Risk-Appropriate Manufacturing for Personalized Gene Editing: Lessons from Baby KJ

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Innovation at the speed of life.

Danaher

Disclosure statement

Vanessa Almendro
Employment: Danaher Corporation
Co-founder and consultant for the Brain Tumor Investment Fund.

Sadik H. Kassim
Employment: Danaher Corporation
SAB Member: nChroma Bio, Aurora Bio
Advisor: RA Capital, SROne, Curie.bio

The First to Be Cured: Stories That Changed the Course of Science

Emily Whitehead

2012 First child to be cured of relapsed leukemia using CAR-T cell therapy, transforming cancer care and launching a new era in immunotherapy.

KYMRIAH® (tisagenlecleucel) Suspension for IV infusion

~8-10K eligible patients/year US

Victoria Grey

2019 First person to be cured of sickle cell disease using ex vivo CRISPR gene editing, marking a historic breakthrough in the treatment of inherited blood disorders.

casgevy™ (exagamglogene autotemcel) suspension for IV infusion

~16K eligible patients/year US

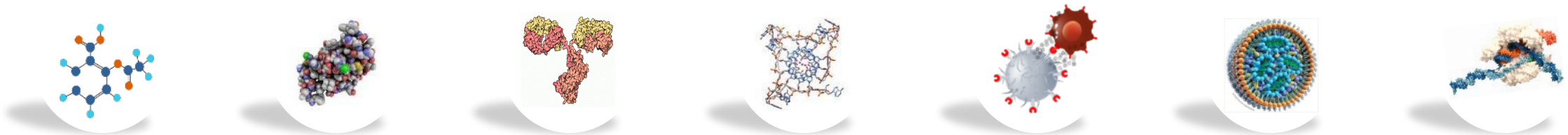
KJ Muldoon

2024 First patient to receive a personalized in vivo CRISPR base editing therapy for a rare metabolic disorder, demonstrating the feasibility of rapid, custom-made genomic medicines.

“kayjayguran abengcemeran” (k-abe)

anaher.

There is an Increasing Complexity of Genomic Medicines and Their Raw Materials



Small molecules	First biologics	mAb	Oligo therapies	C&G therapies	mRNA	Gene editing
1938	1982	1986	1998	2012 / 2017	2020	2023

Manufacturing

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

Supply Chain

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

Regulatory

- ❑ 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

Small molecules	Biologics	AAV or lentivirus	Cell therapy
Aspirin 21 atoms	**Humira** 20 067 atoms	**Viral vector** 10^5 to 10^7 atoms	**CAR T** 10^{22} atoms

Guide RNA · CRISPR Nuclease · Viral Vector · iPSC Line · Cytokines · RNP · mRNA/LNP · Cell Bank · Growth Factors · Plasmid DNA

"The hard part is creating a sufficient CMC and manufacturing package to get these to market."

R&D SVP, top 10 pharmaceutical company

World Medical Innovation Forum: Gene and Cell Therapy, May 2022

Biotechnology

cytiva

Life Sciences

abcam

aldevron

Beckman Coulter Life Sciences

Genedata

IDBS

IDT

Leica Microsystems

Molecular Devices

PALL Corporation

phenomenex

SCIEX

Diagnostics

Beckman Coulter

Cepheid

HemoCue

Leica Biosystems

Mammotome

RADIOMETER

Our family of leading brands in Life Sciences and Diagnostics

Purpose-driven science & technology leader

Built to Solve What's Next:
Danaher's Platform Advantage in Genomic Medicine

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

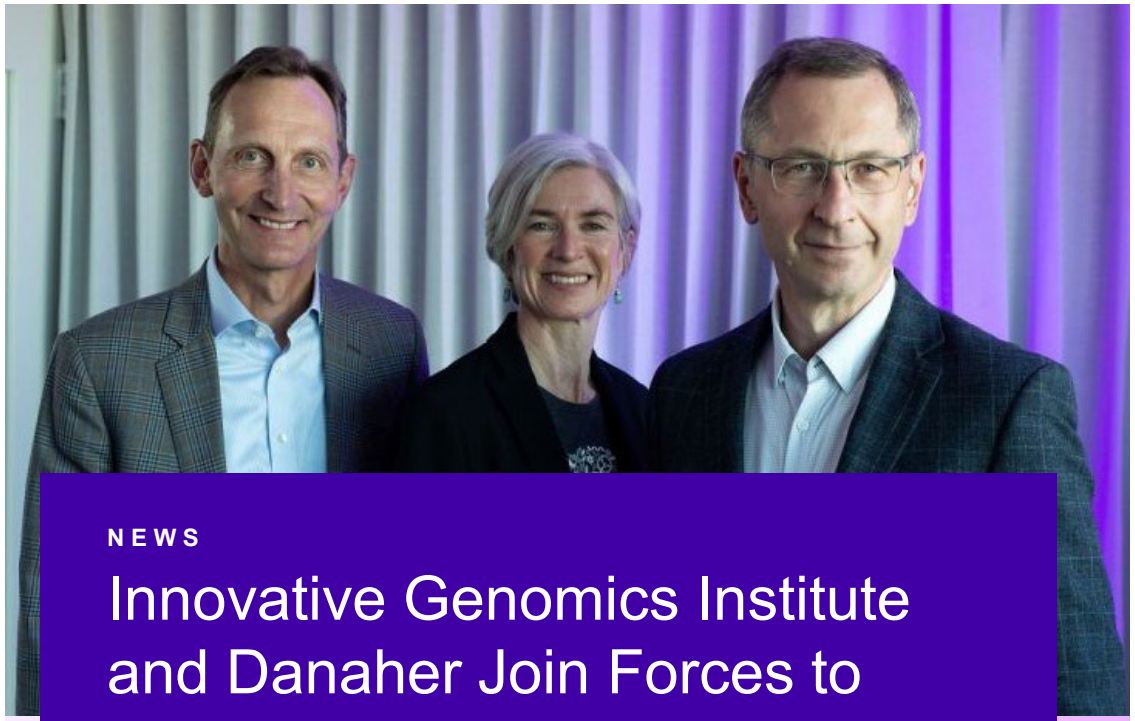
Patients may experience **diagnostic delays** that can averages **4-5 years**

