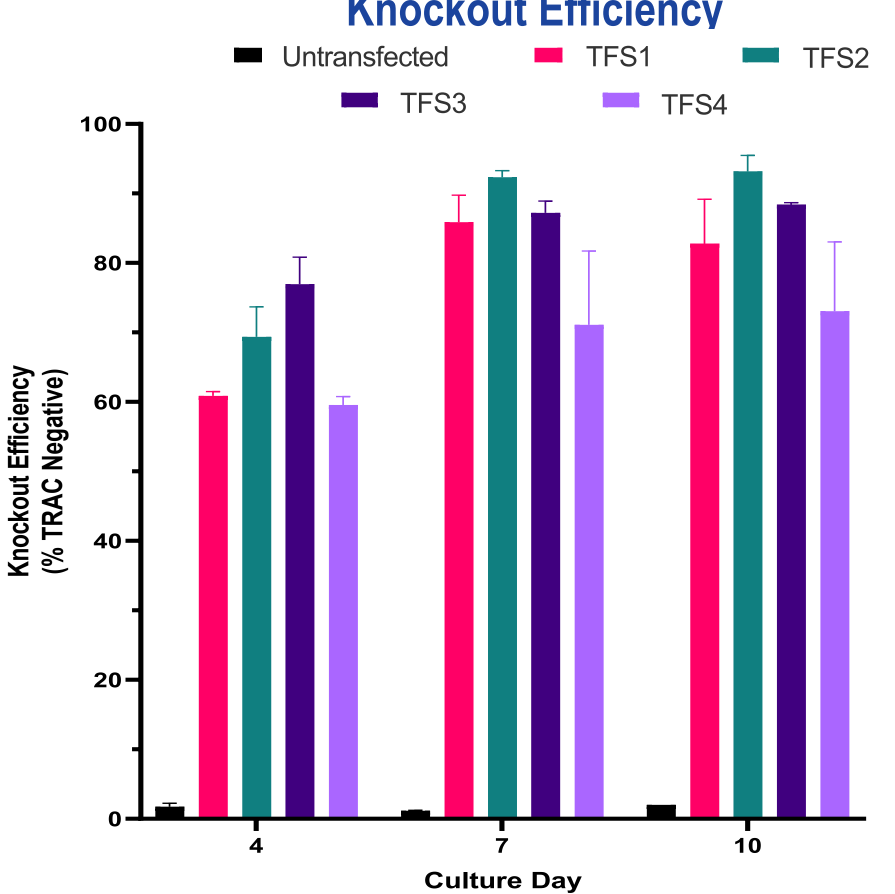




Case Study to Assess Gene Editing Performance

Assess performance of different platforms to enable editing





Opportunities to Streamline CMC

Simplify process to reduce chance of error to increase therapy access

+ **Compile standard CQAs** which can be applied across a platform gene editing program
+ **Standardize precomplexed RNP gRNA** to reduce error risk and increase consistency
+ **Streamline stability & reagent potency studies** by assessing precomplexed RNP gRNA
+ **Platform guide sequence and length for common targets** (i.e. TCR, B2M etc)
+ **Phase-appropriate precomplexed RNP gRNA** for *ex vivo* processes

Plasmid DNA Production Conditions and Options

GMP | HQ | Research

1. Cloning/Synthesis/Mutagenesis of plasmid
2. Scale-up and manufacturing of existing plasmid constructs
3. Proven manufacturing and purification processes for high yield and recovery
4. Standardized manufacturing platform processes
5. Establishment of E. coli master and working cell banks
6. Upstream PD (optimization of transformation and culture conditions, strain, etc.)
7. Downstream PD (optimization of purification and formulation conditions)
8. Full traceability of materials
9. Full room changeover prior to each production
10. Production in segregated and dedicated space
11. Process and change control
12. Aseptic fill and finish
13. Document support for IND and IMPD filing
14. Master Batch Records
15. Stability studies
16. Certificate of Analysis
17. Manufacturing in GMP suite with graded air
18. Environmental monitoring throughout production process
19. QA oversight for production

Alternative CMC Regulatory and Manufacturing Strategies

Leverage academic centers to manufacture rare/ultra-rare indications

Rare/
Ultra-Rare

Yes

Pre-Existing
Therapeutic
Options

Yes

Standard
Regulatory
Pathway

No

Accelerated
Regulatory
Pathway

Yearly
Therapeutic
Need <850
Doses

Yes

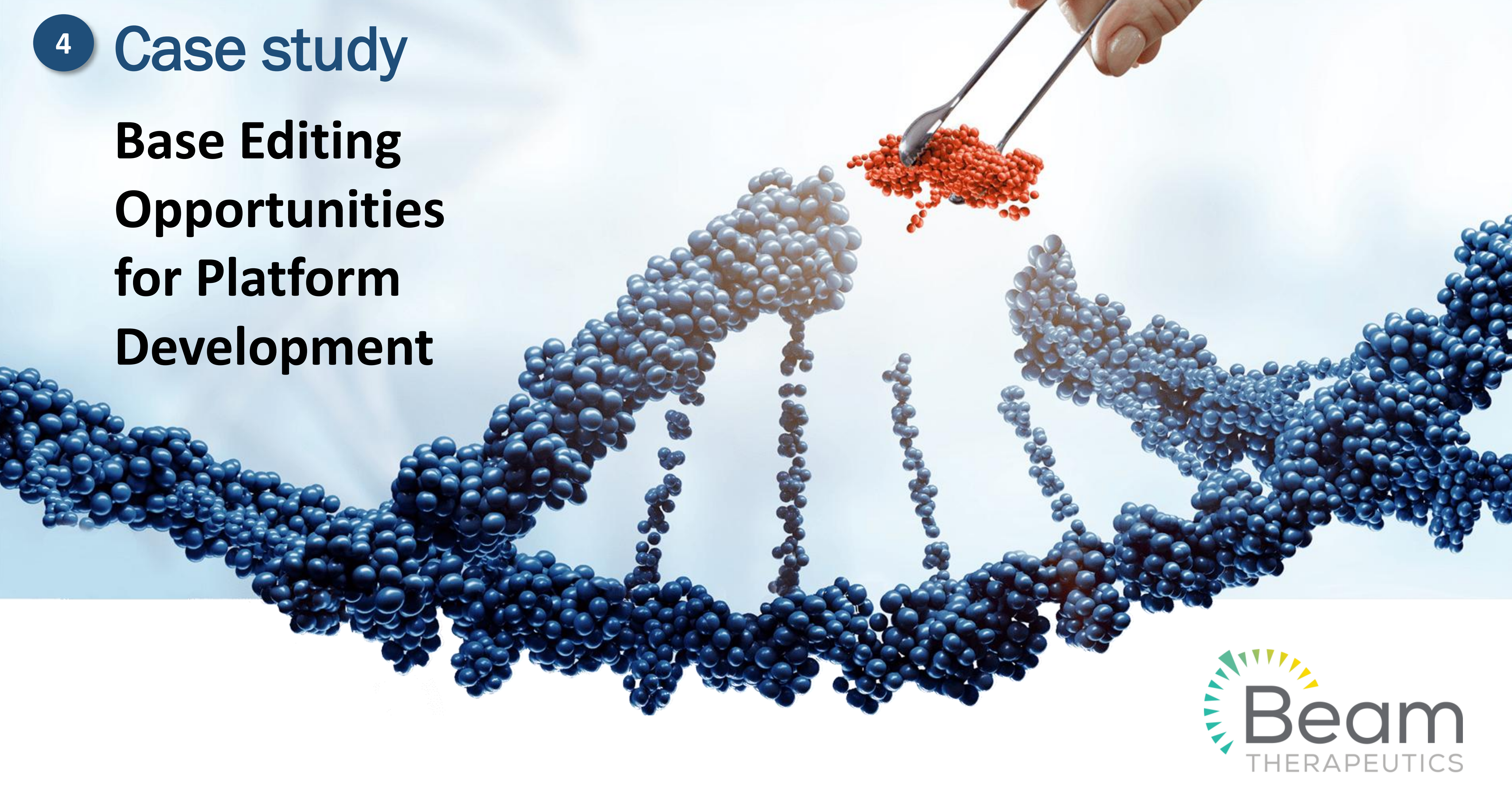
Academic
Center
Manufacturing
Under Modified
Licensure
Similar to Cord
Blood Centers

