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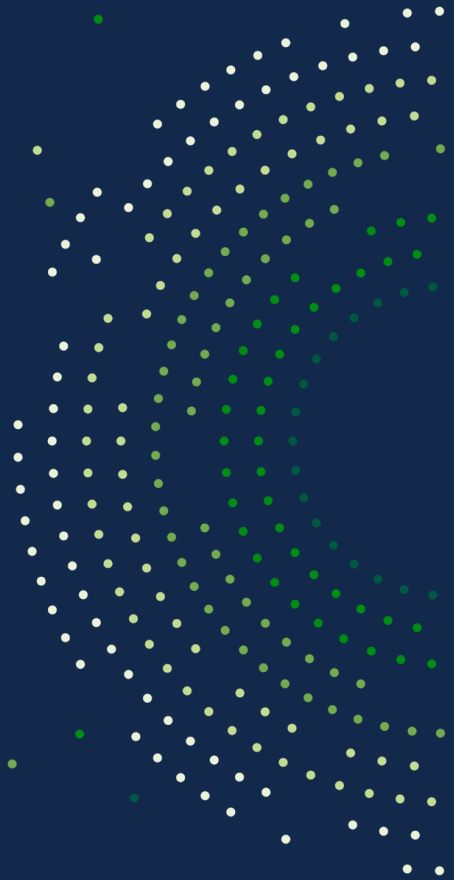
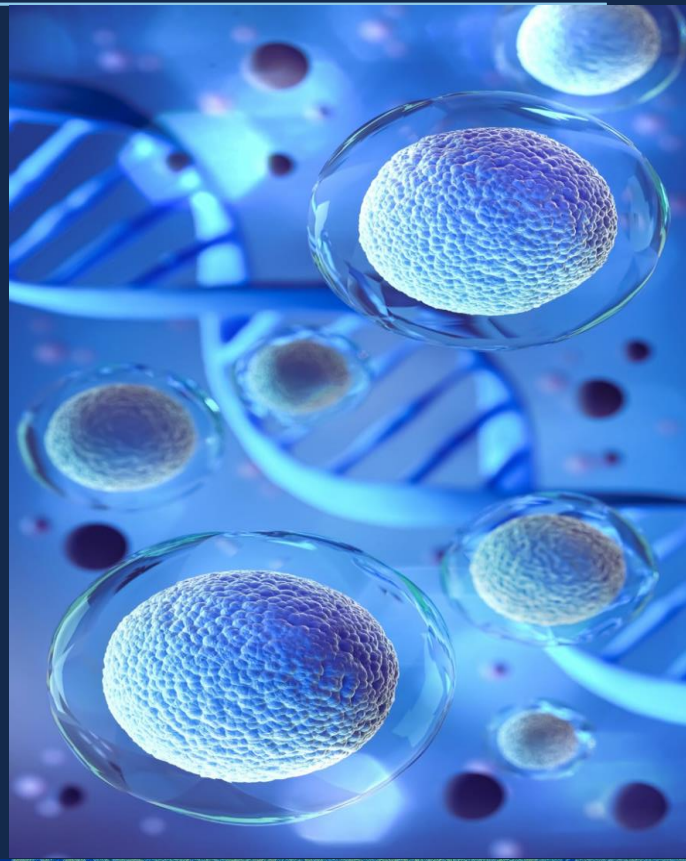
Analytical and Multi-Level Comparability for Genome Editing Components for Allogeneic CAR T Cell Therapy

CASSS' CGTP Summit 2024
Julien Camperi Ph.D.
Sr Principal Scientist, Genentech



OUTLINE

- CAR T cells: Current status, challenges, progress and components of genome editing (GE)
- Assessing Comparability During the CAR T Cell Product Development - Focus on GE component change
- Case study



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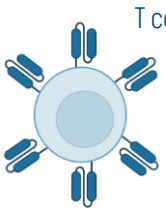
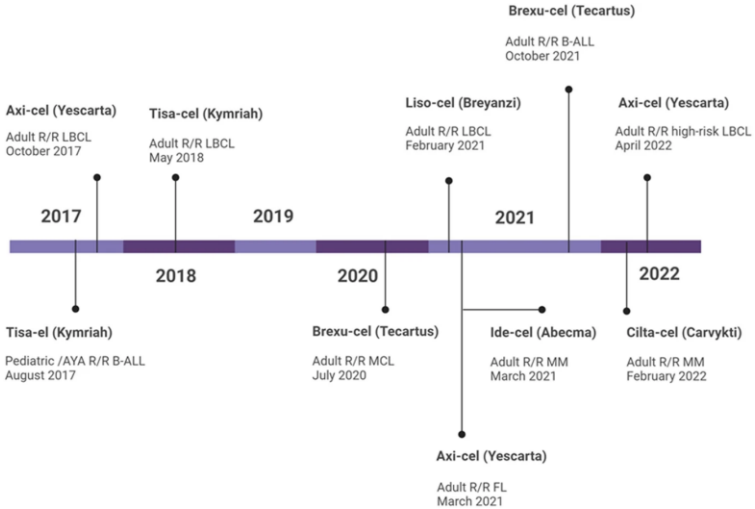
CAR T Cell Therapy: Current State in 2023



- Chimeric antigen receptor (CAR) T-cell therapy
  A real treatment option for patients with B-cell malignancies
- T-cells engineered to express CAR aim to strengthen the power of T-cells to recognize and eliminate tumor cells in a human leukocyte antigen (HLA)-independent manner.
- Multiple efforts are being made to extend this therapy to other malignancies and broader patient populations.
- Since 2017 - Approved Therapies
  - 6 CAR-T products have been approved by the FDA in the United States and other countries,
  - 2 CAR-T products are approved in China by the National Medical Products Administration



