Development of Gene Therapies from a Product Quality Perspective: Gene-modified Cells

Graeme Price, Ph.D.
Research Microbiologist,
Division of Cellular and Gene Therapies, Office of Tissues and Advanced Therapies
CBER | US FDA

REdI Annual Conference – July 23, 2021

Learning Objectives

- Describe key CMC challenges for gene-modified cell products
- Focus on product testing
  - Describe approaches for assay development
  - Emphasize value of characterization studies
  - Outline requirements at key points in lifecycle

Diversity of gene therapy products

- **Oncolytic viruses**
- **Therapeutic vaccines**
- **Vectors expressing transgenes: direct administration to patient**
  - Non-viral vectors (e.g., plasmids, RNA)
  - Replication-defective viral vectors (e.g., AAV, herpes, retro/lentivirus)
  - Attenuated bacterial vectors
- **Gene-modified cells: cell and vector components**
  - Ex vivo gene-modified cells (e.g., cancer vaccines, gene-modified stem cells, chimeric antigen receptor [CAR] T cells)

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2017: First CAR T cell therapies approved

Health

First cancer 'living drug' gets go-ahead

By James Gallagher
Health and science reporter, BBC News website

30 August 2017 | Health -BBC

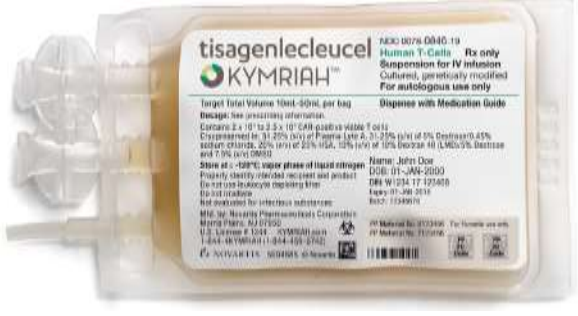
Modified T cells that attack leukemia become first gene therapy approved in the United States

By **Jocelyn Kaiser** | Aug. 30, 2017 , 2:48 PM -Science

- **Kymriah** (Novartis) - 30th August 2017
  - Pediatric relapsed/refractory B cell ALL
- **Yescarta** (Kite Pharma) - 18th October 2017
  - Adult relapsed/refractory DLBCL

2020-21: The second wave

- **Kymriah** (Novartis) - 30th August 2017
- **Yescarta** (Kite Pharma) - 18th October 2017
- **Tecartus** (Kite Pharma) – July 24th 2020
- **Breyanzi** (Juno Therapeutics) – 5th February 2021
- **Abecma** (Celgene) – 26th March 2021

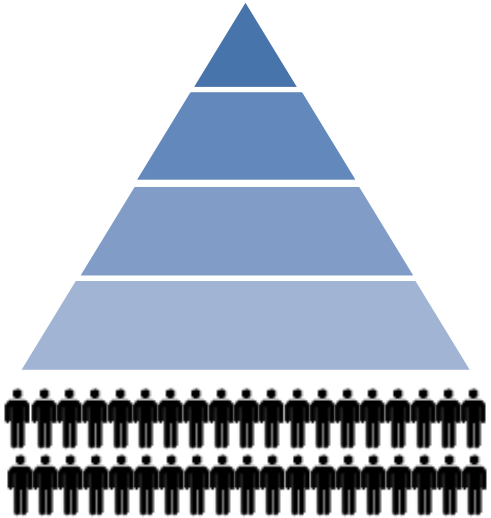


Gene-modified cells:

A different manufacturing and testing paradigm

Conventional Drug/Biologic

1 product lot



Many patients

Autologous GM cells

1 product lot

Raw materials

cGMP Manufacturing

In Process and Lot Release Testing

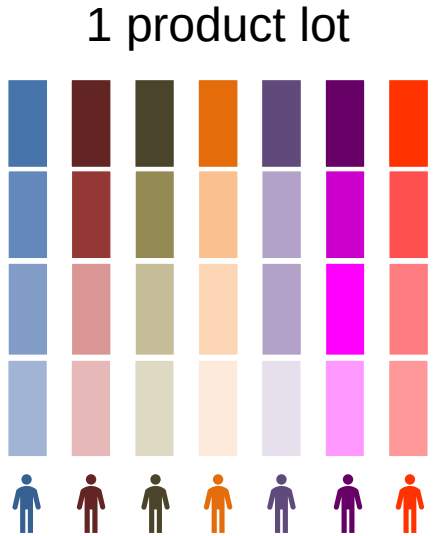
Distribution

One patient

Gene-modified cells:

