U.S. FOOD & DRUG ADMINISTRATION

Off-Target Analysis of Genome Editing Products

CASSS Cell & Gene Therapy Products Symposium 2024

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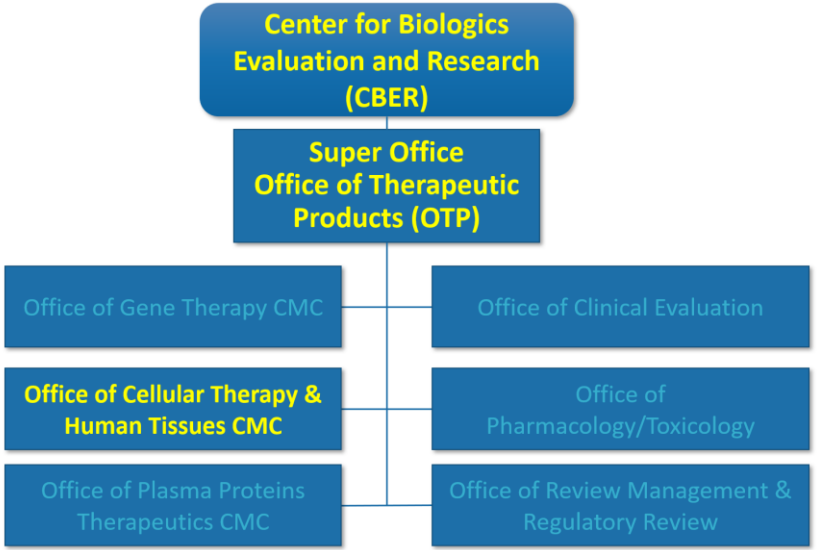
Yongwook Choi, Ph.D.
Bioinformatics Reviewer
Office of Cellular Therapy & Human Tissue
Office of Therapeutic Products
CBER, FDA

Overview

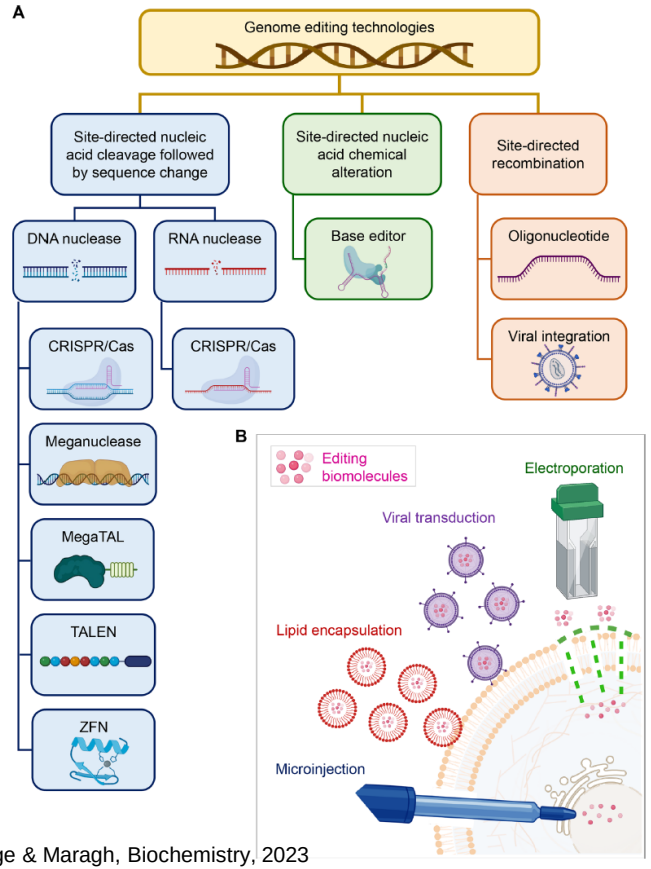
- Bioinformatics review team in CBER/OTP
- Human genome editing products and submissions
- Guidance on off-target editing analysis
- Bioinformatics recommendations

