Regulatory Approach for Gene Therapies Incorporating Human Somatic Genome Editing

Brian Stultz
Chief
Gene Therapy Branch 3
Division of Gene Therapy 1
Office of Gene Therapy CMC
OTP | CBER | FDA

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Learning Objectives

- Understand the basic genome editing technologies

- Describe the important CMC considerations for human gene therapies that incorporate genome editing

What is Genome Editing?

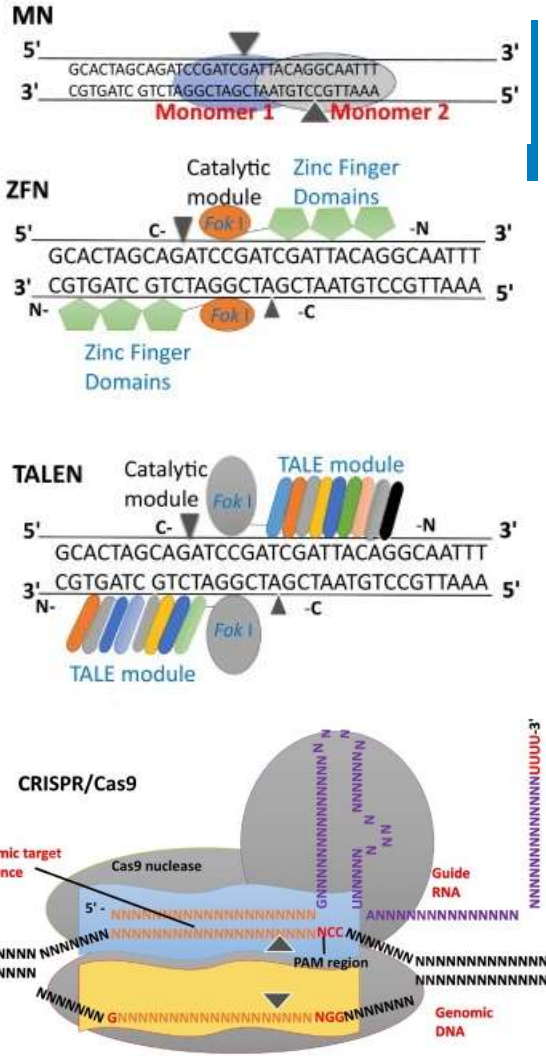
- Process by which DNA is inserted, deleted, or replaced in a site-specific manner in the human genome using nucleases or other methods.

- Nucleases create a site-specific strand break(s), which are repaired by cellular mechanisms.
  - nonhomologous end-joining (NHEJ)
  - homology directed repair (HDR)

