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U.S. FOOD & DRUG ADMINISTRATION

Cellular, Tissue, and Gene Therapies Advisory Committee
BLA 125787

Exagamglogene autotemcel (exa-cel) for the treatment of sickle cell disease in patients 12 years and older with recurrent vaso-occlusive crises (VOCs)

Applicant: Vertex Pharmaceuticals Incorporated

Advisory Committee Working Group
Office of Therapeutic Products, CBER
October 31, 2023

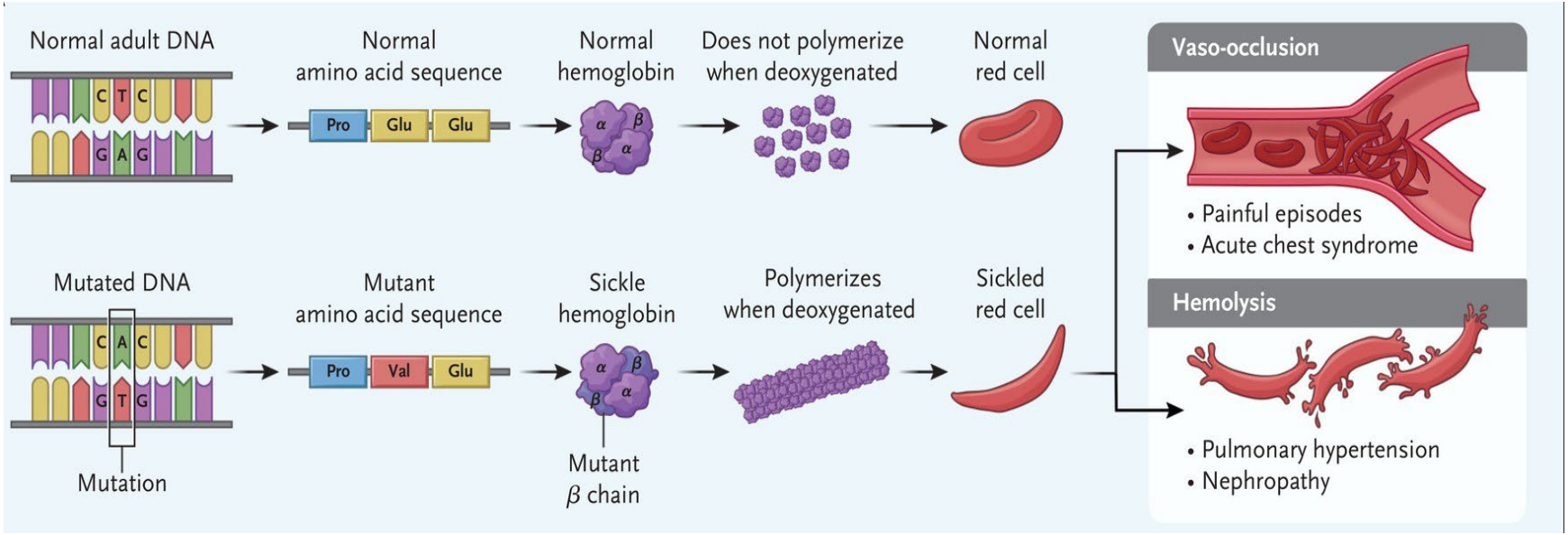
Clinical Assessment of Exa-cel

Karl Kasamon, M.D.
Clinical Reviewer
Office of Clinical Evaluation
Office of Therapeutic Products, CBER

Clinical Outline

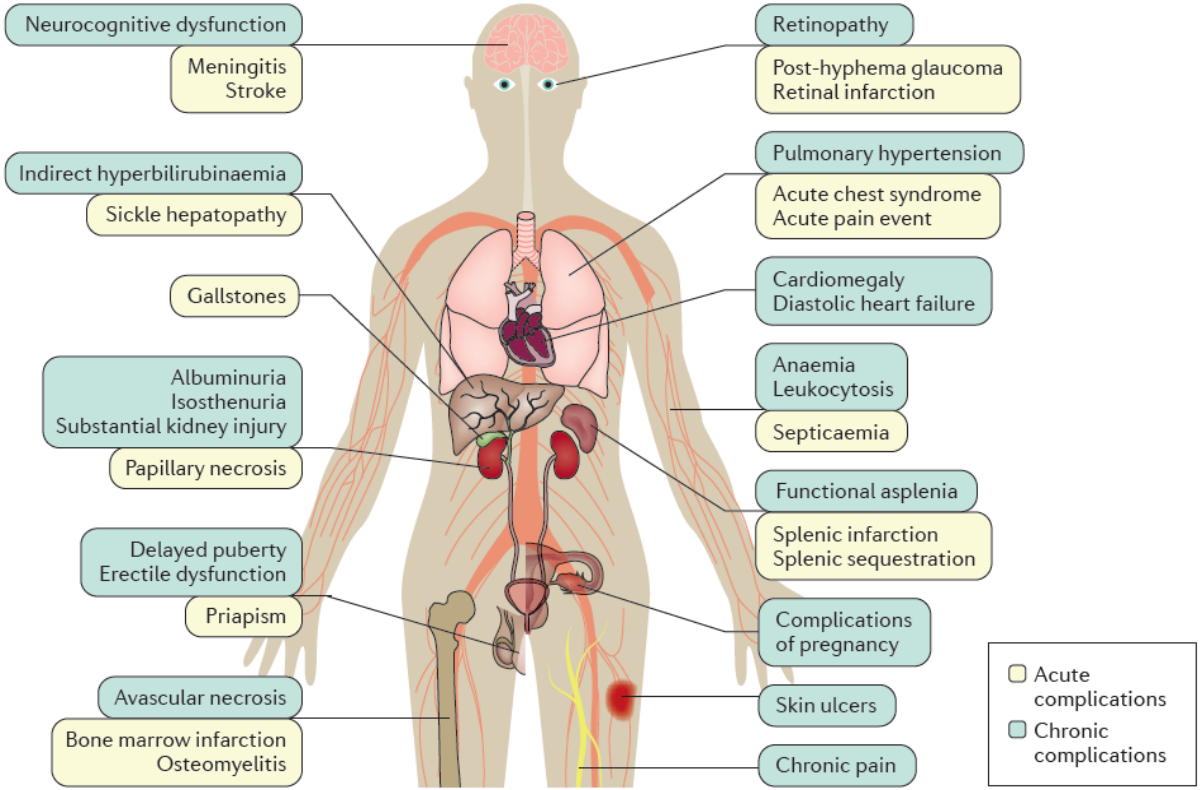
- Sickle cell disease (SCD) and current therapy
- Description of exa-cel mechanism of action and manufacture
- Studies providing evidence of efficacy and safety
  - Study 121 design and results: Efficacy
- Conclusion

