In-use Testing of Cell and Gene Therapies

Securing Patient Well-being: Best Practices for In-Use Stability and Compatibility Studies
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Principles of Cell and Gene Therapy Products (CGTs)

Advanced therapy medicinal products (ATMPs)

Gene therapy

Modify a patient's genes to treat or cure disease

Cell therapy

Introduce cells (patient- or donor-derived) to treat or cure disease

In vivo

Ex vivo
(genetically modified cells)

Unmodified
(cells w/o genetic modification)

https://www.csl-produkte-hemgenix.de/ (accessed: 20-DEC-2023)
https://patienteducation.asgct.org/gene-therapy-101/gene-therapy-basics (accessed: 26-OCT-2023)
https://hemacord.info/ (accessed: 19-DEC-2023)

Considerations for In-use Testing of CGTs

Drive to ensure Pharmaceutical Quality all the way to the patient

- Simulate and evaluate quality from dose preparation to administration
- Define conditions needed for product stability and ensure compatibility with administration materials/diluents

Operational and practical challenges with CGTs

- Diverse product portfolio with different ways of dose preparation and administration
- Limited historic product understanding and development experience
- Analytical challenges that may include low concentration, complex assay setups, multiple chemical entities per CGT (e.g. lipids, nucleic acids, proteins), etc.

As a result …

- No one-size-fits-all strategy for administration and in-use stability testing
- Limited shared understanding of how CGTs are handled at clinical sites → Products often less amenable to small deviations from the handling protocols
- Diverse approaches utilized to conduct in-use stability studies

Blümel et al., J Pharm Sci, 2023 (https://doi.org/10.1016/j.xphs.2023.05.002)

Regulatory Guidance Background

Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs) →	"...measures of both **product quantity and product activity** (e.g., for viral vectors, a measure of physical particles and infectivity (or potency) to assess both adsorption and inactivation). These in-use and in-device stability data should **support recommended hold times and conditions** outlined in the clinical protocol for patient administration."
Draft Guidance for Industry Potency Assurance for Cellular and Gene Therapy Products →	"You should perform studies to **evaluate whether your product's potency will remain acceptable** during preparation of the product and during administration through delivery devices."

In-use stability testing also guided by:

- ICH Q1A(R2): Stability Testing of New Drug Substances and Products
- ICH Q8(R2): Pharmaceutical Development

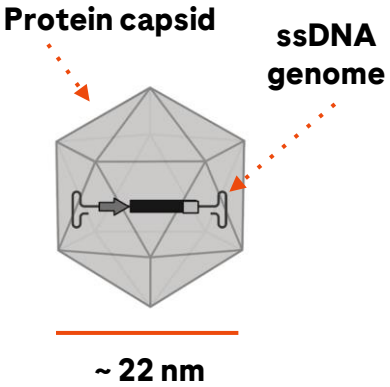
https://www.fda.gov/media/113760/download/ (accessed: 08-JAN-2024)
https://www.fda.gov/media/175132/download/ (accessed: 02-JAN-2024)

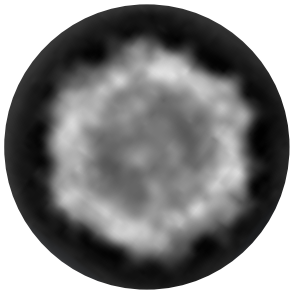
The In Vivo Gene Therapy Landscape

Recombinant Adeno-Associated viral vectors (rAAVv)

In vivo gene therapy

- Requires vector to package gene of interest: **Viral** and non-viral vectors are used
- Different benefit-risk-profiles depending on application





cryo-EM image

Recombinant Adeno-Associated viral vectors (rAAVv)

- Non-pathogenic and replication defective viral vector
- Genes delivered by rAAVv: Episomal, non-integrating, long lasting expression in non-proliferating cells

S.Subramanian et al., Human Gene Therapy, 2019 (https://doi.org/10.1089/hum.2019.041); cryo-TEM image H. Au et al., Front. Med., 2022 (https://doi.org/10.3389/fmed.2021.809118)

Considerations for In-use Compatibility Testing

Recombinant Adeno-Associated viral vectors (rAAVv)

- **Main inactivation mechanisms**
  - Adsorption
  - Aggregation
  - Capsid degradation & unfolding
  - Post-translational modifications (incl. oxidation, deamidation)
  - Genome release & degradation

- **Analytical testing**
  - Analytical methods needed for ssDNA genome, protein capsid and function (potency methods connected to specific mechanism of action)
  - Careful selection of analytical methods based on development phase, product understanding etc.
  - Potency assay setup mostly complex, often progressive implementation of potency assay
  - Potency prediction/assurance and definition of stability indicating methods challenging due to limited historical experience with product class

