Liquid nitrogen-free cryogenic shipping with a new device disrupting cell therapy logistics: the VIA Capsule™

The Challenge

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). However, the fast warm-up profile and dedicated infrastructure required for the re-charge of such devices makes it challenging to manage unforeseen events and delays during transit. Even at the clinical site, there could be potential issues related to scheduling constraints for the administration of the final product post receipt from the manufacturer.

The Solution

To address these challenges, Cytiva developed the VIA Capsule™ system; a smart LN_2-free cryogenic shipping solution for cell therapies. Instead of LN_2, a Stirling engine cryocooler simply uses electricity to cool the Dewar part of the device in which samples will be loaded, and thanks to a high-quality vacuum and insulation, it gives a standby time of at least 5 days below the key threshold of -120°C[1] in transport mode from full charge. In case a longer standby time proved necessary, access to a power socket and the cryocooler will enable to easily re-charge the system which could then safely maintain valuable cryopreserved therapies indefinitely, until for instance, the patient is ready to receive it.

The Results

1. CSL Behring evaluation trial of the VIA Capsule™

CSL Behring is a USA-based biopharmaceutical company which manufactures biotherapies, with a requirement to be shipped over long distances. CSL Behring was interested in evaluating both the VIA Capsule™ and its supportive charging network provided by World Courier, Cytiva's approved logistics partner for temperature and time sensitive shipments.

The VIA Capsule™ was evaluated in two independent shipment trials within the USA, from California to Pennsylvania, to determine any biological quality impact on cryopreserved CD34 enriched fractions in cryobags due to the shipment. The control remained in LN_2 storage in California. Trial 1 consisted of a direct return air shipment from California to Pennsylvania and back, lasting three days, while Trial 2 followed the same route but included a short-term hold of five days with re-charge of the VIA Capsule™

[1] Meneghel J, Kilbride P, Morris JG, Fonseca F. Physical events occurring during the cryopreservation of immortalized human T cells. PLOS ONE. 2019 May 23;14(5):e0217304.

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at CSL Behring's site in Pennsylvania before returning to the original Californian site. The total length of time the samples spent in the VIA Capsule™ was 3.0 days for Trial 1 and 6.2 days for Trial 2.

Acceptance criteria are qualitative, as the number of samples does not allow for definitive statistical analysis. However, there were no clear differences in viability or recovery between both conditions immediately upon thaw. To evaluate any downstream effects of the shipping methods, cells were recovered from cryopreservation overnight, and analyses were performed again on day 2. Similar trends in cell counts and viability persisted, as well as for total viable CD34+ cells. Additionally, there were no differences in relative purity between samples for either trial.

The data gathered from the two evaluation trials suggests no differences in post-thaw cellular parameters between transported and control samples. These results highlight the ability of the VIA Capsule™ system to maintain cryopreserved cellular products within a cryogenic temperature range. Under the conditions in which these 2 shipment tests were undertaken, the VIA Capsule™ therefore emerged to CSL Behring as a viable cryogenic shipping solution for cryopreserved cell products.

Additional advantages of the VIA Capsule™ shipper were highlighted by CSL Behring:

- In case the receiving site does not have LN_2 capabilities, the VIA Capsule™ system is able to maintain cryogenic temperatures indefinitely (provided there is A/C power available for charging the device).
- The smaller physical footprint of the VIA Capsule™ shipper in comparison to a transportation-capacity equivalent dry shipper is significant, allowing quicker and simpler sample handling and loading.
- Not relying on a third party for device availability.
- No safety hazards associated with liquid nitrogen handling.

This work was led by CSL Behring and Cytiva in collaboration with World Courier; data, evidence and conclusions presented with kind permission from CSL Behring. For more information, see: https://www.cytivalifesciences.com/en/se/solutions/cell-therapy/knowledge-center/resources/liquid-nitrogen-free-cryogenic-shipping-of-cell-products.

2. VIA Capsule™ versus dry shipper for the cryogenic transport of a cryopreserved T-cell line suspension

An immortalised T cell line (Jurkat, Clone E6-1, TIB-152) was cultured and cryopreserved in closed-system cryovials (cell density: 10^6 cells/mL; fill volume: 6mL; DMSO concentration: 5% v/v). There were 3 batches, with 3 replicates in each, of which one batch remained in cryogenic storage as un-transported control, the others transported across the MW-ATTC sites in England and Wales (Cambridge – Birmingham – Cardiff – Swansea – Birmingham – Cambridge) either in a VIA Capsule™ or a LN_2 vapour MVE dry shipper. Shipments were managed by World Courier, with a duration of 4 days.