~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

Danaher is uniquely positioned to address researcher and drug developer challenges
But… we can't do it alone



NEWS

Innovative Genomics Institute and Danaher Join Forces to Accelerate and Scale Up the Development of CRISPR Cures

Janurary 9, 2024 – Press Releases, Jennifer Doudna, Innovation / Entrepreneurship

Bz Andy Murdock

The Beacon for CRISPR Cures aims to create a roadmap for rapidly developing genome-editing therapies

A “Beacon” for the gene-edited cell therapy space

“The Innovative Genomics Institute, a nonprofit founded by Doudna, is partnering with life science tools company Danaher for four years to develop gene editing therapies for two rare genetic diseases, and the collaborators plan to share their playbook widely in hopes of spurring more development of gene editing treatments.”

Jennifer Doudna, Fyodor Urnov want to streamline gene editing studies with Danaher's help

Understanding urea cycle disorder in pediatrics

Definition

Urea Cycle Disorder (UCD) is a rare inherited metabolic condition caused by a deficiency in one of six enzymes responsible for converting nitrogen (from protein metabolism) into urea, which is excreted in urine[1]

Pathophysiology

Nitrogen accumulates as ammonia in the bloodstream (hyperammonemia) leading to neurotoxicity which can result in coma, irreversible brain damage, or death[1]

Onset in infants and children

- Symptoms often appear within the first few days of life
- Common signs: vomiting, lethargy, seizures, respiratory distress
- Late-onset forms may present in early childhood with developmental delays or behavioral issues

Prevalence

>100 children are diagnosed yearly with UCD in the United States[2]

1. https://rarediseases.info.nih.gov/diseases/7837/urea-cycle-disorder
2. https://pmc.ncbi.nlm.nih.gov/articles/PMC4364413/

Current treatments manage symptoms; genomic medicines can correct the cause and cure

Current treatments

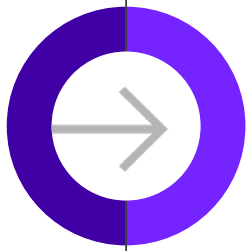
Limited to symptom management and invasive procedures

Acute management[1]

- **Hemodialysis** or **hemofiltration** to rapidly reduce ammonia levels
- **IV nitrogen scavengers** (e.g., sodium benzoate, sodium phenylacetate)
- **Arginine supplementation** to bypass defective enzymes

Chronic management[1]

- **Protein-restricted diet** tailored to minimize nitrogen load
- **Oral nitrogen scavengers** (e.g., glycerol phenylbutyrate)
- **Liver transplantation** in severe or refractory cases