Joy R, et al. . Recent advances and current challenges in CAR-T cell therapy. Biotechnol Lett. 2024 Feb

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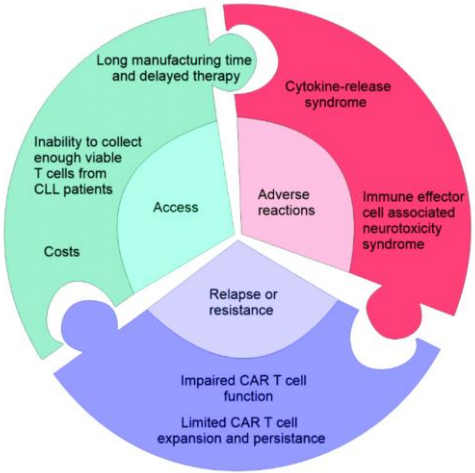
Challenges Remain for these CAR T Cell Therapies

- Adverse events (e.g., cytokine-release syndrome, etc)
- Low durability of responses/effectiveness in the context of solid tumors
- Limitations due to manufacturing of a highly individualized product
- Challenges associated with autologous origin
    - Manufacturing, testing, and release process is time-consuming, and the logistical challenge – poses a significant concern for individuals with rapidly progressive or aggressive cancers.
    - Variability among patients in the cellular starting material (leading to a 2–10% manufacturing failure rate)
    - Logistics, planning and increased expenditures associated with tailored medicines, which necessitate creating and releasing a unique batch for each patient.



Todorovic, Z.et al,. *"CART Cell Therapy for Chronic Lymphocytic Leukemia: Successes and Shortcomings"*. Curr. Oncol. 2022

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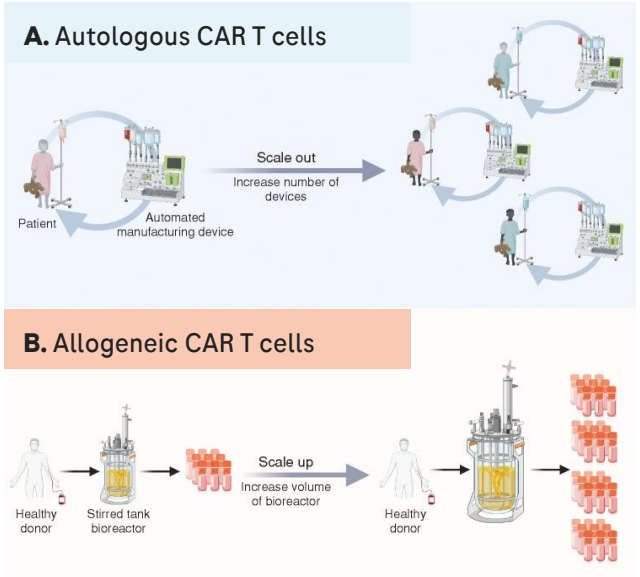
Moving from Autologous to Allogeneic CAR T Cell Therapies: Drivers

Production would be implemented *"in a manner similar to that for conventional biologics"*, with larger quantities produced in a single batch and filled into vials for storage and distribution as needed.

- Scalability and direct access to CAR-T therapies, providing a readily available therapeutic solution for multiple patients.
  - Production large numbers of doses per batch for multiple patients would contribute to lower costs. Est.$7,500–10,000 per dose*.
  - Off-the-shelf alternative is readily available, with no need to modify patient cells, either at a central manufacturing site or at the point of care.



*Jenkins. Michael and Suzanne Farid. *"Cost-effective bioprocess design for the manufacture of allogeneic CAR-T cell therapies using a decisional tool with multi-attribute decision-making analysis."* Biochem. Eng. Journal. 137: 192 (2018).

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Moving from Autologous to Allogeneic CAR T Cell Therapies: Hurdles

- GvHD (graft versus host disease) remains one of the biggest hurdles
  - Immune-mediated rejections, in which the donor cells are attacked as foreign threats, have the potential to be life-threatening.
  - Allogeneic CAR T cells can be rapidly removed from the body, dramatically reducing their therapeutic effects.

- Challenges to the scale-up
  - Access to sufficient quantities of high-quality starting material for genetic modification of the donor cells, single-use manufacturing equipment, and a reliable source of donor cells is essential.
  - Allogeneic cells must be consistent in terms of their properties and quality.
    - Producing high quantity of cells with the same high level of cellular integrity/functionality can be difficult.
    - Extended expansion of cells to the degree required can introduce differentiated phenotypes.
    - Ensuring that all the cells have successfully undergone gene editing must also be achieved.

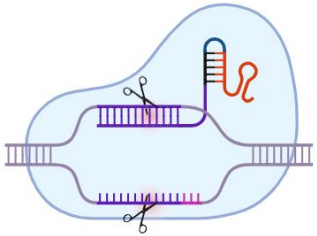
Several Approaches to Overcoming Allogeneic Challenges

- Developing specifications for starting T cell populations and standards for apheresis equipment, procedures, and training should be a focus of the industry as well.

- Gene editing is thought to provide one of the best approaches to eliminating GvHD.

  - Technologies such as CRISPR-Cas9 editing are being used to knock out specific genes that lead the expression of molecules that generate immune responses.

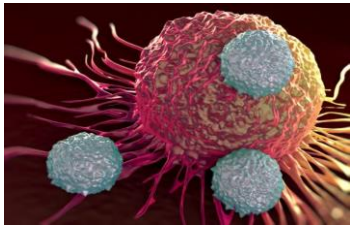
  - Other options include the use immune-privileged mesenchymal stem cells (MSCs) and other immune cell types (virus-specific T cells, memory T cells, natural killer cells, etc.)



