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Comprehensive evaluation of genome editing-associated genetic modifications

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Cellular, Tissue and Gene Therapies Advisory Committee, FDA
31 October 2023

Dana-Farber Cancer Institute | Boston Children's
Dana-Farber/Boston Children's Cancer and Blood Disorders Center

HARVARD MEDICAL SCHOOL
TEACHING HOSPITAL

Disclosures

Patents

- I am a co-inventor of patents related to therapeutic genome editing for blood disorders.
- I hold a licensed patent that is related to BLA 125787 from Vertex Pharmaceuticals, Inc. and it is possible that I could receive future related royalties.

Consulting

- Kytopen

Outline

- Therapeutic genome editing can produce genetic modifications both away from and at the genomic target site (off-target and on-target edits).
- 1. Off-target edits may be influenced by human genetic diversity.
- 2. On-target edits may include short indels and structural variants.
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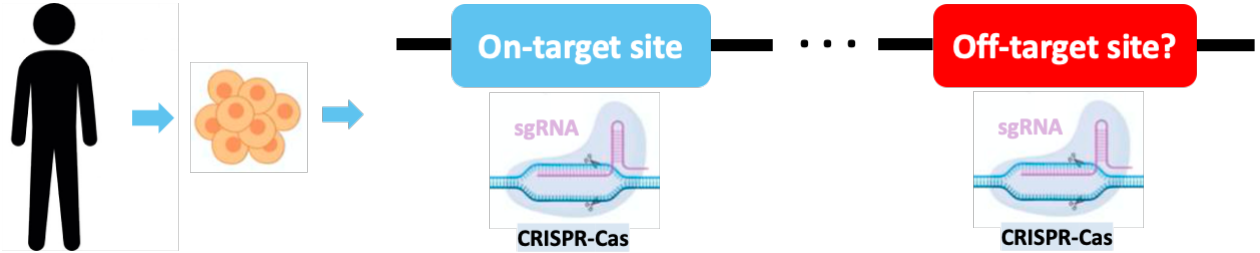
Off-target effects of genome editing



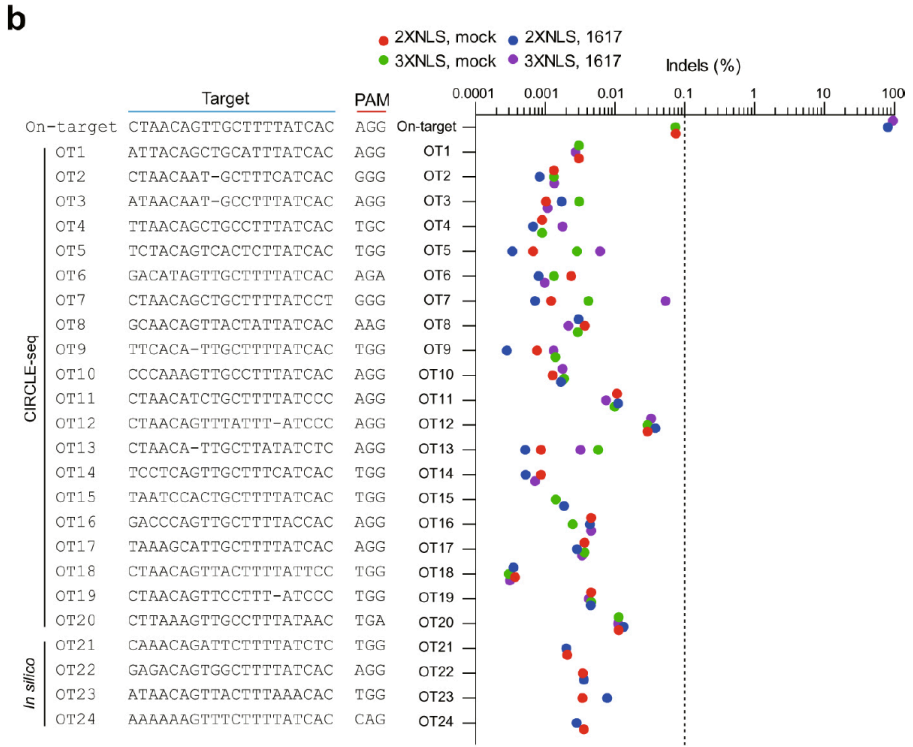
Current methods to nominate candidate off-target sites