Bioinformatics Review Team in CBER/OTP

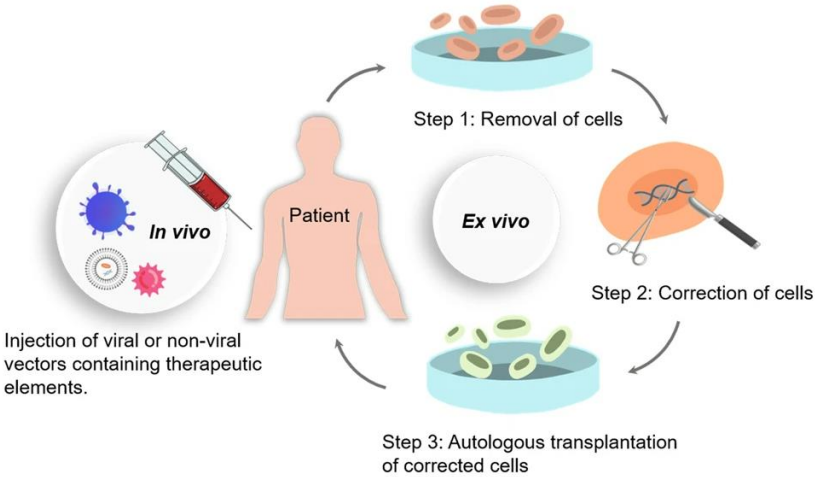
- A team with a diverse skill set
  - Bioinformatics knowledge
  - NGS data analysis
  - Computational skills
  - Domain-specific knowledge in molecular biology, genomics, and epigenomics
- Reviews NGS and bioinformatics information contained in diverse products submitted to OTP
  - Personalized neoantigen cancer vaccines
  - Genome editing products



Genome Editing Products Are Diverse



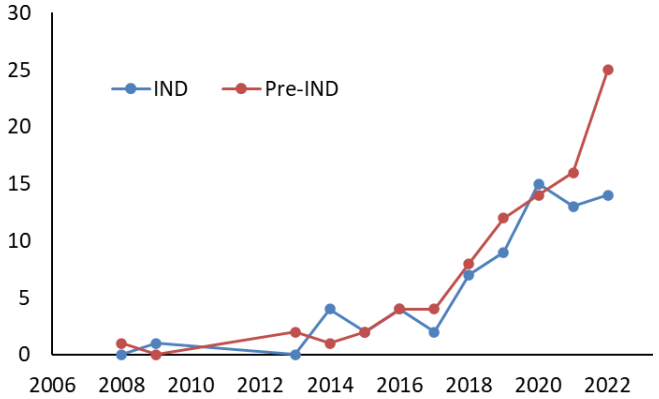
Patange & Maragh, Biochemistry, 2023

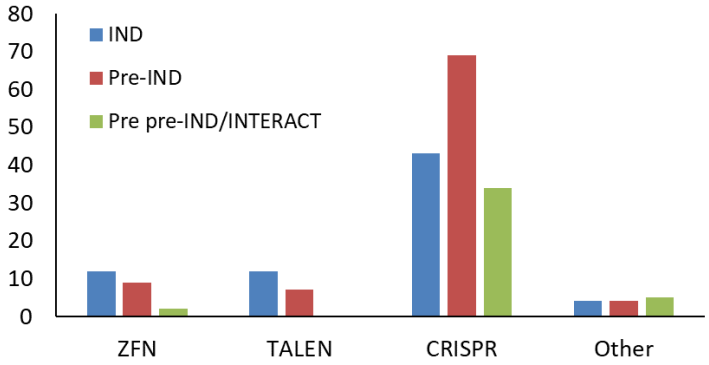


Li, Yang, Hong et al., Sig Transduct Target Ther, 2020

Genome Editing Product Submissions

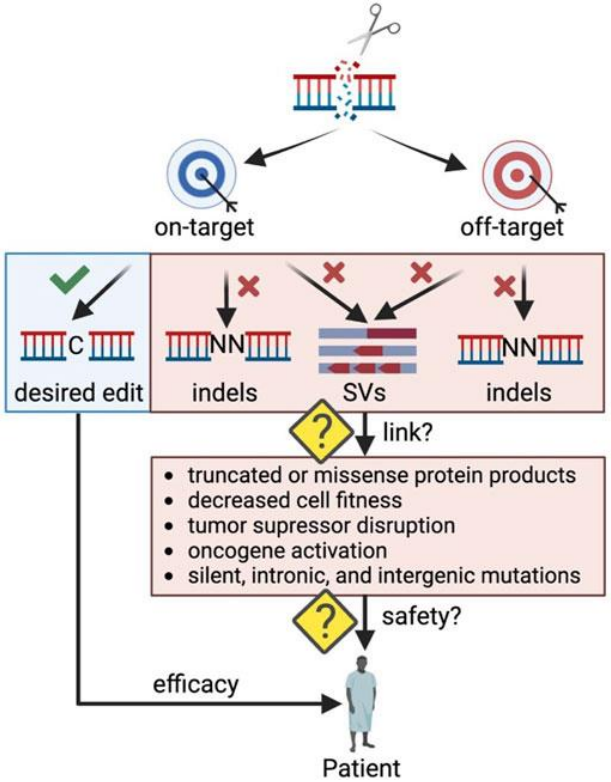
- Increasing trend in the number of submissions
- Number of submissions (as of December 2022)
  - 71 INDs
  - 89 Pre-INDs
  - 41 Pre-pre-INDs/INTERACTs
- INDs
  - 10% in vivo genome editing products
  - 90% ex vivo genome edited cell products