Pathology of Sickle Cell Disease



Source: Steinberg, MH, 2022, Fetal-like Hemoglobin in Sickle Cell Anemia, N Engl J Med, 386(7):689-691

Sequelae of Sickle Cell Disease



Source: Kato, G, FB Piel, CD Reid, et. al., 2018, Sickle cell disease, Nat Rev Dis Primers, 4:18010

Therapy for Sickle Cell Disease

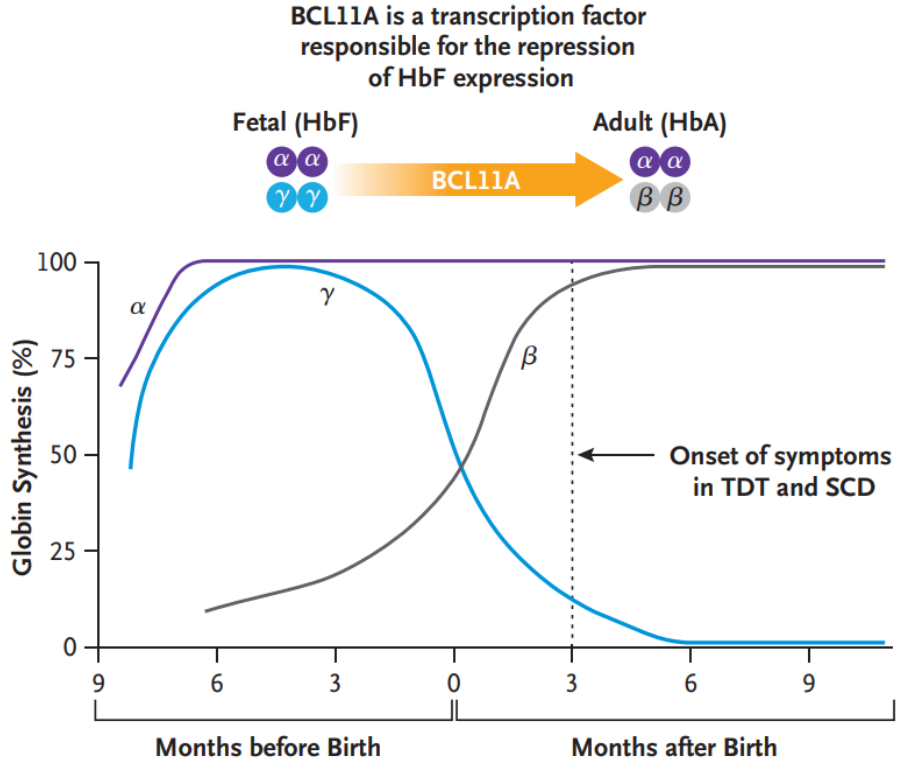
- Supportive care
- Approved drugs
  - Hydroxyurea
  - L-glutamine
  - Voxelotor
  - Crizanlizumab
- Allogeneic hematopoietic stem cell transplantation (HSCT) can be curative but <20% have an appropriate donor

Current treatment for SCD still leaves an unmet medical need

Outline

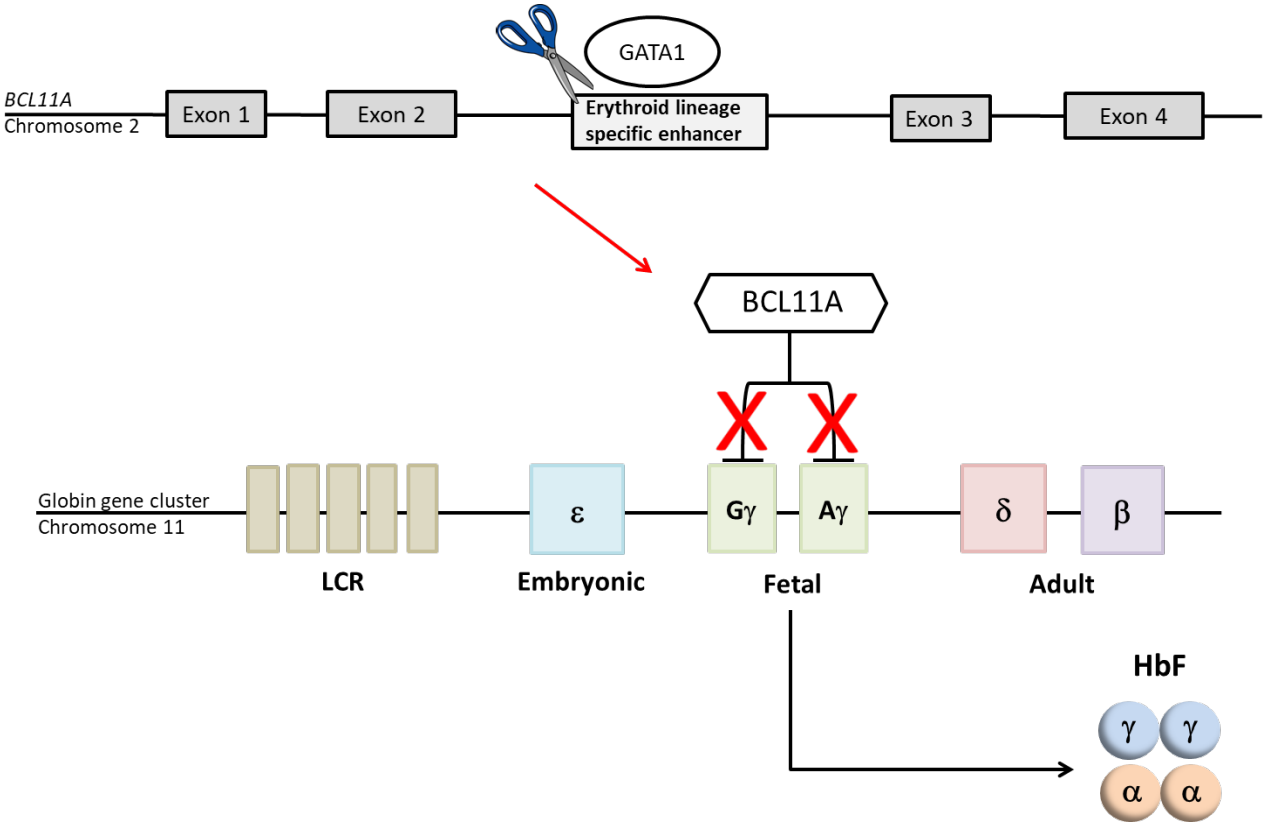
- Sickle cell disease (SCD) and current therapy
- **Description of exa-cel mechanism of action and manufacture**
- Studies providing evidence of efficacy and safety
  - Study 121 design and results: Efficacy
- Conclusions