Genome Editing Technologies

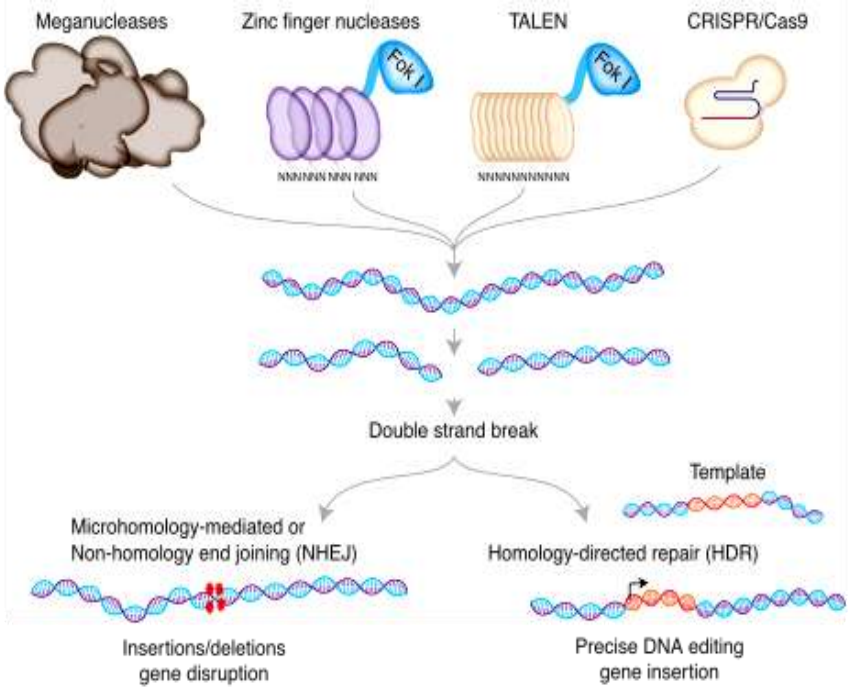
Site Specific Nucleases

- Meganucleases
  - Engineered versions of naturally occurring homing endonucleases
- Zinc Finger Nucleases (ZFNs)
  - Fusion of the DNA cleavage domain from the FokI restriction endonuclease with zinc-finger proteins
- Transcription Activator-Like Effector Nucleases (TALENs)
  - Fusion of the FokI DNA cleavage domain with DNA-binding domains derived from TALE proteins
- Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas systems)
  - Ribonucleoprotein complex composed of a Cas protein and a guide RNA (gRNA)



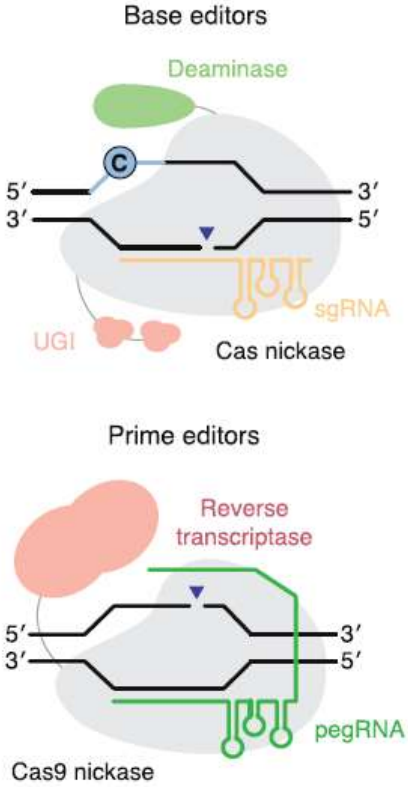
Nuclease-based Editing Mechanism of Action

- Uses inherit DNA repair machinery
- Non-homologous end joining (NHEJ)
- Homology directed repair (HDR)