Patient-specific products = Patient-specific challenges

- Autologous products
- Giving cells from someone else is a safety concern
- Critical to avoid mix-ups during manufacture/shipping
- Procedures must be in place to track and maintain control of product at all times
- **Chain of Identity/Chain of Custody (COI/COC)**

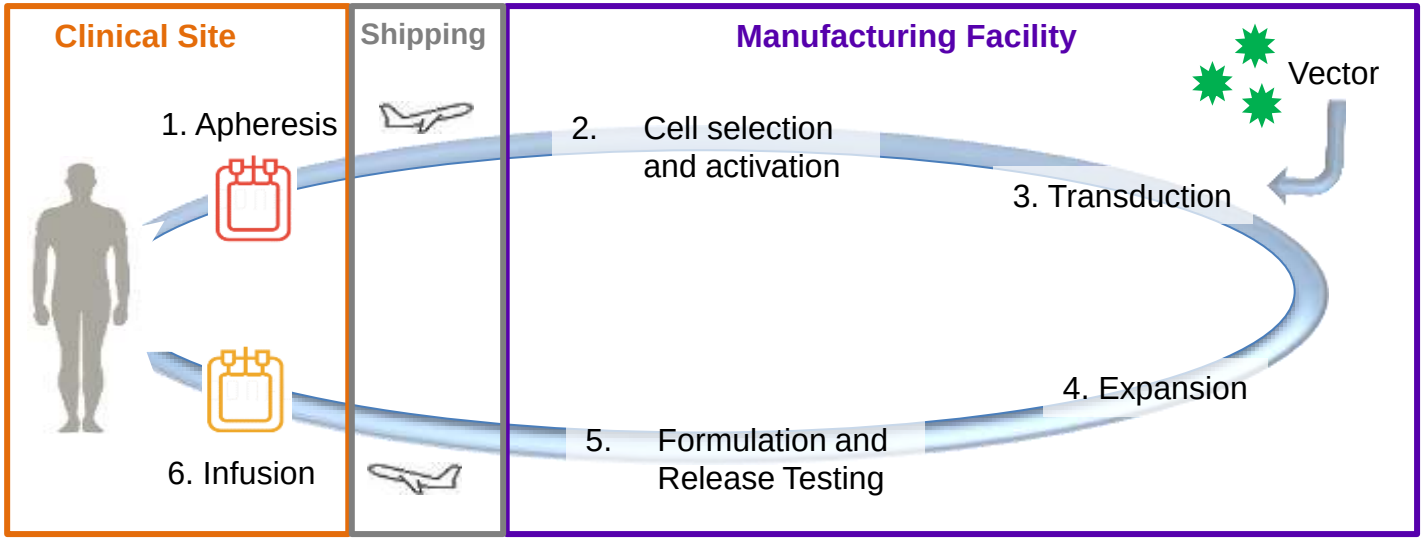


Chain of Identity/Chain of Custody

- Critical for autologous products
- Ensure patient receives correct product
- In place prior to start of Phase I studies
- “Vein-to-vein” tracking (collection to infusion)
- Confirm ≥ 2 unique identifiers by ≥ 2 individuals at set times
- Confirm unique identifiers match patient prior to administration

Complex manufacturing process

- 2 main components:
  - Cells: Autologous (patient-derived) or Allogeneic (healthy donor-derived)
  - Transgene vector: Often retro or lentiviral vectors (also plasmid DNA, mRNA, AAV)
  - Products may also incorporate genome editing