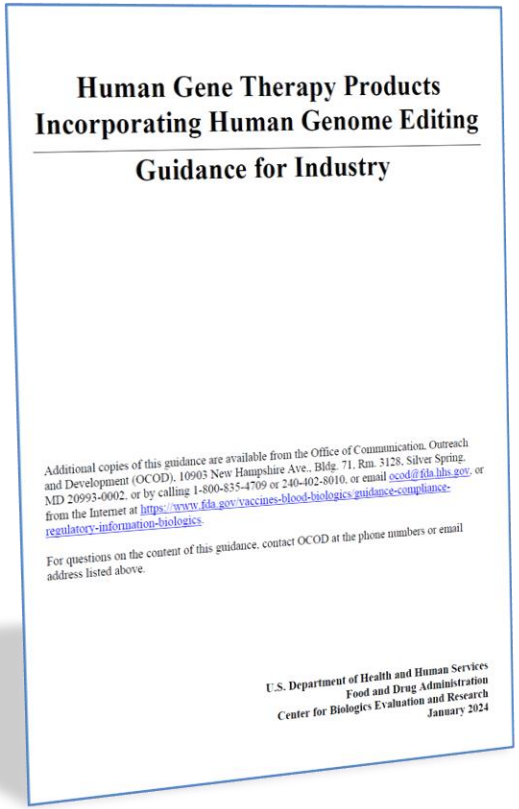
Off-Target Editing

- Off-target editing
  - Unintended genetic modifications in locations other than the targeted site during genome editing process
- Risks associated with off-target editing
  - Disrupting normal gene function
  - Decreasing cell fitness
- A comprehensive study should be conducted to identify off-target editing events



Wienert & Cromer, Frontiers in Genome Editing, 2022

Off-Target Editing Analysis



Human Gene Therapy Products Incorporating Human Genome Editing

Guidance for Industry

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), 10903 New Hampshire Ave., Bldg. 71, Rm. 3128, Silver Spring, MD 20993-0002, or by calling 1-800-835-4709 or 240-402-8010, or email ocod@fda.hhs.gov, or from the Internet at https://www.fda.gov/vaccines-blood-biologics/guidance-compliance-regulatory-information-biologics.

For questions on the content of this guidance, contact OCOD at the phone numbers or email address listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
January 2024

- Identification of potential off-target sites
  - Genome-wide analysis
  - Multiple methods
    - In silico, biochemical, cellular-based assays
  - Relevant human cell type(s) from multiple donors
- Verification of off-target sites
  - Methods with adequate sensitivity
  - Evaluating the final clinical product obtained from multiple donors for ex vivo GE products
  - Including the major cell types in which editing events are detected for in vivo GE products
- Evaluation of the biological consequences associated with off-target editing
- Assessment of genomic integrity

Methods to Identify Potential Off-Target Sites

Method	Description	Advantage	Disadvantage
In silico prediction	• Computationally identify potential off-target sites based on user-provided criteria and assumptions on off-target sites	• Easier to use/implement than in vitro assays • Potential for improvement by incorporating experimental datasets and/or genetic variations	• Biased by the initial assumptions and user-provided criteria
Cell-based assay	• Genome editing in living cells • Identify genomic cleavages by sequencing	• More direct assessment provides higher confidence	• Limited by transfection efficiency and toxicity associated with oligonucleotide tags
Biochemical assay	• Genome editing on the extracted genomic DNA • Identify genomic cleavages by sequencing	• Potential for high sensitivity • Not dependent on transfection/transduction or DNA repair	• May give rise to more false positive hits