Role of BCL11A in Hemoglobin Formation



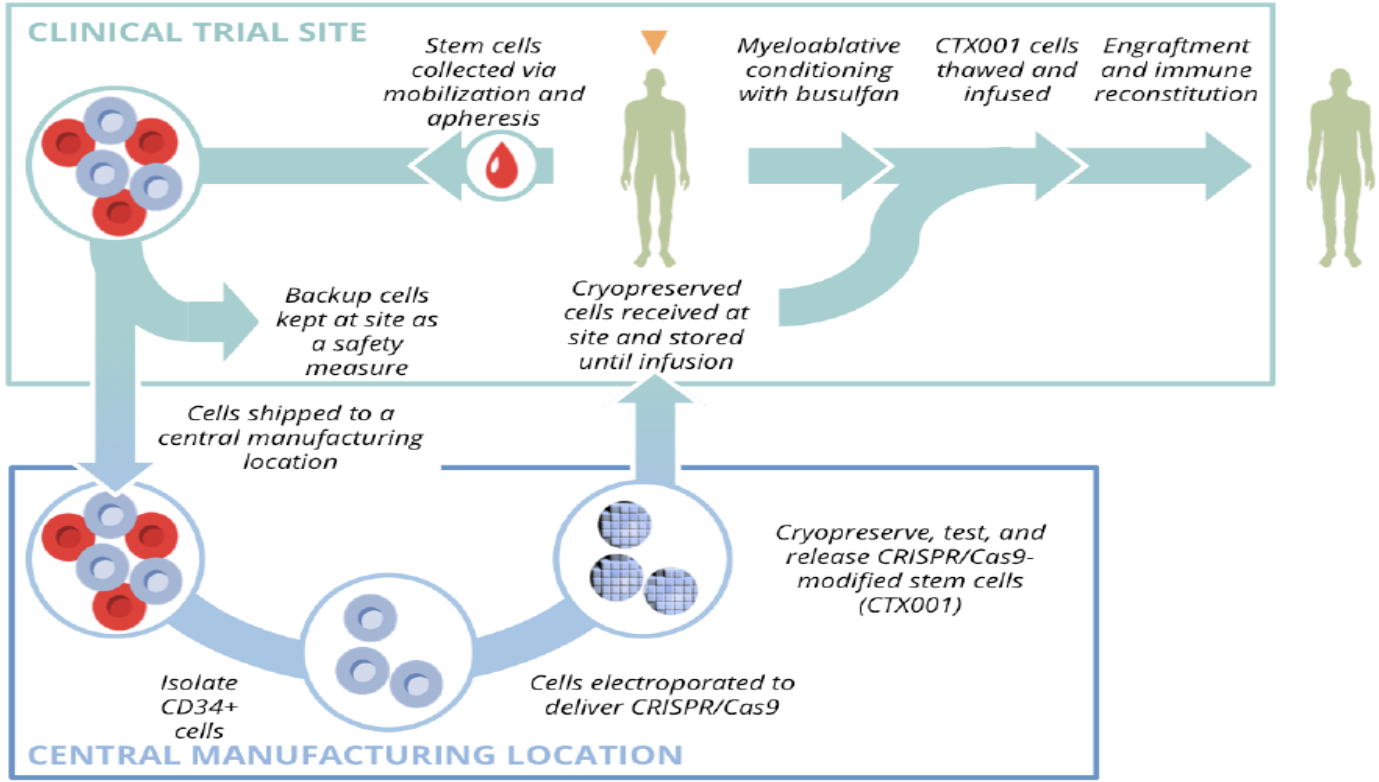
Source: Frangoul, H, D Altshuler, MD Cappellini, et al, 2021, CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia, N Engl J Med, 384(3):252-260
Abbreviations: HbA, adult hemoglobin; HbF, fetal hemoglobin; SCD, sickle cell disease; TDT, Transfusion-dependent β-thalassemia

Exa-cel Mechanism of Action



Adapted from: Bauer, DE, SH Orkin, 2015, Hemoglobin switching's surprise: the versatile transcription factor BCL11A is a master repressor of fetal hemoglobin, Curr Opin Genet Dev, 33:62-70.

Exa-cel Manufacturing



Source: Frangoul, H, D Altshuler, MD Cappellini, et al, 2021, CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia, N Engl J Med, 384(3):Suppl. Appendix, pp. 12

Outline

- Sickle cell disease (SCD) and current therapy
- Description of exa-cel mechanism of action and manufacture
- **Studies providing evidence of efficacy and safety**
  - **Study 121 design and results: Efficacy**
- Conclusions

Sources of Clinical Data on Exa-cel

Study 121

- Phase 1/2/3 multinational single-arm trial started in 2018
- Subjects aged 12 to 35 years with severe SCD
  - Planned study population 45 subjects, 12 adolescents aged 12 to <18

Study 131

- Long-term follow-up rollover study
  - Evaluating safety and efficacy of exa-cel up to 15 years post exa-cel

Study 121 Endpoints

Primary Efficacy Endpoint

- Proportion of subjects achieving absence of sVOCs for ≥12 months (VF 12) at any point on Study 121 following exa-cel infusion. VF12 evaluation started ≥60-day washout following transfusion of red blood cells (RBCs)

Key Secondary Endpoint

- Proportion of subjects achieving freedom from hospitalization for sVOCs ≥12 months (HF12) after exa-cel infusion