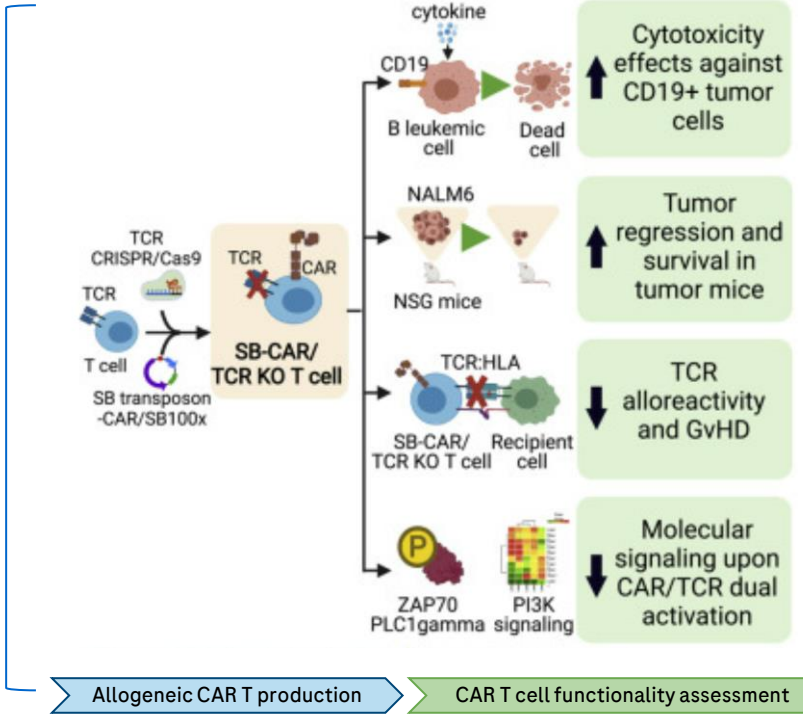
CRISPR-Cas9 System

Advances in Gene Editing technology enabled “off-the-shelf” allogeneic CAR-Ts with low alloreactivity

Cell-based Therapeutics



Gene Editing



Adapted from Jaitip Tipanee, et al. “Universal allogeneic CAR T cells engineered with Sleeping Beauty transposons and CRISPR-CAS9 for cancer immunotherapy.” Molecular Therapy, 2022 June.

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Genome Editing (GE) Components for Allogeneic CAR-Ts

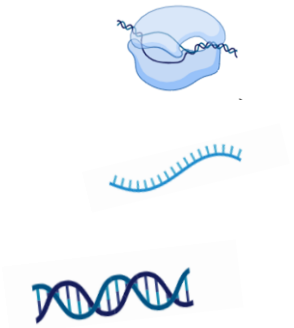
"...GE component is considered any material that is essential for the intended genomic modification, including those that may not appear in the final drug product."

Human Gene Therapy Products Incorporating Human Genome Editing
Guidance for Industry

January 2024

GE components may include, but are not limited to:

- DNA targeting elements (i.e., elements used to dictate the target DNA sequence, such as guide RNA).
- Protein-encoding mRNAs (i.e., Cas9 protein expression for target gene modification (mRNA-encoded designer nucleases), antigen presentation, functional protein expression).
- Donor DNA template (i.e., DNA sequence provided to repair the target sequence).



For CAR T application, the GE components are considered critical components for the manufacture of the final product because without these components, the resulting cell product would not have the same pharmacological activity.

GE Component considered as Starting Materials for CAR T Product Manufacturing

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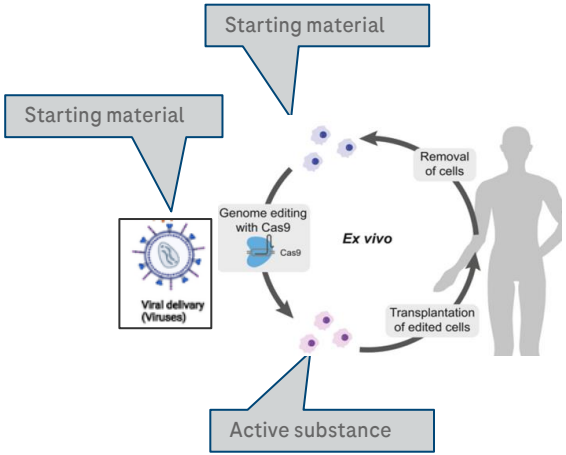
In vivo gene editing approaches: active substance = e.g. RNA, ribonucleoprotein, or other vectors used to deliver gene editing tools

Ex vivo Gene Therapy:
active substance = modified cell
starting materials = unmodified cells, viral vectors or nucleic acids and/or proteins used for modification

The active substance of a gene therapy medicinal product based on gene transfer methods ***in vivo*** is composed of the recombinant nucleic acid and the viral or non-viral vector used to deliver it. In the case of ***in vivo*** genome editing approaches, active substances normally comprise the tools used for the intended genome edition. This can be as diverse as a recombinant nucleic acid, a recombinant protein, a synthetic oligonucleotide or RNA, a ribonucleoprotein, etc. and the viral or non-viral vectors used to deliver them. In the case of gene therapy ***ex vivo*** (i.e. genetically modified cells), the active substance is composed of the modified cells. The unmodified cells, the viral or non-viral vectors and any other nucleic acid and/or protein used in the genetic modification of the cells are considered starting material. The requirements for the gene/vector component should additionally be taken into consideration. In this case of ***ex vivo*** use, viral vectors, plasmids, recombinant proteins and recombinant mRNA, the components to produce them (e.g. plasmids, cells) are also considered starting materials. In this case, the principles of GMP, as provided in the General Principles in the Guidelines for GMP for ATMP, should be applied from the cells bank systems used to produce the starting materials, when applicable.