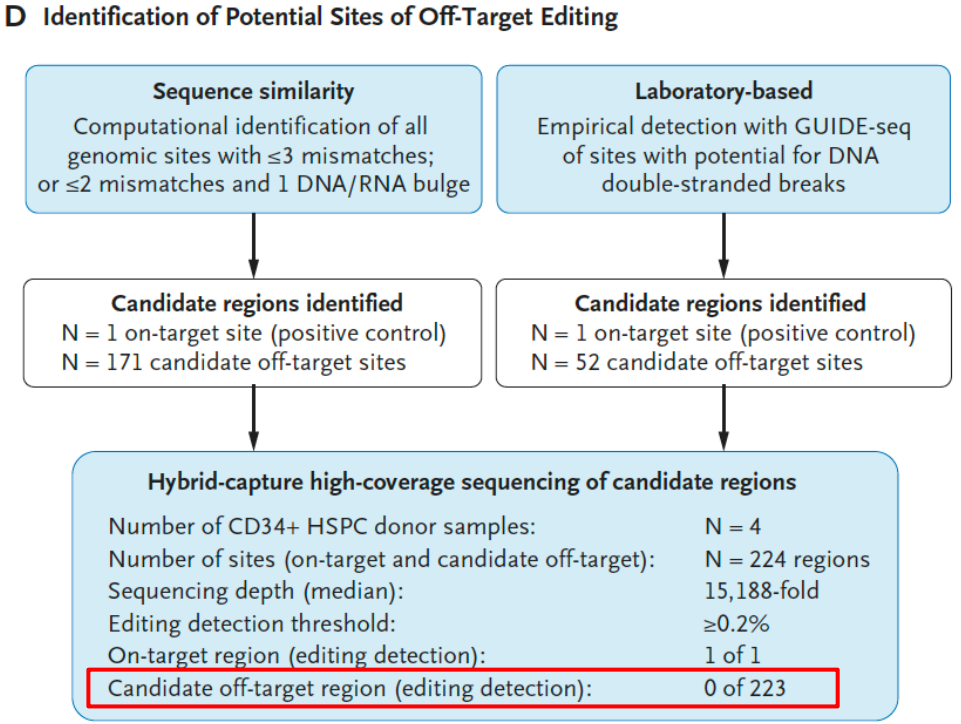
1) In silico: based on sequence homology

2) Cell-based & in vitro: assess modification of genomic DNA

1. Bae et al. *Bioinformatics* (2014), Park et al. *Bioinformatics* (2015), Concordet et al. *Nucleic Acids Res* (2018), Listgarten et al. *Nat Biomed Eng* (2018)
2. Tsai et al. *Nat Biotechnol* (2015), Kim et al. *Nat Methods* (2015), Tsai et al. *Nat Methods* (2017), Lazzarotto et al. *Nat Biotechnol* (2020)

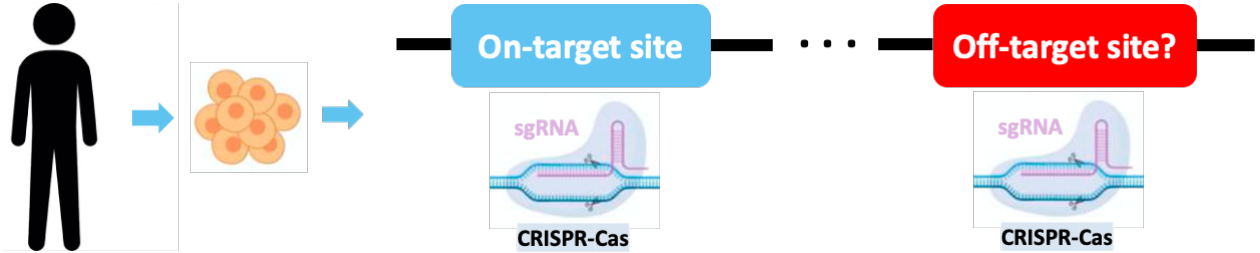
Prior off-target assessment for *BCL11A* +58 sgRNA #1617





Wu *et al*. Nat Med (2019). Frangoul *et al*. NEJM (2021).

Gene editing specificity



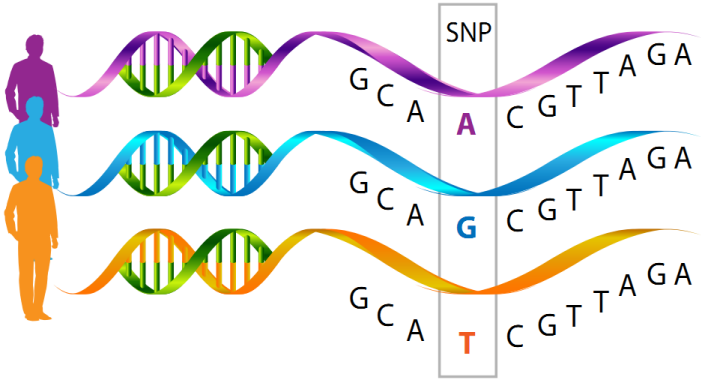
<u>Current methods to nominate candidate off-target sites</u>

1) In silico: predict based on sequence homology
   - Examples: Cas-OFFinder/Cas-Designer/CRISPOR, Elevation[1]
   - Tools analyze the human reference genome

2) Cell-based & in vitro: assess modification of genomic DNA
   - Examples: GUIDE-seq, Digenome-seq, CIRCLE-seq, CHANGE-seq[2]
   - Limited by donor genotype

1. Bae et al. *Bioinformatics* (2014), Park et al. *Bioinformatics* (2015), Concordet et al. *Nucleic Acids Res* (2018), Listgarten et al. *Nat Biomed Eng* (2018)
2. Tsai et al. *Nat Biotechnol* (2015), Kim et al. *Nat Methods* (2015), Tsai et al. *Nat Methods* (2017), Lazzarotto et al. *Nat Biotechnol* (2020)