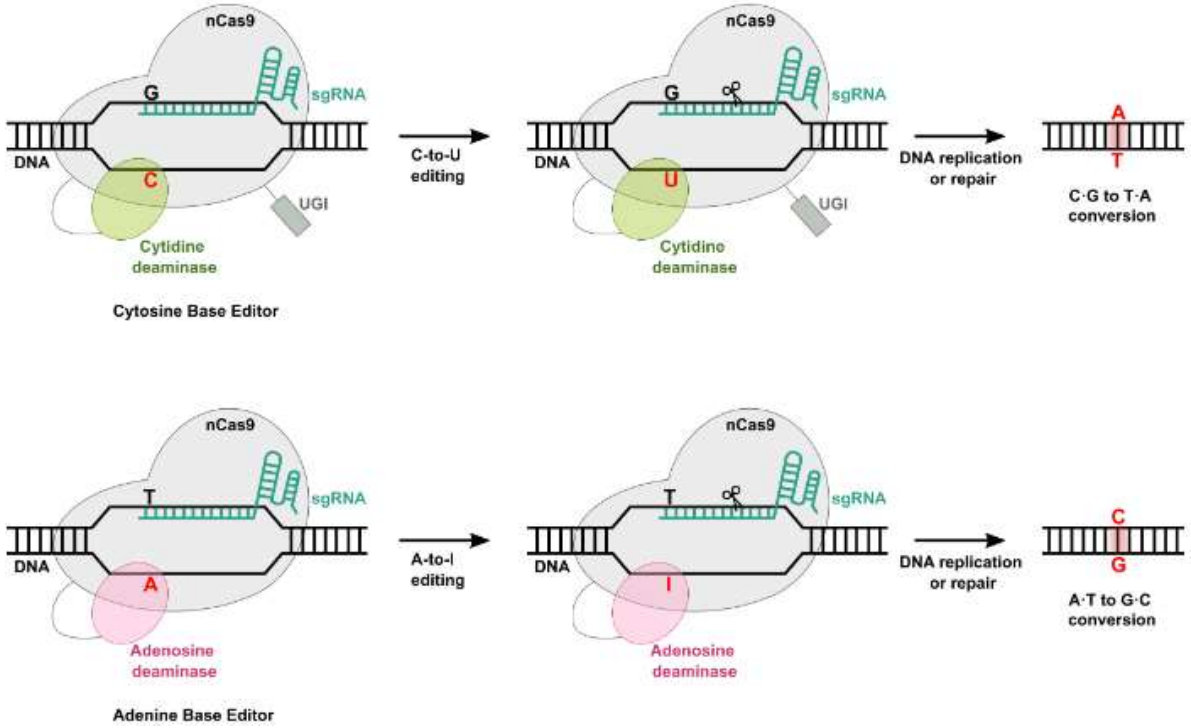


Variations on Nuclease Based Editing

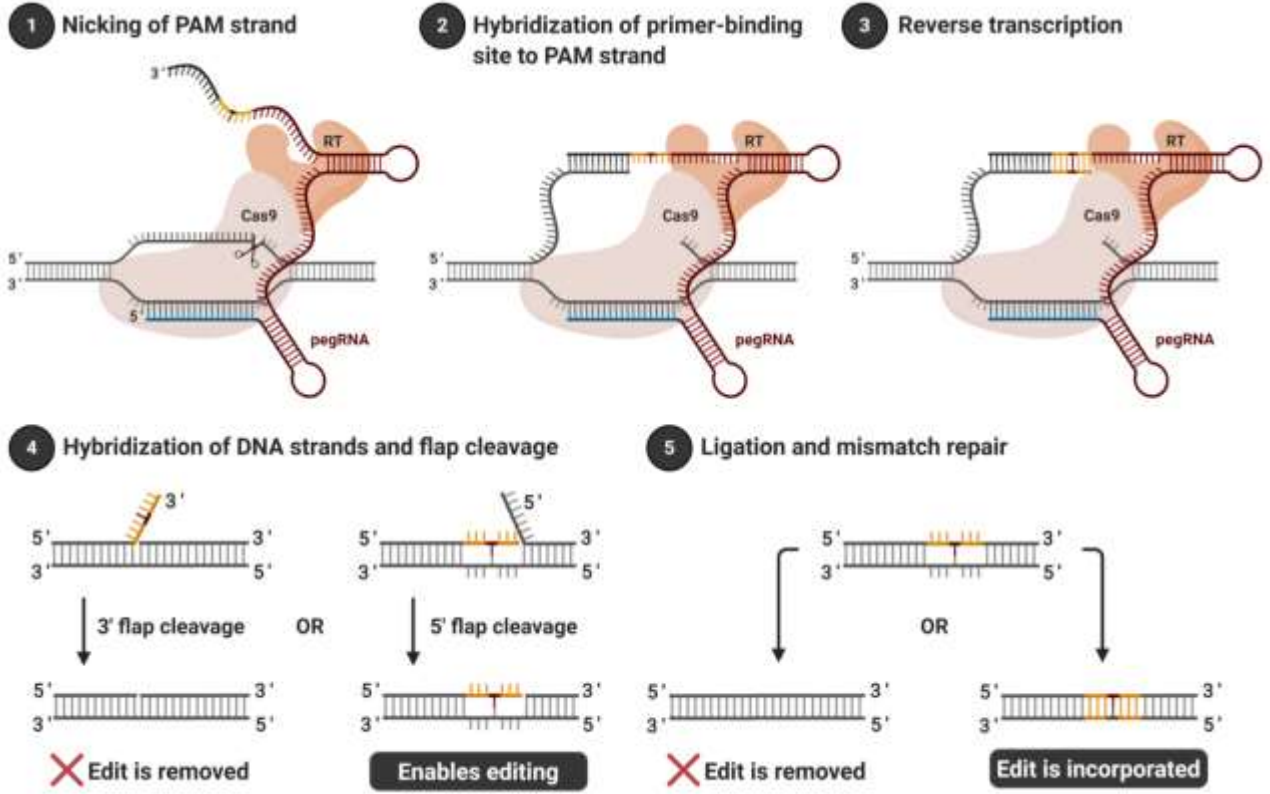
- Base Editors
  - Cas9 nickase fused to a deaminase
- Prime Editors
  - Cas9 nickase fused to a reverse transcriptase
  - gRNA containing a primer DNA template



