Development of Gene Therapies from a Product Quality Perspective: Viral Vector-based Products and Products Incorporating Genome Editing

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Diversity of Gene Therapy Products

- **Oncolytic viruses**
- **Therapeutic vaccines**
- **Vectors Expressing Transgenes**
  - Non-viral vectors (e.g., plasmids, RNA)
  - Replication-defective viral vectors (e.g., AAV, herpes, retro/lentivirus)
  - Attenuated bacterial vectors
- **Ex vivo Modified Cells: cell and vector components**
  - Gene modified stem cells (e.g., iPSCs, $CD34^+$ cells)
  - Gene modified immune cells (e.g., CAR T cells, CAR NK cells, transgenic TCR cells)
- **Products Incorporating Genome Editing**

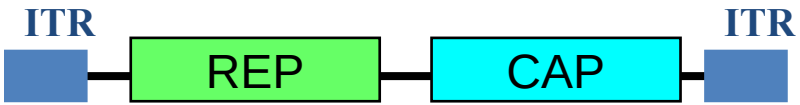
Learning Objectives

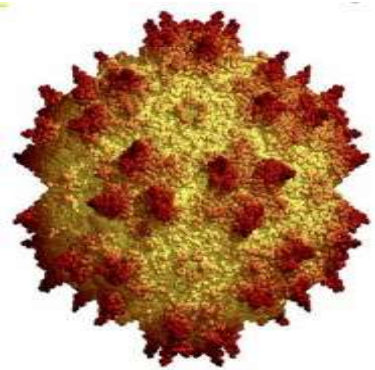
- Describe the important CMC considerations for human gene therapies including:
  - Adeno-associated Virus (AAV) products
  - Products Incorporating Genome Editing

Adeno-associated Virus (AAV)

AAV

- Helper-dependent parvovirus
- Small (18-25 nm) icosahedral capsid; non-enveloped
- Inverted Terminal Repeats (ITRs): only cis elements required for replication and packaging





CMC Considerations for AAV Products

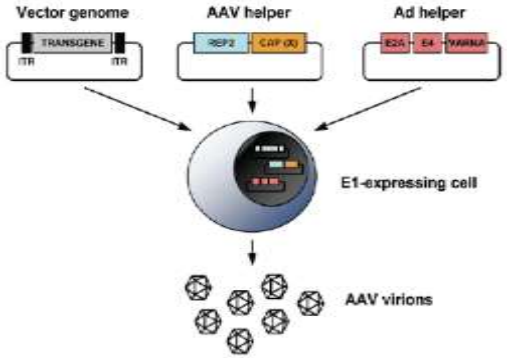
- Vector design
  - Novel serotypes and chimeric/mutant capsids
  - Single-stranded and double-stranded (self-complementary) genomes
  - Dual or split vectors
- Manufacturing Process: production and purification
- Product Testing: safety, identity purity, potency and additional characterization
- Reference materials
- Stability and Comparability

Control of Manufacturing Process

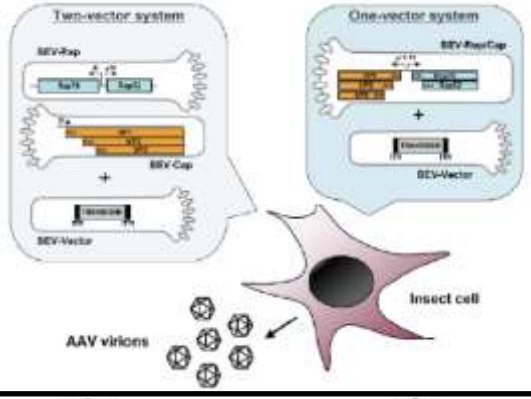
- Type of process and purification scheme
- Component qualification
  - MCB, MVB, reagents
- Quality Assurance/Quality Control Programs
  - Implementation of cGMPs
  - In-process controls
  - Facility controls to prevent cross-contamination