Emerging therapies

Progress towards more precise, rapid, and potentially curative solutions

Genomic medicines

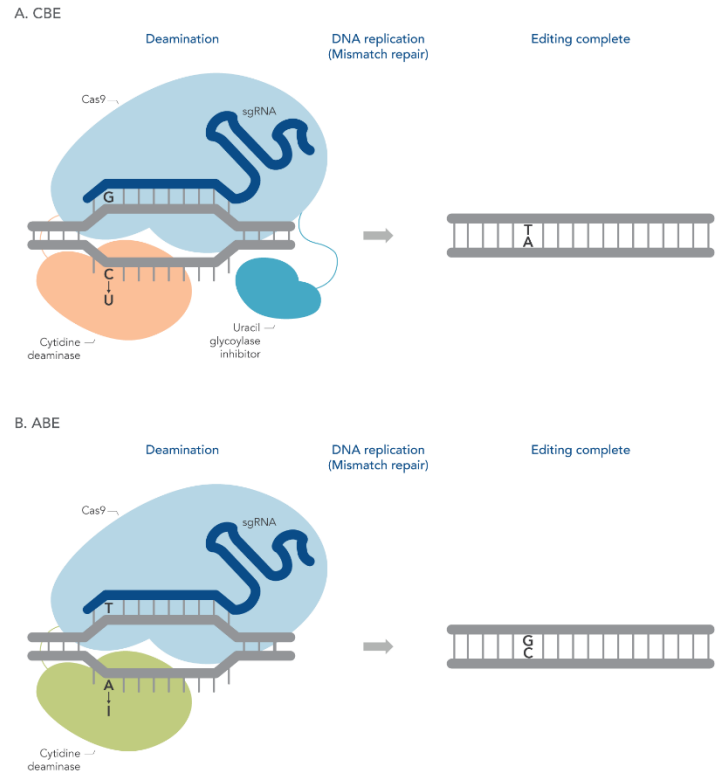
- Gene therapy and mRNA-based approaches under investigation
- CRISPR-based editing offers a potential cure for targeted correction of enzyme deficiencies

1. https://rarediseases.info.nih.gov/diseases/7837/urea-cycle-disorder

Enabling CRISPR-based therapies with base editing

- Base editing enables precise A•T to G•C conversion using an adenine base editor (ABE) or C•G to T•A conversion using an cytosine base editor (CBE) without double-strand breaks
- Uses a deaminase enzyme fused to a Cas9 variant to directly convert a single base in DNA
- Can reduce risk of large deletions or chromosomal rearrangements—ideal for sensitive targets like CPS1[1]
- In UCD, adenine base editors (ABEs) can correct pathogenic point mutations like Q335X, found in the patient, in CPS

Base editing offers an alternative to traditional CRISPR, enabling rapid development of personalized therapies for rare diseases like UCD.