https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-quality-non-clinical-and-clinical-requirements-investigational-advanced-therapy-medicinal-products-clinical-trials_en.pdf

https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01925-5



- Active Substance: CAR-positive T cells
- Starting material (incorporated into the structure of the final product. They are inherently critical)
  a. T cells (apheresis material)
  b. MCB/WCB to produce the lentiviral vector, DNA, etc)
  c. Lentiviral vector, DNA template
  d. Gene editing tools

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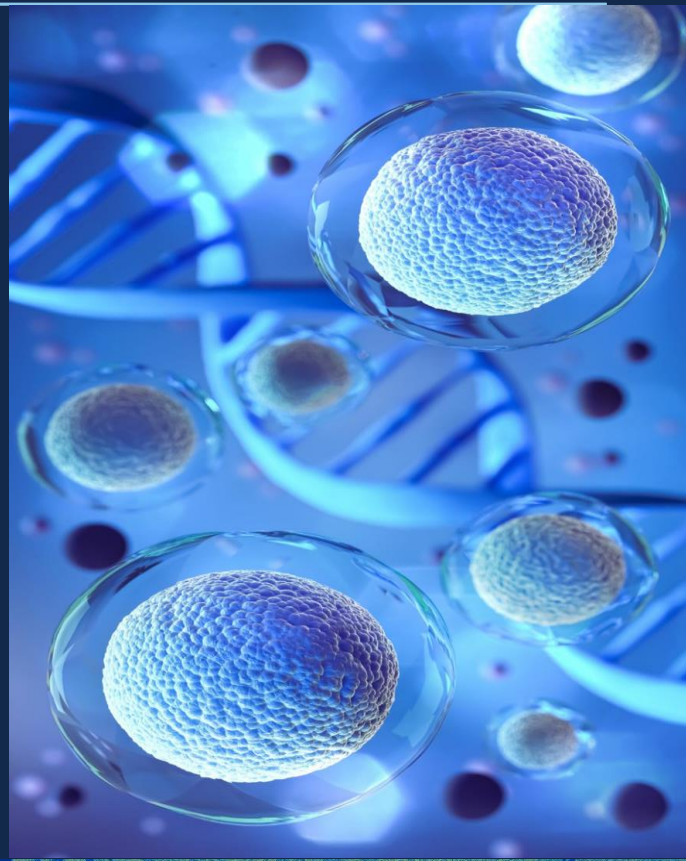

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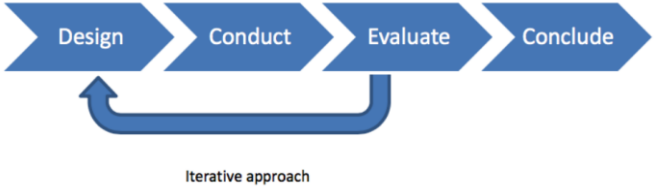
Comparability Framework Introduction

- What is Comparability?

  Comparability Exercise: The activities, including study design, conduct of studies, and evaluation of data, that are designed to investigate whether the pre- and post-change products are comparable. (ICH Q5E)



- How do we approach comparability?

Modular Approach to Comparability

Design

Design the comparability exercise testing strategy with the following steps:

- Compare pre- and post-change state to identify extent of change
- Evaluate the potential risk of the proposed change
- Assess product lifecycle considerations
- Determine the appropriate comparability components and extent of testing

Conduct

Conduct testing as specified during the Design Phase

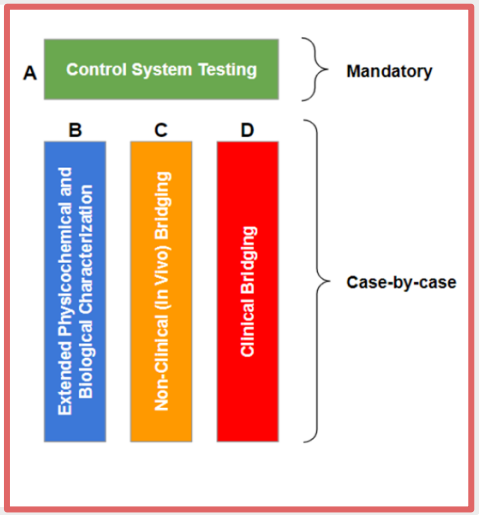
A: Control System Testing

B: Extended Characterization

- Physicochemical Characterization
- Biological Characterization
- Stress Stability

C: Non-Clinical (*In Vivo*) Bridging

D: Clinical Bridging



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Phase Considerations for Comparability

	Before Pivotal Trials	During or After Pivotal Trials
Objective	Comparability exercise are generally performed to demonstrate that nonclinical and clinical data generated with pre-change product are applicable to post change product in order to facilitate further development and, ultimately, to support the marketing authorization (ICH Q5E).	Comparability exercises are performed to demonstrate that manufacturing process changes will not have an adverse impact on the quality, safety and efficacy of the drug product (ICH Q5E).
Comparability conclusion	Comparability exercise demonstrate the quality of the post change product is suitable for the intended clinical study.	Comparability exercise demonstrates that the pre- and post-change products are comparable in terms of Quality, Safety, and Efficacy

- For products in early stages of development, analytical comparability may be sufficient.
- Manufacturing changes made in later stages will require a more comprehensive study.

