New, LN_2-free solution for cryogenic transport of ATMPs

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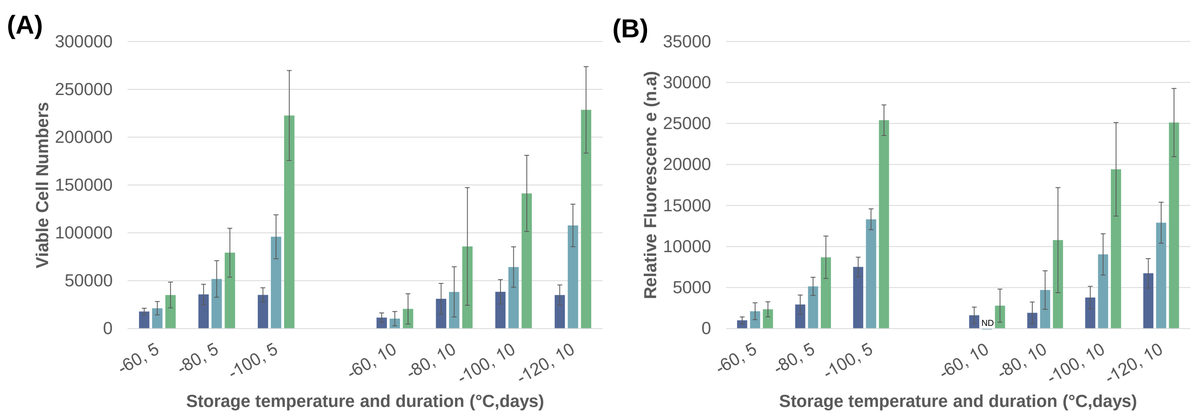
INTRODUCTION

Administration of cell therapies, in clinical trials or as commercial products, involves complex logistics between sometimes numerous partners and sites, making timing critical. Cryopreservation affords extra time and flexibility, but also brings its own challenges. Cryogenic transportation of starting material or finished product is currently carried out in dry shippers that keep contents cold for a period of time using liquid nitrogen (LN_2). If it appears difficult to predict with great confidence the cryogenic standby time offered by such devices, their fast warm-up profile and the requirement of dedicated infrastructure for their re-charge make it challenging to manage unforeseen events and delays during transit. The development of alternative cryogenic shipping devices that are more predictable and easier to prepare, can charge and re-charge during transit, and are hence LN_2-free, is needed. To use such devices, a better understanding of the boundaries not to be crossed, in terms of transit time and temperature, to maintain cell integrity post-thaw is necessary and studied here. We also present the post-thaw cellular outcome following shipping of a cell therapy in an LN_2-free device, the VIA Capsule™ system (Cytiva).

METHODS & RESULTS

Transit time and temperature boundaries definition to maintain cell integrity of cryopreserved samples:

- Storage periods: 5 and 10 days
- Storage temperatures: -60°C, -80°C, -100°C, and -120°C
- Range of cell lines, including an immortalised T cell line (Jurkat) to emulate the behavior of some cell therapies
- Analyses post-thaw: viable cell numbers and metabolic activity after 24, 48 and 72 h of re-culture (*n* = 5)

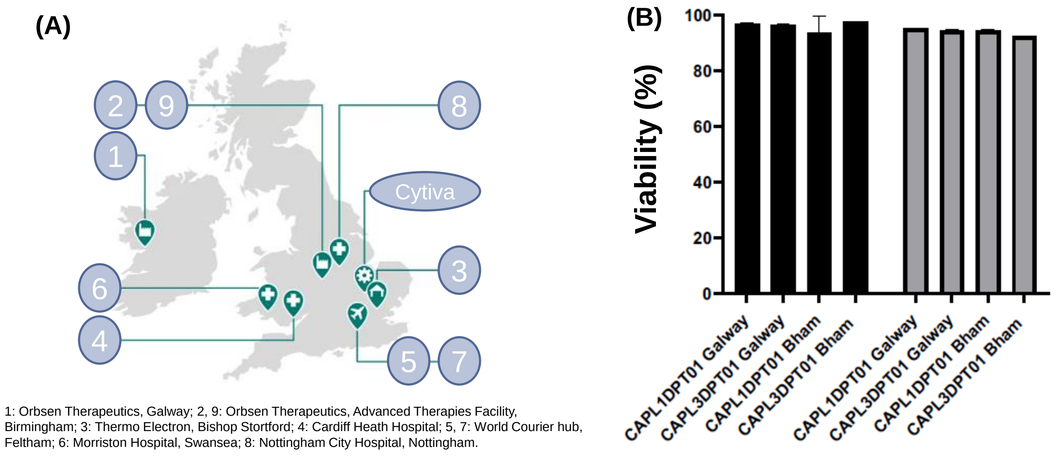


(A) Viable cell number and (B) fluorescence redox functional activity indicating active respiratory metabolism in Jurkat cells, 24 h (dark blue), 48 h (blue), and 72 h (green) post-thaw with simulated transport periods and temperatures. *n* = 5 ± SD; ND: none detected.

A 5-day transit at -100°C did not significantly impair cell parameters post-thaw compared to a 10-day period at -120°C. However, longer transit periods (10 days) at this temperature or at higher temperatures appeared detrimental, and the extent of the impact increased as the temperature gap to Tg' became more important.

Real-life LN_2-free cryogenic shipping test in the VIA Capsule™ system:

- ORBCEL™ patented stromal cell immunotherapy (Orbsen Therapeutics)
- Eight-leg journey across two manufacturing sites and three clinical sites in Great Britain via air and road, between Galway and Birmingham
- Transportation managed by our privileged specialist courier partner, World Courier.
- Cell viability post-thaw measured on untransported versus transported samples (*n* = 2)



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(A) Overview of the eight-leg, multi-party, multi-process journey trial across the UK and Ireland and (B) post-thaw viability of ORBCEL™ measured by nucleocounter (NC-200™, black) or flow cytometry (Sytox™, grey) for un-transported (Galway) versus transported (Bham) samples.

Cryogenic shipping of the ORBCEL products in the VIA Capsule system did not lead to significantly different cell viabilities post-thaw compared to untransported products.

CONCLUSION

Molecular mobility is greatly slowed down in frozen samples, until it comes to a complete halt below glass transition temperature (Tg'), which is approx. -120°C in DMSO-containing cell suspensions and theoretically allows indefinite storage.[1,2] Although greatly reduced, some residual molecular mobility still exists above Tg', which may lead to cellular damage over time and impaired cell integrity post-thaw.

The extent of this damage depends on the time and temperature gap to Tg'. The VIA Capsule system is a safe and predictable LN_2-free cryogenic shipping solution which ensures a 5-day fully passive shipping window below -120°C. Moreover, it is extremely easy to handle and re-charge anywhere using electricity to mitigate any unforeseen event or delay in transit. [3,4]

VIA Capsule system
with cryocooler on and wheels unfolded

VIA Capsule system
with cap on and wheels folded for transport

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