No

CDMO/
Industry for
Manufacturing

No

Standard
Regulatory
Pathway


4
Case study
Base Editing
Opportunities
for Platform
Development
Beam
THERAPEUTICS

The Precision of Base Editing Presents New Opportunities

- Base editing can be used to precisely correct single nucleotide variants that cause disease
- Many inborn errors of metabolism can be corrected by gene editing in the liver through LNP-mediated delivery (e.g., BEAM-301 for glycogen storage disease 1a to correct G6Pase)

BEAM-301

1. Formulate LNP	2. Infuse	3. Correction
Lipid Base editor mRNA gRNA		Non-functional G6Pase (A / T) A>G Wild type G6Pase (G / C)

Base-editing strategy to treat a severe pathogenic variant underlying Glycogen Storage Disease Type Ia

Wild type G6Pase

Non-functional G6Pase

Glucose

Non-functional G6Pase

Glycogen

Glucose-6-phosphate

Glycolysis

Pyruvate

Acetyl-CoA

Lipogenesis

Fatty acids, triglycerides

Acute drops in blood glucose levels can result in fasting hypoglycemia, seizures, and death

No treatment beyond dietary management with slow-release glucose

Metabolic and long-term complications include,

- Elevated cholesterol, triglycerides, lactic and uric acid
- Enlarged livers (glycogen, fats)
- Hepatic adenomas, HCC
- Growth impairment

- GSDIa is a genetic disease caused by mutations in the G6PC gene encoding G6Pase, a predominantly liver-expressed enzyme vital to glucose metabolism
- G6PC-p.Arg83Cys (R83C) is a prevalent pathogenic variant associated with severe manifestations of GSDIa
- Beam's base-editing technology has the potential to permanently correct this mutation and restore glucose metabolism

The Precision of Base Editing Also Presents New Challenges in Drug Development

- Monogenic diseases often have a diverse mutational spectrum
- GSD1a has ‘founder’ mutations which vary by ethnic background and region
- Within ethnicities there is also a long tail of rare variants

Caucasian	Chinese	Japanese	Korean	Hispanic	Caucasian Ashkenazi Jewish
p.R83C (33%)	p.R83H (26%)	c.648G>T (91%)	c.648G>T (75%)	c.380_381insTA (54%)	**p.R83C (98%)**
p.Q347X (18%)	c.648G>T (54%)			p.R83C	p.Q347X (2%)

Hum Mutat. 2008 Jul; 29(7): 921–930.

- **Each disease variant requires its own drug product**
  - Common LNP composition
  - Unique gRNA (required)
  - Customized base editor mRNA (maybe…)

Challenges at the top of the funnel: lead discovery and optimization

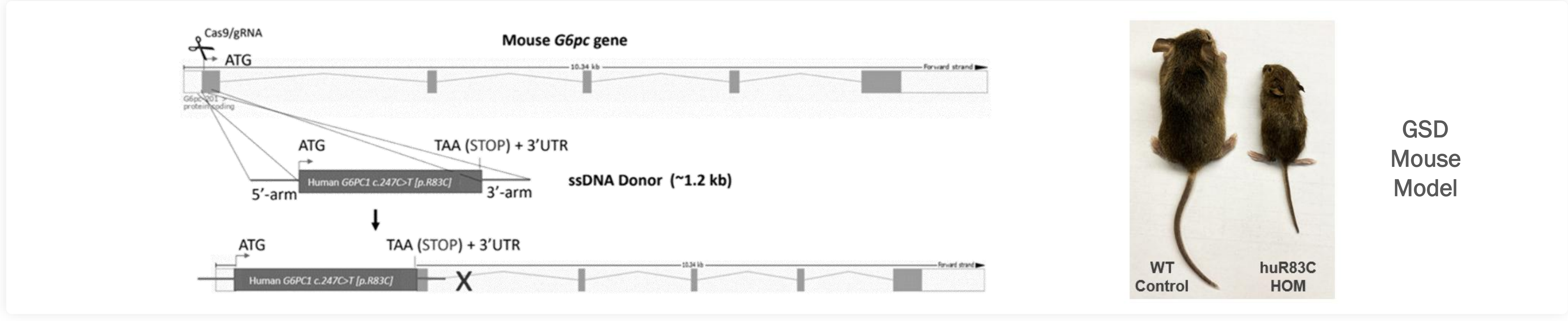
- gRNA screens are limited to the sequence immediately surrounding the disease causing SNV
- This densely tiled gRNA screen requires use of Cas orthologues and variants that accept alternative PAM motifs

GCATTCATTCAGTAACCCCAGAAACTTGTTCTGTTTTTCCATAGGATTCTCTTTGGACAGtGTCCATACTGGTGGGTTTTGGATACTGACTACTACAGCAACACTTCCGTGCCCCTGATAAAGC
CGTAAGTAAGTCATTGGGGTCTTTGAACAAGACAAAAAGGTATCCTAAGAGAAACCTGTCaCAGGTATGACCACCCAAAACCTATGACTGATGATGTCGTTGTGAAGGCACGGGGACTATTTCG
78 80 82 84 86 88 90 92 94 96 98 100 102 1
I L F G Q C P Y W W V L D T D Y Y S N T S V P L I K
42900877-42911426: Region 2
R83C 7
BEAM-301
R83C 6
R83C 5
R83C 4
R83C 3
R83C 2
R83C 1

- Preliminary hits are often evaluated with a panel of editors with varying deaminases and linkers
  - Optimizing for editing of the target base while minimizing undesirable bystander edits.
  - **Thus far, Beam's development stage programs each use a unique base editor**

Overcoming barriers to achieving proof-of-concept

- Base editing correction require models that harbor the disease-causing variant of interest
- *In vitro* models may exist in biorepositories or can be engineered (CRISPR, BE, PE, lentivirus)
- *In vivo* models rarely exist but unique knock-in mouse model can be generated, characterized and used to support an IND-enabling nonclinical data package



- Mice compound heterozygous for mutation A and B may better reflect patient population and permit study of up to two products in a common model species
- This approach to *in vivo* models is not scalable, and at a certain point may not be necessary?
  - We expect models of the same disease to behave similarly despite having different mutations
  - *In vitro* potency assays could be developed to compare potency of distinct drug products