Analytical Comparability Study Design (Cell-focused)

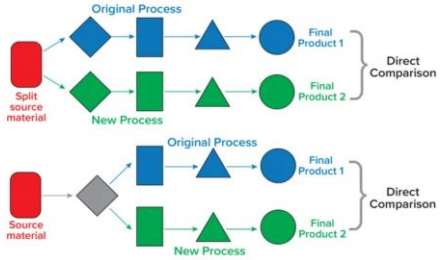
Manufacturing Changes and Comparability for Human Cellular and Gene Therapy Products

Draft Guidance for Industry

July 2023

- Selection of product lots for the study
  - prefer lots manufactured at full scale
  - perform data-driven risk assessment of CPPs, CQAs, and other relevant drug product characteristics to justify that scaling down
  - requires that the analytical test methods are equivalent across product lots to provide interpretable data. (i.e., development of potency assay using pre-change material relevant for post-change material)

- Special considerations for products derived from a variable cellular starting material and other starting materials
  - split source study design whenever possible
  - results from split runs should in-process and release specifications and be representative of relevant historical data
  - material comparability



K.R. Poudel, et al. "Comparability for Cell and Gene Therapy Products: A Challenge and an Opportunity". Bioprocess International, 2022

Common GE Changes that require a Comparability Study

Manufacturing Changes and Comparability for Human Cellular and Gene Therapy Products

Draft Guidance for Industry

July 2023

"Changes during the CAR T cell product lifecycle (e.g,. starting material, final container, cytokines used during culture, or duration of cell expansion), may impact product quality, safety, efficacy, or stability."

Common GE Changes:

- Manufacturing site changes
- Scale-up
- Improve manufacturability by switching format (e.g. liquid/lyophilized format).
- Change made to optimize GE design and stability (e.g. optimize sgRNA to reduce the potential for off-target genome modification or mRNA to increase stability/persistence.)
- Switch from RUO to GMP for Later Phases (Phase 2/3 and/or registration studies).

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*Minor changes (e.g., such as a GE formulation buffer change) may not require a comprehensive comparability study.

Managing GE Component Changes During the CAR-Ts Product Development

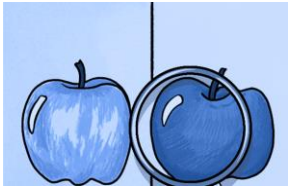
"Due to the critical impact on role of GE components in CAR T cell activity, the impact of such changes should be assessed as follows":

Human Gene Therapy Products Incorporating Human Genome Editing

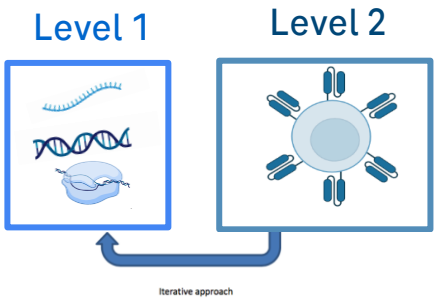
Guidance for Industry

January 2024

- Studies should include side-by-side analyses of the pre- and post-change GE component.
- Additionally, CAR T cells manufactured with pre- and post-change GE component should be assessed using side-by-side analysis by using the same cellular starting material (e.g., splitting the leukapheresis starting material from the same donor).
- When the CAR T cells or GE manufacturing facility is changed, product comparability from the pre- and post-change manufacturing facilities should also be assessed.



Approach to Assessing Comparability: As per ICH Q5E



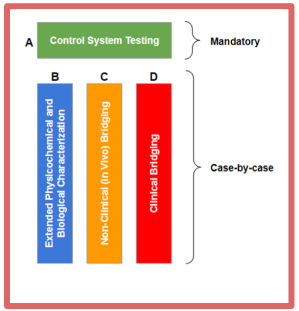
- Different levels, what to compare
  - GE components (i.e. RNA, mRNAs, DNA) and drug products (i.e. CAR Ts)
  - Comparison of pairs of GE and drug product batches from existing and new facility, if applicable.

- Parameters to compare:

Analytical Comparability

  - A: Release testing of both GE component and drug product – within specifications and statistically determined ranges (mandatory)
  - B: Extended characterization of both GE component and drug product + Stress stability of each GE components (if multiple) and drug product (with initial read-out based on accelerated and stressed conditions)
  - C: Non Clinical (in vivo) Bridging and D; Clinical Bridging

Modular Approach to Comparability



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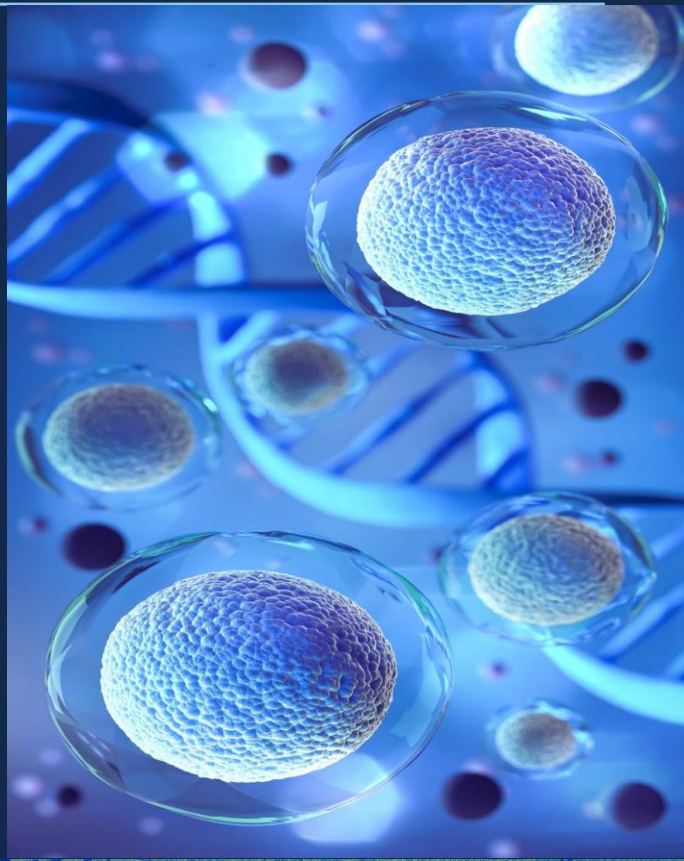