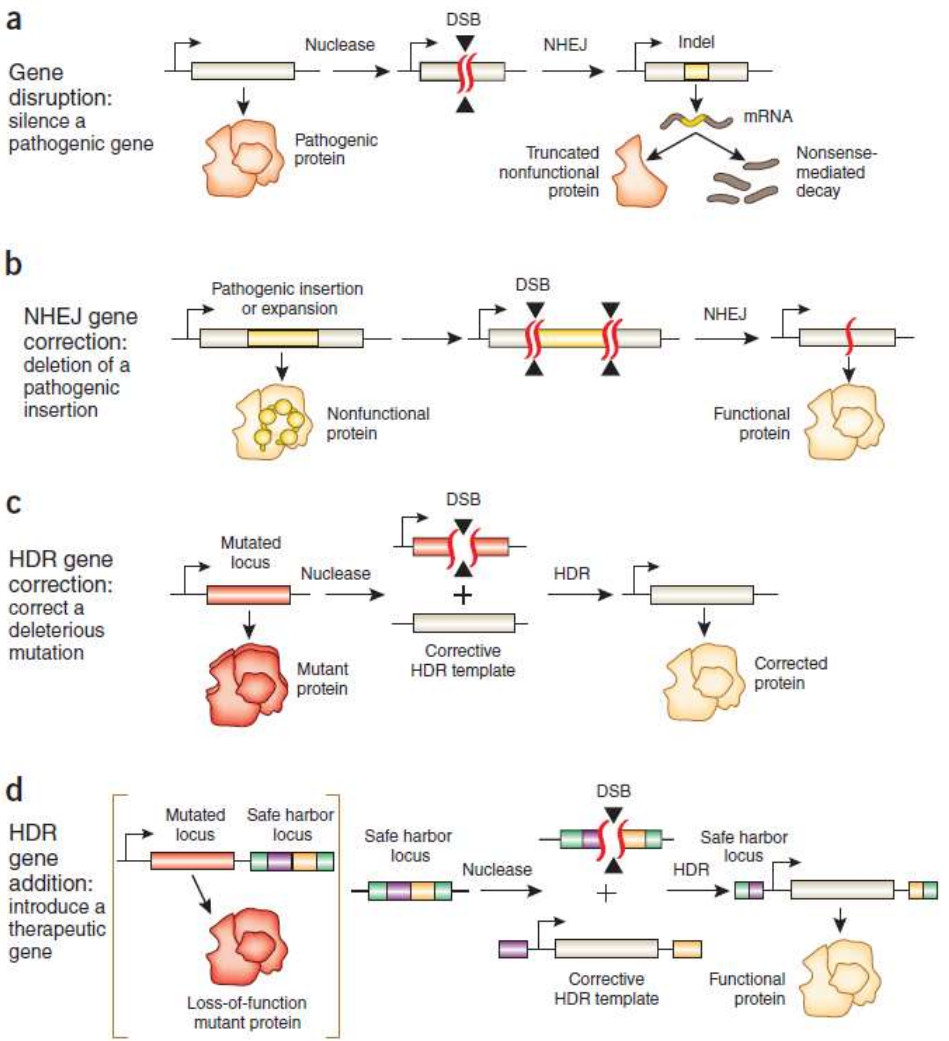
Base Editor Mechanism of Action



Prime Editor Mechanism of Action



Examples of Therapeutic Genome Modification



www.fda.gov

Potential Therapeutic Applications of Genome Editing

- Hematologic disorders
  - SCID, SCD, hemophilia, β-thalassemia
- Neuromuscular disorders
  - Muscular dystrophy, SMA, ALS, Huntington's
- Ocular diseases
  - Leber Congenital Amaurosis type 2, retinitis pigmentosa
- Skin disease
  - Dystrophic epidermolysis bullosa
- Lysosomal storage disorders
  - Fabry, Pompe, MPS
- Viral infections
  - HIV, HBV, HPV
- Cancer

Challenge Question #1

Which technology uses a nickase?