Streamlining the Nonclinical Toxicology Package

	Study	Species	Purpose
LNP Platform Studies	Non-GLP Tolerability Studies	NHP and rodent	Non-GLP Tolerability Studies: Initial exploratory toxicity and DRF studies confirming rodent is most sensitive species for LNP safety assessment and establish tolerability across wide dose range
	Single dose GLP Toxicology study	Rodent	Single dose GLP Toxicology study: LNP safety in wild-type animal (may or may not contain editable gene)
	GLP Cardiovascular safety pharmacology study	NHP	GLP Cardiovascular safety pharmacology study: CV safety assessment for novel lipids
	GLP *In vivo* micronucleus study	Rat	GLP *In vivo* micronucleus study: *In vivo* genetox assessment for novel lipids
	GLP Ames	*In vitro*	GLP Ames: Mutagenicity assessment for novel lipids
	GLP *in vitro* micronucleus assay	*In vitro*	GLP *in vitro* micronucleus assay: Clastogenicity assessment for novel lipids
	GLP Progeny and Fertility Study	Rodent	GLP Progeny and Fertility Study: Show lack of germline transmission of edit and fertility assessment
Product Specific Studies	GLP Pharmacology/Toxicology Study	Disease model	LNP and PD-related safety assessment in pharmacologically-relevant (containing editable gene) model

Streamlining Nonclinical Pharmacokinetics and Biodistribution

	Study	Species	Purpose
LNP Platform Studies	Non-GLP Biodistribution Study	Rat	Tissue and cellular (mRNA and editor protein) distribution of mRNA, novel lipid, and gRNA distribution
	Non-GLP PK Study	NHP	Systemic and liver mRNA and lipid PK in higher order species to enable FIH dose prediction modeling
Product Specific Studies for some diseases that could impact product uptake and/or clearance	Non-GLP Biodistribution Study	Disease model	Tissue distribution of on-target editing in pharmacologically relevant model and limited, confirmation of mRNA and/or novel lipid distribution
	Non-GLP PK Study	Disease model	Systemic and liver mRNA and lipid PK in pharmacologically relevant model to enable FIH dose prediction modeling

Off-target Assessments Have Product-Specific Components

Study	Experimental Assays	What is assessed?
gRNA-independent off-target editing	RNA-seq scWGS	Editor itself (pairing with a different gRNA should not change result)
Genomic rearrangements	UDiTaS	gRNA/editor pair
gRNA-dependent off-target editing	ONE-seq Digenome-seq rhAmpSeq	gRNA/editor pair
gRNA-dependent off-target risk assessment	RT-qPCR Targeted RNAseq	Individual off-target sites/edits

Maintaining product quality while maximizing CMC efficiencies across related drug products

Manufacturing Process

- Multiple products (variants of same disease or different indication), could be developed using same or highly similar manufacturing processes
- Efficiencies could be gained through qualification and validation of one manufacturing process for multiple products using a platform approach

Analytical Methods

- Where possible, analytical method development and validation can be assessed to use a platform approach
- Platform Assay Validations
  - Purity
  - Safety
- Product Specific Assays
  - Potency
  - Identity

Stability

- Products will be evaluated to determine stability indicating methods
- Stability programs will be developed with scientific intent and compliance but maximizing efficiency
- In-use stability may be another area of efficiency

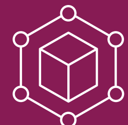
Clinical considerations, precision medicine via base editing

Opportunity to *treat* rare and ultra rare diseases, correct disease-causing mutation in a targeted, biologically-rationale fashion, enabling genetic outcome approvable endpoints

Potential for large clinical effects that could not be explained by chance variability, enabling a reduction in patient numbers required to demonstrate efficacy and/or potentially eliminating need for placebo cohort

Potential for biomarker serving as basis of accelerated or full approval given correction of disease pathophysiology at it's most proximal and root cause, the DNA mutation

Potential for umbrella trial to treat one disease which can be caused by a variety of mutations with the corresponding based editing therapy



Opportunity to leverage knowledge of components of platform technology to understand dosing, safety, benefit-risk profile and enable more efficient study designs

Opportunity for Integrated Summary of Safety across different platform products to generate a larger safety database

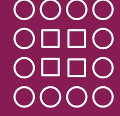
Opportunity to have one long-term follow up study across multiple mutations for one disease

Leverage synergies to support clinical development package and understanding of safety and dosing

Drug component	Contribution to potential safety profile	Safety	Study design (stagger)	Clinical pharmacology to inform dosing
LNP (delivery)	IRR, increase LFTs, immunogenicity	Since same LNP, understanding risks from 1 indication will inform safety monitoring, mitigation and characterization in other indications(s)	Timing of acute toxicity in 1 indication can inform stagger duration for other indications; no need to wait the standard 28d period if acute tox seen within 14d	BioD is determined by delivery method PK from one indication can inform modeling and dose selection. Use safety and PK data from one indication to enable higher starting dose in a different indication; shorter staggering; limit number of dose levels to evaluate
mRNA encoding for base editor	guide-independent off-targets, immunogenicity	If same mRNA, safety profile from 1 indication can be used to support the other indications(s)	Doesn't contribute to acute toxicity so would not impact stagger	
gRNA directing editor to precise genetic location	guide-dependent off-targets	Unique gRNA – dependent risks need to be considered separately for different indications	Doesn't contribute to acute toxicity so would not impact stagger	Unique potency; need to generate PD data to understand dose-response or efficacy.

Clinical synergies achievable via a master protocol

Same investigators/sites involved (disease-driven)



Same inclusion/exclusion criteria (except for genotype)

Same endpoints for safety (LNP-mediated)

Same endpoints for efficacy (disease-driven)

Same schedule of assessments (disease-driven)

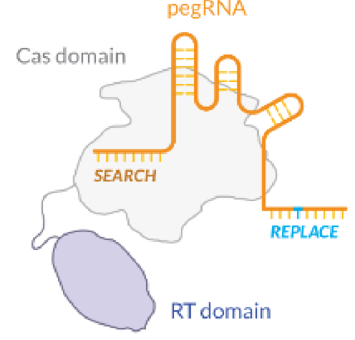
- PK will require methods appropriate to the drug substance(s) that the patient received

Case study
Modular platform for in vivo
LNP Formulated Prime Editors
Targeting Rare Genetic
Diseases of the Liver
prime
medicine_

Prime Editing: A Gene Editing Technology That Allows Personalized Therapy That is Mutation Specific

Prime Editor Machinery Overview

Key Components



1. Prime Editor protein (Cas domain + reverse transcriptase domain)
2. Prime Editing guide RNA (pegRNA)
3. +/- nicking guide RNA (ngRNA)

Key Attributes

- Programmable to different DNA targets
- Versatility enables correction of broad mutation classes (point muts, ins, del)
- Flexibility in PAM targeting through small changes to Cas domain
- Precise edits without "bystanders"
- No requirement for double stranded breaks or donor DNA
- Minimal or no detectable off targets
- Guide discovery needed for each mutation

Steps to Gene Correction

SEARCH	FIND & NICK	PRIME	REPLACE	GENE CORRECTED
Prime editor complex initiates search for target DNA	Prime editor complex finds DNA with target mutation, nicks one strand	Nicked DNA strand primes the RT domain for DNA synthesis	Prime editor complex copies in corrective DNA sequence	3' flap preferentially incorporated, excess flap repaired, gene fully corrected

Abbreviations: pegRNA = prime editing guide RNA; RT = reverse transcriptase; Cas = CRISPR associated protein

Source: Anzalone, et al (David R. Liu). Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature*, 2019.