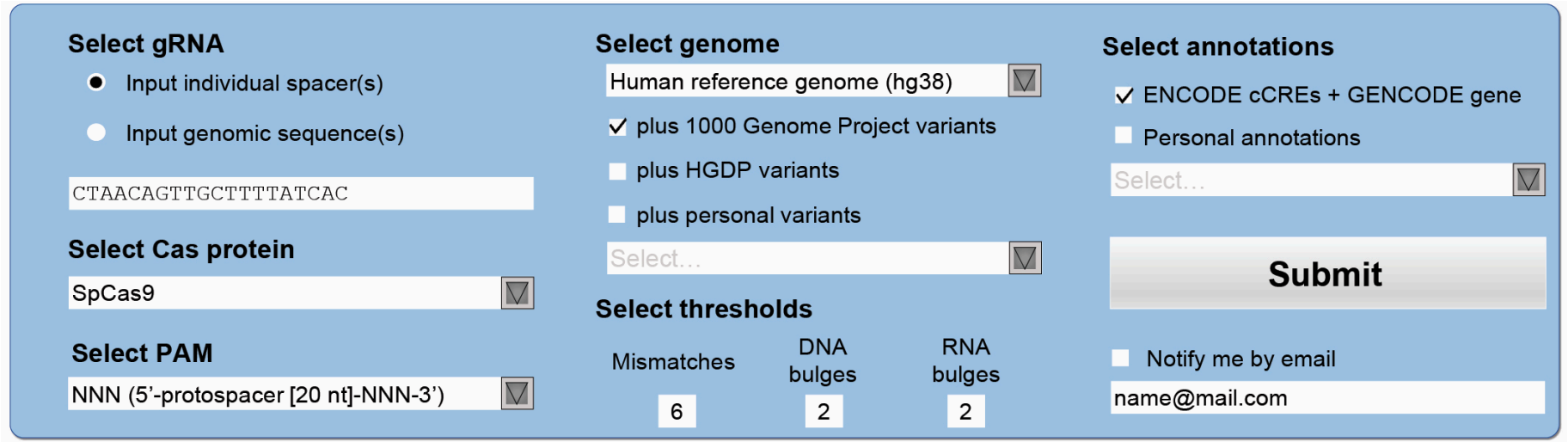
Off-target assessment for therapeutic gene editing



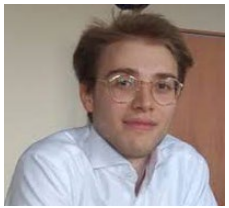
What about off-target sites that are <u>not found</u> in the human reference genome, but may be found in …

- Populations?
- Individual patients?

CRISPRme: comprehensive, variant-aware off-target assessment tool



Samuele Cancellieri
Linda Lin
Rosalba Giugno
Luca Pinello



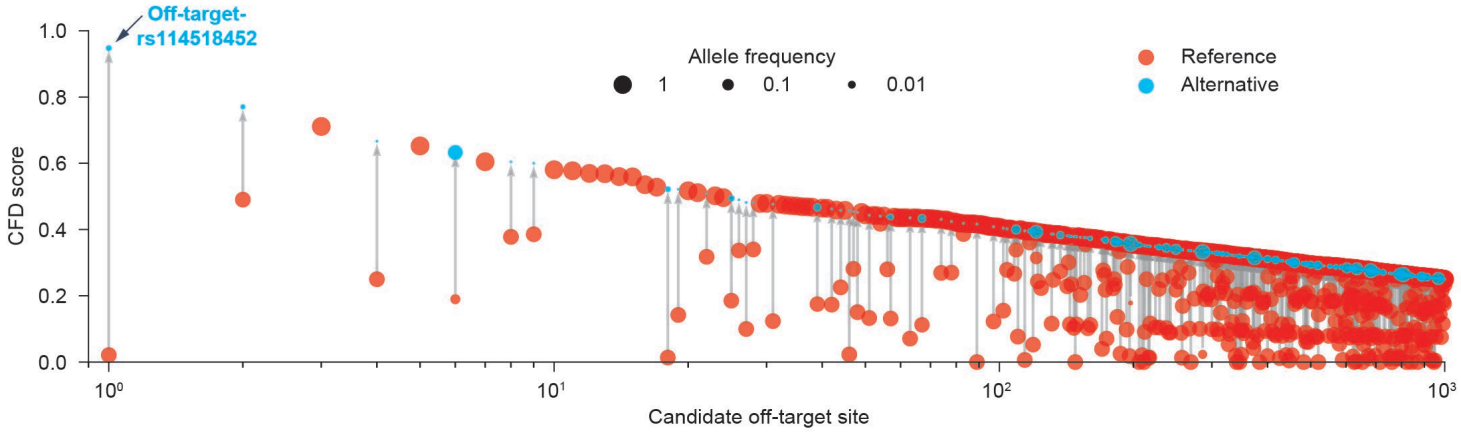

- Website: crisprme.di.univr.it
- Code (for offline installation): github.com/pinellolab/CRISPRme

Cancellieri *et al.* Nat Genet (2023) 55:34.