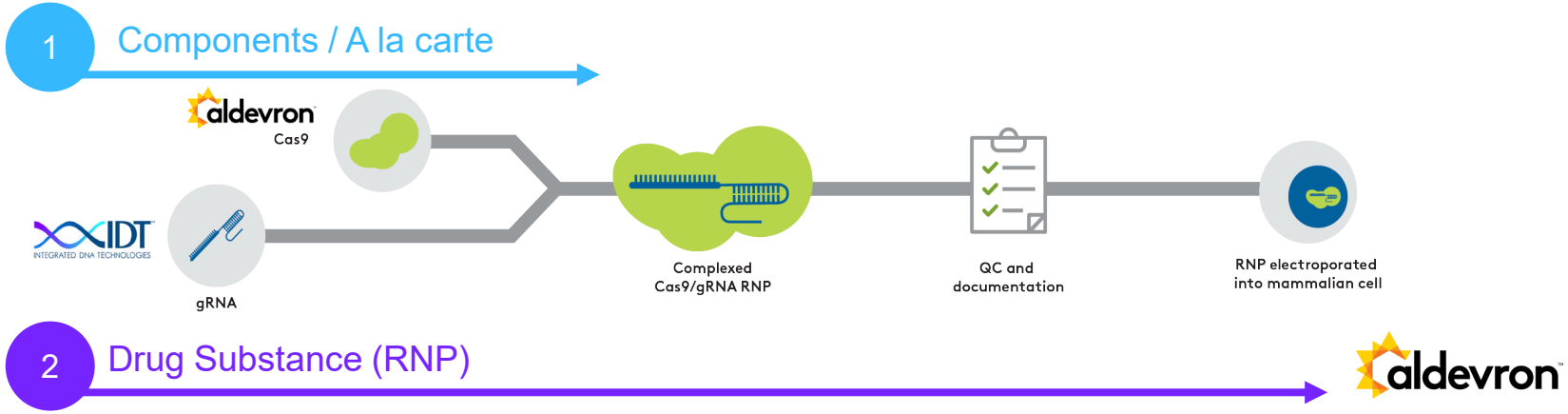


Simplified illustration of CBE and ABEs

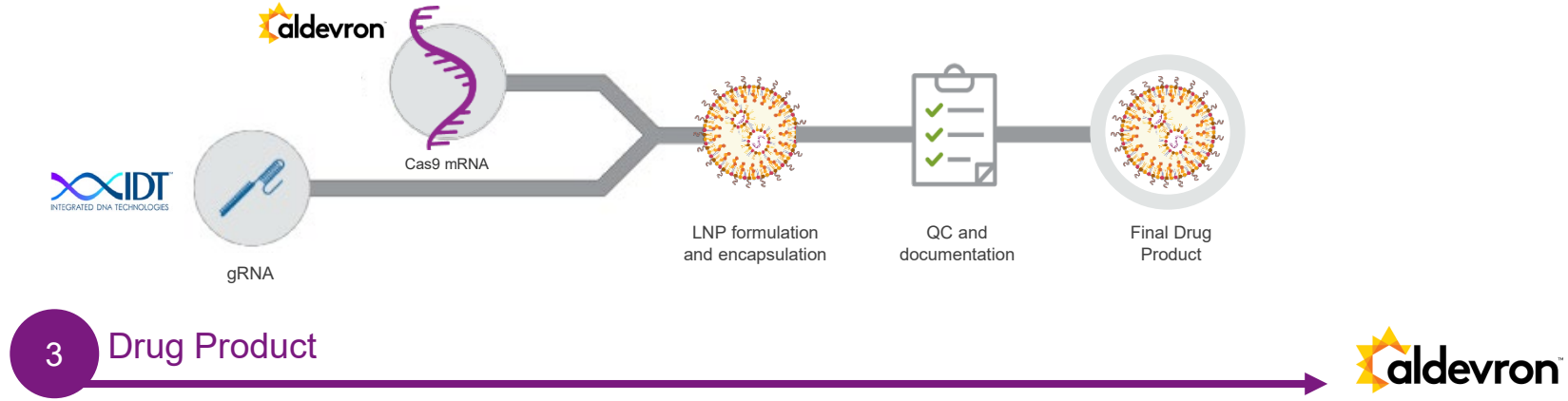
1. https://www.idtdna.com/pages/education/decoded/article/single-base-editing

In vivo mRNA-based CRISPR gene editing in the liver chosen to treat UCD

RNP Modality



mRNA Modality

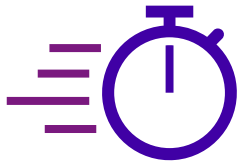


*Aldevron provides RNPs only to customers who are duly licensed, including to make and have made RNPs, for their intended use

The opportunity

Manufacture a first-in-class CRISPR therapy for UCD

September 2024: Can we be ready to treat a pediatric patient with a novel CRISPR therapy by February 2025?



Through the Innovative Genomics Institute (IGI)-Danaher Beacon Program, Aldevron, IDT, and Acuitas Therapeutics partnered to deliver a gene editing drug product for a pediatric patient with UCD

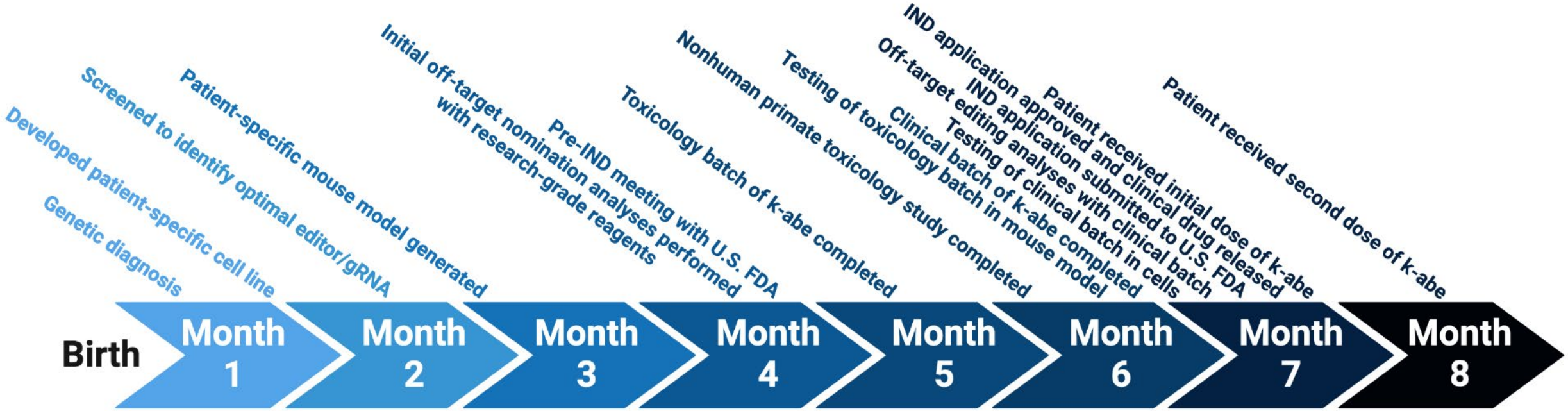
Challenges to overcome

Manufacture a **new guide RNA sequence** tailored to the patient's mutation	Synthesize a novel **mRNA base editor**	Design an **LNP formulation** for targeted liver delivery	Conduct **comprehensive off-target analysis**
Align the efforts of four partners	Expedite **regulatory and quality** by working with the FDA	**Condense timeline** – execute in less than 1/3 of the typical timeline because the patient didn't have more time	

Needed to compress a **1.5-year process** into **<6 months timeframe**

How can we compress a 1.5-year process into a **<6** months timeframe?

