Viral safety in cell therapies

A suggested approach for what to include in viral safety packages for cell therapies.

1. Summary

Regulatory agencies expect cell therapy (CT) product manufacturing processes to have viral safety and contamination risk control strategies in place to ensure patient safety.

Viral safety measures using the orthogonal approaches of preventing, detecting and clearing potential viral contaminants is a regulatory expectation provided there is no negative impact on product quality and safety. For cell therapy, these options are limited due to the nature of these products. In most cases, CT product sponsors have to rely more heavily on the prevention and detection pillars of the viral safety strategy as opposed to the clearance pillar. Currently, CT specific guidance related to viral safety is limited and there is uncertainty around what is required for viral safety strategy for cell therapies.

A collaboration of CMC experts in CT manufacturing (18 participants across 10 organisations) has reviewed current viral safety regulations/guidance, discussed critical viral safety aspects in the manufacturing process of CT products, and provided a risk assessment template and structure to assess and mitigate risks for cell therapies with CAR–T product as an example.

Problem

- Regulatory agencies expect cell therapy sponsors to have viral safety and contamination controls strategy in place to assure patient safety.
- Viral clearance options are limited due to the nature of these cell therapy products.
- No clear guidance or approach for viral safety and clearance for cell therapy products.

Activity

A team formed to discuss experiences, challenges and possible solutions.

Publicly available Q3 2025

Output

- Publication with best practices for what to include in viral safety package for cell therapies.
- Will include a matrix of an exemplary cell therapy model. This matrix will map out risk factors and mitigations associated with the cell therapy products.

2. The approach

Scope Cell–based ATMPs and genetically modified cells

01 Review the regulatory background (US/EU) for cell therapy products with respect to viral safety requirements

02 The different viral safety risk factors relevant for cell therapy product manufacturing

03 The evaluation matrix

04 Viral safety risk evaluation of a typical cell therapy product: CAR–T cells

3. Regulatory landscape for viral safety of cell therapy products

The most relevant guidance documents for viral safety of cell therapy products can be found across three different groups:

1. Guidance documents addressing cell–based products directly
2. Guidance documents covering critical raw/ancillary materials used in the production of cell–based products (raw/ancillary materials).
3. General and other product–related viral safety documents frequently referenced in the CT product guidelines and providing suitable details not outlined in the CT product guidelines.

Conclusion

- Most cell therapy specific guidance documents, including related raw/ancillary materials documents, do not provide much specifics on viral safety. They typically make general statements and refer to other documents which contain more detailed viral safety considerations.
- Most frequently referenced guidance documents with more detailed information on viral safety testing:
  - *ICH Q5A (R2): Viral Safety Evaluation of Biotechnology Products derived from Cell Lines of Human or Animal Origin (2023)*
  - *FDA Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications (2010)*
  - *Ph. Eur. 2.6.16: Tests for Extraneous Agents in Viral Vaccines for Human Use (2024)*
  - *Ph. Eur. 5.2.3: Cell Substrates for the Production of Vaccines for Human Use (2018)*

Note: these documents are all part of the "general viral safety" documentation
Exception: *Draft FDA Guidance for Industry: Safety Testing of Human Allogeneic Cells Expanded for Use in Cell–Based Medical Products (2024) Chapter V: detailed information on viral safety testing requirements for the banked product*

4. Viral safety risk factors

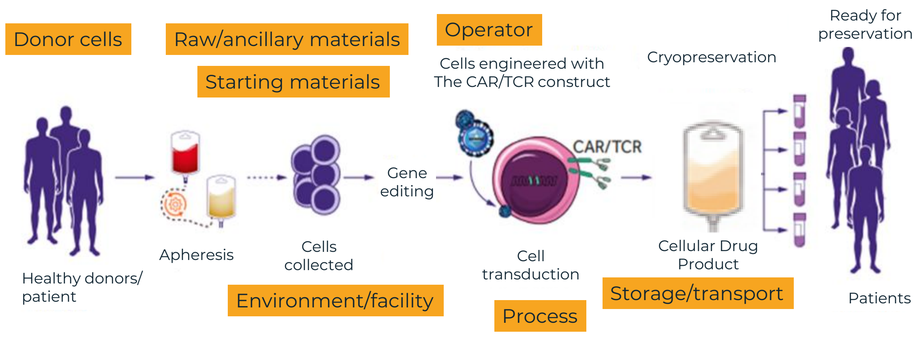
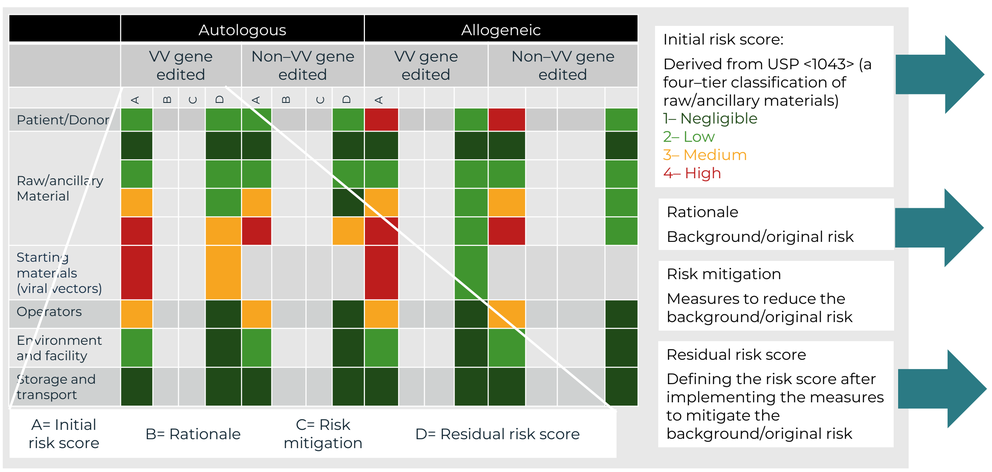