How to make it economically viable to ensure that even the subset of patients with the rarest mutation of an ultrarare disease may benefit from a Prime Editing cure

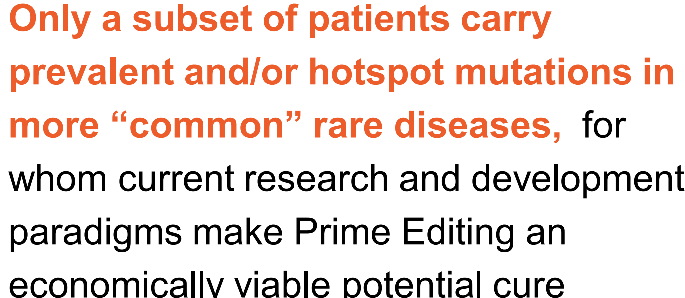
Call to Action

There needs to be a way to **leverage preclinical, CMC/manufacturing** and **clinical data across mutations and diseases addressed by the same tissue delivery platform** to offer every patient a potentially curative once and done bespoke genetic medicine

Non-Sustainable Model

After addressing the prevalent and/or hotspot mutations, **additional patients do not have access to potential Prime Editing cures** due to the rarity of their mutations or ultrarare nature of their disease which would require significant development time and costs based on current regulatory paradigms

Where we are now



Only a subset of patients carry prevalent and/or hotspot mutations in more "common" rare diseases, for whom current research and development paradigms make Prime Editing an economically viable potential cure

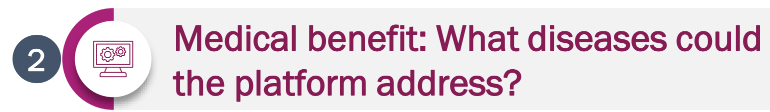
Prime Editing Platform Modularity Goals

Category	Element	Fixed	Variable
Therapeutic target	Disease / syndrome		X
	Specific indication / target gene		X
	Target organ / cell	X	
Therapeutic platform	Delivery	X	
	Gene editing method	X	
	Gene editor	X	
	Guide RNA		X
Quality	Manufacturing (APIs / DP)	X	
	QA / QC / analytical validation		X
	CQAs	X	
Trial	Route of administration	X	
	Endpoints	X	X
	Trial protocol	X	X

Prime Editing Platform Modularity

Goal	Bringing Prime Editing (PE) technology with liver targeted *in vivo* LNP to more patients rapidly and cost-effectively
Modularity	For an *in vivo* LNP program targeting different mutations in the same gene, and even different genes causing liver specific disease, the delivery vehicle and editing cargo remain constant except for the guide RNAs
Rationale	Expect similar critical product attributes, toxicity profile, biodistribution, manufacturing process and many analytical assays, clinical safety and pharmacology profile
Considerations	Only potential differences are off-target edits and, for different diseases, disease-specific biomarkers and assessments of clinical efficacy; some CQAs reduced w/ platform progression
Patient Impact	A modular platform is a solution to most efficiently and expeditiously develop mutation-specific Prime Editors for the treatment of patients with diverse genetic mutations

Potential to Reach All Patients Through Gene Correction Regardless of Mutation and Population Size



>1K U.S. patients
0.1-1K
<0.1K

Mutation
Wilsons' Disease
GSD1b

Exemplary Gene Correction Patient Accessibility

Illustrative, not to scale

	I Initial development candidate(s)	II Next iteration	III Final iteration
Mutation Frequencies Distribution	Height represents frequencies of mutations		
Case Study Example	I	II	III …
Framework for Expanding *in vivo* LNP formulated Prime Editor	Prime editor targeting the **prevalent or hotspot mutation** with the **largest patient population** for **one disease** (*in vivo* LNP)	Prime editor targeting **multiple mutations** that meet a patient **population threshold** across **more than one disease** (*in vivo* LNP)	Potential to efficiently develop Prime Editors targeting **all mutations**, addressing the **entire patient population** in **more than one disease** (*in vivo* LNP)

Framework for Expanding Liver Targeted LNP-Formulated Prime Editors

Medical benefit: What diseases could the platform address?

Legend: Low / High

		I. Initial development candidate(s)	II. Next iteration	III. Final iteration
Treatment Scope	Disease	1 disease	2 diseases	2 diseases
	Gene and Mutation	1 mutation in 1 gene	Multiple mutations[1] in 2 different genes	Many very rare mutations[1] in multiple genes
	Delivery Method	*In vivo liver targeting* LNP	*In vivo liver targeting* LNP	*In vivo liver targeting* LNP
Prime Medicine Development	Development Effort	High, guide discovery (in silico, hit to lead, optimize), preclinical, CMC/mfg, standard clinical trial for rare disease	Medium, guide discovery, preclinical, mfg, clinical platform trial w/ biomarkers	Low, guides discovery, mfg, minimal preclinical, non-clinical study
	Platform Modularity (Leveraging Prior Data)	No leverage, generate comprehensive data	Medium, leverage prior data for preclinical, CMC, and clinical	High, leverage all prior data
Case Study Example[2]	1st Disease	Wilson's Disease H1069Q	Wilson's Disease R778L	Wilson's Disease remaining rare mutations
	2nd Disease	N/A	GSD1b L348fs and G339C	GSD1b many remaining rare mutations

1. Exceptions for scientific/clinical reasons, such as situations in which certain mutations have contradictory effects to other sets of mutations
2. Case Study Example may not reflect Prime Medicine's actual clinical path for exemplified diseases

Clinical Overviews of Wilson's Disease and Glycogen Storage Disease Type 1b

Medical benefit: What diseases could the platform address?

Wilson's Disease

Disease severity and opportunity
- Common liver and systemic disease, typically appearing in teens to 20s
- Causes liver failure, neurocognitive decline, premature death
- Affects over 20,000 patients in the US and Europe, with 30-50% carrying the H1069Q mutation
- R778L is most common mutation in the Asian population

Unmet need
- Liver transplant is only curative option, but with high morbidity/ mortality
- No approved disease-modifying therapies
- Current care focuses on preventing copper buildup with chelating agents and a low-copper diet

Human biology
- Autosomal recessive disorder caused by loss-of-function mutations in ATP7B
- Disrupts copper homeostasis, causing toxic buildup in the liver and brain
- Correcting 20-30% of hepatocytes could be curative

Glycogen Storage Disease Type 1b (GSD1b)

Disease severity and opportunity
- Rare metabolic disorder, typically presenting in infancy
- Causes life-threatening hypoglycemia, hepatomegaly and growth delay
- Affects fewer than 1,000 patients in the US and Europe
- Patients often experience neutropenia, leading to increased infection risk, as well as inflammatory bowel-like syndrome

Unmet need
- Patients rely on lifelong dietary management and frequent cornstarch supplementation to prevent hypoglycemia
- No curative treatments, only dietary and infection management
- Care focuses on blood sugar control and infection prevention with G-CSF

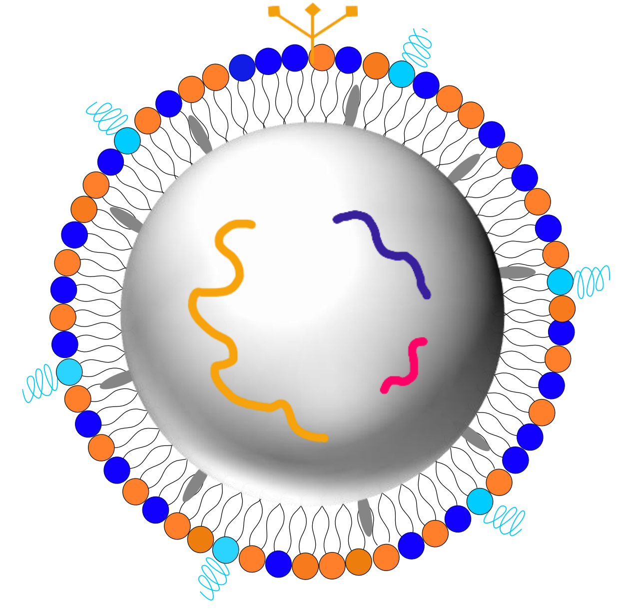
Human biology
- Autosomal recessive disorder caused by SLC37A4 mutations
- Disrupts glucose-6-phosphate transport, leading to glycogen buildup
- Restoring this pathway could normalize glycogen metabolism and improve outcomes

Delivery Vehicle: Liver targeted LNPs are unchanged across products

Performance assessment: How is the platform characterized?