A. Meganucleases

B. Prime Editors

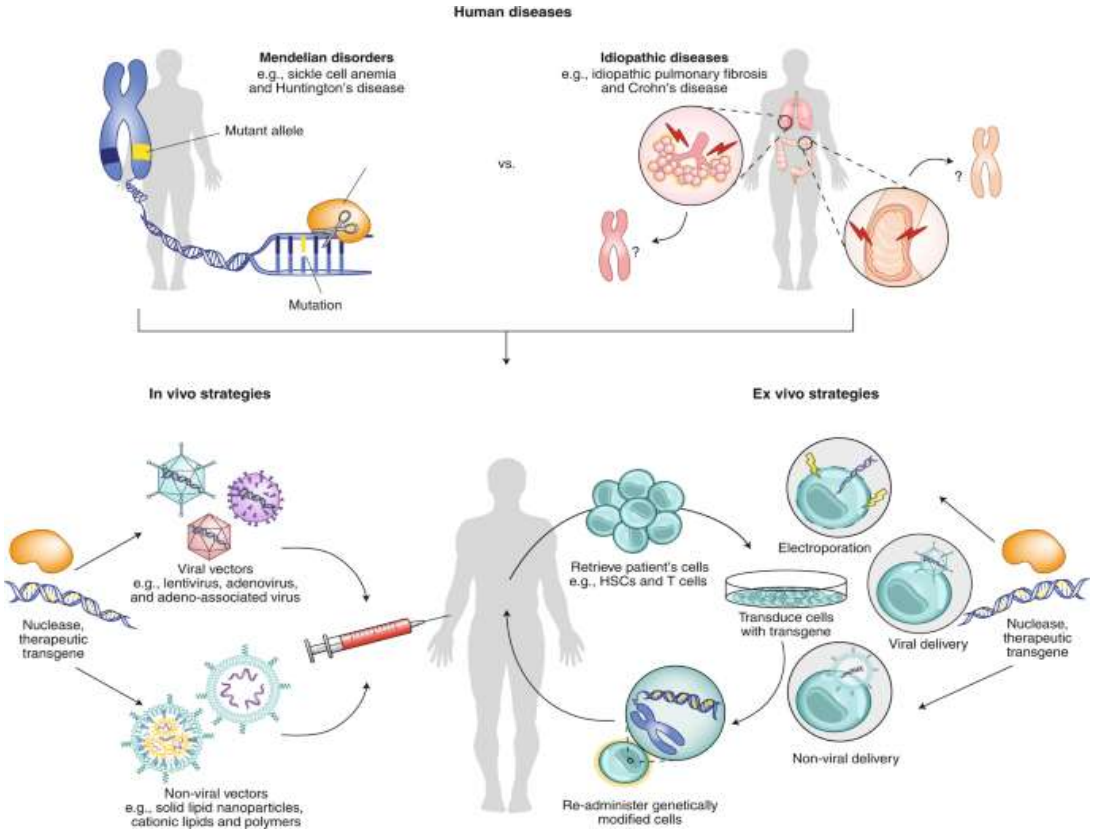
C. TALENs

D. CRISPR/Cas9

Gene Therapy Products Incorporating Genome Editing (GE Products)

GE Products

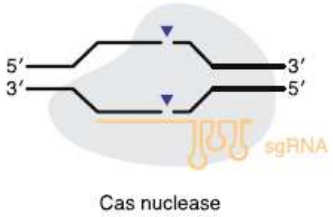
GE products include both directly administered (in vivo) and ex vivo modified cell products.

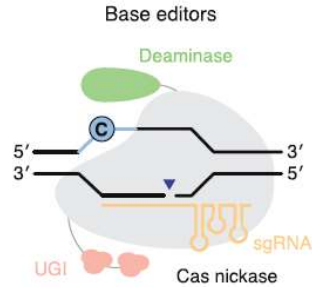


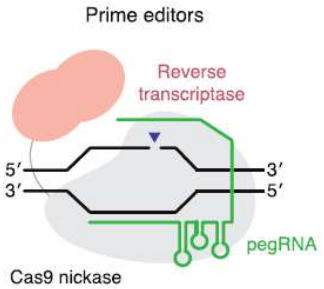
van Haasteren, J., Li,. et al., Nature Biotechnolgy, 2020

Considerations for Developing GE Products

- Type & degree of modification needed
- Mechanism of DNA sequence change
- Product design and delivery method
  - In vivo versus ex vivo
  - Viral vectors, nanoparticles, plasmid DNA, mRNA, protein (RNP)
- Optimization of genome editing component expression
- On- and off-target editing studies
  - What assays and models are available/appropriate?
  - What will you monitor – sequence, expression, function?
- Clinical trial design, patient monitoring, long-term follow-up







Anzelone, Koblan, and Liu; Nature Biotechnolgy, 2020

Regulation of GE Products

Science-based approach

- Characterization of the product mechanism of action and safety attributes, using current knowledge and tools in the field