Development of World's First Custom mRNA Based Gene Editing Drug Product



aldevron
IDT INTEGRATED DNA TECHNOLOGIES
ACUITAS THERAPEUTICS
amplifybio
The Jackson Laboratory Leading the search for tomorrow's cures
Penn University of Pennsylvania
The Children's Hospital of Philadelphia
Innovative Genomics Institute
BROAD INSTITUTE
MGH 1811

Patient-specific surrogate models and genotoxicity package were used to gain regulatory approval

1. Patient-specific sequence was used to generate *in vitro* and *in vivo* models

3. A multi-platform, off-target analysis framework enabled FDA approval

Editing efficacy assays

In vitro	In vivo
HuH-7 Q335X	*Rosa26* Q335X
	Cps1 Q335X KI

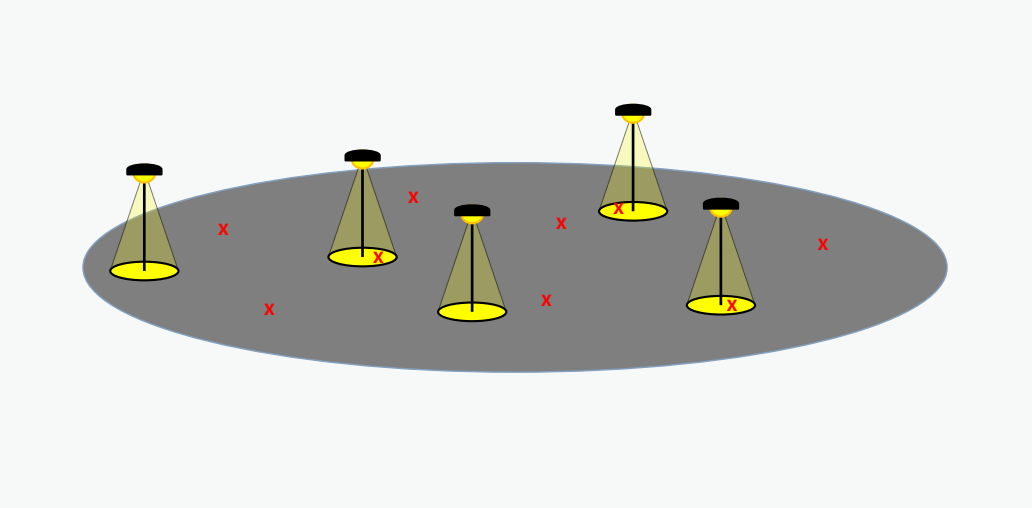
Genotoxicity risk assessment

GLP studies	Off-target nomination			Off-target confirmation
	In silico	In vitro	In cellulo	
NHP GLP studies	GuideScan 2	ONE-Seq	UncoverSeq	rhAmpSeq™ CRISPR Analysis System
		ChangeSeq		

2. No functional efficacy studies performed (known pathogenic mutation with evidence of genotype-phenotype associations)

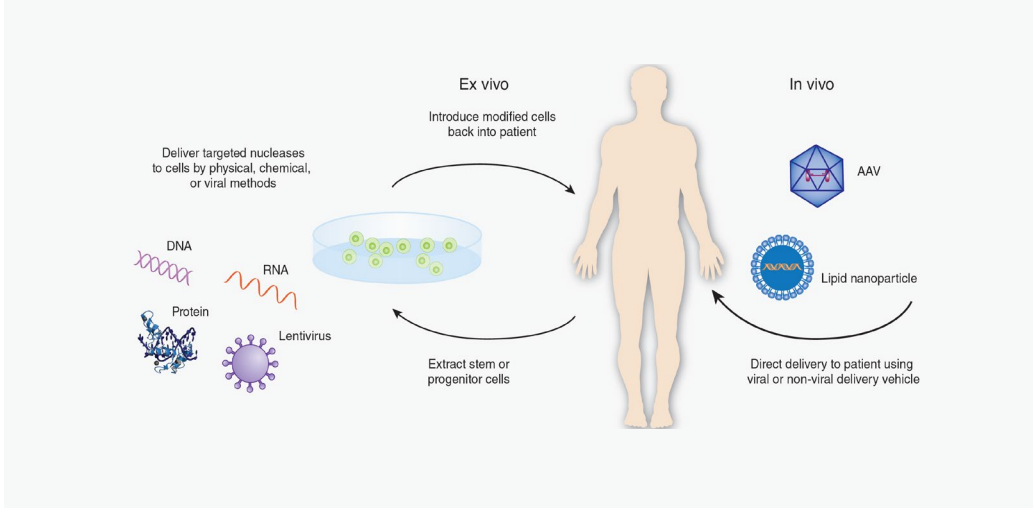
Off-target genotoxicity assessments are a 2-step process

Step 1: **Nomination**



- Nominate potential sites of cleavage/mutations in a genome
  - Need a sensitive and genome-wide method
- Sensitivity is critically important because it defines the sites examined in the subsequent confirmation step
- Various types described to date: Computational, cell-based, biochemical