LNP Modularity:

6 out of 8 components within the LNP are the same for liver programs



Component	Function
Ionizable Lipid	• Nucleic acid encapsulation and endosomal escape
Helper Lipid	• Stabilize and improve LNP pharmacokinetics • Facilitate membrane fusion and endosomal escape
PEG Lipids	• Control particle size and stability • Stealth coating reduces serum interactions and increases half-life
Cholesterol	• Improve intracellular delivery • Increase LNP stability
Targeting Ligand	• Improve biodistribution of LNPs to hepatocytes
PE mRNA	• Prime editor enzyme
pegRNA	• pegRNA is disease & mutation specific
ngRNA	• ngRNA is disease & mutation specific; usage is dependent on the Prime Editing strategy applied

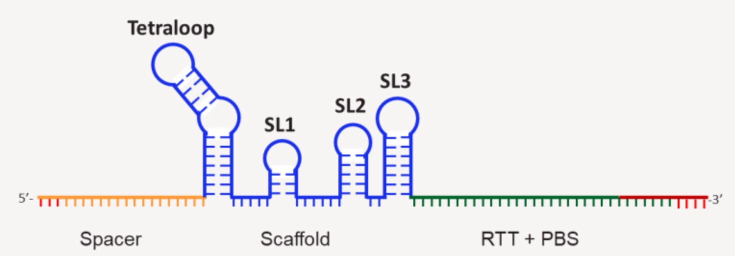
LNP vehicle drives biodistribution and toxicity, and is the major contributor to any immunogenicity

Prime Editing Components Platform Modularity

Each RNA component making up the LNP cargo is designed with modularity

Exemplary Prime Editing Components

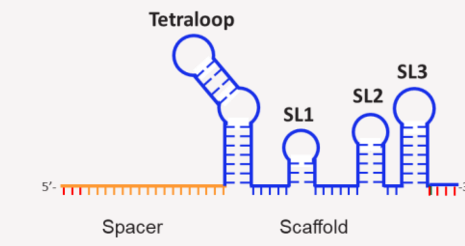
Prime editing guide (peg) RNA



pegRNA complexes with translated **Prime Editor (PE) Protein** *in vivo* to form active **Prime Editor Complex**

- **Scaffold** is considered *fixed*, enabling Prime Editor loading
- **Spacer**, **RTT**, **PBS** uniquely target the desired gene loci
- **RTT** serves as a template to reverse transcribe the correct genetic sequence

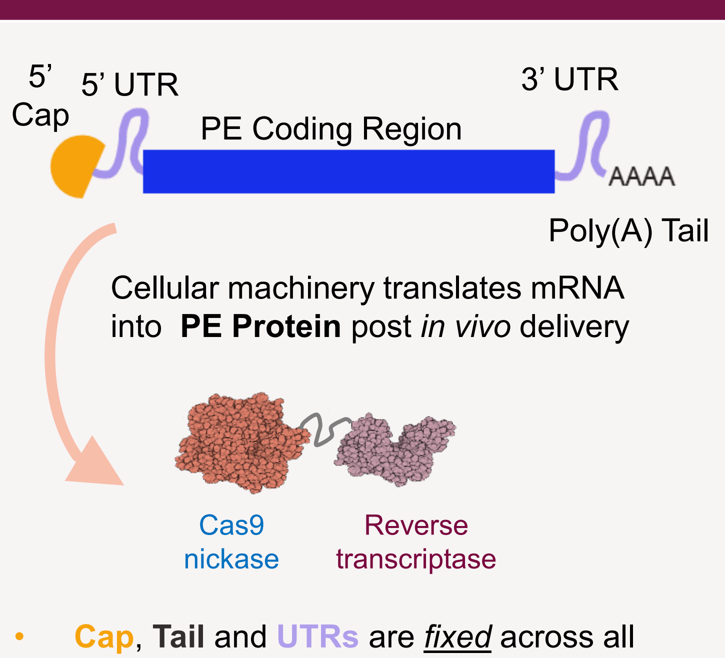
nick guide RNA



ngRNA complexes with translated **PE Protein** *in vivo* to form Prime Editor Complex which enhances efficiency of edit

- **Scaffold** is considered *fixed* and common to the pegRNA scaffold sequence, enabling Prime Editor loading
- **Spacer** uniquely target the desired gene loci

Prime editor mRNA



- **Cap**, **Tail** and **UTRs** are *fixed* across all Prime Editor mRNAs for a target tissue
- **Coding region** is *fixed* except for minimal changes required for a different PAM

Platform Performance to be Characterized in the Initial Development Candidate(s)

Performance assessment: How is the platform characterized?

Preclinical

- *In vitro assay* using patient cells for potency
- Humanized mouse model for PK/PD modeling/efficacy/biodistribution
- Rat toxicology for safety
- NHP surrogate: PK/PD modeling for dose determination, biodistribution, safety
- Comprehensive suite of off-target assays for detection at base pair scale, chromosome scale or structural off targets

CMC

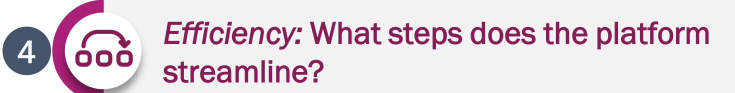
- Certificates of analysis (COA) from suppliers of key raw materials, and from manufacturers of critical reagents/DS, and LNP DP all meeting specifications.
- Continuous verification of manufacturing processes for PE components and drug product achieving pre-specified acceptance criteria based on process performance established during process development and prior GMP lots
- Continuous trend analysis for CQAs (critical quality attributes) and CPPs (critical process parameters)

Clinical

- Safety: Treatment related AEs compatible with benefit-risk considerations
- Surrogate biomarker: Reductions in serum copper (WD) and prevention of hypoglycemia (GSD1b)
- Clinical Efficacy: Ability to wean off SOC therapy without recurrence of clinical phenotype

Progression of PE platform development paradigm from comprehensive to streamlined

Exemplary Platform Development Paradigm[1]



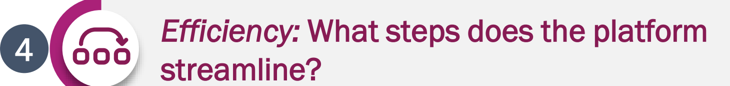
Fixed / Variable

		I Initial development candidate(s)	II Next iteration	III Final iteration
Therapeutic target	Disease / syndrome	Wilson's Disease (WD)	WD / GSD1b	WD / GSD1b
	Specific indication / target gene	ATP7B, H1069Q	ATP7B, R778L / SLC37A34, L348fs, G339C	ATP7B, rare variants / SLC37A34, rare variants
	Target organ / cell	Hepatocytes (liver)	Hepatocytes (liver)	Hepatocytes (liver)
Therapeutic platform	Delivery	LNP *in vivo*	LNP *in vivo* (same formulation)	LNP *in vivo* (same formulation)
	Gene editing method	Prime editing	Prime editing	Prime editing
	Gene editor	PE mRNA	PE mRNA (possibly different PAM)	PE mRNA (possibly different PAM)
	Guide RNA	peg and ngRNA for H1069Q	Multiple guides (for common mutations)	Multiple guides (for rarer mutations, and for different disease)
Preclinical	Pharmacology / DMPK / Tox.	***Expanded section for preclinical on next slide***		
Quality	Manufacturing and QC	***Expanded section for Quality (CMC/Mfg) on next slide***		
Trial	Route of administration	IV	IV	IV
	Surrogate endpoint	Copper level	Copper level / Glucose level	Copper level / Glucose level
	Trial protocol	Typical dose finding	Abbreviated dose finding; pooled safety (across all liver), and pooled efficacy (across indication)	No clinical investigation for rare variants

1. Exemplary Platform Development Paradigm may not reflect Prime Medicine's actual clinical path for exemplified diseases

Progression of PE platform development paradigm from comprehensive to streamlined (cont.)