Benefit-risk analyses

- Potential to correct genetic causes of disease
- Risk of unintended genome modification
- Unknown long-term effects of on- or off-target genome editing

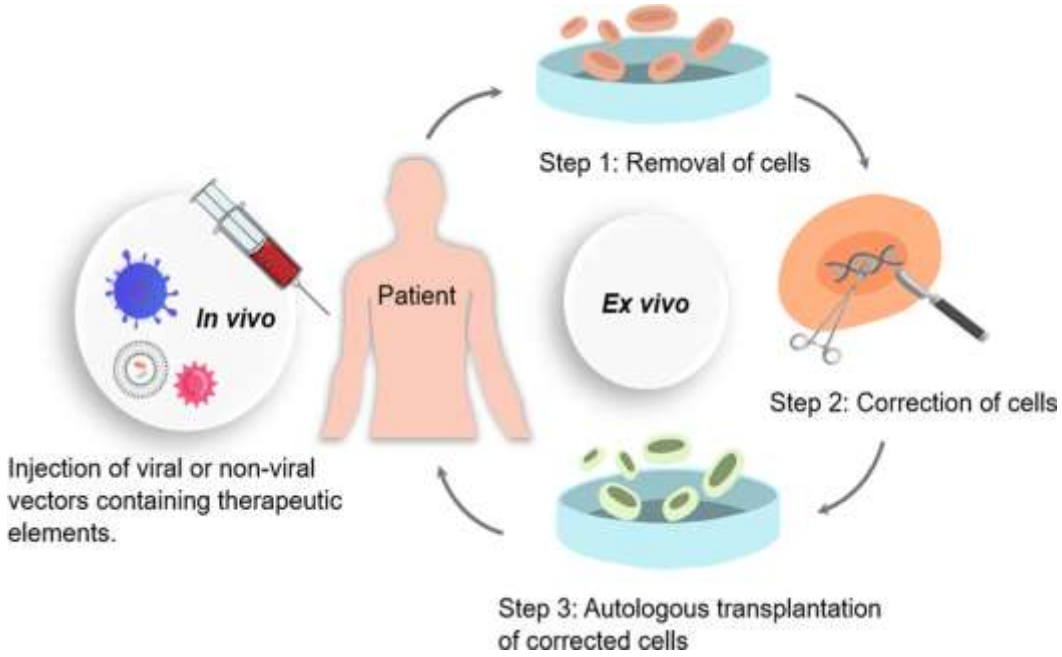
Ensuring GE Product Quality

- Suitable qualification of starting materials & components
- A well-defined process and process controls
- Appropriate product testing & characterization

GE Components

Genome editing components (e.g., nuclease, targeting elements, donor template) are considered:

- Drug substances when they are formulated into the final drug product (API) that is directly administered to perform genome editing in vivo
- Critical components when they are used to perform genome editing in cells ex vivo and the autologous/allogeneic cells are the drug product