Step 2: **Confirmation***



- Confirm nominated sites in editor-treated target cells/tissue or appropriate surrogate cells
  - Need to ensure appropriate editing (acceptance criteria) to ensure it is a representative process/surrogate
- Assess for indels but also larger-scale alterations (translocations, inversions, large deletions)
- Important: Not all off-targets nominated are bona fide in the ultimate therapeutic context due to actual treatment conditions

*Image above from Maeder and Gersbach, *Mol Ther*. 2016

Comprehensive off-target analysis assessment with orthogonality

Completed off-target nomination and confirmation services for a novel base editor in <6 weeks compared to a standard time of 12 weeks

- *In silico*, *in vitro*, and *in cellulo* **orthogonal nomination strategies** used to nominate sites using the parent's whole genome sequence
  - Used custom Cas9 variant and base editor for precision targeting
- **300+ sites prioritized** using empirical and biological annotations
- **Off-target confirmation** was performed using rhAmpSeq's multiplexed targeted deep sequencing with no concerning off-target edits in primary human hepatocytes
  - Translocation detection was also performed using the PASTA pipeline

A multi-platform, off-target analysis framework enabled FDA approval

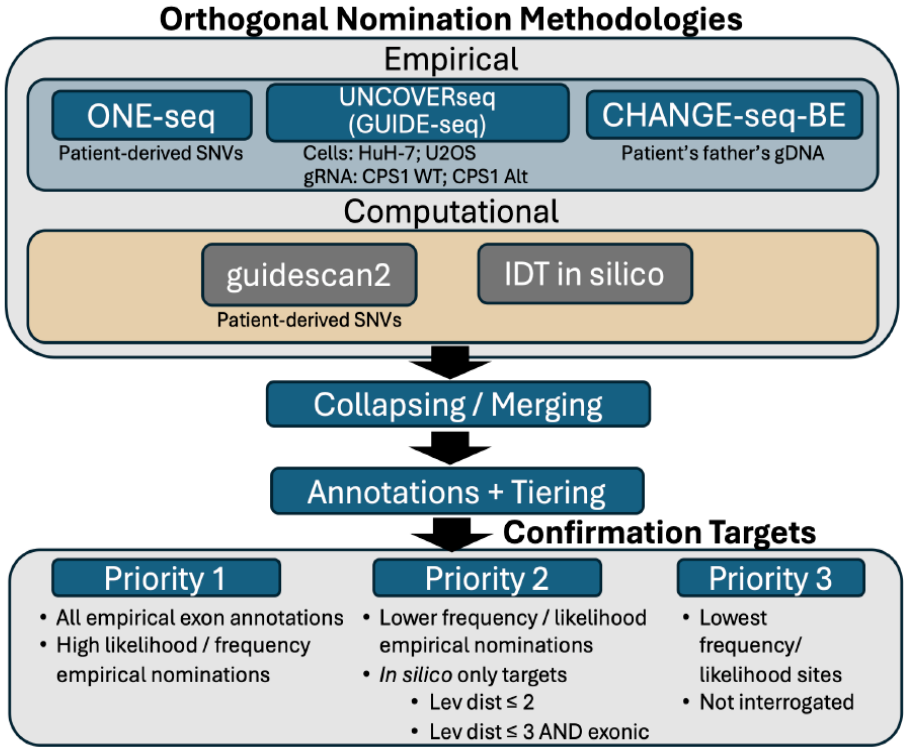


Figure S15. Off-target Nomination Workflow

Overview of the workflow consolidating and prioritizing off-target site lists from three empirical and two computational off-target nomination methodologies based on impact/risk and event likelihood.

Manufacturing quality under compressed timelines

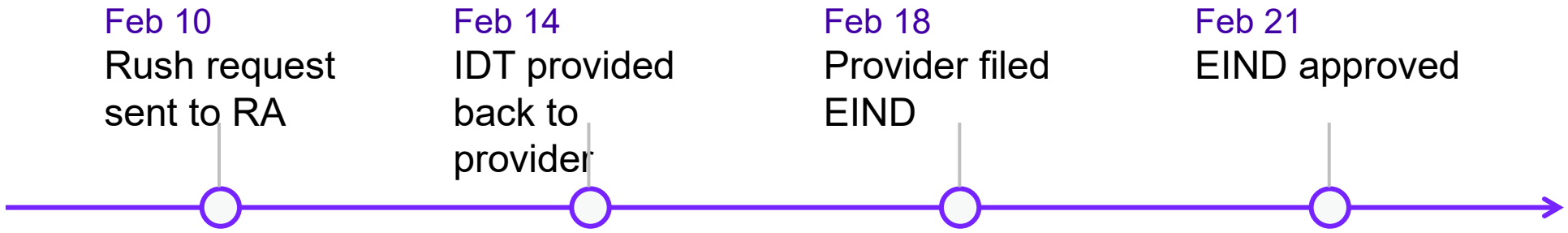
Aldevron and IDT’s unified quality framework under Danaher ensured compliant, drug product delivery under urgent timelines