Exemplary Platform Development Paradigm[1]



Leveraging prior data

		I Initial development candidate(s)	II Next iteration	III Final iteration
Preclinical	In vitro assay with patient cells	In vitro assay for efficacy and dose	In vitro assay for efficacy and dose	In vitro assay for efficacy and dose
	Potency assays	Run for initial development	Next iteration of guides tested in potency assay	Run only CMC functional potency assay on DP
	Humanized mouse model	Run for initial development	Next iteration guides will be tested in humanized mouse model to measure editing	No additional mouse studies to be run;
	Rat tox	Run for initial development	Leverage prior data; toxicity driven by LNP which is the same	No Rat Tox needed; same LNP clinically validated
	NHP surrogate (PK/PD dose modelling, BD and safety)	Need monkey surrogate guide for NHP PK//PD dose modelling, biodistribution and safety	Leverage previous data	No additional NHP studies
	Off-target detection	Comprehensive suite of off target detection assays – leveraging from previous programs	Leverage prior knowledge: minimized package, focus new data generation using data driven risk assessments	New pegRNAs can be derisked using largely computational approaches
CMC	Guides and mRNA manufacturing process	Robust, scalable and well-controlled process, same manufacturing facility, defined equipment	Same process, mfg facility and equipment; add ligation/conjugation step for very long guides	Reduce scale of equipment to produce smaller lot of guides for personalized patient population
	LNP formulation process	Robust, scalable and well-controlled process, same manufacturing facility, defined equipment	Same process, mfg facility and equipment	Reduce scale of equipment to produce smaller lot of formulated LNPs for personalized patient population
	Design space around CPPs	Comprehensive QbD design space for initial product to gain understanding of process; structure data to use AI/ML	Capture structured data for lots generated and feed into QbD[2] design space algorithm.	Leverage previous data
	Process validation	1 PPQ lot, use data from GLP and GMP lot(s) for continuous verification protocol and acceptance criteria.	Include GMP lot for new guides into the process characterization and trend process data.	Periodic continuous process verification and trend data
	# of assays for QC, stability and characterization	Full suite of assays	Risk-based reduction in number of assays, eliminate orthogonal assays	Significant reduction in assays to focus on safety, identify and potency
	Stability data	Build from prior data from other programs (eg. guide, mRNA) and conduct stability protocol per ICH guidelines	Leverage prior GMP data while continuing to build expanded stability database in tech dev and feed AI/ML	Broad stability dataset for leverage, no new stability data generation

1. Exemplary Platform Development Paradigm may not reflect Prime Medicine's actual clinical path for exemplified diseases

2. QbD = quality by design

Benefits of Prime Editing Platform Modularity for Preclinical Development

Efficiency: What steps does the platform streamline?

Leveraging the shared modular components streamlines NCD across programs and diseases

Pharmacology

Same prime editing mechanism across all mutations, tissues/organs, indications:

- Standardized NGS assay measures biological activity (editing) across all mutations
- Target engagement, pharmacodynamic, and efficacy biomarkers within diseases
- PK/PD modeling for dose projection

DMPK

Toxicokinetic and pharmacokinetics are driven by shared modular delivery components:

- Limited biodistribution to non-target tissues
- Bioanalytical assays are tailored to each modality
- ADME properties for the shared components of each modality

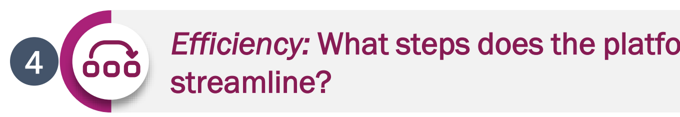
Toxicology

Safety expected to be driven by shared modular delivery components:

- Target tissue safety
- Germline transmission
- Safety assessments and biomarkers
- TK/TD modeling is tailored to each modality
- Immunogenicity of delivery components and cargo

DP: Drug Product; PK/PD: Pharmacokinetic/Pharmacodynamic; TK/TD: Toxicokinetic/Toxicodynamic; ADME: Absorption, Distribution, Metabolism, and Elimination; Drug metabolism and pharmacokinetics; NCD: Nonclinical Development; NGS, Next Generation Sequencing

Leveraging PE Platform Modularity in Preclinical Development to Accelerate Next and Final Iteration Candidates



Preclinical Development Streamlined Across Three Phases Exemplified[1]

I — Initial development candidate(s)

Patient cells: efficacy/dose

Potency assay: potency

Humanized mouse H1069Q model: PK/PD modeling/efficacy/biodistribution (BD)

Rat tox: safety

NHP surrogate: PK/PD modeling, BD, safety

Off-target dataset: extensive

II — Next iteration

Patient cells: efficacy/dose

Potency assay: potency

Humanized R778L and GSD1b mouse model: PKPD/efficacy

Off-target dataset: extensive

Leverage: NHP PKPD and BD, H1069 BD & all toxicology datasets

III — Final iteration

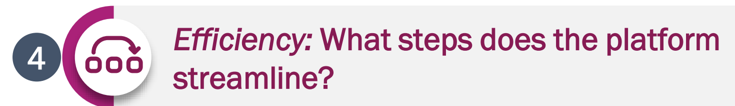
Patient cells: efficacy/dose for subsequent mutations

Potency assay: potency

Leverage: previous data packages

1. Exemplary Preclinical Development Paradigm may not reflect Prime Medicine's actual preclinical path for exemplified genes

Identifying the meaningful risks in gene editing



Efficiency: What steps does the platform streamline?

Off Targets (bp scale)

GGCCCAGACTGAGCAC GTGA

PE2

On-target

genome

GGCCCAG-CTGAGCTCGTGA

PE2

Off-target

Chromosome Scale or Structural Off Targets

cDNA synthesis

Translocations

Large Deletions

Assays for off-target detection

Targeted Nick Detection Assay	Genome wide DSB detection	Genome wide Nick Detection Assay

Potential off-target editing sites

Targeted Sequencing

Identified Off-targets

Reverse Trans. Assay

mRNAseq PEG integration

Chromosomal integrity assays

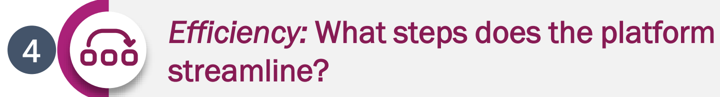
Imaging based	Sequencing based

Whole Genome Sequencing	Long read sequencing

Identified Off-targets

Comprehensive suite of off-target assays applied to initial product(s) powers data-driven risk assessment for later products

Off-target Assessment Paradigm[1]



Efficiency: What steps does the platform streamline?