CRISPRme

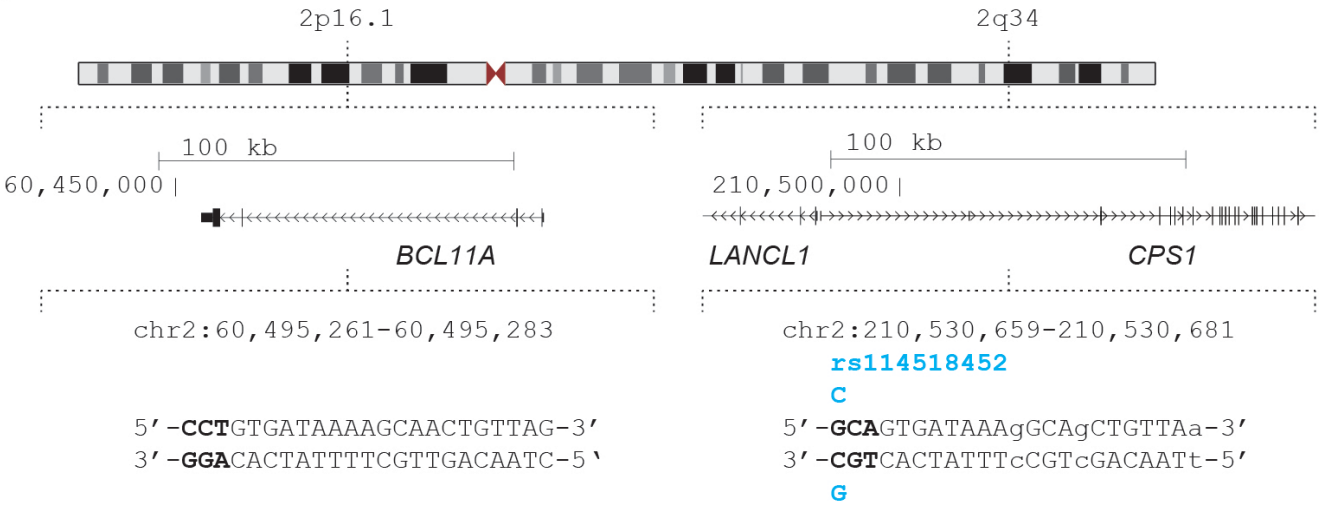
BCL11A enhancer sgRNA #1617 with 1000 Genomes Project variants

Cutting frequency determination score
- Range: [0, 1]
- 1 for on-target site



Cancellieri *et al.* Nat Genet (2023) 55:34.

Top predicted off-target for *BCL11A* enhancer sgRNA #1617 created by variant common in African ancestry populations



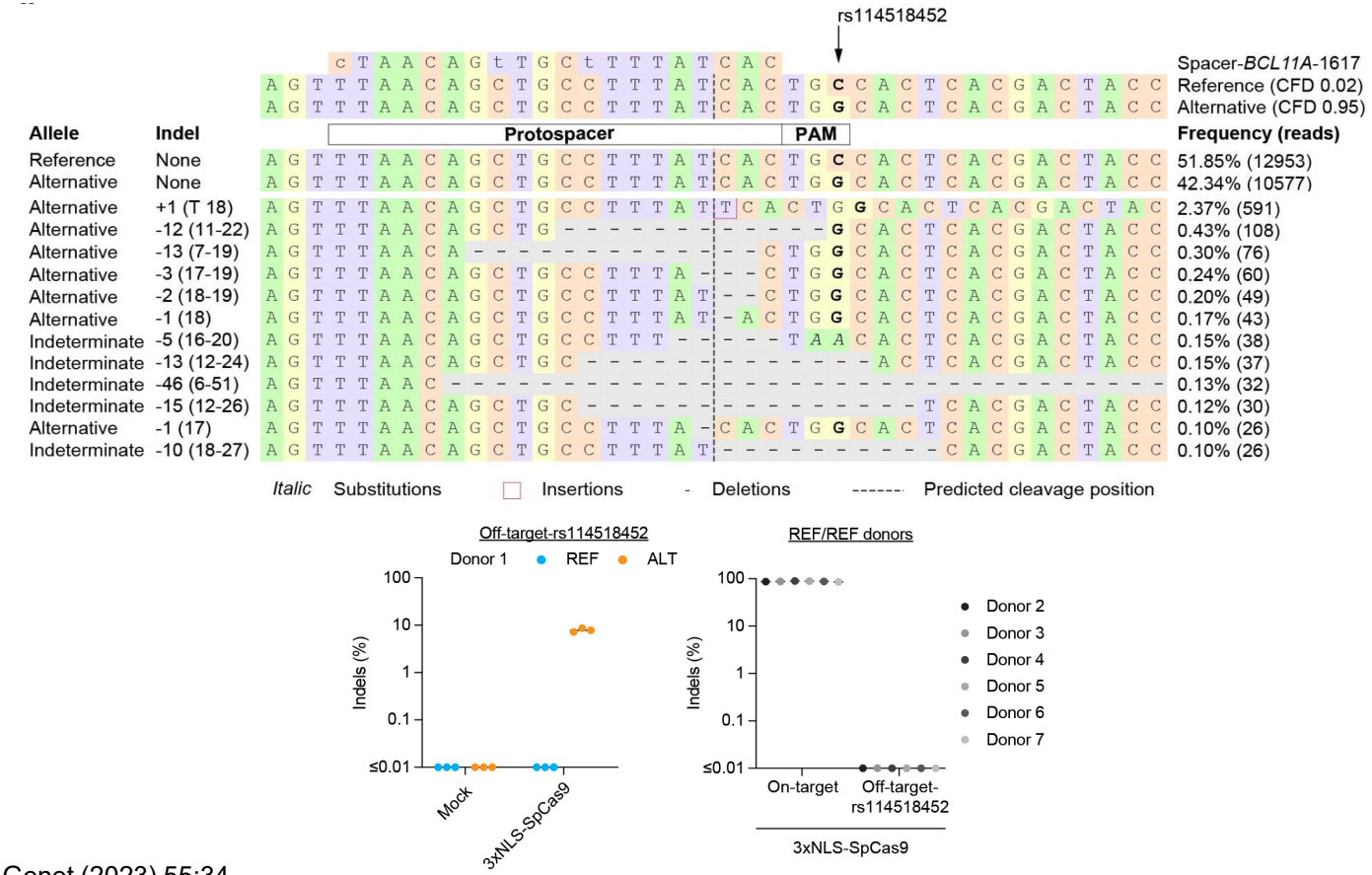
rs114518452 2-210530659-G-C

MAF (%)	Population
4.55	African/African American
0.91	Other
0.66	Latino/Admixed American
0.12	South Asian
0.01	European (non-Finnish)
0.00	Finnish/Amish/East Asian/ Middle Eastern/Ashkenazi Jewish

Cancellieri *et al.* Nat Genet (2023) 55:34.