Figure adapted from: DM Bedoya , V. Dutoit, D. Migliorini: Allogeneic CAR T Cells: An Alternative to Overcome Challenges of CAR T Cell Therapy in Glioblastoma. Front. Immunol Vol. 12 –03 Mar 2021 DOI=10.3389/fimmu.2021.640082

The figure above illustrates generic unit operations of an ex–vivo gene–edited CAR–T manufacturing process when starting with primary T cells obtained from a healthy donor or patient. Orange rectangles highlight viral safety risk factors.

5. Overall structure of general evaluation matrix



6. CAR–T risk evaluation matrix (below is an example of the viral vector edited autologous portion of the matrix)

Risk factor	Examples	Initial risk score	Rationale	Risk mitigation	Residual risk score
Patient/donor	N/A	2	Patients own cells; low risk of virus being activated	N/A	2
Raw materials	USP 1043–tier 1 (sterile fluids for injection)	1	• Lowest risk category as per USP<1043> • Low–risk, highly qualified materials with intended use as therapeutic drug or biologic, medical device, or implantable material Note: assumes controls in place for these materials	No mitigation required with exception of check of the Certificate of Analysis	1
	USP 1043–tier 2 (recombinant growth factors, cytokines, tissue culture media)	2	Low–risk, well-characterized materials with intended use as AMs, produced in compliance with GMPs	If satisfied that material is "well characterized", we can accept risk score of 2 and further testing / mitigation activity not required	2
	USP 1043–tier 3 (tissue culture media)	3	Moderate–risk materials not intended for use as AMs (frequently produced for in vitro diagnostic use or reagent grade materials)	• Try to avoid where possible and use tier 1/2 materials if available. • If must be used, consider virus testing of material and implement of viral clearance steps (i.e. filtration/irradiation/heat treatment) before using material. Treat and test impact of treatment on viral reduction potential.	2
	USP 1043–tier 4 (Feeder cells, FBS, Animal or human cells used as feeder layers, animal derived (including human extracts)	4	High–risk materials	• Same as in tier 3, plus • Verify traceability to country of origin • Assure country of origin is qualified as safe with respect to source–relevant animal diseases, including TSE • Adventitious agent testing for animal source–relevant viruses	3
Starting materials (viral vector)	N/A	4	Risk of residual replication competent retro virus and site of integration, human derived Viral vector produced in human cells, manipulated by humans	• Apply ICH Q5A R2 guidance (test viral vector for adventitious agents and recombinant Lentivirus), test raw materials used in process. Apply viral clearance where possible. • Test and characterize cell banks used for manufacturing of Lentivirus viral vectors, in–process testing to be performed, Replication competent testing on bulk harvest or final lot.	3
Operators	N/A	3	• CAR–T cells are human cells– highly susceptible to human virus. If operator has virus, risk virus transmitted to cells. • Manual handling process	• Hygiene measures (gowning, disinfection, engineering controls) • Closed systems if possible • Standard operating procedure	1
Environment and facility	N/A	2	Operators present higher risk for viral entry but still opportunity from environment	• Engineering controls (appropriate ISO/clean room grades based on risk) • Closed processing • Disinfectant of surfaces • Monitoring/routine testing • Humidity and temperature • Pest control	1
Storage and transportation	Frozen product	1	• Frozen product and transported frozen so low risk of contamination • Containers tested for integrity, so risk for contamination is limited • Sterile containers used for storage	• Correct storage temperature (temperature tracking device) • Integrity testing of primary and secondary packaging	1
	Refrigerated	1	Still considered low risk as integrity of packaging will prevent viral entry	• Correct storage temperature • Integrity testing of primary and secondary packaging	1

Note; The above matrix is is a preliminary outcome of discussions. The final content is still in a comprehensive review process and hence may be updated in final publication.

7. Conclusions

- Viral safety and viral safety risk mitigation for cell therapy products need to be defined on a case–by–case basis:
- Individual risk assessments are required for each product/process
- Mitigations can be implemented based on these identified risks
- There may be a significant risk of viral contamination from the starting and raw materials for both autologous and from the donor additionally for allogeneic modalities
- There is virtually no opportunity of risk mitigation steps during the manufacturing process (remove). Viral clearance capabilities are not applicable except for critical raw material production.
- Risk mitigation is achieved by controlling raw material quality, closed processing, operator training (prevention) and testing starting materials and potentially in process.
- The wide variety of cell therapy products (from stem and non–stem cell sources) requires tailored manufacturing and viral safety strategies, with CAR–T principles offering a useful framework for evaluating diverse approaches despite significant differences in materials and processes.
- Special considerations are needed for iPSC–based CAR–T. Although their manufacturing process poses increased viral safety risks due to potential contamination during reprogramming and differentiation, these risks can be effectively mitigated through comprehensive testing of intermediate cell banks using advanced methods like NGS.

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