- Final drug product manufactured in GMP-compliant cleanrooms under **phase-appropriate conditions**
- Real-time batch record review enabled faster resolution and documentation turnaround
- IDT and Aldevron jointly expedited COA, batch record, and sgRNA sequencing for EIND inclusion
- QA/QC teams adapted analytical methods for compassionate use manufacturing
- Demonstrated ability to scale quality systems for future personalized therapies

Regulatory acceleration: Fast-tracking EIND submission and approval

Strategic alignment across Danaher, IDT, and Aldevron enabled regulatory speed without compromising rigor

FDA approved EIND in 7 days (vs. 30-day standard) following Danaher-led INTERACT meetings



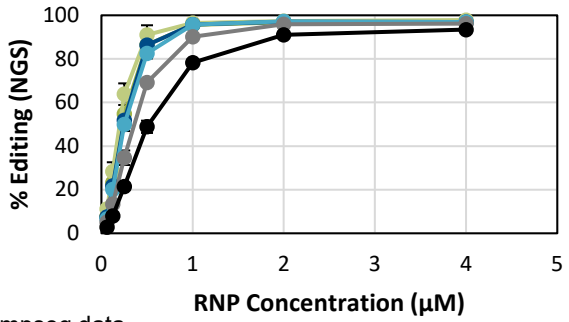
- Joint Aldevron–IDT regulatory framework aligned on phase-appropriate GMP expectations
- IDT RA created a CMC template to streamline documentation while protecting IP
- QA/RA team delivered 300-page technical package in 4 days, enabling rapid FDA review
- Direct communication with FDA to enable real-time issue resolution

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

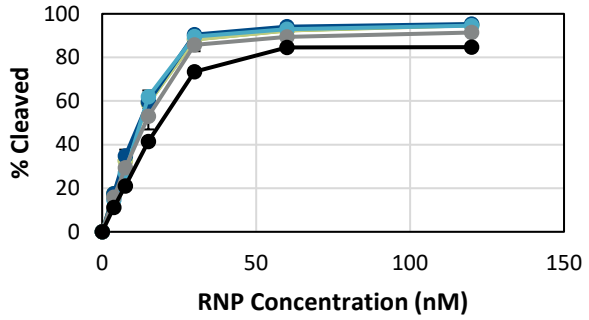
Quality	Manufacturing technology	Time to manufacture	Purification method	Purity (%)	Specificity
GMP	AKTA	6 to 18 months	HPLC	92.75	99.9
RUO	IDT	2 days	HPLC	84.8	99.9
RUO	IDT	2 days	Std Desalt	44.5	99.9

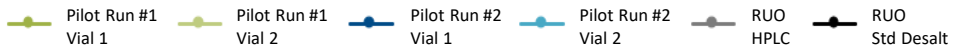
Cell-Based Editing Activity:
Cas9 RNP Electroporation, K562 cells



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.

Danaher UCSF Health Innovative Genomics Institute

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

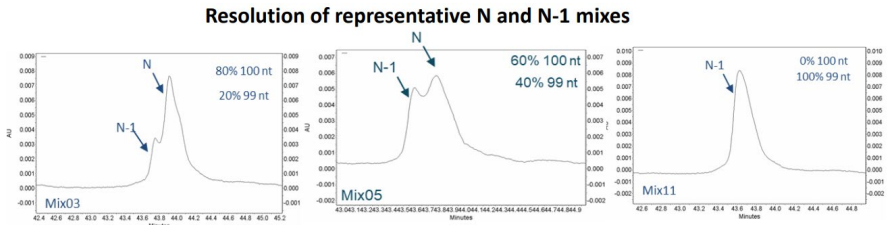
chr	start	end	strand	hg38_seq	Levenshtein_distance
chr8	42379946	42379966	+	CTTGTACCCAGCACTTCGTG	3
chr3	100929120	100929140	+	CTTATTTCCAACACTTAGTT	3
chr11	84719391	84719411	-	TTTATATAAAACACTTCATG	4
chr14	74725529	74725549	+	ATAATTCCCAACACTTCGTG	4
chr7	133980572	133980592	-	CTTACATACAACACTTCTTT	4
chr5	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