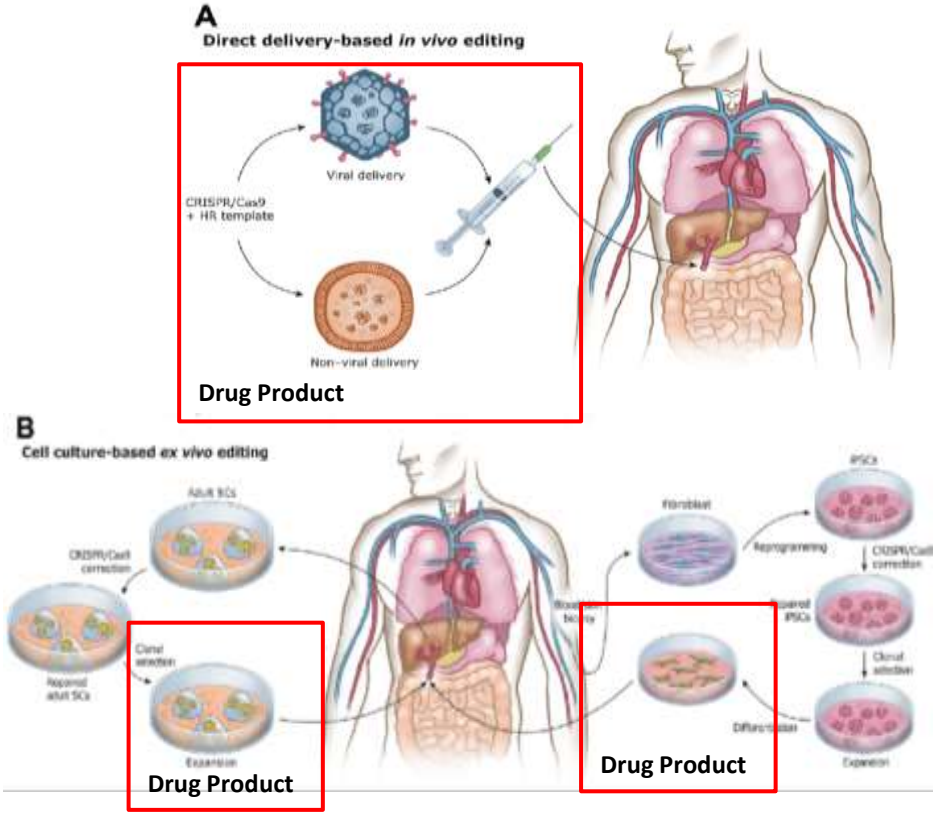
GE Component CMC Considerations

- Detailed descriptions of how the components are designed, manufactured, and tested should be included in an IND
- In most cases, components should be manufactured according to CGMPs
  - Phase 1: FDA Guidance for Industry: CGMP for Phase 1 Investigational Drugs
  - Full CGMPs are expected for BLA supporting clinical trials and licensure
- GE components should be tested for safety, identity, purity, activity, and residuals based on their manufacturing process
  - Specifications should be determined based on manufacturing experience and what has been shown to be safe and effective in preclinical/clinical studies
- Stability of GE components should be assessed

GE Product Testing

- Test drug product for safety, identity, purity, and potency
  - Set specifications based on manufacturing experience and what has been shown to be safe and effective in preclinical/clinical studies

- For ex vivo modified cell products
  - Characterize the presence of residual genome editing components
  - The need to test each batch for off-target modifications, translocations, etc. will be considered on a case-by-case basis
  - Allogenic cell products need thorough characterization to ensure safety



Savić, Nataša & Schwank, Gerald, Translational Research, 2015

Human Genome Editing Safety Concerns

- Off-target genome editing
  - Type and sensitivity of off-target screening methods
  - In vivo: off-target cells/tissues
- Unintended biological consequences of on-target editing
  - Mutagenesis from imprecise DNA repair following on-target editing
- Adverse effects of genomic DNA cleavage at on- and off-target sites
  - Chromosomal translocations, inversions, etc.
- Immunogenicity
  - To GE components, editing outcome, or the delivery system

Assessing the Potential for Unintended Genome Modifications

- Select appropriate methods for predicting and identifying unintended genomic modifications
  - Use orthogonal methods that best suit your product
    - Some modalities may require specific analysis methods: Base editors or epigenome editors
  - Provide analysis details including experimental parameters and sequencing depth and quality
  - Provide an annotated list of unintended modifications observed

- Account for genomic variation between individual human subjects
  - Use appropriate:
    - Databases based on intended patient population
    - Variant frequency cut-offs based on a risk assessment
    - Methods for confirmatory testing, if needed

- Determine the biological impact of identified unintended genomic modifications to the extent possible

Methods for Identifying Off-Target Modifications

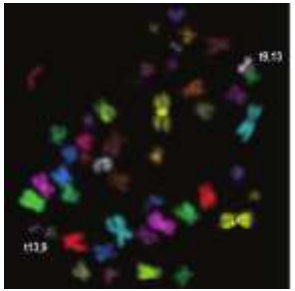
Method	Description	Examples	Concern
In silico	Identifies areas of homology to targeting sequence	COSMID Cas-Off-Finder CHOP-CHOP	Platforms are based on a reference genome and different algorithms that may yield different results
Cellular	Sequencing of tagged, edited sequences	Guide-seq BLESS/BLISS IDLV Capture	Off-target editing events may be cell type specific
Biochemical	Sequencing of edited, fragmented DNA	Circle-seq DiGenome-seq SITE-seq	May give rise to more false positive hits
Whole Genome sequencing	Next generation sequencing	Illumina PacBio	Helpful for clonal populations but has difficulty identifying sites that are cleaved at low frequencies in non-clonal cell populations

