Regulatory considerations for manufacturing and testing of investigational chimeric antigen receptor (CAR) T-cell products

Xiaobin Victor Lu
Product Reviewer
Gene Therapies Branch
DCGT/OCTGT/CBER/FDA

MEASUREMENT CHALLENGES FOR CAR-T BIOMANUFACTURING
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Overview

• General description of CAR T-cell products and the manufacturing process
• Challenges in manufacturing CAR T-cell products
• Manufacturing controls and product consistency
• Key CAR T-cell product attributes and testing
• Managing manufacturing process changes and product comparability
• Reference standards
• Summary
Chimeric Antigen Receptor (CAR) T-cells

Chimeric Antigen Receptors

$mAb \rightarrow \text{scFV}$

1$^{st}$ $\rightarrow$ 2$^{nd}$ $\rightarrow$ 3$^{rd}$

Generation

Retrovirus

CAR-T cell

$\text{CD3\zeta} \quad \text{CD28} \quad \text{4-1BB} \quad \text{TCR}$

Michael S. Magee and Adam E. Snook, *Discovery Medicine*, Volume 18, Number 100, November 2014
None, or CTX, or BENDA, or CTX + PENT or CTX + FLU

Tumor Burden, Chemo-sensitive vs. Chemo-resistant

Patient Selection

Conditioning

Apheresis

T cell activation

Fixed-dose vs. Escalation

T cell dosing

Expansion & Formulation

CD3±CD28 beads, OKT3+IL2, PBL vs. T cell subsets

Electroporation γ-retroviral Lentiviral

Epitope, scFv, CAR-affinity, Signaling domains

Davila et al, Oncoimmunology, 2012
CAR T-cell characteristics

• The binding domain is derived from antibodies with higher affinities than T cell receptors.
• Not dependent on HLA
  • Recognize cell surface proteins
  • Insensitive to tumor escape mechanisms related to HLA loss variants
• Autologous adoptive T cell transfer is independent of host immune system (works for immune deficient host)
Relevant guidance documents

• Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)

• Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications

• Gene Therapy Clinical Trials – Observing Subjects for Delayed Adverse Events

• Supplemental Guidance on Testing for Replication Competent Retrovirus in Retroviral Vector Based Gene Therapy Products and During Follow-up of Patients in Clinical Trials Using Retroviral Vectors

• Potency Tests for Cellular and Gene Therapy Products

• Process Validation: General Principles and Practices

• Analytical Procedures and Methods Validation for Drugs and Biologics
Challenges in product development

- Difficult to control manufacturing process for a consistent product.
- Develop meaningful biological potency assays
- Difficult to set tight lot release specifications
- Lack of reference standard materials
- Limited material for testing
- Process changes - insufficient comparability data
Manufacturing process control

Goal: minimize variations to consistently produce a safe, pure, and potent product:

- Product safety and quality should be built into the manufacturing process; testing alone is insufficient.
- Controlling starting materials and critical reagents
- Facility, equipment, training etc.
- Defining processes and procedures (e.g. detailed SOPs, CPP)
  - Process qualification (engineering runs) and formal process validation
- Testing intermediates, drug substances, drug products
  - Control of critical quality attributes (CQA)
  - Analytical assay qualification and validation
Control starting materials and critical reagents
Vector is a critical reagent

• Vector design and derivation/construction
• Cell bank qualification/certification for producer cells or for transfection substrate for vector production
• Vector manufacturing process description
• Sequence verification: entire vector if < 40 kb; relevant therapeutic transgene and regulatory regions if > 40 kb
• In-process and lot release testing for sterility, identity, adventitious agents, purity, endotoxin, mycoplasma, titer, potency (activity), physical viral particles, etc.
Starting cells — Apheresis products

- Apheresis machines are FDA cleared medical devices. Collection of PBMC are performed according to institutional SOPs and policies.
- For multicenter trials, sponsors should develop detailed procedures to minimize variations of the apheresis process.
- If shipping is necessary, the packing condition, shipping and handling, and storage conditions should be validated.
- For allogeneic donor cells, additional donor eligibility screening and testing are required.
Reagents

Ancillary reagents and excipients:

• Highest grade available
  – FDA-approved or cleared reagents
  – Compendial reagents
  – CGMP grade reagents

• Provide CoAs

• May need to be further qualified for its intended purpose.
Human and animal derived reagents