Bioinformatics Recommendations

- Experimental parameters
  - How was the concentration of editing components/oligonucleotide tags determined?
- Criteria used to determine potential and confirmed off-target sites (along with justification)
  - Examples:
    - Number of mismatches/bulges between gRNA protospacer and target genome sequences
    - Protospacer adjacent motif (PAM) sequences used
    - Number of supporting reads
    - Threshold for statistical significance comparing unedited and edited groups
- On-target editing characterization
  - Was the intended on-target editing rate achieved?
  - Were there any significant unintended on-target editing events such as large deletions, insertions, and chromosomal translocations?
- Limit of detection

Quality Control of Next-Generation Sequencing Data

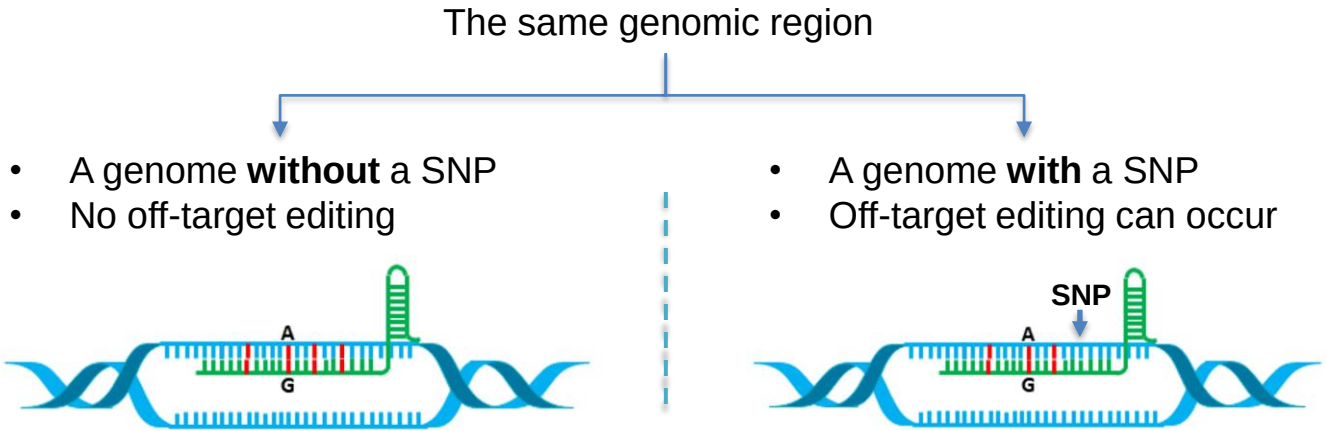
- NGS data quality control (QC) metrics examples
  - Total number of reads, or number of reads per site (raw and deduplicated)
  - Base quality
  - Read duplication rate
- Reporting QC results for individual samples
- NGS raw and processed data may need to be submitted to the Agency when necessary

Describing Bioinformatics Analysis Procedures

- A comprehensive description is needed so that the analysis can be reproduced by the Agency if needed
  - Overall workflow
  - Bioinformatics tools and databases
  - Individual computational steps
  - Statistical testing method
  - Analysis results

Genetic Variation Affects Off-Target Editing

- Human genomes are diverse
  - Individual genomes have about 4 to 5 million variant sites



- How much assurance can we have that no deleterious off-target editing will occur in a patient's cells?

Assessing Impact of Genetic Variations on Off-Target Editing

- Collecting genetic variation information
  - Genetic variation databases
  - Genetic ancestry of the target patient population
  - Variant types
  - Variant frequency thresholds
- In silico prediction of potential off-target sites accounting for genetic variations
- In vitro testing of the potential off-target sites

Summary

- A comprehensive study should be conducted to identify off-target editing events
  - Sensitive, genome-wide analysis to identify and evaluate the type, location, frequency, and biological consequence of off-target editing events
  - Relevant human cell type(s) from multiple donors
  - Genetic diversity should be considered
- The off-target analysis methods should be justified and thoroughly described
  - Comprehensive details of NGS data quality control and bioinformatics analysis procedures

Contact Information

- **Yongwook Choi, Ph.D.**
  OCTHT/OTP/CBER/FDA
  Yongwook.Choi@fda.hhs.gov

- **Regulatory Questions:**
  OTP Main Line – 240 402 8190
  Email: OTATRPMS@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