AAV Production Methods

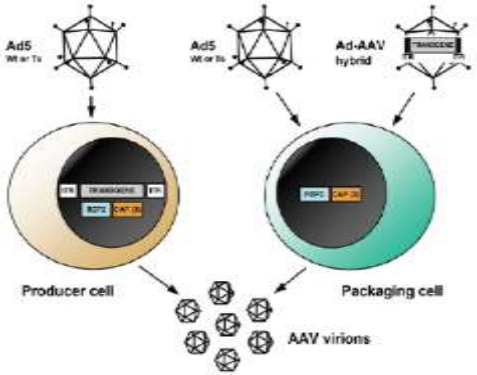
Transient Transfection

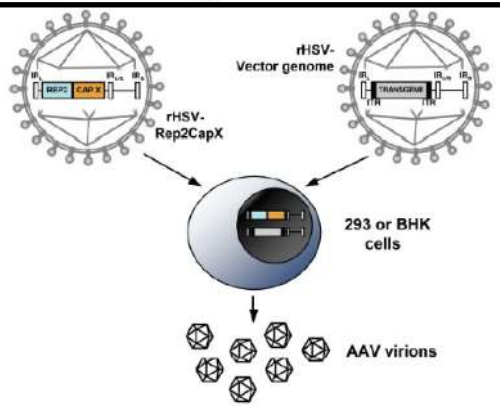


Baculovirus



Helper Virus Based: Adenovirus or HSV



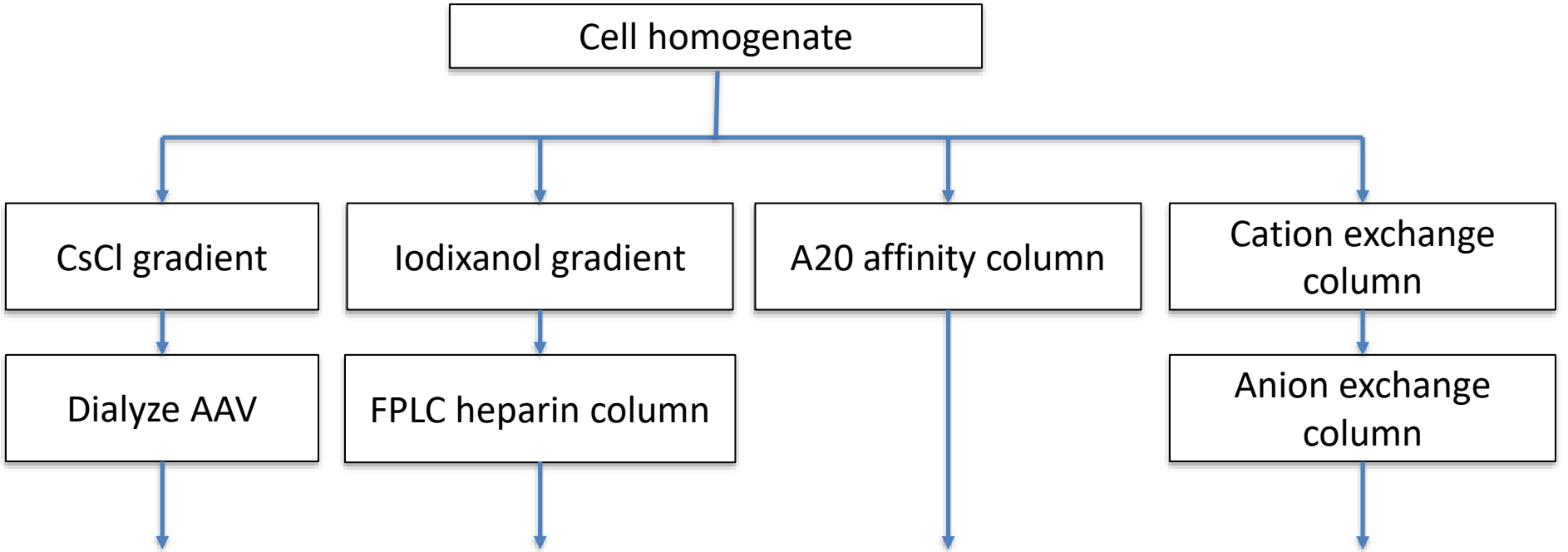


Ayuso, Mingozzi and Bosch, Current Gene Therapy, 2010

Starting material and Reagent Qualification

- Cell lines used to produce AAV should be well qualified and chosen to reduce product risk from packaged non-AAV DNA
- Descriptions of plasmid or virus bank manufacturing and purification procedures, the reagents used, and specifications for use should be provided
  - Specifications should include testing to ensure the identity, purity, potency, and safety of the final product
- Serum (BSE-free source, tested per 9 CFR.113.53, irradiated)
- Trypsin (tested per 9 CFR.113.53, parvovirus free, PCV free)

AAV Purification Methods



Modified from Grieger, et al, Nature Protocols

Testing of AAV Products

- Safety
  - Bulk harvest: mycoplasma, adventitious virus
  - Final vialed product: sterility (bacteriostasis and fungistasis), endotoxin (LAL or equivalent)
- Identity
  - Minimum one-time sequence analysis
  - Genetic integrity (e.g., restriction digest/southern blot/PCR)
  - Transgene specific (e.g., RT-PCR, western blot, ELISA)
  - Capsid specific (e.g., ELISA, protein digestion)