Gene transfer vectors

For vectors used in ex vivo gene-modified cell manufacturing

- **Vector quality impacts gene-modified cell quality**
- Describe vector in a separate Drug Substance (DS) section in eCTD
- Fully test master and working cell banks used in vector production
- Vector test results available prior to gene-modified cell manufacture
- Recommend using constant amount of vector (fixed MOI) for gene-modified cell manufacture
- Establish stability program for vector

Gene transfer vector testing

Parameter	Tests
Safety	• Sterility • Endotoxin • Mycoplasma • Adventitious agents (end-of-production [EOP] cells) • Replication Competent Retrovirus/Lentivirus (RCR) if used (EOP cells and vector supernatant)
Identity	• Presence of transgene sequence (PCR, Southern blot etc.)
Purity	• Process and product-related impurities (residual BSA, antibiotics, etc.)
Dose	• Vector concentration/titer (e.g., transducing units/ml)
Potency	• Cytokine production, tumor cell killing, phenotype, etc.

Cellular starting material

- **Autologous (patient derived) cells:**
  - Patient-related variability (age, disease state, prior treatment)
  - Recommend sterility test before initiating manufacture (or retain sample for post hoc testing)
- **Allogeneic (healthy donor derived) cells:**
  - Donor eligibility determination, testing for relevant communicable disease agents (21 CFR 1271 subpart C)
  - Additional testing for adventitious agents etc. on expanded cell banks
- Establish acceptance criteria for incoming material
- Recommend additional characterization studies

Gene-modified cell manufacture

- Use high quality raw materials
- Monitor process: In-process testing
  - Sample at appropriate times in manufacture
  - Monitor cell proliferation/cell quality in real time
  - Cell counts, viability, phenotype, in process sterility
- Valuable for establishing process validation parameters
- Ensure product quality and consistency

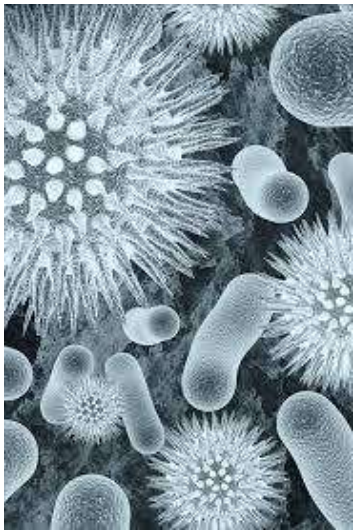
Gene-modified cells: lot release testing

Parameter	Tests
Safety	• Sterility • Endotoxin • Mycoplasma • Rapid RCR (e.g., qPCR for VSV-G) if applicable • Vector copy number (generally <5 copies per transduced cell) for integrating vectors • Additional viral safety testing for allogeneic products
Identity	• Presence of transgene sequence • Cellular phenotype
Purity	• Transduction efficiency • Process-related impurities (residual BSA, antibiotics, activation/selection reagents etc.) • Product-related impurities (cellular contaminants [flow cytometry])
Dose	• Number of viable gene-modified cells (flow cytometry)
Potency	• Biologically relevant function (cytokine production, tumor cell killing, etc.)

Testing challenges

- Complex, variable products
- Mode of action may not be fully known
- Time constraints for release testing
- Limited material available for testing
- Limited availability of reference standards and controls

Safety assays



- Focus on microbiological safety:
  - Sample at point of maximum sensitivity
- Sterility
- Mycoplasma
- Endotoxin
- Replication Competent Retrovirus
  - Co-culture assay for vector and EOP cells: > 4 weeks
  - Alternate methods for transduced cells (qPCR/enzyme based)
- Adventitious viruses
  - Testing required for cell banks (including for vector production)

Identity, purity, and dose assays

- Test final product at appropriate time
  - Issues with rapid manufacture
  - Transgene expression etc. may take time to become stable
  - Can affect dose determination
  - May need to culture aliquot of final product for additional time after harvest before testing

Potency: Regulatory definitions

- 21 CFR 600.3(s): The word *potency* is interpreted to mean the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result.
- 21 CFR 610.10: Tests for potency shall consist of either in vitro or in vivo tests, or both, which have been specifically designed for each product so as to indicate its potency in a manner adequate to satisfy the interpretation of potency given by the definition in §600.3(s) of this chapter.