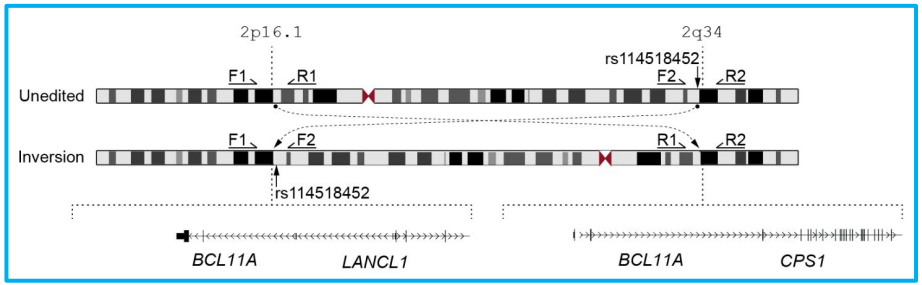
MAF from gnomAD v3.1

Allele-specific off-target editing in heterozygous HSPCs



Cancellieri *et al.* Nat Genet (2023) 55:34.

sgRNA #1617 off-target editing results in allele-specific pericentric inversions



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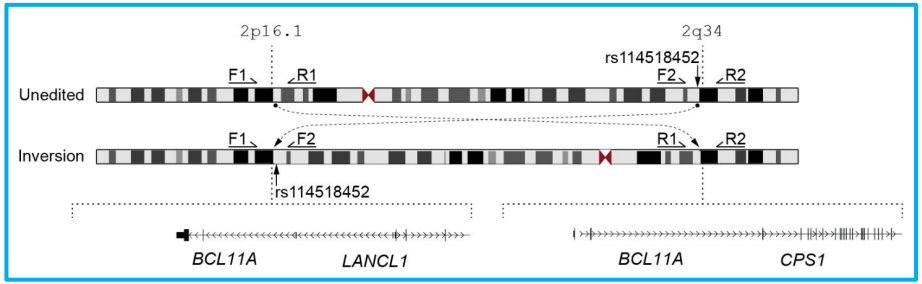

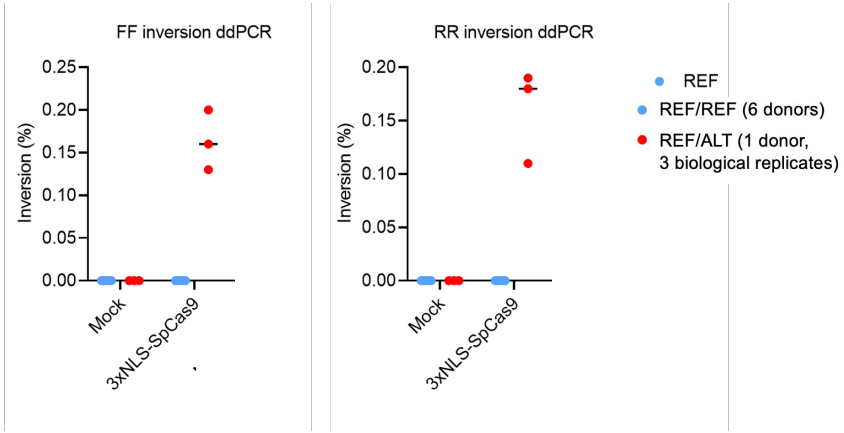

- Biological significance of these off-target indels and pericentric inversions is uncertain and may be negligible

Cancellieri *et al.* Nat Genet (2023) 55:34.

Outline

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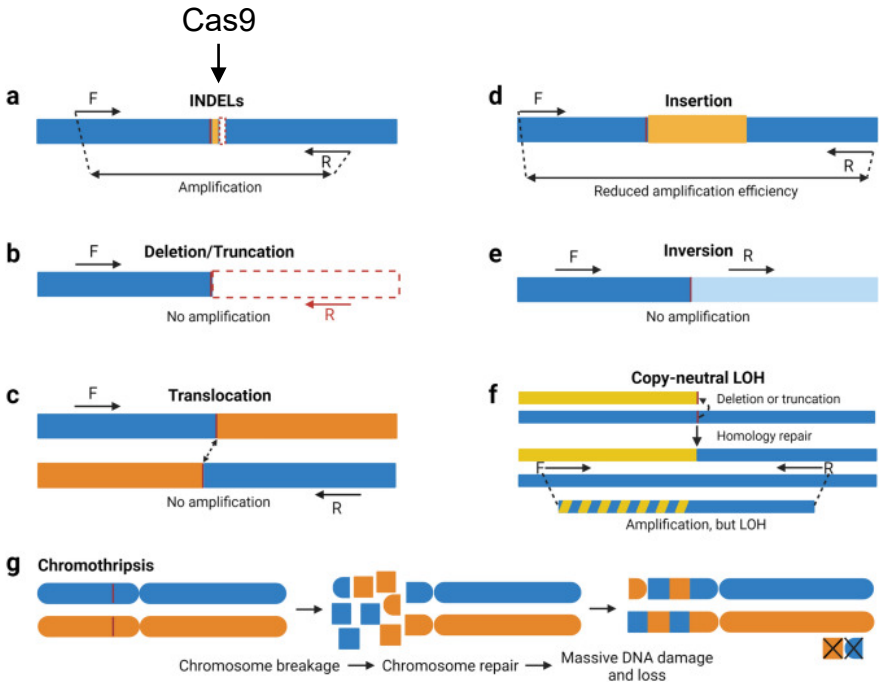
On-target edits include short indels and structural variants