Eligibility

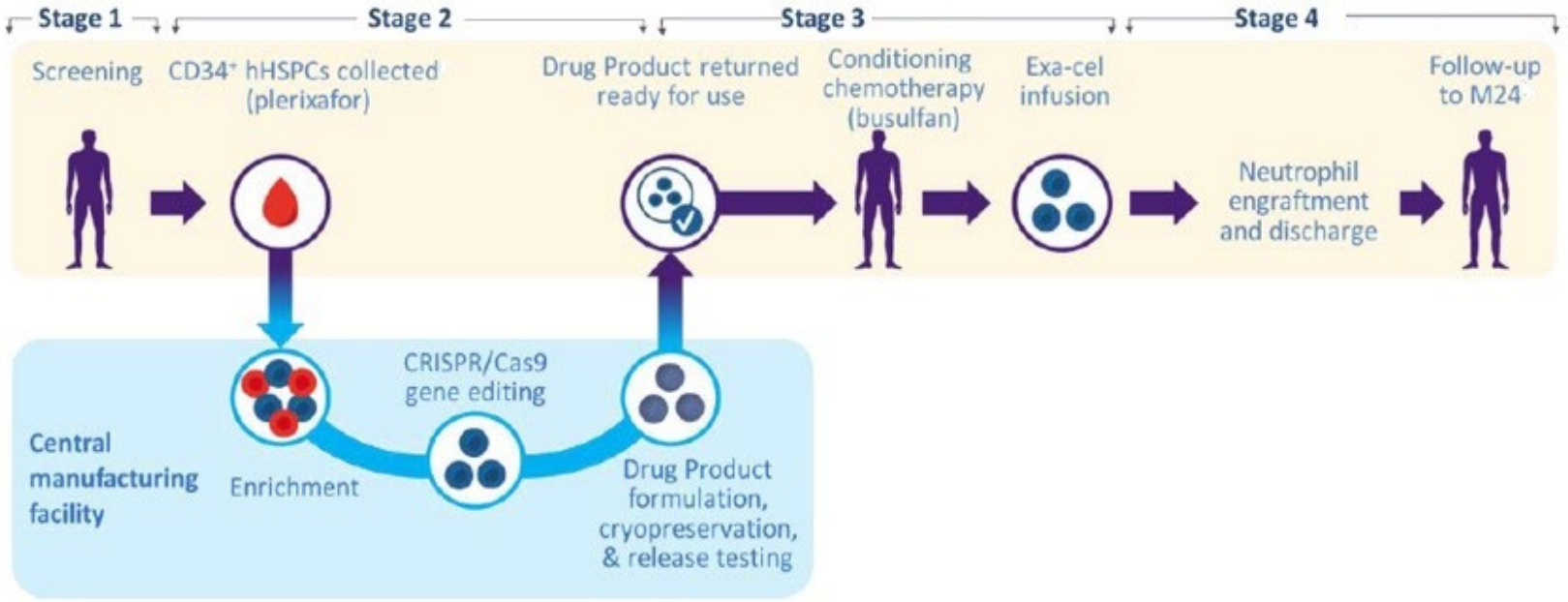
Subjects aged 12 to 35 years with genotypes βS/βS, βS/β0, or βS/β+, and severe SCD based on ≥2 of the following events per year during 2-year period before screening:

- Acute pain event requiring medical facility visit and administration of opioids or IV NSAIDs or RBC transfusions
- Priapism lasting >2 hours and requiring a visit to a medical facility
- Splenic sequestration
- Acute chest syndrome

Key Exclusion Criteria:

- Available donor for HSCT or history of prior HSCT
- Abnormal transcranial Doppler ultrasound / Moyamoya disease, active infections, abnormal organ function, fetal hemoglobin (HbF) >15%

Schematic of Study 121



Source: Adapted from Study 121 protocol version 6.11 US, Appendix 16.1.1
Abbreviations: hHSPC, human hematopoietic stem and progenitor cells; M24, month 24

Key Baseline Characteristics

N=30 primary efficacy set; 44 subjects dosed

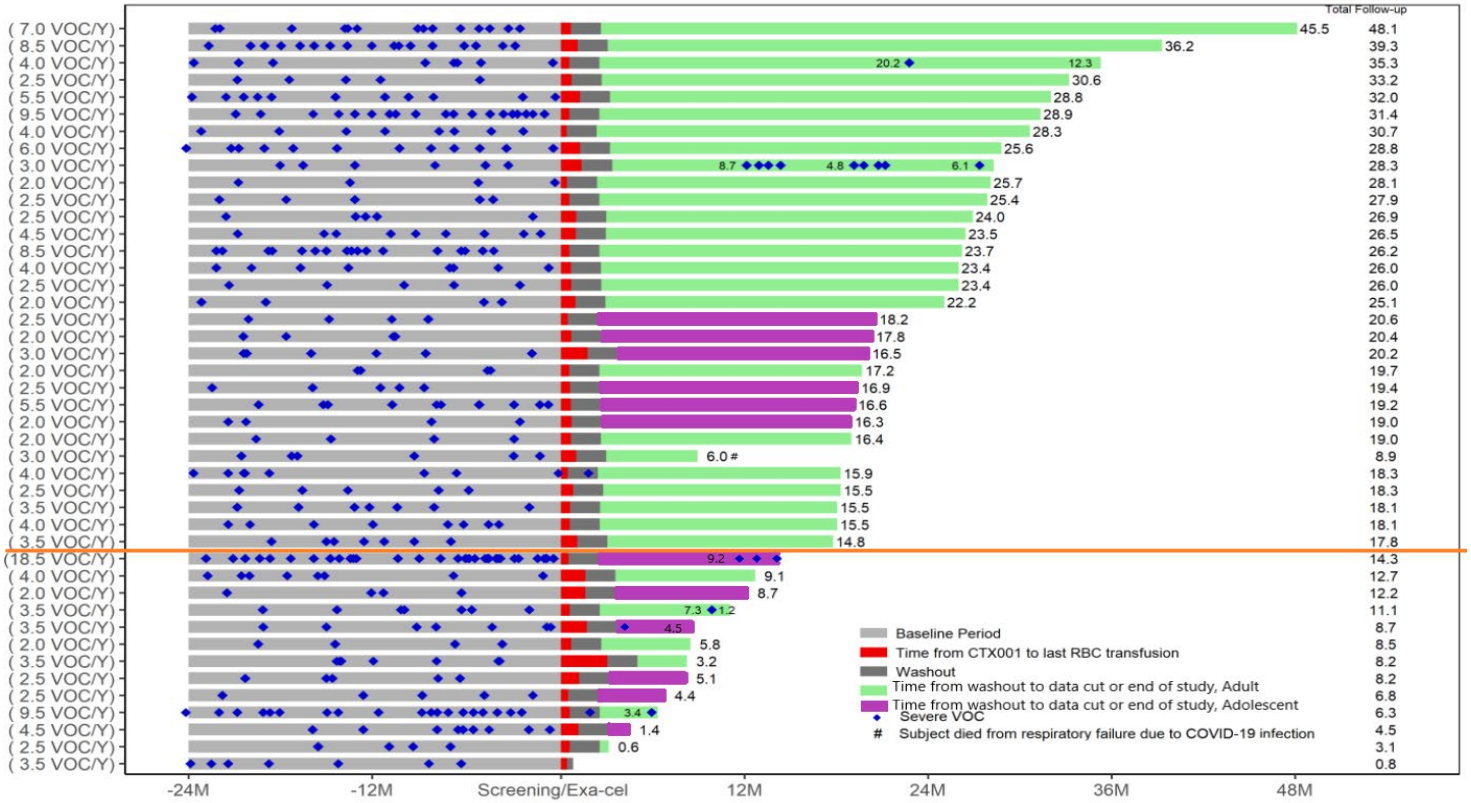
Parameter	PES, N=30
Age in years, median (range)	21 (12-34)
Adolescents <18-year-old (%)	6 (20%)
sVOC rate (annualized, median [range])	3.3 (2-9.5)
Hospitalization rate (annualized, median [range])	2 (0.5-8.5)
Hospitalized days (annualized, median [range])	12.3 (2-65)
RBC units transfused for SCD (annualized, median [range])	3.3 (0-75.5)
HbF concentration (% median [range])	5 (0-14.7)