		I Initial development candidate(s)	II Next iteration	III Final iteration	Key Findings
Treatment Scope	Disease	Wilson's Disease 1069Q	WD R778L GSD1b L348fs, G339	WD and GSD1b rare variants	
Preclinical Assays	Fixed Components[1]	Comprehensive assay set	Common components derisked	Common components derisked	PE protein expression in human cells has defined and acceptable risks
	Variable Components[2] (pegRNA)	Comprehensive assay set	Minimized Assay set addressing identified risks[2]	Predictive risk assessment framework, supplemented by experiment[3]	New pegRNAs can be derisked using largely computational approaches

1. Exemplified Off-target Assessment may not reflect Prime Medicine's actual plan for Off-target assessment
2. Fixed components would be materials used across multiple editing reagents such as Prime Editor mRNA or Prime Editor protein
3. As data driven risk assessment models of gene editing improve and more population level whole genome data is available risks associated with unexpected editor-genome interactions is expected to decline

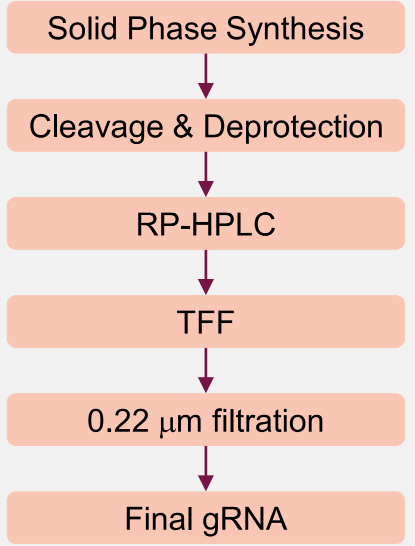
Platform Manufacturing Processes are robust, well-defined and fixed

Defining new CMC regulatory expectations to improve speed and access to potentially curative genetic editing therapy for ultrarare disease

Efficiency: What steps does the platform streamline?

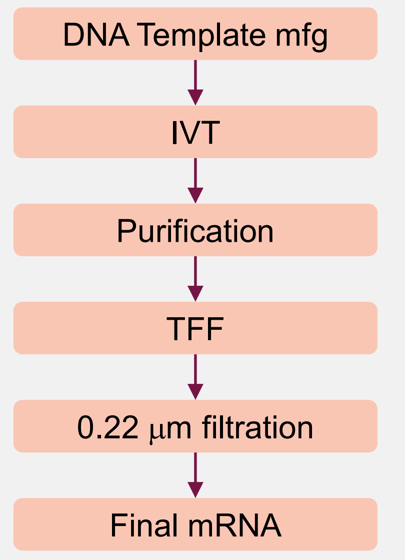
Solid phase synthesis and /or ligation for long guide RNAs

- Same semi-automated equipment, operations, and process used for all mutations across programs



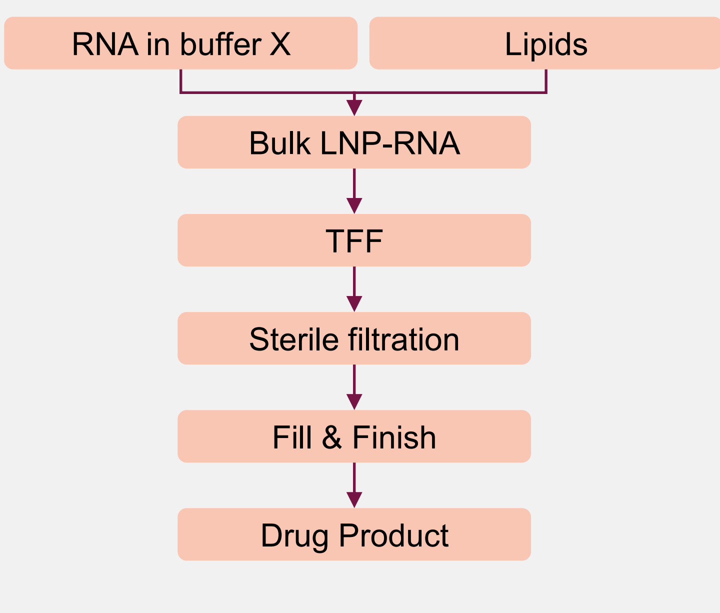
mRNA manufacturing by In Vitro Transcription

- Platform enzymatic process for mRNA transcription used for all programs



Liver Targeted PE-LNP

- Platform process for LNP-formulated PE drug products across all mutations



Utilizing Modularity to Streamline CMC Data

Defining new CMC regulatory expectations to improve speed and access to potentially curative genetic editing therapy for ultrarare diseases

Efficiency: What steps does the platform streamline?

Key Opportunities

Solid phase synthesis

Can the production of guide fragments for split synthesis via ligation/conjugation be carried out in well-documented, controlled laboratory operations to meet high quality attributes?

Design space for CPPs

Establish design space using QbD principles for the initial development candidate. Robust process characterization ensures strong process knowledge and appropriate design of control strategy with data leveraged for future programs

CDMO GMP

Given the small lot sizes required for RNA and LNP components in ultrarare diseases, many oligonucleotide and mRNA CDMOs are oversized. Can these RNA components be produced using semi-automated equipment in a well-controlled lab setting?

PPQ[1] lot

Given the ultra-rare disease and even rarer mutations, it seems practical to produce a single lot of guides vs 3 lots for PPQ for commercialization

Stability data

Collect comprehensive stability data on similar long guide RNAs per ICH guidelines. Build a database by generating additional stability data from both development and GMP processes, leveraging for other components and drug products.

Key Observations

Leverage platform modularity to accelerate CMC/Manufacturing and reduce the costs

- Carry out CMC CQA and CPP identification for Initial Development Candidates and QbD design space once, then leverage data for next and last iteration candidates
- Consistent quality parameters across products enable faster approvals
- Rethink traditional Quality cGMP requirements (e.g., facility, equipment, validation) and use risk-based approach for personalized gene editing ultra-rare diseases
- Use continuous process verification approach to minimize PPQ lot requirements to 1 lot instead of 3 lots to support commercialization

1. PPQ is process performance qualification

Hypothetical case study: Opportunity for risk-based reduction in number of assays to address all mutation specific products

Efficiency: What steps does the platform streamline?

Illustrative Analytical Assay Reduction Strategy for *in vivo* liver targeting LNP-Formulated PE DPs (across 3 iterations)

	I. Initial development candidate(s)			II. Next iteration			III. Final iteration		
pegRNA (ngRNA[1])	Safety/ General (4)	Identity (2)	Strength (1)	Safety/ General (2)	Identity (1)	Strength (1)	Safety/ General (1)	Identity (1)	Strength (1)
	Purity (3)	Impurity (3)	Activity (1)	Purity (1)	Impurity (2)	Activity (1)	Purity (1)	Impurity (0)	Activity (0)
mRNA	Safety/ General (4)	Identity (1)	Strength (1)	Safety/ General (2)	Identity (1)	Strength (1)	Safety/ General (1)	Identity (1)	Strength (1)
	Purity (4)	Impurity (4)	Activity (1)	Purity (3)	Impurity (1)	Activity (0)	Purity (1)	Impurity (1)	Activity (0)
LNP-formulated PE DP	Safety/ General (>10)	Identity (3)	Strength (4)	Safety/ General (6)	Identity (2)	Strength (3)	Safety/ General (3)	Identity (2)	Strength (1)
	Purity (3)	Impurity (3)	Potency (2+)	Purity (2)	Impurity (1)	Potency (1)	Purity (0)	Impurity (0)	Potency (1)

I. Initial development candidate(s)

- Large suite of >69 assays developed for **release, stability and characterization** (includes ngRNA)
- Many assays developed as **platform methods** and have been/are used in other non-liver products

II. Next iteration

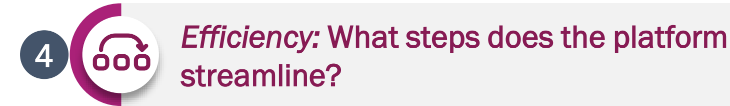
- Only **new assay needed** is for **DP functional potency**; otherwise leverage platform assays and adapt and/or product-specific qualification
- Selectively reduce # of assays, **eliminate** mainly **orthogonal assays**, and leverage data across mutations/products.