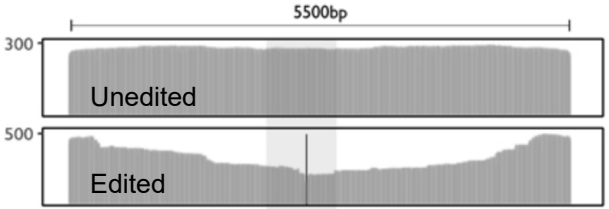


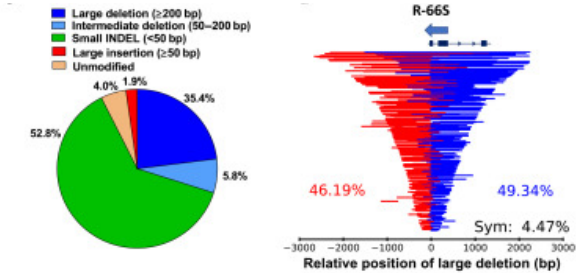
- Standard short-amplicon sequencing cannot capture structural variants
- Biological significance of any individual structural variant is uncertain and may be negligible

Hunt *et al.* Hum Genet (2023) 142:705.

On-target edits include short indels and structural variants

Long-read sequencing





Kosicki *et al.* Nat Biotechnol (2018) 36:765. Park *et al.* Sci Adv (2022) 8:7676. Hunt *et al.* Hum Genet (2023) 142:705. Newby *et al.* Nature (2021) 595:295.

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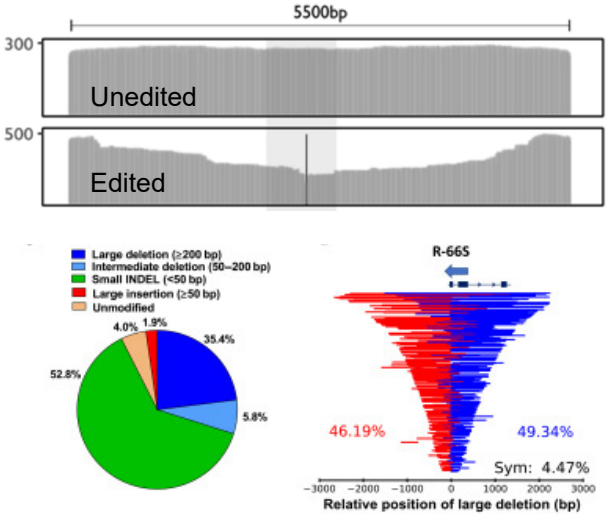
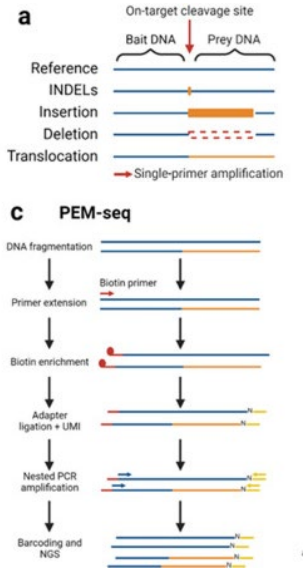


Single-primer amplification



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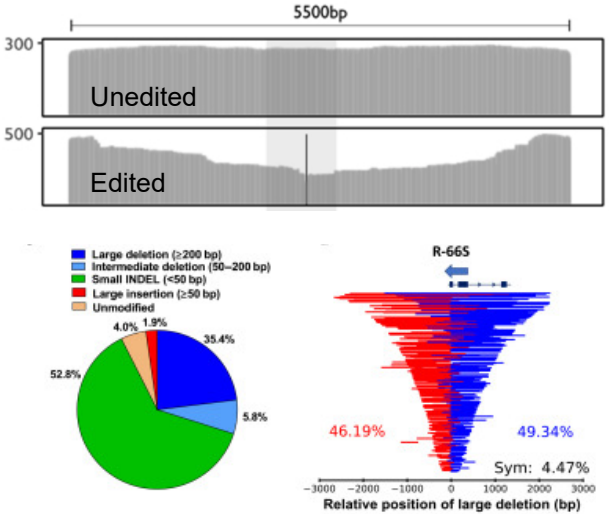