Source: Derived by reviewer from ADSL dataset from 90 Day Update.
Abbreviations: HbF, fetal hemoglobin; FAS, full efficacy set; PES, primary efficacy set; RBC, red blood cell; SCD, sickle cell disease; sVOC, severe vaso-occlusive crises

Efficacy Analysis

- 29 of 30 exa-cel recipients with ≥16 months follow-up achieved VF12, including 6 adolescents
  - All 30 subjects achieved HF12
  - All 30 subjects had sustained HbF ≥20% for at least 12 consecutive months
- One additional adolescent with 14.3 months of follow-up experienced 3 sVOCs from month 11.6 to 14.1, thus failing to achieve VF12

Distribution of sVOC Before and After Exa-cel, N=44



Source: Modified from SCD Clinical Overview Addendum: Efficacy and Safety Update 14 June 2023, page 19

Conclusion

- The study of patients with SCD treated with exa-cel achieved its primary and secondary efficacy endpoints
- Further discussion is needed regarding long-term safety of genomic editing using CRISPR-Cas9 technology, with respect to off-target edits

Off-Target Safety Assessment of Exa-cel Using Bioinformatics Methods

Komudi Singh, Ph.D.
Bioinformatics Reviewer
Office of Cellular Therapy and Human Tissues
Office of Therapeutic Products, CBER

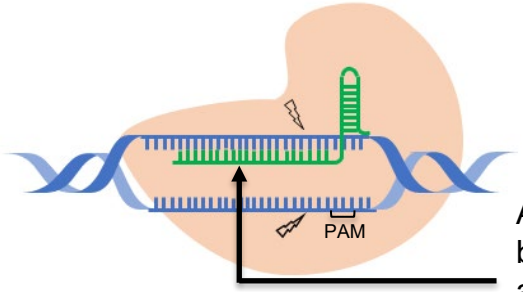
Bioinformatics Outline

- CRISPR-Cas9 technology
- Off-target editing and risk assessment
- Methods of off-target analysis
- Applicant's off-target analysis of exa-cel
- Potential issues and discussion topics

CRISPR-Cas9 Gene Editing Technology

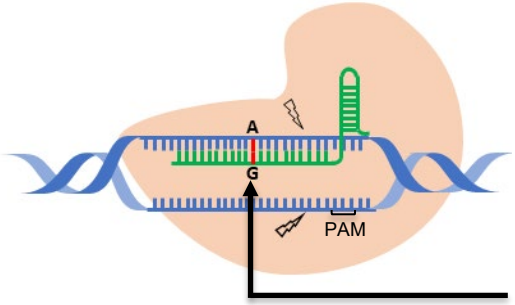
- Naturally occurring microbial defense system that has been engineered to introduce DNA breaks
- A double strand DNA break occurs upon base pairing between the guide RNA and the target sequence in the genome near protospacer adjacent motif (PAM)
- Precise editing at target sequence leads to on-target edits
- Unintentional editing at other loci leads to off-target editing

On-target editing



A perfect base-pairing between the **guide RNA** and the **genomic DNA**

Off-target editing



An imperfect base-pairing between the **guide RNA** and the **genomic DNA** withstanding mismatches

CRISPR-Cas9 Gene Editing Technology

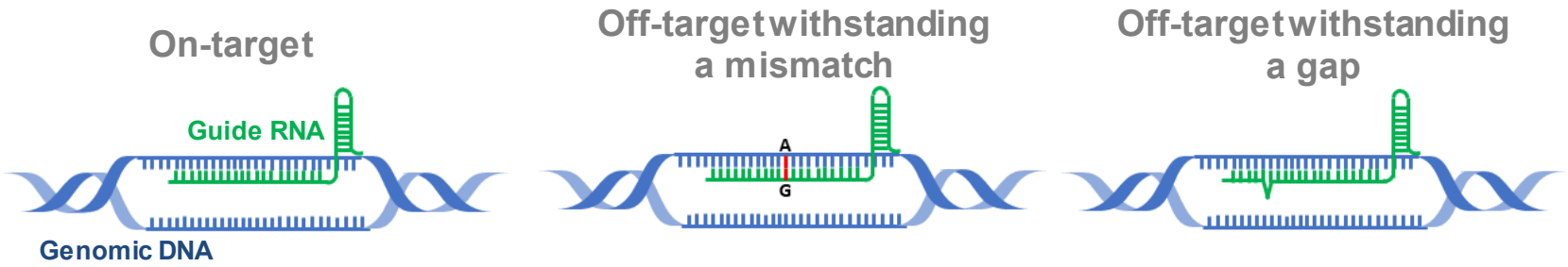
- An off-target edit can be deleterious if it occurs at:
  - Genomic regions with regulatory element(s)
  - Coding region of a gene
- Therefore, an adequate off-target analysis would allow for safety assessment of such genome editing products intended for therapeutic purpose.

Off-Target Editing Analysis

- Rapidly expanding CRISPR-Cas based genome editing tools.
- Development of many bioinformatics tools to assess off-target editing.
- Quantitative bioinformatics tools:
  - Use reference genome sequence information
  - Use sequencing data to perform off-target analysis
- Bioinformatics methods are broadly divided into three categories.

In Silico Methods for Off-Target Editing Analysis

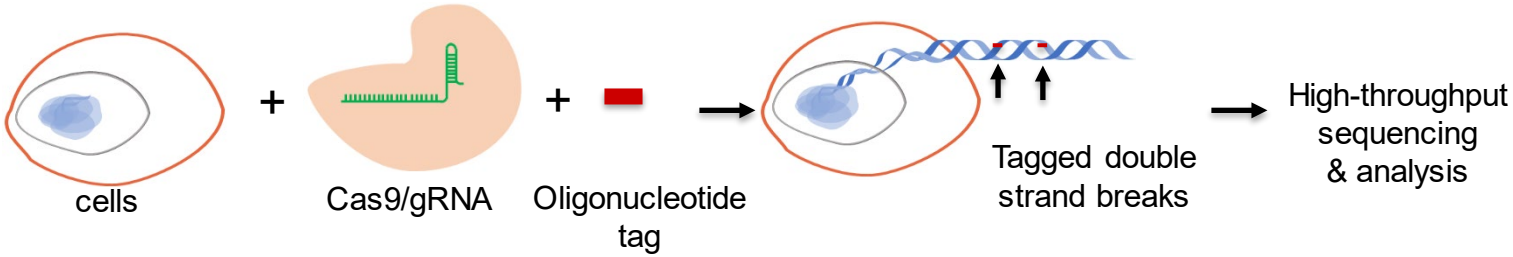
In silico methods use computational algorithms to scan the reference human genome sequence and identify off-targets withstanding user provided mismatch limit



- Straightforward to implement
- Biased by user provided mismatch criteria
- Cannot account for cell-type specificity arising from the unique chromatin landscape within a cell