• Examples: Human AB serum, FBS, Cytokines, Growth factors, trypsin, etc.
• Use clinical grade reagents if available.
• Reagent qualification programs
  – Source of origin
  – Certification of analysis
  – Adventitious agent testing
  – Purity
  – Potency (activity or function)
Defining processes and procedures
Critical process parameters (CPP)

- CPPs: Key variables that impact the manufacturing process
- Independent process parameters most likely to affect the quality attributes of a product
- Determined by sound science and manufacturing experience
- Controlled and monitored to confirm that the quality attributes of the product are maintained or improved
Critical process parameters (CPP)

• Examples for CAR T-cells
  – Cell growth and expansion conditions (e.g. growth factors, cytokines, etc.)
  – Selection of intended target cells (e.g. CD4, CD8, T_{em}, T_{cm}, etc.)
  – Pre-stimulation conditions (e.g. bead-antibody, cytokines)
  – Transduction conditions (e.g. multiplicity of infection (MOI), length of incubation time, etc.)
Control of vector transduction

• A critical step for controlling the product potency

• Transduction efficiency
  – Determine upper and lower limits in early phases and refine the acceptance criteria towards late phase studies.

• Vector copy numbers (VCN) (for RV or LV vectors)
  – For RV or LV: < 5 copies per cell for safety reason (e.g. oncogenicity)
  – Set a lower limit

• Time the tests for transduction efficiency and VCN close to the final product (to reflect true values and avoid overestimation due to pseudo-transduction).
Control of intermediates, drug substances, drug products
Critical Quality Attributes (CQA)

• A physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality.

• Evaluate many attributes early during development and prune during lifecycle to those that can discern process-related changes in product safety, quality and efficacy.
Safety

- **Sterility**: 21CFR610.12 (recently updated), USP <71> Sterility tests, alternative rapid test methods, Gram stain method.
- **Mycoplasma**: 21CFR610.30, Points to Consider 1993, USP <63> Mycoplasma tests
- **Endotoxin**: 21 CFR 610.13, LAL test, USP <85> Bacterial endotoxin test
- **Replication competent retrovirus (RCR) or replication competent lentivirus (RCL)**: “Supplemental Guidance on Testing for Replication Competent Retrovirus in Retroviral Vector Based Gene Therapy Products and During Follow-up of Patients in Clinical Trials Using Retroviral Vectors”
Identity

• Detection of specific CAR sequences (e.g. PCR or FACS)
• Additional cell surface markers (e.g. central memory or effector T cells)
• Product chain-of-custody (e.g. labelling, tracking, segregation and shipping/handling from collection of donor cells to administration of the product)
Purity

• Product related impurities
  – Define intended target cell population (e.g. $T_{em}$, $T_{cm}$, CD3, CD4, CD8, CD62L, etc.)
  – Evaluate subtypes of cell populations in the final product.
  – Remove/deplete irrelevant contaminating cell types

• Process related impurities
  – Residual ancillary materials (e.g. antibody, beads, cytokines, growth factors, serum, etc.)
  – Typically removed by washing multiple times.

• Dynamics of cell populations may change during cell expansion, but final cell product should be well defined.
Potency

• Measures multiple product CQAs
  – Transduction efficiency (i.e. % of cells express CAR) (e.g. flow cytometry)
  – Level of CAR expression (e.g. mean intensity of flow cytometry analysis)
  – Cytokine production upon stimulation (e.g. IFN-γ)
  – Relevant biological functions based on MOA (e.g. target tumor cell killing)
  – Potential to persist/engraft post infusion

• Progressive implementation of potency assays
  – Early phase studies: transduction efficiency and transgene expression
  – Relevant biological function assays should be in place prior to phase 3 and pivotal studies that support BLA.
  – Validated prior BLA submission

• *Guidance for industry — Potency Tests for Cellular and Gene Therapy Products*”
Stability programs

- Apheresis materials— shipping, handling, and storage validation
- Vector - shipping, handling, and storage condition validation
- Final product stability studies:
  - Cryopreserved CAR T-cell products
  - Fresh CAR T-cell products
  - Shipping, handling and storage validation
- Thawed product: time between thaw and administration
- Preliminary stability data to support initial IND
- Formal real-time and real condition stability studies with clinical material during product development
- Data from validated methods to support expiry dating for BLA

*ICH Q5C Stability Testing of Biotechnological/Biological Products*
Manufacturing process changes

• Manufacturing process improvement and optimization based on accumulated experience
• Addition or removal of manufacturing steps
• Manufacturing scale-up for commercialization
• Critical raw material and reagent changes (e.g. cell banks, antibodies, beads, vector source, etc.)
• Facility or contract manufacturer changes
• Major equipment changes
Managing changes

• Understand the nature of the changes (significant changes and minor changes) and potential impact on the final product.