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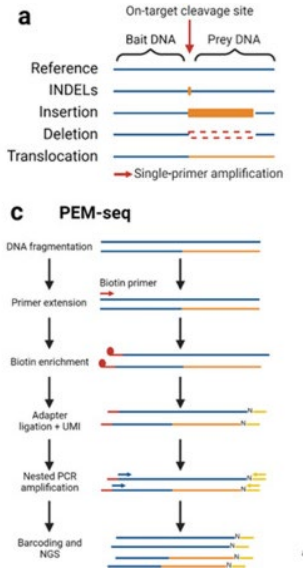
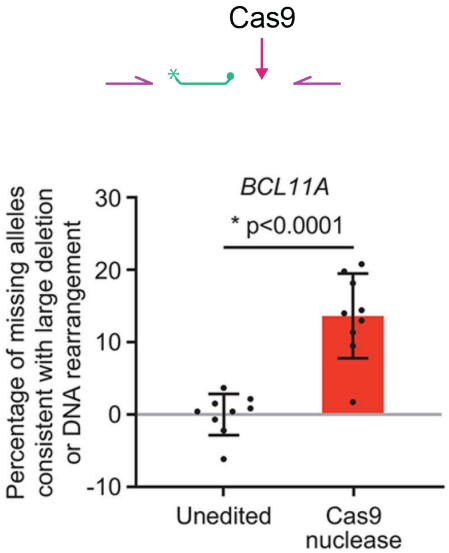


Droplet digital PCR



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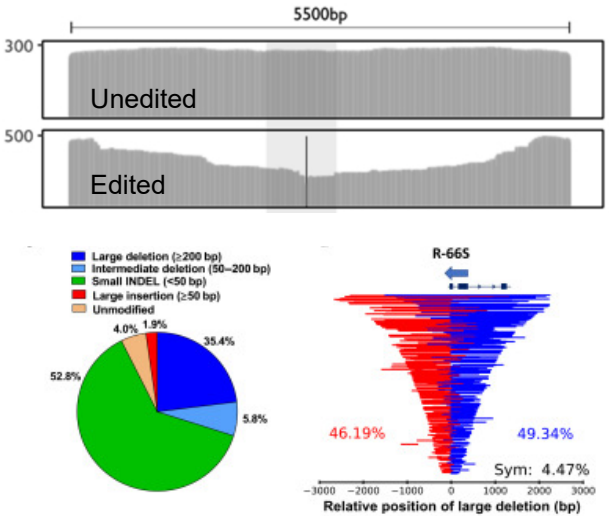

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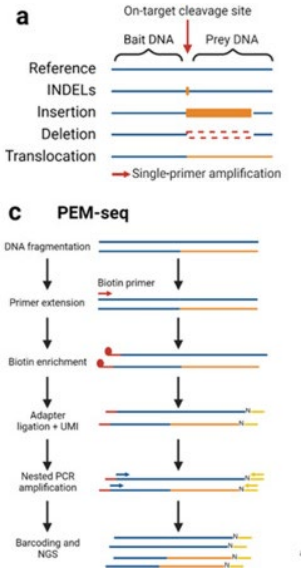

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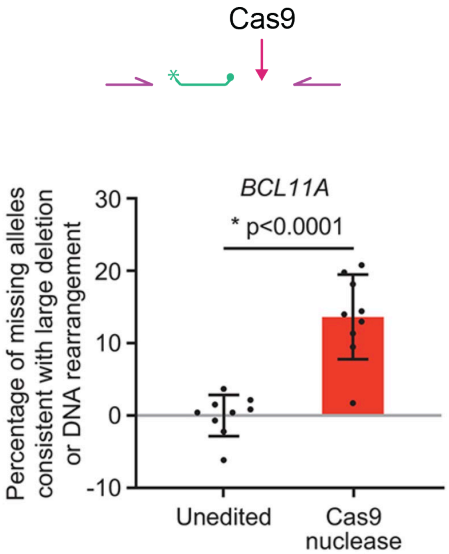

- Numerous assays exist to detect on-target structural variants, although no single assay may fully characterize all classes

Kosicki *et al.* Nat Biotechnol (2018) 36:765. Park *et al.* Sci Adv (2022) 8:7676. Hunt *et al.* Hum Genet (2023) 142:705. Newby *et al.* Nature (2021) 595:295.

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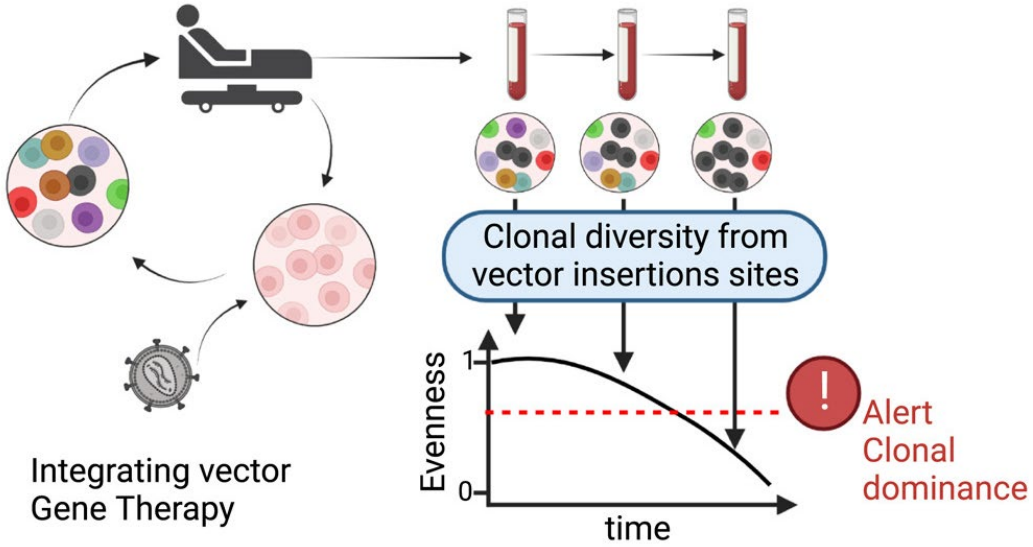
Edit distribution reflects clonal composition of hematopoietic graft



- Monitoring clonal composition may inform safety (clonal dominance) and efficacy (therapeutic edits)
- Analogy to integrating vector gene therapy, although gene edits may not be as diverse as vector integrations (different clones may share same edits)

Corre and Galy. Mol Ther Methods Clin Dev (2023) 29:418.