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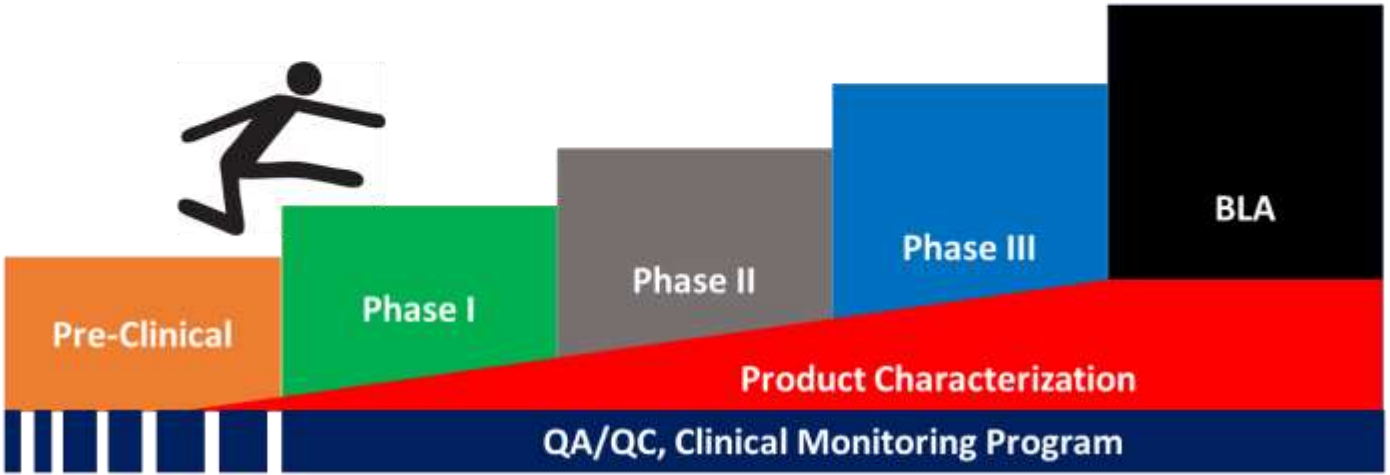
Lifecycle approach to potency measurement (ideal version)

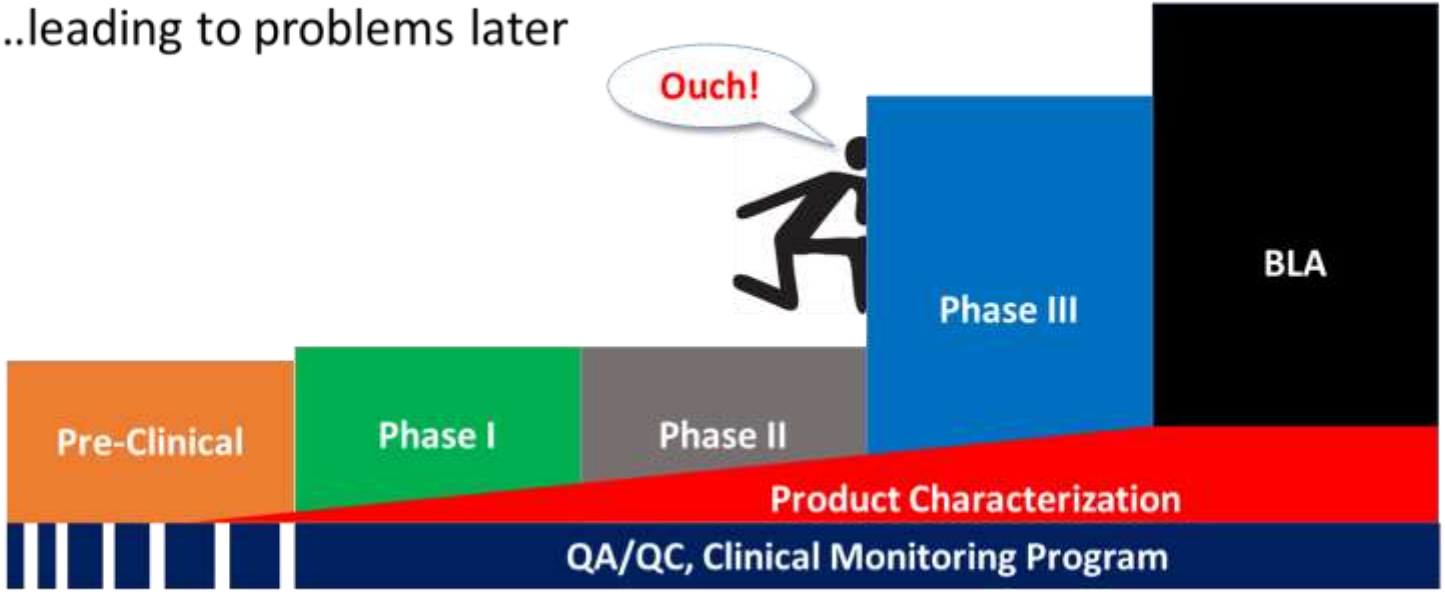
- Stepwise assay development
  - Investigation of biological activity
  - Development of relevant potency assay



