Define and address the challenges and barriers to effective end to end supply chain management: LN2-free shipping project

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Funded by

UK Research and Innovation

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CATAPULT Cell and Gene Therapy

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1. Introduction

This plan of work investigates how implementation of liquid nitrogen-free (LN2-free) shipping technology could simplify the logistics of shipping Advanced Therapy Medicinal Products (ATMPs) between NHS sites and the cell therapy manufacturer.

1.1 Purpose

This report describes the field work conducted in collaboration between Barnsley's NHS Blood and Transplant (NHSBT), the Pharmacy department of St. James's University Hospital (St. James') part of Leeds Teaching Hospitals NHS Trust (LTHT), and Cytiva to explore and test Cytiva's LN2-free shipping technology in the CAR-T workflow. And ultimately, to determine whether the use of novel shipping technology improves the supply chain related to cryo shipments of ATMPs between the hospital and cell therapy manufacturer, via NHSBT.

1.2 Background

Currently, the CAR-T vein-to-vein process flow involves the shipment of starting material either fresh from the hospital to the cell therapy manufacturer or, in case it needs to be cryopreserved, this is the stem cell lab's responsibility [1]. In our case, the stem cell lab is NHSBT Barnsley, where the starting material therefore needs to be shipped for analysis, processing and cryopreservation. The cryopreserved starting material is then shipped to the manufacturer in a dry shipper. Once the final therapeutic product is manufactured and cryopreserved, it is shipped back to NHSBT for short-term cryogenic storage until the hospital sends a request for the issue of a CAR-T product. Then, NHSBT issues the CAR-T product in a dry shipper and their internal courier service delivers it to the clinical area 'just in time' for administration to the patient. Pharmacy has the responsibility to verify and issue the CAR-T product for infusion, before it is thawed and administered to the patient. See Appendix 1: 'CAR-T vein-to-vein process flow' [1] for more details.

- The earlier part of the workflow, when cryopreservation is deemed necessary (shipping starting material out of the hospital, to the stem cell lab and then to the cell therapy manufacturer), presents a level of complexity that it is difficult to consider making any changes to it. For instance, if the stem cell lab's role was moved back to the clinical site with a LN2-free controlled-rate freezer (VIA Freeze™), sample analysis, processing, labelling etc. would still need to be performed, which require the stem cell lab's facilities and expertise.
- The later part of the workflow concerning the shipping of the final product from the manufacturer back to the clinic via the stem cell lab may however potentially benefit from substituting steps with Cytiva's LN2-free shipping technology, the VIA Capsule™ system.

1. Introduction

A more detailed investigation into the cryo logistics of returning the final product back to the hospital includes 3 major steps:

- Leg 1: from manufacturer to NHSBT, using manufacturer's dry shipper fleet to transport the cryopreserved CAR-T product
- Short-term storage at NHSBT, where NHSBT transfers the CAR-T product from manufacturer's dry shipper to cryo tank
- Leg 2: from NHSBT clinical area, using NHSBT's dry shipper fleet to transport the cryopreserved CAR-T product.

Transferring samples from one device to another carries a risk of breaks in the products' chain of identity, as well as staff burden in terms of preparation of the dry shippers, and procedures to follow. Performing these 3 steps using a single device capable of transporting and storing cryogenic samples would therefore constitute a significant simplification to the current workflow. Cryobiologists at Cytiva recognised this challenge when designing the VIA Capsule™ system. Cooled using a mains powered cryocooler, the VIA Capsule™ shipper is a smart, liquid nitrogen-free cryogenic shipper for autologus cell therapies. Able to hold cryogenic temperature whilst 'on-charge' with a cryocooler adds the capacity to use the VIA Capsule™ shipper for temporary cryogenic storage, as shown in Figure 1. Chronicle™ automation software allows monitoring of the VIA Capsule™ shipper remotely in real time (e.g. the temperature inside the chamber and other shipping parameters) thanks to a data logger connected to the VIA Capsule™ shipper. It also allows the booking of shipments and supports the use of electronic Standard Operating Procedures (eSOP), thereby logging information related to the sample loading and unloading in/from the shipper in a GMP-compliant manner. Tasks associated with eSOPs are indeed automatically stamped with the operator's name, the date and time; a second operator can verify each task, and in case any deviations are recorded, 2 review teams may check and comment on these. The VIA Capsule™ system and Chronicle™ software from Cytiva were made available to collaborators from NHSBT and St James' to explore the suitability of these products to ship and receive cryopreserved CAR-T products.