Edit distribution reflects clonal composition of hematopoietic graft

CRISPR–Cas9-mediated gene editing of the *BCL11A* enhancer for pediatric β^0/β^0 transfusion-dependent β-thalassemia

- 2 patients with TDT treated with Cas9:sgRNA-#1617 ex vivo editing
- Edit distribution tracked in cell products and serial patient samples

Fu *et al.* Nat Med (2022) 28:1573.

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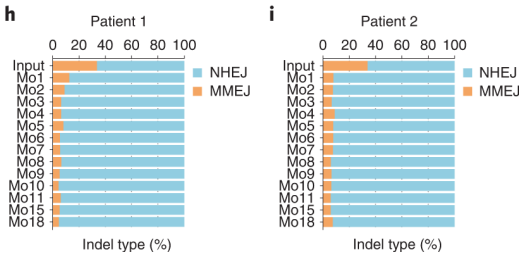
Reduction of MMEJ alleles in engrafting cells



- Edit distribution may differ substantially between cell products and engrafting cells

Fu *et al.* Nat Med (2022) 28:1573.

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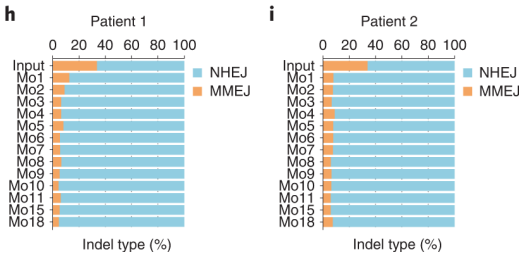
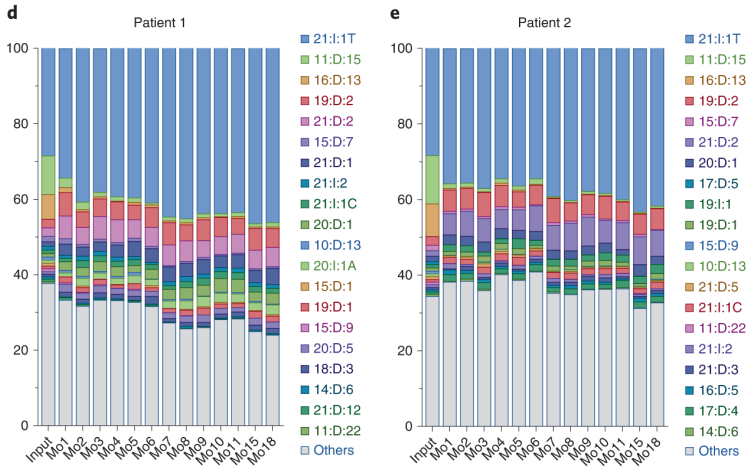


Stable edit distribution suggests polyclonal hematopoiesis



- Edit distribution may differ substantially between cell products and engrafting cells
- Tracking edits enables monitoring clonal dynamics

Fu *et al.* Nat Med (2022) 28:1573.

Conclusions

- Therapeutic genome editing can produce genetic modifications both away from and at the genomic target site (off-target and on-target edits).
- 1. Off-target edits may be influenced by human genetic diversity.
  - For the #1617 gRNA targeting the *BCL11A* +58 enhancer, there is a likely off-target site due to the rs114518452 variant, with ~5% minor allele frequency in African ancestry populations, including a risk of a rearrangement (pericentric inversion) between on-target and off-target site.
  - A risk assessment could include uncertainty about the biological relevance of indels or rearrangements at the off-target site.
  - Patients could be screened and/or patient samples could be monitored.

Conclusions

- Therapeutic genome editing can produce genetic modifications both away from and at the genomic target site (off-target and on-target edits).
- 2. On-target edits may include short indels and structural variants.
  - Short amplicon PCR with short read sequencing will miss structural variants.
  - Assays exist to characterize and quantify structural variants, although more than one assay may be needed for comprehensive measurement of these on-target edits.
  - A risk assessment could include uncertainty about the biological relevance of structural variants.

Conclusions

- Therapeutic genome editing can produce genetic modifications both away from and at the genomic target site (off-target and on-target edits).
- 3. Edit distribution reflects clonal composition of hematopoietic graft.
  - The distribution of edits in the cell product may not mimic the distribution of edits in engrafting cells over time, which could impact safety and/or efficacy.
  - Gene edits that do not impact cell fitness (i.e. *passengers*) nonetheless mark engrafting stem cells and their progeny (clones) so offer opportunity to track clonal dynamics.
  - Gene edits that do impact cell fitness, if any exist, (i.e. *drivers*) would be expected to cause clonal loss or expansion which might be detected by tracking edit distribution.
  - Tracking gene edit distribution over time is akin to vector integration site analysis in integrating vector gene therapy studies.