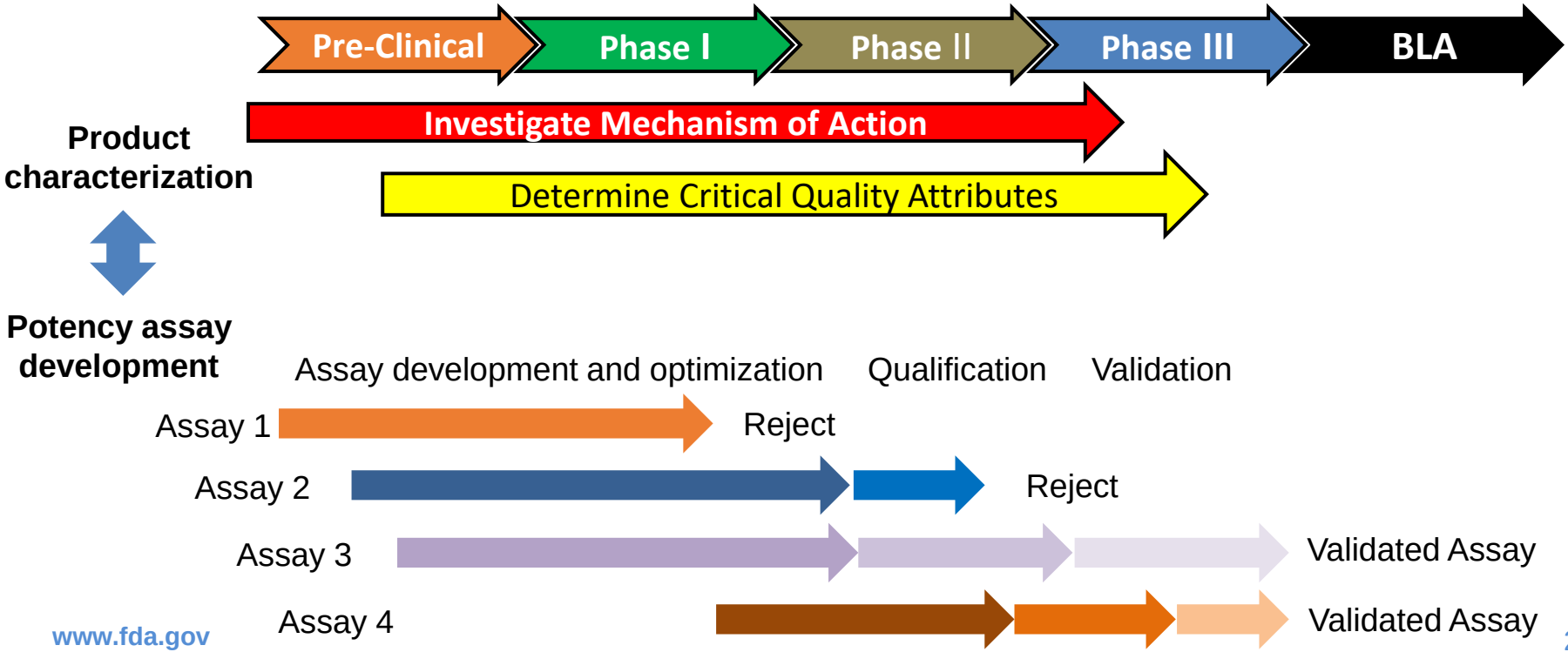
Lifecycle approach to potency measurement (typical)

