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REGULATORY WRITING & CONSULTING

CASE STUDY

Fresh vs. Cryopreserved Autologous Cell Therapy Release Testing

Therapeutic Area: Oncology

Product Type: Autologous Cell Therapy

Document: Module 3 - CMC

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Our **Regulatory CMC Strategists** develop sound and defendable strategies to help clients navigate the complex regulatory landscape.

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ABOUT THE CLIENT

Client C developed autologous cell therapy derived from peripheral blood mononuclear cells (PBMCs), activated and expanded ex vivo with an indication for advanced-stage cancer under compassionate use and early-phase clinical trial setting. The manufacturing time was approximately 48 hours from leukapheresis to final formulation with an administration mode of fresh (non-cryopreserved) infusion within 6 hours post-manufacture.

ABOUT THE PROJECT

Challenge: Fresh administration does not permit completion of traditional 14-day sterility testing prior to patient dosing. The client looked to compare the testing strategy and regulatory considerations for fresh, same-day administered autologous product, with no sterility results at the time of administration as well as cryopreserved product, where full sterility and other release criteria are available before administration.

ABOUT THE PROJECT CONTINUED

Fresh Product – Constraints and Additional Testing
Challenge: Sterility testing not available before administration.

- Traditional sterility testing (USP <71>) takes 14 days
- Fresh product has a 6-to-8-hour shelf-life post-manufacture
- Cannot be held back for test results

Additional tests and risk mitigation required - see table on following page for details.

Regulatory Strategy

- Risk-based approach (per FDA and EMA guidance on advanced therapies)
- Justification for release without sterility based on:
- Aseptic process validation
- Rapid testing results
- Clinical need and autologous nature
- Label includes: "Sterility testing in progress at time of release"

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Category	Test or Strategy	Purpose
Rapid Micro Methods	Gram Stain, BacT/ALERT (6-24 hours), endotoxin (LAL)	Early indication of contamination
Environmental Monitoring	Cleanroom enviromental monitoring, personnel monitoring logs	Ensure aseptic conditions were met
In-Process Controls	Media fill validation, aseptic process and simulations	Process validation and batch-specific quality assurance
Donor Screening	Serology for HBV, HCV, HIV, Syphillis	Infection risk reduction
Final Product Testing	Mycoplasma (PCR), Endotoxin (LAL), Gram Stain	Partial assurance of product safety
Retrospective Testing	Sterility test post-infusion (14 day culture)	Informative; does not prevent release
Chain-of-Identity and Custody	Barcoding, reconciliation, dual verification	Prevent administration errors

ABOUT THE PROJECT CONTINUED

Cryopreserved Product – Full Release Panel Possible

Benefits

- Allows:
- Full 14-day sterility test
- Mycoplasma PCR
- Endotoxin
- Potency, identity, and viability
- Greater regulatory confidence in safety profile

Trade-Offs

- Viability loss due to freeze and thaw:
- Approximately 15 to 30 percent viability reduction in some cell types
- Potential potency loss (especially in T-cell therapies)
- May require:
- Cryoprotectants (such as DMSO), which have toxicity concerns
- Thaw logistics and specialized handling at clinical sites

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OPERATIONAL IMPLICATIONS

Factor	Fresh Product	Frozen Product
Viability	High (approximately 90-98%)	Lower (approximately 70-85%) post-thaw
Potency	Preserved	May be reduced
Sterility Assurance	Lower (no results at infusion)	Higher (full results at release)
Logistics	Complex, just-in-time delivery	Easier inventory management
Regulatory Risk	Higher (conditional release)	Lower (full testing completed)

POTENCY AND VIABILITY TESTING CONSIDERATIONS

Test Type	Fresh Product	Cryopreserved Product
Viability	Flow cytometry (7-AAD or PI)	Same, post-thaw
Potency Assay	ELISpot, cytokine release, cytotoxicity	Same, but may require adjustment
Identity	Cell surface markers (CD3, CD4, CD8)	Same
Stability Testing	Not applicable - short shelf-life	Required for thawed product

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CONCLUSIONS

Fresh autologous therapies offer maximum biological activity but pose regulatory and microbial risk due to limited release testing prior to administration.

Cryopreservation allows for full release testing but may compromise potency and viability, affecting clinical efficacy.

Recommendation: Where sterility testing cannot be completed pre-release:

- Employ robust in-process controls
- Use rapid microbial detection methods
- Perform post-release sterility testing
- Justify release through a risk-based approach with regulatory authorities

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