Cellular Methods of Off-Target Editing Analysis

Cellular methods use genome edited cells where the double strand breaks are "marked" by oligonucleotide tags and subsequently assessed

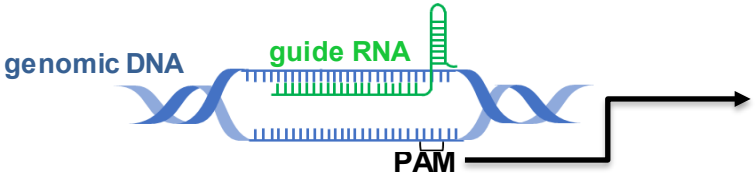


- Provide high-confidence off-target candidates
- Toxicity associated with oligonucleotide tags observed in certain cell types

Off-Target Editing Analysis of Exa-cel

In silico method

- Used three in silico tools to perform homology-based off-target nomination
  - Scan the human genome reference sequence



- Performed homology searches that were inclusive of native and suboptimal PAM sequence

Cellular method

- Performed GUIDE-seq analysis
  - Healthy donor-derived CD34+ hematopoietic stem and progenitor cells (HSPCs)
  - SCD donor-derived CD34+ HSPCs
- The genomic material of Cas9/SPY101 edited cells were extracted
- Performed high throughput sequencing
- Implemented GUIDE-seq pipeline

In Silico Methods for Off-Target Editing Analysis of Exa-cel

- Used 3 different algorithms and 2 different mismatch criteria of 3 and 5
- Used a less stringent mismatch criterion of 5 when including cognate PAM sequence pattern
- More stringent mismatch criterion of 3 was used for suboptimal PAM sequences

- The number of mismatches used can impact the number of off-targets sites nominated

No. of Mismatches	No. of Off-Targets
3	171
5	5,007

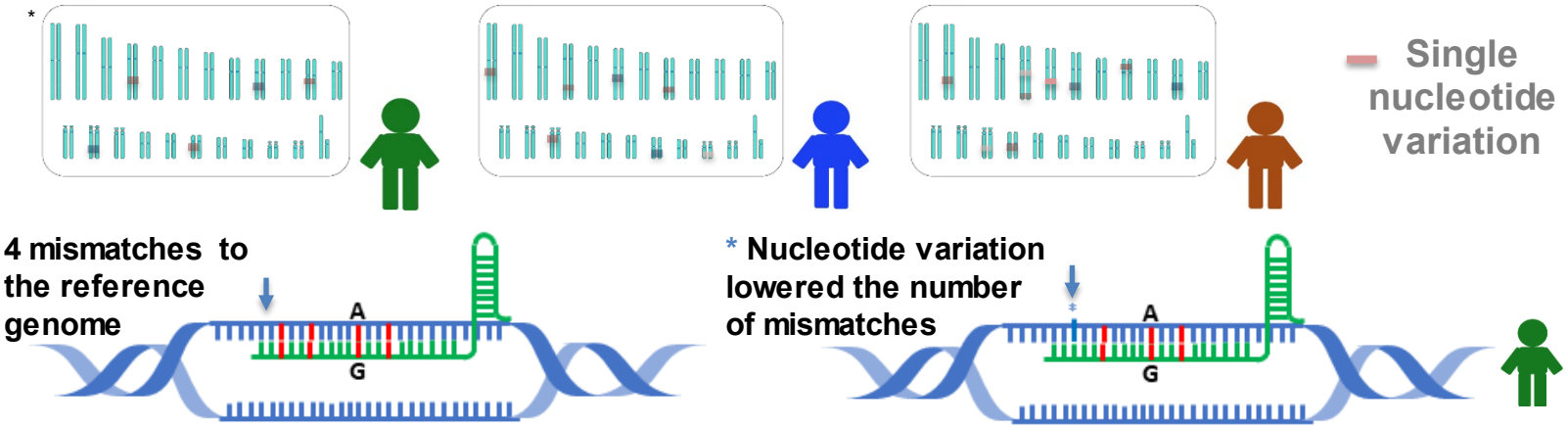
Confirmatory Testing

- Several of these nominated sites were composed of different PAM sequence patterns.
- Therefore, the sample used to empirically test editing potential at these sites should comprise appropriate sequence in the presence of all PAM sequence patterns used in the nomination step.

In Silico Off-Target Editing Analysis of Exa-cel Accounting for Heterogeneity

- Scanning the reference genome sequence cannot account for individual genetic variations that may result in off-target editing at a locus harboring the variation

Cartoon of individual genomes with nucleotide variations contributing to heterogeneity

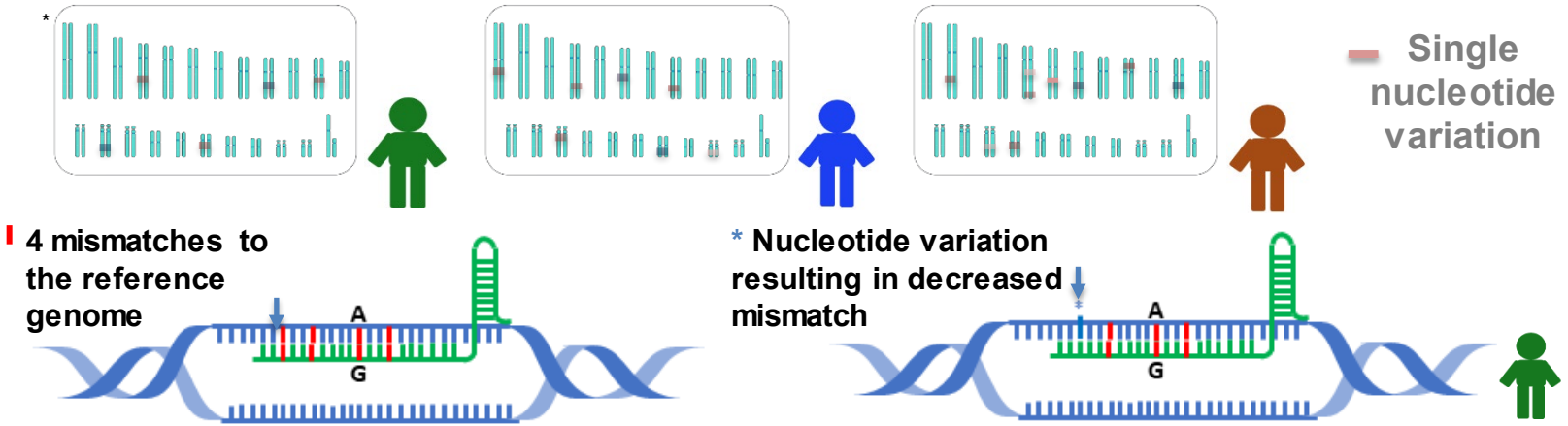


*Genome pictures modified from https://www.genome.gov/genetics-glossary/Genome

In Silico Off-Target Editing Analysis of Exa-cel Accounting for Heterogeneity

- Scanning the reference genome sequence cannot account for individual genetic variations that may result in off-target editing at a locus harboring the variation