III. Final iteration

- **Further reduce** number/type of analytical assays
- **Critical assays** include **safety, identity, potency, and strength**
- Leverage **aggregated stability data**

1. same concept and assays apply to ngRNA, doubling the # of assays for guides indicated

Clinical Modularity for Platform Trial



Steps to Enable Clinical Modularity

- Multiple mutations and diseases studied in parallel or sequentially
- Safety aggregated across liver-directed LNP products
- Clinical experience in initial product leveraged to expedite dose finding in subsequent products
- Biomarker/ efficacy outcomes pooled for products for same disease
- Approval of products for rare variants based on non-clinical and CMC comparability

	I Initial development candidate(s)	II Next iteration	III Final iteration
Indication / Mutation	Wilson's Disease H1069Q[2]	WD R778L GSD1b[2] L348fs, G339C	WD and GSD1b rare variants
MRSD[1] / Dose Finding	MRSD based on toxicology; conventional dose finding	MRSD **informed by initial program**; abbreviated dose finding	**No clinical investigation required**; leverage nonclinical mutation-specific assays, CMC comparability on key quality attributes (including potency), and use clinical safety and efficacy data from studies on common variants
Surrogate Biomarker	Urinary and/or serum copper	Wilson's Disease: **Urinary** and/or **serum copper** GSD1b: **Fasting duration** to hypoglycemia	
Safety	Aggregate across all liver LNPs	Aggregate across all liver LNPs	
Additional Efficacy	Aggregate across WD variants	Wilson's Disease: Aggregate across WD variants GSD1b: Aggregate across GSD1b variants	

1. Maximum recommended starting dose
2. Wilson's Disease and GSD1b are used as hypothetical examples in this platform schematic and may not reflect Prime Medicine's actual clinical path

Exemplary integration of preclinical, CMC and clinical modularity for streamlined development paradigm

Efficiency: What steps does the platform streamline?

Hypothetical case study: LNP formulated Prime Editors for Wilson's Disease and GSD1b[1]

Wilson's Disease H1069Q

I Initial development candidate

Preclinical:
- Standard toxicology and off-target assessments
- Establish target editing efficiency thresholds in relevant cellular and/or animal models
- PK/PD and dose projection
- Full off-target assays

CMC:
- Comprehensive CMC practice, including identifying important DP attributes, full suite of assays

FIH-to-POC:
- Standard dose finding
- Demonstrate in vivo gene correction sufficient to drive surrogate biomarker correction

Pivotal:
- Combine safety and efficacy data across different editors to support aggregate risk-benefit profile
- Demonstrate critical DP attributes/release criteria sufficient to support safety and efficacy of treatment

Wilson's Disease R778L and GSD1b G339C and L348fs

II Next iteration

Preclinical:
- Demonstrate editing efficiency meets target editing in cellular and/or animal models
- In silico off-target only

CMC:
- Demonstrate consistency and comparability of critical DP attributes; reduce release criteria for RNA drug substances by eliminating orthogonal assays used for common variants

FIH-to-POC & Pivotal:
- Abridged dose finding, leveraging toxicity information from Phase 1. Surrogate biomarker with shortened durability requirement

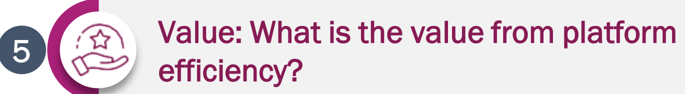
Additional mutations in Wilson's Disease and GSD1b

III Final iteration

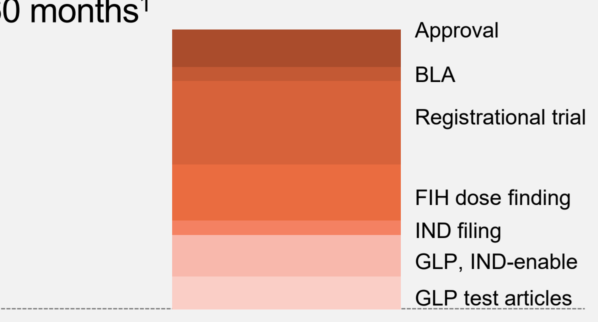
Preclinical & FIH-to-POC & Pivotal : Leverage from previous phases. No clinical study

1. Exemplary development paradigm may not reflect Prime Medicine's actual development plans

Exemplary case study: No Patient Left Behind and Shortening Time-to-Licensure by Unlocking Platform Modularity



Hypothetical development and iterations of therapeutics

	I. Initial development candidate(s)	II. Next iteration	III. Final iteration
Therapeutic Access Implications	Only patients with most prevalent mutations of more prevalent diseases have access to therapy, largely due to the extensive effort required to develop the therapy	Patients with less prevalent mutations in rare disease or with prevalent mutations of ultrarare indication gain access to therapy, made possible by platform modularity streamlined in preclinical and CMC stages	Most patients gain access to therapy, with accelerated timelines leveraging the full potential of platform modularity
Exemplary Fastest Development Time per Therapy (DC to licensure)	60 months[1] 	39 months[1]	6 months[1, 2] + time needed for FDA BLA review
Patient Access to Therapy	>5K Wilson's Disease patients in U.S. and EU with H1069Q	~2K Wilson's Disease patients worldwide across a few mutations ~200 GSD1b patients in U.S. across a couple mutations	~2K Wilson's Disease patients in U.S. across dozens of mutations ~200 GSD1b patients in U.S. across dozens of mutations
Sustainability to Drug Manufacturer	Sustainable	Not sustainable without efficiencies due to platform modularity	Not sustainable without efficiencies due to platform modularity

1. Wilson's Disease and GSD1b development plans are used as hypothetical examples and do not reflect Prime Medicine's actual CMC, clinical paths or timelines
2. Assuming there will be a standardized "Personalized PE Therapy Performance Criteria Protocol

Perceived Risks with Mitigation Strategies and Potential Areas for Regulatory Flexibility

Risk mitigation: How viable is the proposed platform?

Methods to Mitigate Editing Efficiencies

- **Translatable *in vitro* pre-clinical models:** Using models applicable to clinical settings
- **Machine Learning (ML):** Leverage ML to enhance predictions of on-target activity

Regulatory Tools to Oversee the Platform

- **Pre-clinical Editing and Off-target Profiles**: Ensure regulators have access to tools that track editing efficiency and off-target edits
- **Surrogate or Clinical Assays**: Use pre-clinical models to evaluate the performance
- **Monitoring Clinical Adverse Events**: Monitor any potential AEs from previous data

Regulatory Flexibility

- **GMP Manufacturing:** Maintain strong process and analytical controls while streamlining
- **1 BLA for 1 Disease:** Simplify approval with one BLA per disease
- **Alternative to full BLA:** Propose standardized “Personalized PE Therapy Performance Criteria Protocol” that is pre-approved (aka comparability protocol); if Final Iteration candidates meet performance criteria, then the lot can be used and commercialized
- **Continuous Verification:** Implement ongoing checks on lots for process performance verification
- **1 IND Filing for 1 Disease:** Use one IND per disease to streamline the process
- **Unified Clinical Trials:** Execute 1 umbrella clinical trials for each disease, shared SOC