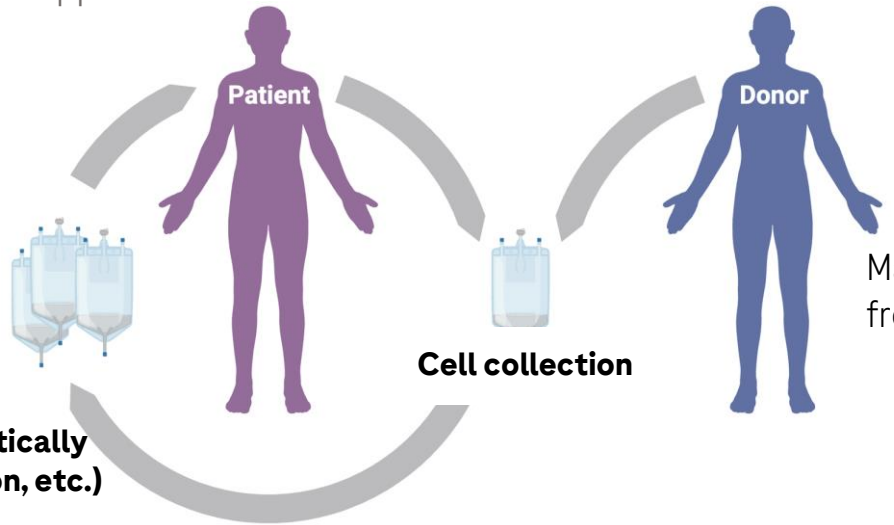
- **Dose preparation**
  - Mostly deep frozen (≤ - 60 °C) product storage and shipment
  - Often complex dose preparation procedures (including product thawing, short hold times, pooling of multiple vials, use of multiple syringes, special injection devices)

The Cell Therapy Landscape

Autologous and Allogeneic Approaches

Autologous

Manufactured as a single lot from the patient being treated



Allogeneic

Manufactured in large batches from unrelated donor tissues

- Administered cells are “living drugs” that require novel analytical methods and approaches
  - The starting material is a dominant source of variability
  - CQAs may not yet be well understood
- Limited product/process understanding
  - Need for extended characterization early in the process (invest early to ensure success)
  - Deeper biological understanding throughout product life cycle

Considerations for In-Use Compatibility Testing

Cell Therapies

- **Mechanisms impacting product quality**
  - Adsorption and settling in administration components
  - Aggregation of cellular components
  - Cell death/ loss in viability

- **Analytical testing**
  - Monitor cell health and function (analytical methods to evaluate cell viability, recovery, as well as potency methods connected to specific mechanism of action)
  - Product-specific potency method → reflective of the mechanism of action of the drug product

- **Dose preparation and administration**
  - Frozen long-term product storage and shipment in vapor phase of liquid nitrogen (cryoprotectant required, ≤-135°C storage)
  - Specific product thawing procedures which may impact product quality if not done correctly
  - Often complex dose preparation procedures (washing and buffer exchange required for some products, special administration devices)
  - Limited shelf-life during room temperature and refrigerated storage (short in-use hold times)

FDA & EMA approved Cell and Gene Therapies

Gene therapies for IV infusion (products for non-IV infusion not included)

Product	Primary packaging (fill volume)	In-use stability	Route of administration	Containers per dose	In-use handling
Hemgenix (2022)	Glass vial (10 mL)	24 h at 15-25 °C (bag)	IV infusion infusion bag	10-48 vials	• Dilution in saline and transfer into infusion bag • Infuse using an infusion pump and in line filter (0.2 µm)
Roctavian (2022)	COP vial (8 mL)	10 h at 15-25 °C (syringe)	IV infusion syringe	~ 8-30 vials (27 for 70 kg)	• Thaw vials and hold for max. 3 days at 2-8 °C • Prime tubing and in-line filter with ROCTAVIAN, flush infusion line with saline • Infuse using a syringe pump and in line filter (0.2 µm)
Zolgensma (2019)	CZ COP vial (5.5 & 8.3 mL)	8 h at 15-25 °C (syringe)	IV infusion syringe	2-14 vials	• Thaw vials and hold for max. 14 d at 2-8 °C • Prime tubing with saline • Infuse manually or using a syringe pump
Elevidys (2023)	CZ COP vial (10 mL)	6h at 15-25 °C (syringe)	IV infusion syringe	Up to 70 vials (for 70 kg)	• Thaw vials and hold for max. 14 d at 2-8 °C • Infuse using a syringe pump and in line filter (0.2 µm)

https://labels.fda.gov/ (accessed: 09-JAN-2024)

FDA & EMA approved Cell and Gene Therapies

CD19-directed genetically modified T cell immunotherapies

Product	Primary packaging (fill volume)	In-use stability	Route of Administration	Containers per dose	In-use handling
Breyanzi	vial (5 mL)	2 hours (from frozen storage to patient administration)	IV bolus - syringe	1-4 vials	• Flush the infusion tubing with normal saline prior to and after each CD8 or CD4 component administration • Administer all CD8 component first followed by CD4 component • Flush with normal saline
Kymriah	Infusion bag (10 - 50 mL)	30 minutes at room temperature	IV infusion - gravity/peristaltic pump	1 - 3 bags	• Prime the tubing prior to infusion with sodium chloride 9 mg/mL (0.9%) solution for injection • Infuse all contents of the infusion bag • Rinse the infusion bag with 10 mL to 30 mL sodium chloride 9 mg/mL (0.9%) solution for injection
Tecartis	Infusion bag (68 mL)	3 hour at room temperature	IV infusion - gravity/peristaltic pump	1 bag	• Prime the tubing with normal saline • Infuse within 30 minutes • Rinse the tubing with normal
Yescarta	Infusion bag (68 mL)	3 hour at room temperature	IV infusion - gravity/peristaltic pump	1 bag	• Prime the tubing with normal saline • Infuse within 30 minutes • Rinse the tubing with normal saline

https://labels.fda.gov/ (accessed: 20-OCT-2023)

Where do we go from here?

- In-use stability requires close collaborations (Pharmaceutical companies: CMC and Clinical Teams, suppliers for administration devices, regulatory agencies, clinical sites)
- Create shared understanding and expectations on in-use compatibility
- Develop phase-appropriate strategies for in-use testing
- Stability-indicating methods that can be used for in-use testing
- Harmonized in-use strategies across the industry (where appropriate) to support these new modalities