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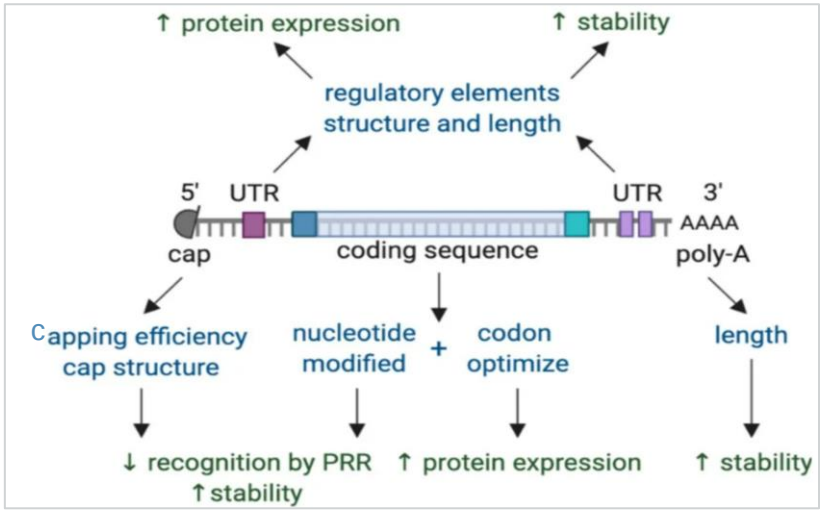
Understand the Structural Features of each GE component

=> mRNA-encoded designer nucleases - commun genome editing tool for allogeneic CAR T cell products



- mRNA integrity is calculated as the proportion of RNA that is full intact, active, intact, and effective
- Coding region of the mRNA must be corrected to ensure that the intended protein is expressed
- Structural features quality: As critical quality attributes of mRNA control over the degree of consistency of i) capping efficiency and ii) 3′ poly-A length should be determined and quantified.

Focus on Structural integrity/Purity and Identity (sequence confirmation)

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Common Analytical Tool Box for mRNA-based applications

Material level comparability - mRNA 1 vs mRNA 2

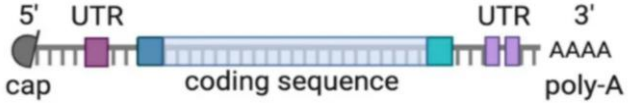
- GE component be tested appropriately purity, identity, sterility and activity, in a phase appropriate manner.
- Additional testing, such as that for process residuals, should be included, depending on the manufacturing process.

Integrity and purity
- Fragment analyzer (R, S)
- Capillary Gel electrophoresis (CGE)
- IPRP-LC

Identity
- RT-PCR, Sanger Sequencing (R)
- Oligo Mapping by UHLC-MS/MS
- Direct RNA sequencing

Quality

Capping efficiency: HPLC-UV(R,S)/MS
Poly(A) tail: HPLC-UV (R,S)/MS



Safety

Endotoxin USP <85>, Bioburden USP <61>, <62>, <1115>, Sterility (R)

Content
- UV-Vis Spectroscopy (R, S)
- RT-qPCR
- IPRP-HPLC

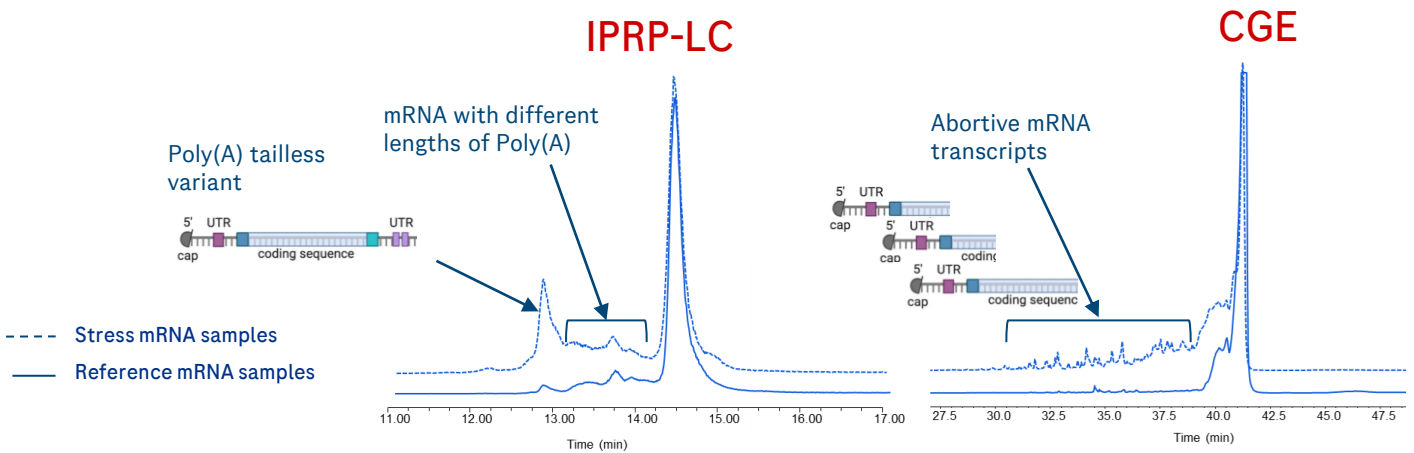
Functionality (challenging for GE)
- In vitro translation/Western blot
- Cell-based assay