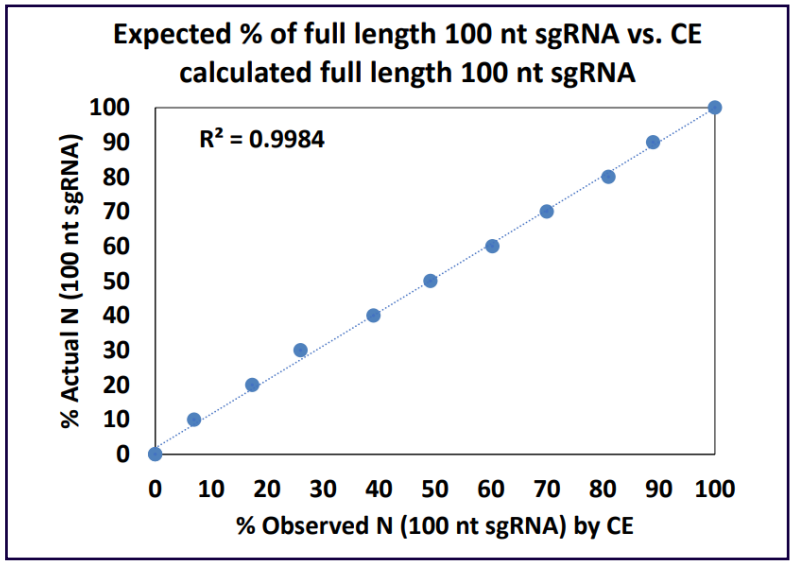
A C T G = mismatched NT

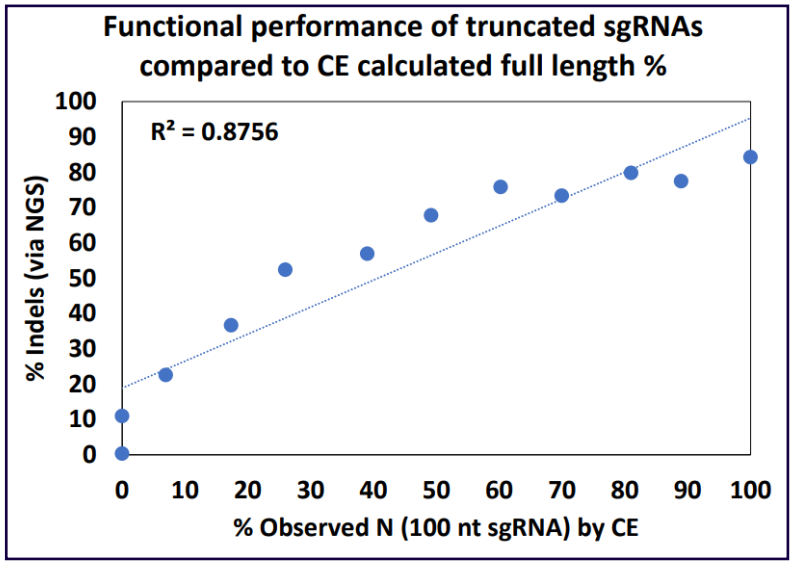
What is the Relationship Between sgRNA Purity and Activity?

N: 5'-AATTATGGGGATTACTAGGA sgRNA scaffold -3' **100 nt**

N-1: 5'-AATTATGGGGATTACTAGG- sgRNA scaffold -3' **99 nt**







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).

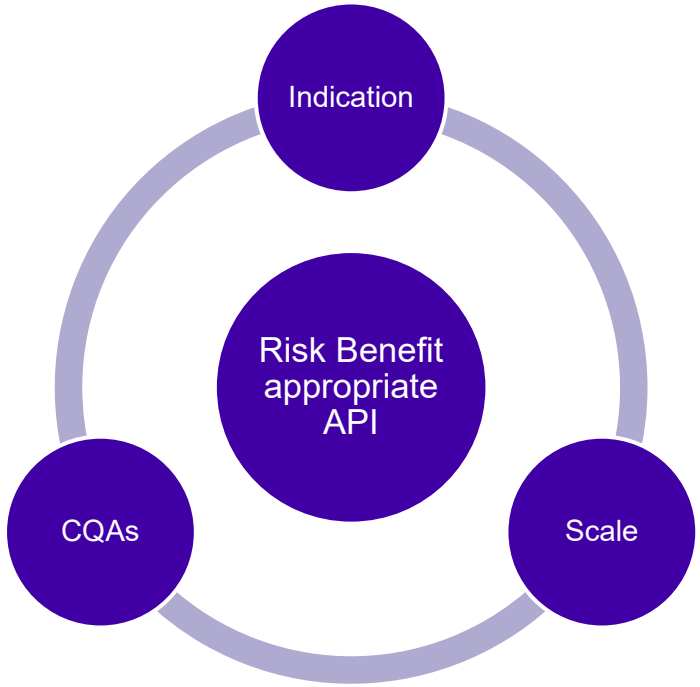
Opportunity for the Future: Risk-Benefit Appropriate Products

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: Benefit Risk 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 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

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Specific recommendations

1 Platformization is Essential	Standardization reduces cost, accelerates timelines, and ensures reproducibility
2 Risk-Appropriate GMP Unlocks Feasibility	**Phase-appropriate, risk-tailored quality standards** are needed for ultra-rare and personalized therapies for rapidly progressive diseases.
3 Patient-Centered CMC Analytics	Incorporating the **patient's own genome** into off-target analyses represents a new paradigm of individualized quality assessment. Centralized repository of data will be needed for scalability.
4 Collaborative Ecosystems Accelerate N-of-1	No single entity can deliver N-of-1 alone; **ecosystem models** are the future.


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