Cartoon of individual genomes with nucleotide variations contributing to heterogeneity



- To account for heterogeneity, the Applicant used the 1000 Genomes Project database to include variants that were present at frequency >1% in the database

*Genome pictures modified from https://www.genome.gov/genetics-glossary/Genome

In Silico Off-Target Editing Analysis of Exa-cel Accounting for Heterogeneity

- The 1000 Genomes Project database had ~83 million single nucleotide variations
- Of these, ~21 million variations were present at a frequency >1%
- Variant-aware homology search:
  - Identified sites with either reduced mismatches in the presence of variants
  - Identified sites with a PAM site created in the presence of variants

- **Summary of off-target analysis result**

No. of Mismatches	No. of Off-Targets	No. of Off-Targets Accounting for Heterogeneity
3	171	**50**
5	5,007	—

Confirmatory Testing of In Silico Nominated Loci Using Hybrid Capture Sequencing

- Generate a library of biotinylated RNA fragments that act as bait
- These baits or probes would enrich DNA fragments from the loci nominated by in silico off-target analysis
- To ensure optimal capture of target DNA, the baits were tiled around the off-target loci
- The genomic material from edited and matched unedited cells were used
- The sequencing data after DNA capture were aligned to the reference genome and deduplicated
- Reads with indels 3 bp from the potential cleavage sites were counted

Confirmatory Testing of In Silico Nominated Loci Using Hybrid Capture Sequencing

- Used genomic materials from the cells of 4 healthy donors that were either Cas9/SPY101 edited or were unedited (controls)
- Captured DNA and analyzed sequencing data
- Excluded sequences that have suboptimal coverage, high GC content, high background indel frequency, and/or homopolymers
- Confirmatory testing of 4,340 loci was done in 4 samples for which no metadata was provided
- Confirmatory testing of 171 loci was done in 4 samples: 1 from an individual of African American ethnicity and 3 from individuals of Hispanic ethnicity

Confirmatory Testing of In Silico Nominated Loci Using Hybrid Capture Sequencing

- Several of these nominated sites were composed of different PAM sequence patterns. It is unclear if the 4 samples tested comprised of all PAM sequence patterns used in the nomination process.
- No editing was detected at either 171 or 4,340 off-target loci nominated using different mismatch criterion.
- Summary of hybrid capture sequencing results:

No. of Mismatches	No. of Off-Target Loci	No. of Sites With Sufficient Quality	Total Sites Confirmed
3	171	171	0
5	5,007	4,340	0

Confirmatory Testing of Off-Target Loci Identified From Variant Aware Search

- One SCD and two Transfusion-dependent β-thalassemia (TDT) donor samples were used
- 50 off-target loci nominated from variant aware homology search were tested
- Appropriate samples harboring variants contributing to potential off-target edits should be used
- 13 variants were confirmed to be present in at least 1 sample that was used in confirmatory testing

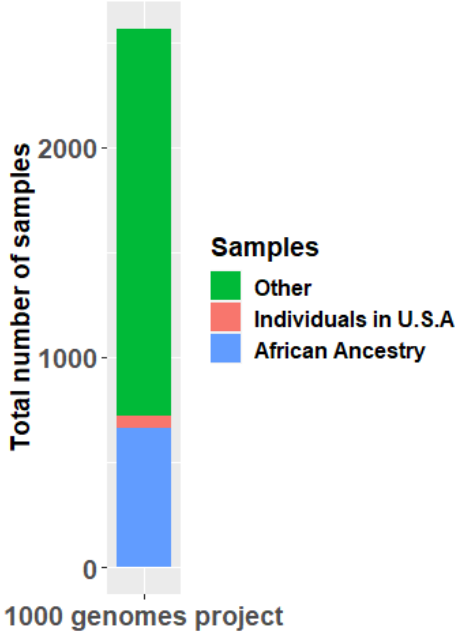
Potential unknowns

- 20 loci annotated to 18 genes whose intronic/exonic locations were identified as potential off-target loci
- Adequate risk assessment of potential disruption in these genomic sequences may be needed

In Silico Off-Target Editing Analysis of Exa-cel Accounting for Heterogeneity

- To adequately account for heterogeneity, variant information used from a reference database should contain:
  - Adequate number of samples
  - Adequate data from individuals representative of the intended patient population
  - Good quality sequencing data
  - Suitable allele frequency cutoff

Adequate sampling of variants

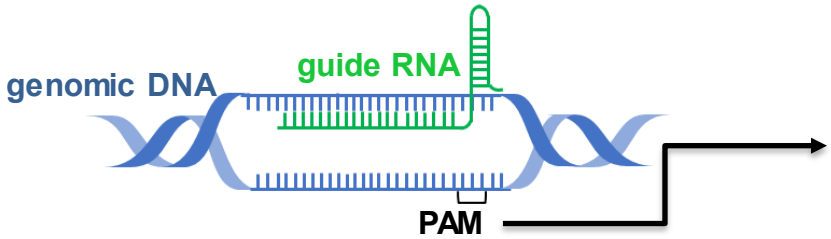


Applicant used the 1000 Genomes Project database (total 2,504 samples)

- Limited data from the intended patient population:
  - 661 individuals were representative of the intended patient population of exa-cel (blue and red bar combined in the graph)
  - In this group, data from 61 individuals were from the United States* (red bar)

* May not adequately represent patients across United States

Intronic Variant Contributing to an Off-Target Locus



SpCas9 can recognize different PAM sequences: **NGG,** NAG, NGA, *etc.*

Sequence	Alignment	Chr	Position	Strand	Variant ID	CFD	MAF	Annotation
Spacer+PAM	CTAACAGTTGCTTTTATCACNNN							
Reference	tTAACAGcTGCcTTTATCACTGC	2	210530658	-		0.021		Intron:*CPS1*
Alternative	tTAACAGcTGCcTTTATCACTGG				rs114518452 2-210530659-G-C	0.947	0.02	

Source: Cancellieri, S, J Zeng, LY Lin, et. al., 2022, Human genetic diversity alters off-target outcomes of therapeutic gene editing, Nat Genet, 55(1):34-43

Variant allele frequency in the indicated study & groups

Variant	Cancellieri *et al.*	Applicant reported data
rs114518452	2% (global) 4.55% (African/African American)	1.58% (global) 5.6% (African/African American)

- Confirmatory testing was performed in samples harboring the TGC PAM sequence only.
- Editing potential of this genomic locus with TGG PAM sequence was not empirically tested.