At the end of the shipment period, the transported samples were returned to cryogenic storage until thaw and subsequent analyses for viable cells counts and metabolic activity after 24, 48 and 72h of culture post-thaw (a detailed experimental procedure can be found in Meneghel *et al*. 2019[1]). Statistical analyses were performed using the R software (version 4.0.2) and the Rcmdr package as follows: distributions were first tested for normality and homogeneity of their variances (Shapiro-Wilk and Bartlett tests, respectively). The means of normally distributed groups were compared according to one-way ANOVAs, using the Welch T-test for groups with heterogeneous variances, while groups that did not

meet the normal distribution criterion were compared using the Kruskal-Wallis rank sum test. Confidence level for all tests was set at 0.95. The results obtained are displayed in Figure 1 below (n=3 ± SD).

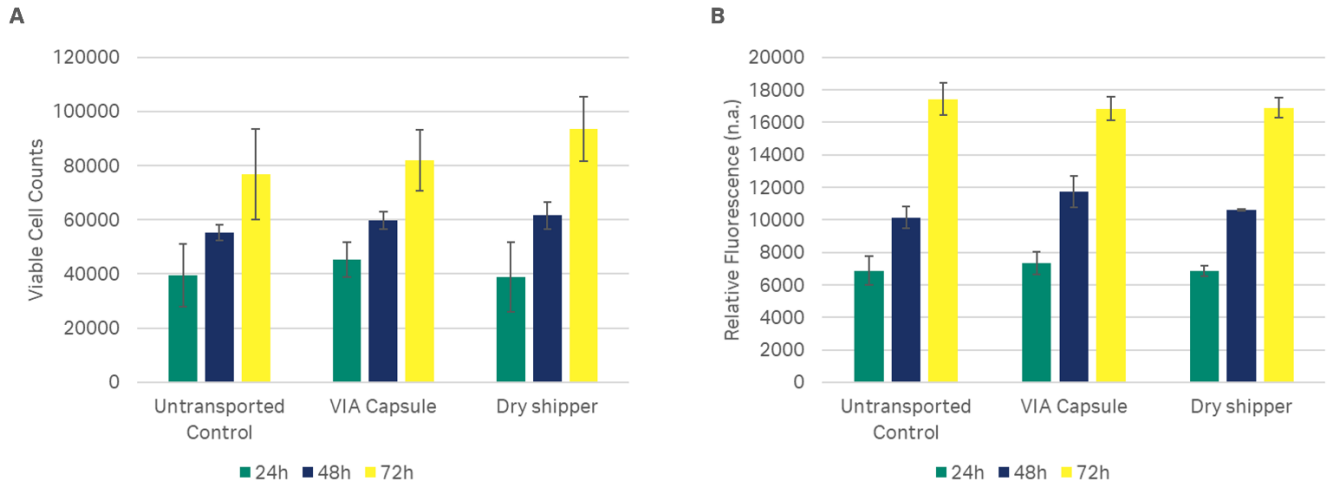

Figure 1. Impact of cryogenic shipping condition on cryopreserved Jurkat cell samples: **(A)**, average viable cell counts and **(B)**, average metabolic activity. Both parameters were measured after 24h (green), 48h (blue) or 72h (yellow) of culture post-thaw (n=3 ± SD; *p*-values > 0.06).

There were no statistically-significant differences observed for viable cell counts and metabolic activity between the shipping conditions tested at each timepoint of analysis (after 24h, 48h or 72h of culture post-thaw; *p*-values > 0.06). This indicates that these parameters were not impacted by cryogenic transport, whether it be in a traditional LN_2 dry shipper or in the LN_2-free VIA Capsule, and neither was the cells' proliferation ability over 78h of culture post-thaw.

This demonstrates that the LN_2-free, electrically powered VIA Capsule™ is a viable alternative to conventional LN_2 dry shippers for cryogenic shipping of biological material.

This work was conducted to support an ATTC Industry partner (Orbsen Therapeutics) during transport tests in preparation for clinical trials; with thanks to contributing NA-ATTC & MW-ATTC partners, especially World Courier and the University of Birmingham. This Industry partner will utilise the VIA Capsule as part of the POLARISE clinical trial taking place across ATTC clinical sites including Newcastle and Birmingham.

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Making Further Impact

The unprecedented collaboration between industry and NHS partners across the ATTC Network has enabled Cytiva to identify barriers associated with cryogenic shipping and successfully develop an appropriate technological solution. This has ultimately enabled the accelerated commercial launch of the VIA Capsule and creation of a formal partnership with World Courier. A pilot to establish the routine use of the VIA Capsule in the NHS for receipt and storage of cellular products is being rolled out with an NA-ATTC partner; this will address an NHS capacity constraint which ultimately should support adoption of licenced products that rely on cryopreservation.

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