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

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MEASUREMENT CHALLENGES FOR CAR-T BIOMANUFACTURING

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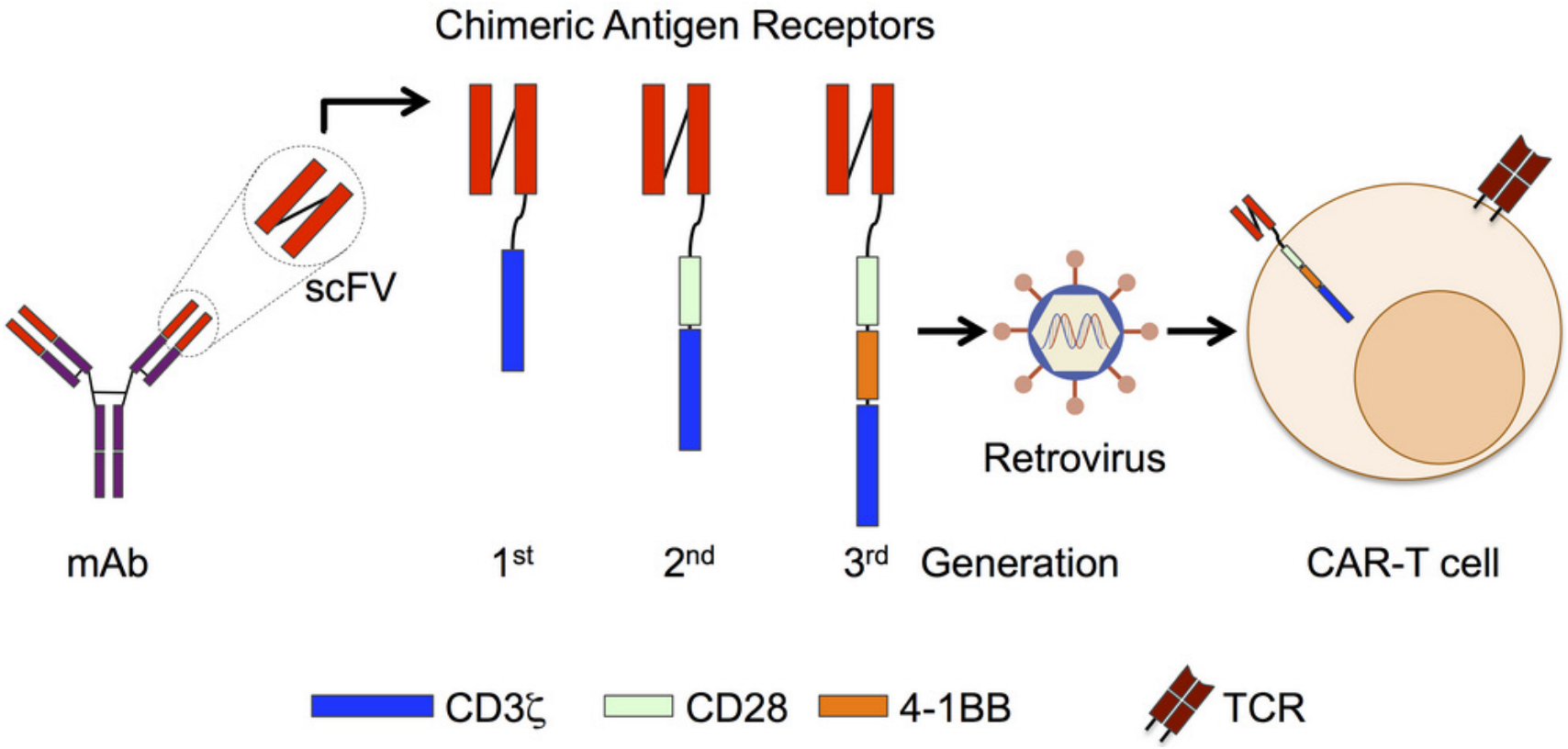
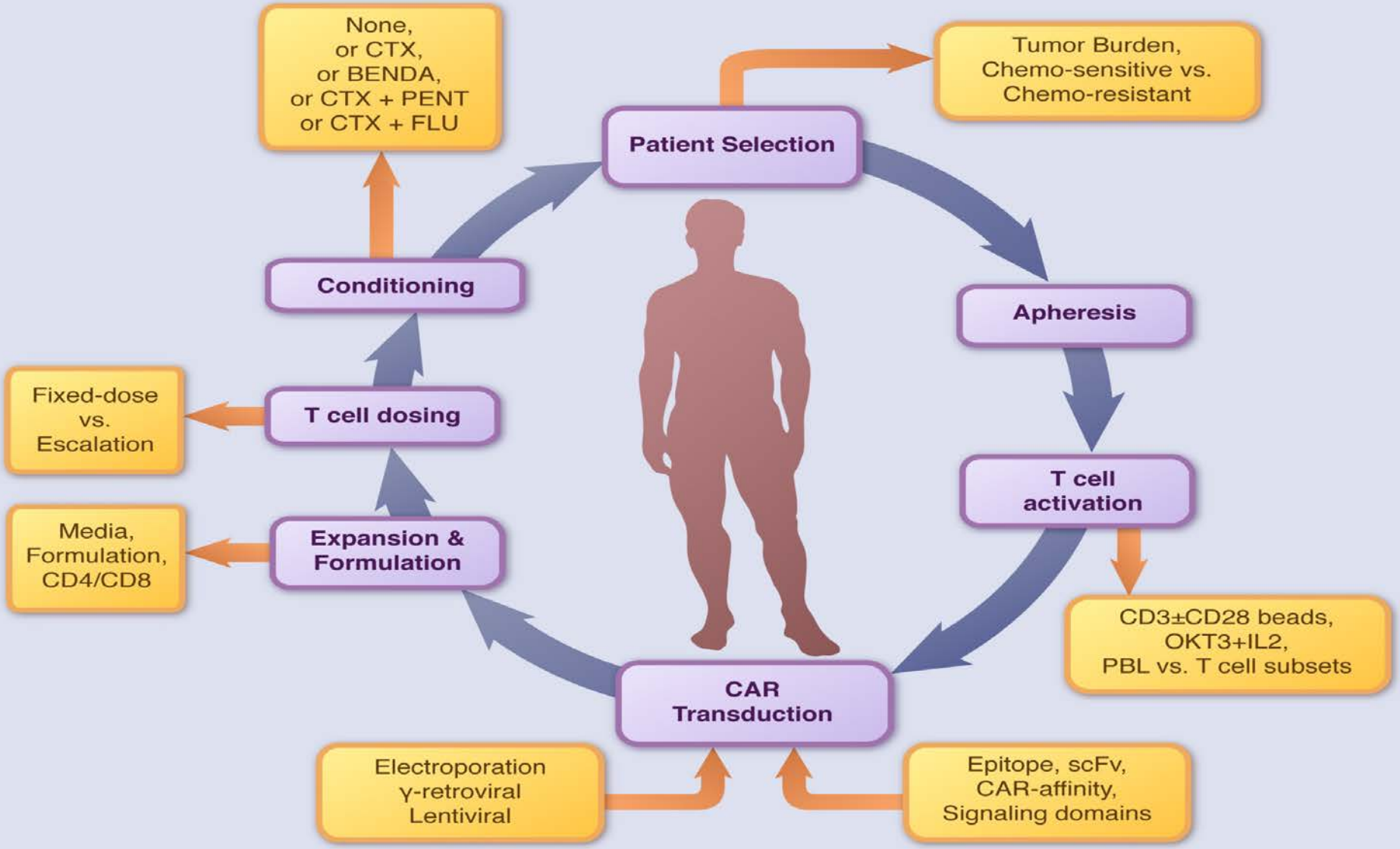
Overview

- 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



Michael S. Magee and Adam E. Snook, *Discovery Medicine*, Volume 18, Number 100, November 2014



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

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- **References for the regulatory process for OCTGT**
  http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/OtherRecommendationsforManufacturers/ucm094338.htm
- **OCTGT Learn Webinar Series:**
  http://www.fda.gov/BiologicsBloodVaccines/NewsEvents/ucm232821.htm

Public access to CBER

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- http://www.fda.gov/BiologicsBloodVaccines/default.htm
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THANK YOU!

QUESTIONS?