• Develop formal strategies to control and document the manufacturing changes and comparability studies.

• Perform comparability studies to show the post change product is comparable.

• If comparability not shown, additional preclinical or clinical studies may be required.
What are significant changes?

- Changes that impact product safety, quality, purity and potency
  - Adverse impact on product attributes
  - Or positively improve product attributes
  - Or combination of above
Examples of significant changes

- **Product characteristics**: vector design, composition of the final CAR T-cell subpopulations, and intended active T cell subtypes, etc.

- **Process**: conditions for transduction, cell expansion, manufacturing facility/sites, cell selection method, introduction of automation, transition from cell culture flasks to bioreactors, etc.

- **Materials/reagents**: vectors source, critical reagents (e.g. mAb, beads, cytokines, growth factors)
What does “comparable” mean?

• Highly similar quality attributes before and after manufacturing change
• No adverse impact on product quality, safety or efficacy
• Products do not necessarily have to be identical.
• If comparability not demonstrated, additional preclinical or clinical studies may be needed to bridge the gap.
Comparability study

- Sufficient lots should be compared
  - e.g. 3 pre- and post-change lots (or statistics based)
  - Lots may be developmental lots, non-GMP
- Acceptance criteria should be set prospectively
- Comparability testing is typically more extensive than lot release testing.
- Vector equivalence should be demonstrated.
- Side-by-side analysis of pre- and post-change products.
- Stability comparison should be included.
- Retention samples should be archived.
- Reference standard materials should be developed.
Adequate methods for assessing change

- Robust, sensitive and relevant methods
- Biological and analytical assays
- Suitable for manufacturing changes
- Not necessarily validated assays
- Scientifically sound and provide results that are reliable (i.e. appropriately qualified)

ICH Q5E Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process, 2005.
Reference standard considerations

• CAR T-cell products are relatively new; reference standards are yet to be developed.
• In-house reference standards should be developed.
• Available reference standards from ATCC
  – Adenovirus type 5
  – Retrovirus vectors
  – Adeno-associated virus type 2
• Other helpful reference standards
  – fluorescent bead/antibodies and particle size reference materials for calibration of FACS machines (NIST)
Reference standard considerations

- Clinical lot material should be used if possible.
- Characterization, qualification and validation
- Expiration dates based on stability data
- Side-by-side comparison of new lot to the original lot when perform re-qualification or re-validation.
- Retain samples of each lot for future comparison
- Consult with your CBER review team.
Summary

• Manufacturing processes for autologous CAR T-cell products are complex and require process controls to minimize product variability.

• Ensure a consistently safe, pure and potent product by controlling CPPs and CQAs.

• Stability studies during all phases of product development.

• Manufacturing process changes are managed appropriately through comparability studies.

• Reference standards should be developed for analytical assay qualification/validation and product comparability studies.

• Consult with FDA early for challenging manufacturing issues.
Additional resources

• Preclinical Assessment of Investigational Cellular and Gene Therapy Products
• Considerations for the Design of Early – Phase Clinical Trials of Cellular and Gene Therapy Products
OCTGT contact information

• Xiaobin Victor Lu: Xiaobin.lu@fda.hhs.gov
• Regulatory Questions:
  Contact the Regulatory Management Staff in OCTGT at CBEROCTGTRMS@fda.hhs.gov or Lori.Tull@fda.hhs.gov
• References for the regulatory process for OCTGT
• OCTGT Learn Webinar Series:
  http://www.fda.gov/BiologicsBloodVaccines/NewsEvents/ucm232821.htm
Public access to CBER

- CBER website:
  - http://www.fda.gov/BiologicsBloodVaccines/default.htm
- Phone: 1-800-835-4709
- Consumer Affairs Branch (CAB)
  - Email: ocod@fda.hhs.gov
- Manufacturers Assistance and Technical Training Branch (MATTB)
  - Email: industry.biologics@fda.gov
- Follow us on Twitter
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THANK YOU!

QUESTIONS?