In process Impurities
- dsRNA
- Residual DNA, proteins, NTPs, and solvents

(R) : Common Release methods for mRNA-based applications (USP mRNA Vaccine Chapter)
(S) : Common methods used for mRNA stability study

Why Release Testing Is Just One Piece Of The Analytical Puzzle

- An extensive data set should be generated evaluating critical stages in the process that could impact the final product characteristics, including in-process controls, drug substance release testing, drug product release testing, and extended characterization.
- Important to evaluate impurity profiles and to include stability data in the comparability exercise to identify differences in product degradation. Note: GE components need to be assessed for stability if being stored.
- Importance of using orthogonal methods to evaluate a single CQA. e.g., Purity.



Complementary information - detection of different types of impurities

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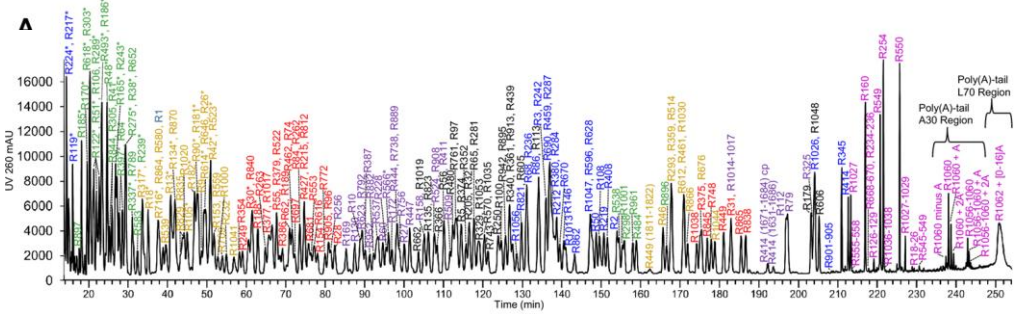
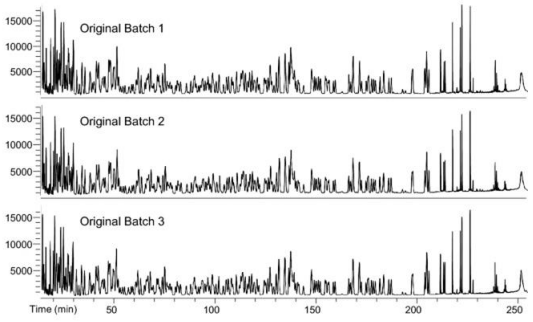
Multi Attribute Method can be Beneficial for Ensuring Comparability

Nucleic acid mapping via Mass Spectrometry (MS)

ID sequencing



Batch to batch comparison



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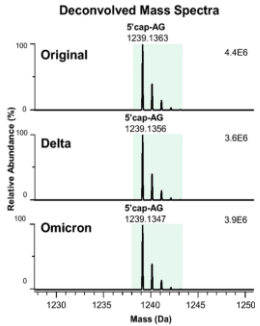
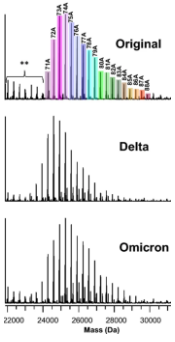


PolyA tail



Nucleic acid mapping by MS can provide important information on :

- sequence ID, polyA tail length and purity/impurities.
- mRNA chemistry, including the detection of nucleoside modifications (e.g., modified nucleobase N1-methylpseudouridine (m1Ψ). *Note: Potential change made to optimize GE design*

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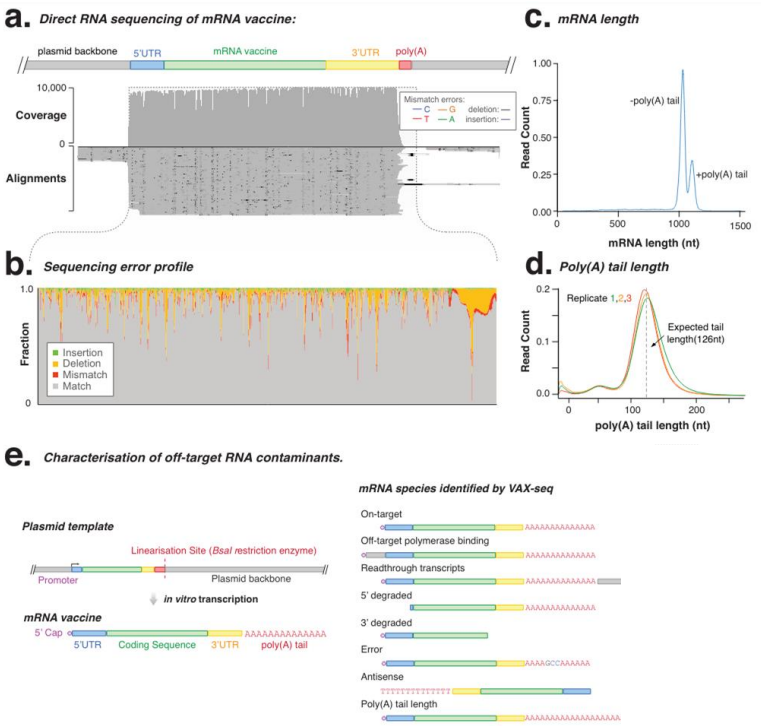
Gau, B.C., Dawdy, A.W., Wang, H.L. *et al.* Oligonucleotide mapping via mass spectrometry to enable comprehensive primary structure characterization of an mRNA vaccine against SARS-CoV-2. *Sci Rep* 13, 9038 (2023).