Differences Between the Two Study Findings

- Applicant did not report the CPS1 variant, but they had reported this locus in their in silico study
- However, in other instances, the Applicant reported variants from loci that were likely included in their in silico study (Table 1)
- Other variants may have been excluded from the Applicant's heterogeneity analysis due to their set criteria
- Genetic variations reported in a specific database that was not used by the Applicant may be excluded
- Different variant allele frequency cutoff used in the two studies may also result in exclusion of variants from the Applicant's study (Table 3)

Table 1: Mismatches in the Indicated Variant Locus

Variant	Without Variant	With Variant
rs186390458 (BBS9 intron)	4mm	3mm
rs73264600 (FBXO38 intron)	4mm	3mm

Table 2: Applicant's Homology Based in silico nomination

No. of Mismatches	No. of Off-Targets
3	171
5	5007

Table 3: Variant Frequency in the Indicated Study/Dataset

Variant	Cancellieri *et al.*	1000 Genomes Project Database
rs148421996	0.6% (global)	0.6% (global)

Comparison of Heterogeneity Analysis Studies

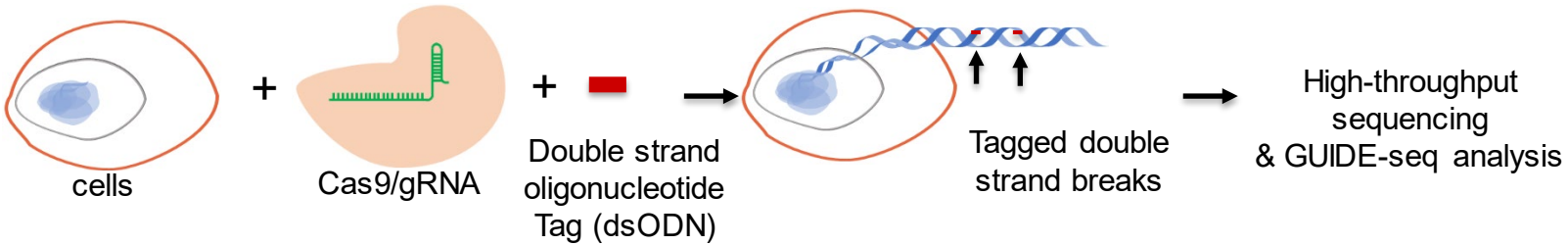
	Exa-cel Off-Target Assessment	Study Published by Cancellieri *et al.*, 2022
Method	Implemented a variant aware homology search	Developed and implemented an in silico off-target analysis tool
Database	1000 Genomes Project comprised of sequencing information from 2,504 individuals	Human Genome Diversity Project (HGDP) comprised of sequencing information from 929 individuals and Genome Aggregation Database (gnomAD) comprised of sequencing information from 76,156 individuals
Off-target loci nominated	Identified 50 potential off-target loci that were contributed by one or two variants	Identified a variant that resulted in creation of a PAM site and a potential off-target locus

Summary of In Silico and Heterogeneity Analysis

- Different number of off-target loci identified after accounting for heterogeneity is concerning
  - A limited number of sequencing information from individuals who are representative of target population in the two databases (1000 Genomes Project and Human Genome Diversity Project)
  - Different databases used in two studies
  - Different in silico algorithms used
  - Inadequate sampling of variants due to limited amount of sequencing data
- Confirmatory testing requires cells that harbor the variants contributing to an off-target loci
  - Only 13 variants out of 50 variants were confirmed to be present in at least 1 sample
- A small subset of in silico nominated loci were experimentally tested
- Lack of clarity on the adequacy of heterogeneity accounted in exa-cel safety assessment

Cellular Method Using GUIDE-seq for Off-Target Editing Analysis of Exa-cel

- The Applicant used GUIDE-seq to identify off-targets in SPY101 guide RNA edited CD34+ HSPCs.



- The Applicant performed this experiment using three healthy donor cells and three SCD donor cells.

Cellular Off-Target Editing Analysis of Exa-cel and Confirmatory Testing

- The Applicant used GUIDE-seq on three healthy donor cells and three TDT donor cells and identified several off-targets.

Healthy Donor Samples	dsODN Concentration (micro molar)	Cell Viability (%)	Number of Off-Target Loci	Total On-Target Reads
Donor 1*	1	88	11	>10,000
Donor 1*	1	94	25	>10,000
Donor 2	0.5	71	16	12,095
Donor 3	0.5	75	5	11,336
Donor 4	0.5	75.2	5	23,468
Donor 5	0.5	78.1	11	23,938
Donor 6	0.5	81	6	18,807

*Different concentration of oligonucleotide tags used

- Several off-target loci were identified in each of the samples tested
- The Applicant used hybrid capture sequencing on four independent healthy donor cells and did not detect editing at these loci in these samples

Cellular Off-Target Editing Analysis of Exa-cel and Confirmatory Testing

- The Applicant used GUIDE-seq on three SCD donor cells and identified several off-targets

SCD Donor Samples	dsODN Concentration (micro molar)	Cell Viability (%)	On-Target Editing Frequency (%)	Total On-Target Reads	Number of Off-Target Sites
Donor 1	0.5	78.7	83.8	16,508	12
Donor 2	0.5	82.8	93.5	28,879	13
Donor 3	0.5	78	93.6	20,857	17

Cellular Off-Target Editing Analysis of Exa-cel and Confirmatory testing

- Manual assessment of DNA break regions and hybrid capture analysis identified a DNA break hotspot

SCD Donor Samples	Total On-Target Reads	Number of Off-Target Sites at Hotspot	On-Target Editing Frequency (%)
Donor 1	20,278	2	71.8
Donor 2	22,075	0	66.2
Donor 3	22,004	1	71.9

- Tested if these loci were false positives using false positive filtering step

SCD Donor Samples	Number of Off-Target Sites	Number of Filtered Off-Target Sites
Donor 1	12	0
Donor 2	13	0
Donor 3	17	0

Summary of Cellular Off-Target Editing Analysis of Exa-cel

- The Applicant performed two GUIDE-seq experiments, one using three healthy donor-derived CD34+ HSPCs and another using three SCD subject-derived CD34+ HSPCs.
- The off-target loci identified from the GUIDE-seq experiments did not overlap with 171 off-target loci nominated using in silico methods.
- It is not clear if off-target analysis using healthy donor cells adequately inform off-target editing in exa-cel.
- We are concerned about the adequacy of a small number of samples used in the cellular off-target analysis in understanding potential risks of off-targets in exa-cel.

Potential Issues With Exa-cel Off-Target Editing Analysis

- In silico off-target analysis was performed using the 1000 Genomes Project database to account for heterogeneity; however:
  - The database contains a small amount of sequencing data from the target population of exa-cel
  - Sampling of variants may be inadequate
  - Confirmatory testing was performed in samples harboring a fraction of the variants nominated
- Cellular off-target analysis was performed in a small number of samples from healthy donors and SCD donors
  - Adequacy of the sample size
  - Use of limited number of SCD samples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