Methods for Identifying Chromosomal Modifications

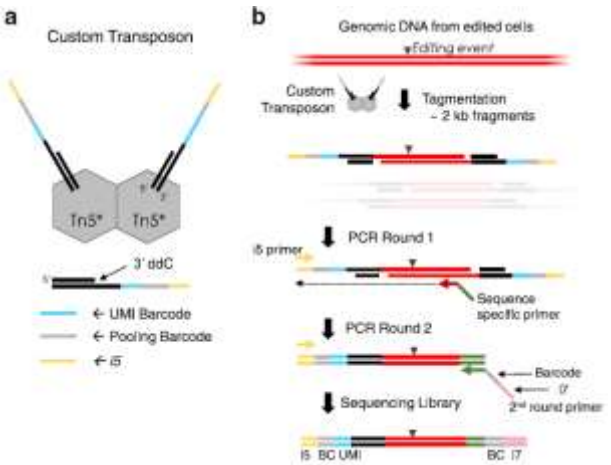
- In silico modeling
- Cellular approaches
  - Imaging-based genome analysis

    (e.g., BioNano, FISH, karyotyping)
- Biochemical approaches
  - Unidirectional sequencing

    (e.g., HTGTS, AMP-seq, UDiTaS)



Zeegers, D., et al.; Genome Integrity, 2017



Giannoukos, G. et al.; BMC Genomics, 2018

Summary

- GE products include directly administered (in vivo) and ex vivo modified cell products
- GE components can be considered drug substances or critical components
  - Tested for safety, identity, purity, activity, residuals and stability
  - Manufactured according to phase appropriate CGMPs
- GE products should be thoroughly tested depending on the product type

Challenge Question #2

Which of the following statements is NOT true?

A. GE components can be considered drug substances or critical components.

B. Detailed descriptions of how the GE components are designed, manufactured, and tested should be included in an IND.

C. In most cases, GE components do not need to be manufactured according to CGMPs.

D. If GE components are modified during the product life cycle, comparability studies may be necessary.

Additional Guidance

- **Human Gene Therapy Products Incorporating Human Genome Editing**
  - Provides recommendations for information that should be provided in an IND application to assess the safety and quality of the investigational GE product**.**
  - Human Gene Therapy Products Incorporating Human Genome Editing

- **FDA CBER Webinar**
  - FDA CBER Webinar: Human Gene Therapy Products Incorporating Human Genome Editing

Early Communication with CBER/OTP

- INTERACT meetings
  - INTERACT - INitial Targeted Engagement for Regulatory Advice on CBER producTs
  - Non-binding, informal discussions between nonclinical review disciplines (P/T & CMC) and the sponsor
  - Initial targeted discussion of specific issues after obtaining preliminary data from pilot studies but prior to conducting extensive animal studies
  - https://www.fda.gov/BiologicsBloodVaccines/ResourcesforYou/Industry/ucm611501.htm
- Pre-IND meetings
  - Non-binding, but formal meeting between FDA and sponsor
  - Meeting package should include summary data and sound scientific principles to support use of a specific product in a specific patient population
  - Draft Guidance for Industry: Formal Meetings Between the FDA and Sponsors or Applicants of PDUFA Products (December 2017) https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM590547.pdf
- CATT meetings
  - Conducted with the CBER Advanced Technologies Team
  - Early discussions involving novel manufacturing platforms or analytical methods
  - https://www.fda.gov/vaccines-blood-biologics/industry-biologics/cber-advanced-technologies-team-catt

CBER Contact Information

- **Brian Stultz**
  Chief, Gene Therapy Branch 3
  Division of Gene Therapy 1
  OGT/OTP/CBER/FDA
  WO Bldg. 71 Room 6006
  10903 New Hampshire Ave.
  Silver Spring, MD 20993
  Tel: 240-402-7405
  brian.stultz@fda.hhs.gov

- **Regulatory Questions:**
  OTP Main Line – 240 402 8190
  Email: OTPRPMS@fda.hhs.gov

- **References for the CBER/OTP regulatory process and interactions with CBER/OTP**
  http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/OtherRecommendationsforManufacturers/ucm094338.htm
  https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/interactions-office-tissues-and-advanced-therapies

- **OTP Learn Webinar Series:** http://www.fda.gov/BiologicsBloodVaccines/NewsEvents/ucm232821.htm