Testing of AAV Products

- Purity
  - Process related impurities: cell substrate protein/DNA, helper virus, plasmid DNA, transfection reagents, affinity ligands
  - Product related impurities: rcAAV, non-infectious particles, empty capsids, aggregates
- Potency
  - Measures intended biological activity (product specific)
  - Quantitative (whenever possible)
  - Matrix approach may be needed (e.g., genome titer, particle titer, infectious titer, transgene expression, transgene function)
  - Surrogate measures may be acceptable with sufficient correlative data

Dose Determination of AAV Products

- Dose is usually based on vector genomes (vg)
  - Multiple methods are available for determining vg concentration (e.g., qPCR, ddPCR, dot-blot, size exclusion chromatography)
- Assay should be well-qualified prior to initiating clinical studies (demonstrate assay linearity, accuracy, precision, range, sensitivity and specificity)
  - Accuracy: spike recovery acceptance criterion of ~ 75-125% of the expected value is reasonable and achievable for early phase studies
  - Precision: acceptance criterion of ≤ 15% CV is reasonable and achievable for early phase studies
  - Specificity: testing should utilize an unrelated AAV vector/plasmid not only buffer alone

Dose Determination of AAV Products

Additional assay recommendations:

- Understand sources of variability
- Use product specific primers/probes when possible (PCR-based methods)
- Use appropriate controls: similar composition and formulation as the clinical sample (e.g., linear versus dsDNA for PCR-based methods)
- Establish suitable reference material
- Use the same assay to quantitate vector used in preclinical and clinical studies
- Qualification should utilize vector under study, not only plasmid standards or a platform vector

AAV Reference Materials

- Similar composition and formulation as the clinical sample
- Thoroughly characterized using appropriately qualified/validated methods
  - Titer assessed by orthogonal methods
- Stability assessed
- Plan for bridging between lots established
- AAV2 and AAV8 reference standards available
  - Can be used to develop and qualify in-house reference material that is specific to your product

AAV Stability Testing

- Stability results determine storage conditions, expiration date, shipping conditions and product handling procedures
- Stability testing should include assessment of vector titer, purity, potency and sterility at appropriate timepoints
  - Stability during any manufacturing hold steps should also be assessed
  - Results should be compared to that of the Reference Standard
- Delivery device compatibility
  - Compatibility with the final product: product absorption to device, product activity after exposure to device
  - Studies should mimic clinical delivery as closely as possible

Comparability

- Multiple changes can occur during clinical development (e.g., reagents, starting materials, manufacturing/purification procedures, scale, facility, analytical procedures, etc.)
- A comparability study should include:
  - A risk assessment of the extent and nature of the change
  - Prospective assessment of product attributes and critical process parameters that have a potential to be impacted by the change using appropriate methods
  - Split manufacturing and side-by side testing of multiple pre- and post-change lots should be performed when appropriate and possible
  - Predefined acceptance criteria for comparability for each attribute being evaluated using appropriate, robust statistical methods

Summary

- AAV manufacturing process and purification schemes may dictate the starting materials, reagents and product testing necessary
  - Choose AAV starting materials and reagents carefully to reduce product risks
- AAV product quality testing includes assessment of safety, identity, purity, potency, stability, and delivery device compatibility
  - Potency assessment should be tailored to the individual product
  - Proper qualification of the AAV dose determining assay is necessary prior to initiating clinical studies
- All reference materials should be appropriate and well qualified
- Well planned comparability studies should be conducted when needed

Challenge Question #1

The AAV dose determining assay:

A. Is usually based on capsid particles

B. Should be well qualified prior to initiating clinical studies

C. Should be qualified using a platform vector, whenever possible

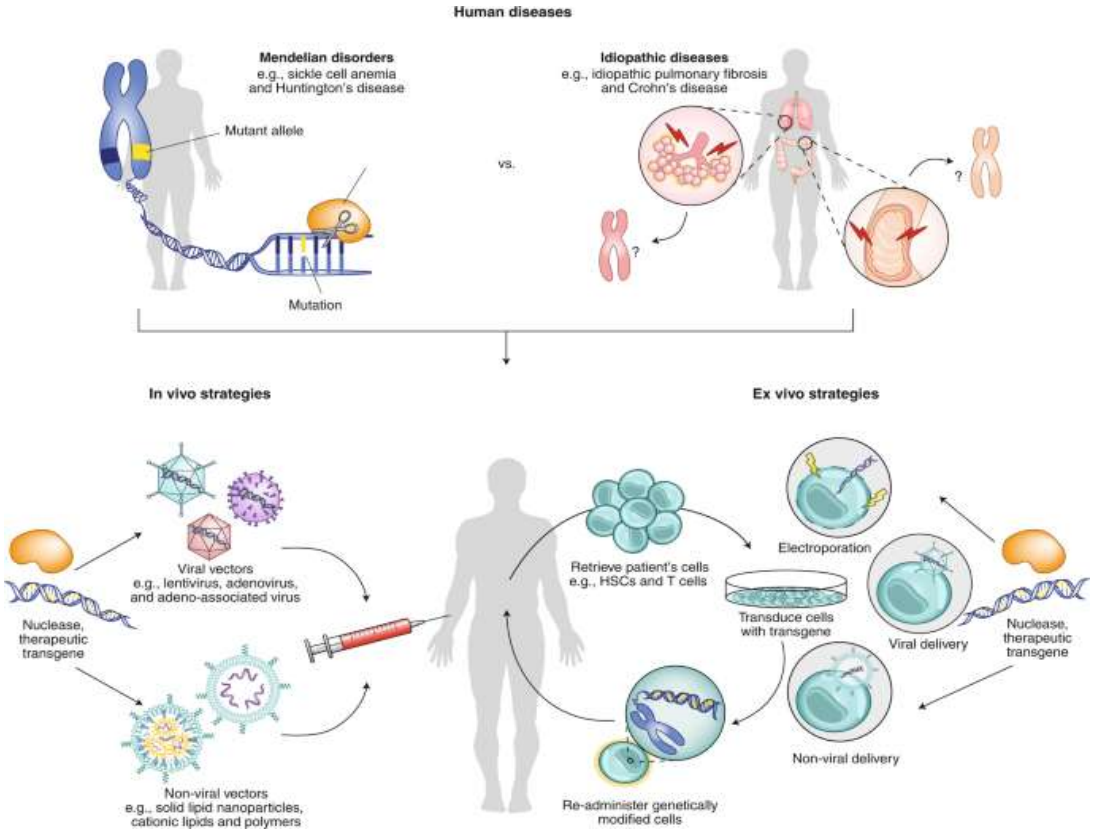
D. Specificity should be determined using buffer alone

Gene Therapy Products Incorporating Genome Editing (GE Products)

GE Products

Human genome editing is a process by which DNA is inserted, deleted, or replaced in the human genome using engineered site-specific nucleases or non-nuclease based methods.

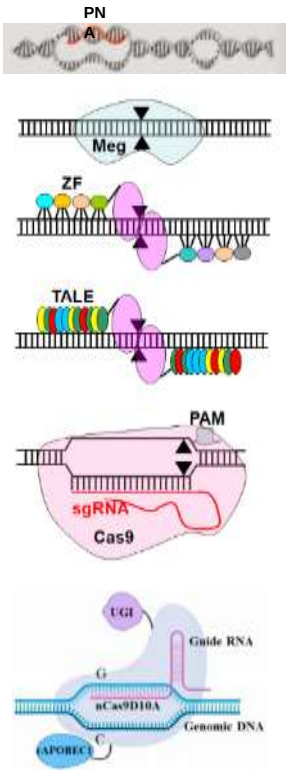
GE products include both directly administered (in vivo) and ex vivo modified cell products.



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

Considerations for GE Products

- Type & extent of modification needed
- Editing platform
- Optimization of targeting elements
- Delivery method
  - Viral vectors, nanoparticles, plasmid DNA, mRNA, protein (RNP)
    - Direct administration
    - Modification of cells ex vivo