Multi Attribute Method can be Beneficial for Ensuring Comparability

Sequencing-based approach to measuring key mRNA quality shows great promise




Comment

https://doi.org/10.1038/s41592-022-01633-w

Advances in nanopore direct RNA sequencing

Miten Jain, Robin Abu-Shumays, Hugh E. Olsen and Mark Akeson

Check for updates

Article

https://doi.org/10.1038/s41467-023-41354-y

mRNA vaccine quality analysis using RNA sequencing

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Check for updates

Helen M. Gunter[1,2,6], Senel Idrisoglu[1,2,6], Swati Singh[1,2], Dae Jong Han[2], Emily Ariens[2], Jonathan R. Peters[2], Ted Wong[3], Seth W. Cheetham[1,2], Jun Xu[4], Subash Kumar Rai[4], Robert Feldman[5], Andy Herbert[5], Esteban Marcellin[1], Romain Tropee[2], Trent Munro[1,2] & Tim R. Mercer[1,2]

Direct RNA sequencing can also provide important information on :

- sequence, length, integrity, polyA tail length and purity/impurities.
- mRNA chemistry, including the detection of nucleoside modifications (e.g., modified nucleobase N1-methylpseudouridine (m1Ψ).

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Gunter, H.M., Idrisoglu, S., Singh, S. et al. mRNA vaccine quality analysis using RNA sequencing. Nat Commun 14, 5663 (2023).

Functionality Testing Should Be Used, Wherever Possible

FDA guidance presents "a potential phased approach to potency testing, specifically with respect to the genetic modification. In early phases, demonstration of the sequence modification may be an adequate measure of potency, whereas for late-phase studies, a functional consequence of the sequence modification may be required.

Case study: GE component = mRNA-encoded Cas9.
This GE is supposed to knock-out (KO) TCR signaling or expression in CAR Ts product (functional consequence).

- Material level-comparability - TCR KO % measurement should be performed during CAR-Ts manufacturing process for ensuring the quality of the post change mRNA provide equivalent or superior process performance to the pre change starting material.

- Potential caveat - Using part of the process to test mRNA functionality may lead to inconclusive data due to multiple sources of variability in the manufacturing process. Therefore, functionality testing must be adequately backed up by release assays (e.g. confirmation of correct sequence and integrity).

- A key aspect of demonstrating analytical comparability is to ensure that the clinical data generated pre-change continues to be relevant to the safety and efficacy of the post-change product. If there is insufficient evidence to demonstrate analytical comparability, then new non clinical or clinical studies need to performed (iterative apporach).

Incorporation of multi GE starting material

Human Gene Therapy Products Incorporating Human Genome Editing
Guidance for Industry
January 2024

One item that is frequently overlooked in comparability study designs is the cumulative impact of individual changes. While individually these changes have minimal impact, when taken together there may be a significant impact on drug product quality, safety, or efficacy."

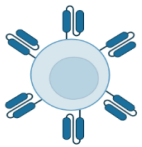
- Stepwise approach

Objective(s):

- Evaluation of each of the post GE components and its impact on process performance.
- To show comparable DP attributes in pre vs post change GE component.

Case study 2: GE1 = mRNA-encoded Cas9; GE2 component = gRNA

- All pre change GE1/GE2 components
- Upgraded GE1 component, pre change GE2 component
- Upgraded GE2 component, pre change GE1 component
- All upgraded GE1/GE2 components



DP attributes testing: residual mRNA, on-target, off-target, translocation, in-vitro potency, identity, etc

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Some key considerations for drug product level comparability

In addition to Release testing of CAR Ts drug product - It is important to complete data-driven drug product risk assessment to evaluate potential risk of the GE change and extent of the testing.

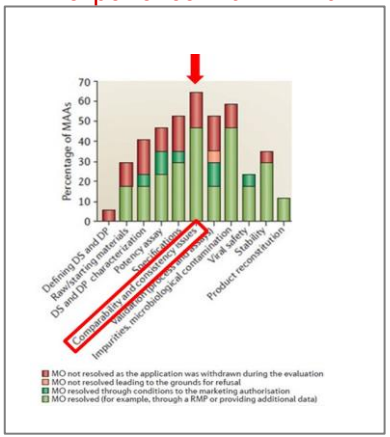
- Within the context of assessing product safety, characterization of off-target and on-target editing capacity of GE components should be included. Determination of GE efficiency (e.g., the degree of editing at the on-target site) and may include an assessment of specificity (e.g., the degree of editing at off-target sites)

- Important to assess the level of risk associated with each impurity of GE component on drug product.
    - Low levels of host cell DNA, residual RNA, and protein do end up in drug products, but it is important to determine, what acceptable and the risk level. The acceptable levels of impurities may vary depending of the intended use of the mRNA.
    - Important to pay attention to the endotoxin levels. Additional purification step at the material level may be required to reduce endotoxin to acceptable levels in drug product.

Final thoughts

- Comparability is one of the most common CMC concerns regulatory agencies have at late stages of product development.
- Well designed and executed comparability studies are essential as drug products advance through the development life cycle.
- Comparability studies should start with quality data and then continue as appropriate with non-clinical and clinical studies, as needed.
- The extent of the studies needed will depend on where in the manufacturing process the changes are being made, the potential impact on product quality attributes, safety and efficacy, and the suitability of analytical techniques to detect potential product modifications.
- The unique challenges developing allogeneic CAR T products add complexity to the standard approach to comparability.
- Assessing comparability of GE component by comparing different levels (e.g. material and drug product level)

European regulatory experience with ATMPs



Adapted from: Barkholt et al., 2019

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