- Assay development and product characterization is often postponed once product enters clinical studies…

…leading to problems later



Product characterization supports potency assay development



What makes a good potency assay?

- Reflects mode of action (MOA)
- Reproducible
- Accurate
- Robust
- Stability indicating
- Practical
- **Potency assays are product specific**
- **A single assay may be insufficient – explore orthogonal methods and matrix approaches**

Potency assay criteria

- What attributes reflect product performance?
- What potency assays are most appropriate
  - Cytokine production? (CAR T cells)
  - Enzyme activity? (gene replacement)
  - Phenotype? (flow cytometry)
- Limit-based specifications may be appropriate
  - Lower limit for efficacy
  - Upper limit for safety
- Set commercial release criteria using data from lots that are safe and effective

Challenge Question #1

Potency assays should be performed:

A. On vector stocks for lot release

B. On vector stocks for stability studies

C. For cellular product lot release

D. For cellular product stability studies

E. All of the above

Assay validation

- **During assay development**
  - Evaluate assay performance and suitability
- **Analyze and validate all relevant assay parameters**
  - Accuracy
  - Detection limit
  - Precision (repeatability, intermediate precision)
  - Specificity
  - Linearity and range
  - Robustness
  - System suitability
- **Not all assays are created equal**
  - Some will be harder to validate than others

See also: ICH Q2(R1) Validation of analytical procedures

Reference materials

- Qualification
  - Demonstrate suitability (antibodies, assay kits, cell lines)
  - Lot-to-lot variability of reagents
- Availability
  - Don’t be over-reliant on one supplier
  - Ensure sufficient supplies of critical materials
  - Cell banks
- Retention samples and reference standards
  - Important for qualifying new reagents/lots

Accelerated development

- Promising clinical results and accelerated clinical studies…

  … give less time for product development
- Assay development can lag behind clinical studies
- **Requirements for licensure are unchanged**
- Especially problematic for stability studies
  - Initiate stability studies earlier

    …but need potency assay for stability
  - Potential issues if manufacturing or testing methods change
- **Plan early for BLA and commercialization**

Challenge Question #2

Which of the following statements are NOT true?

A. Compendial assays are always appropriate for gene modified cells.

B. Compendial assay methods are just guidelines. It's no big deal to change them.

C. There is always plenty of time and material for lot release testing of gene modified cells.

D. Gene transfer vector quality doesn't matter so long as the final cellular product meets specifications.

Resources

- Cellular & Gene Therapy Guidances | FDA
  - Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs) | FDA
  - Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up | FDAPrescription Drug Labeling Resources
  - Potency Tests for Cellular and Gene Therapy Products | FDA

Summary

- Gene-modified cell manufacturing and testing is challenging
- Start working on assays early
  - Test different assays, discard ones that don't work
  - Continue through product development
- Product characterization informs assay development
- Validated assays for BLA submission
- Talk to regulators about challenging issues

Closing Thought

For gene therapy products, the traditional development lifecycle is changing.

With accelerated programs and effective products, the challenge for CMC is to keep pace with clinical development.

Contact Information

- **Graeme Price, PhD**
  graeme.price@fda.hhs.gov

- **Regulatory Questions:**
  OTAT Main Line – 240 402 8190
  Email: OTATRPMS@fda.hhs.gov and
  Lori.Tull@fda.hhs.gov

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

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- **Consumer Affairs Branch**: ocod@fda.hhs.gov
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FDA Headquarters
Federal Research Center at White Oak
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002


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