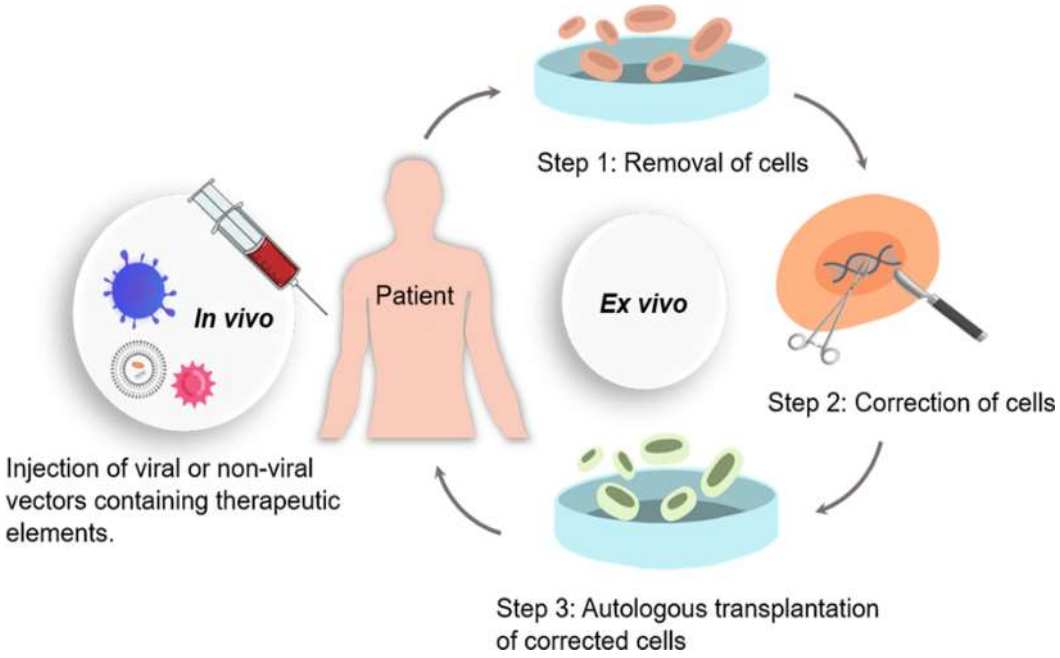
Ensuring GE Product Quality

- 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 nanoparticles to produce the drug product 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

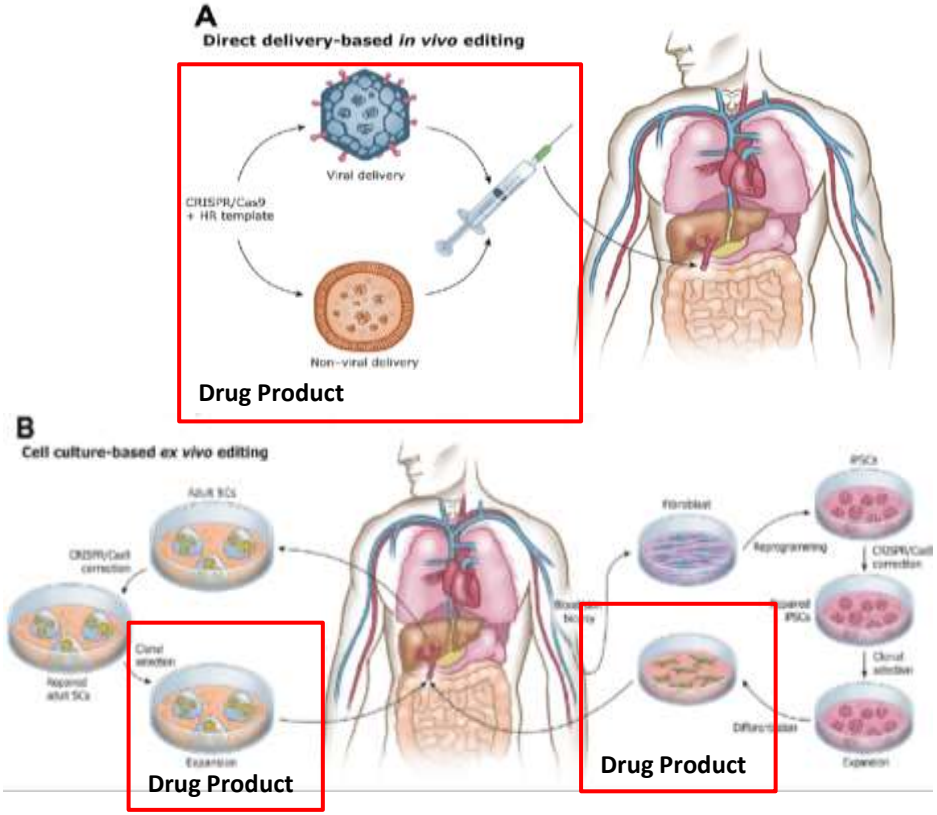


GE Component CMC Considerations

- 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 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 final product for safety, identity, purity, potency, and residuals based on the manufacturing process
  - 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

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 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.

Early Communication with CBER/OTAT

- INTERACT meetings
  - INTERACT - INitial Targeted Engagement for Regulatory Advice on CBER producTs
  - Non-binding, informal scientific discussions between CBER/OTAT 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 (with minutes generated)
  - 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

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

- **CBER website:** www.fda.gov/BiologicsBloodVaccines/default.htm
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