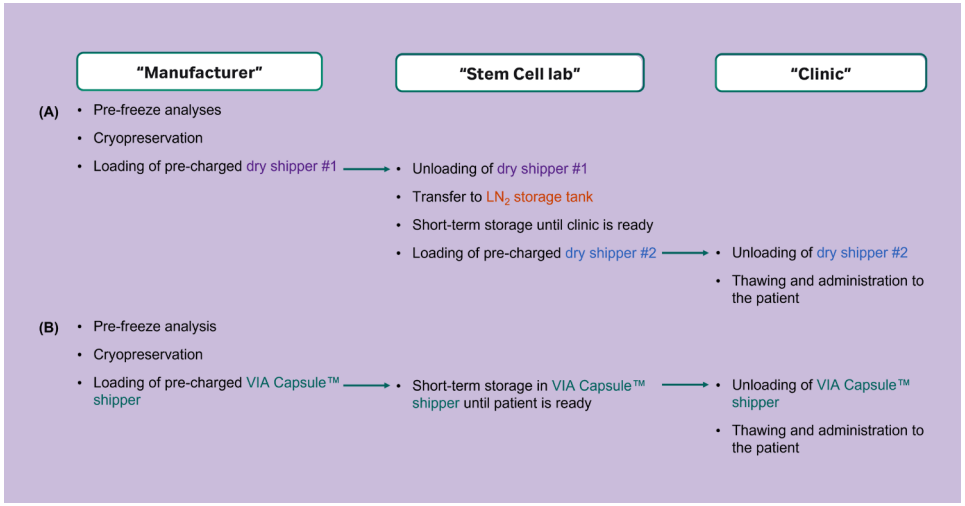


Figure 1: Logistics elements of the shipping of final, cryopreserved CAR-T products from Manufacturer to Clinic via Stem Cell lab according to (A) the current, 'CAR-T vein-to-vein process flow' presented in Appendix 1 with products transitting from a dry shippers to a liquid nitrogen (LN2) storage tank and to a second dry shipper, or (B) an alternative, LN2-free shipping process flow using the VIA Capsule™.

1. Introduction

1.3 Disposition of the study

A VIA Capsule™ system (Dewar + cryocooler) equipped with a data logger, and Chronicle™ software with logistics eSOPs for loading and receiving samples were used for this study.

A face-to-face introduction and training on how to operate the VIA Capsule™ system, navigate Chronicle™ software and use its eSOPs was given to the partners before starting the field work. In addition, a mock shipping run was organised with an empty VIA Capsule™ shipper at ambient temperature.

The VIA Capsule™ shipper was loaded with cryopreserved samples from cryostorage at NHSBT by NHSBT's cell therapy advanced specialist by following an eSOP, then held onsite for short-term storage for a few hours to up to a few days until transported by NHSBT's courier service to the Pharmacy department of St. James'. There, it was received by St. James' pharmacist following another eSOP containing NHS's CAR-T product receipt checklist [2]. The following day, the VIA Capsule™ shipper was shipped back to NHSBT using 2 other eSOPs to log these processes. Upon delivery back to NHSBT, the samples would be transferred back to cryostorage (tank).

This run was performed 3 times, with different samples being shipped each time. For each run, a matching cryobag and a cryovial were maintained in cryostorage, thereby acting as non-transported controls. All samples were thawed and analysed altogether upon completion of all 3 shipping runs.

1.4 Terminology

Term	Comment
7-AAD	7-aminoactinomycin D
ATMP	Advanced Therapy Medicinal Product
CCM	Complete culture medium
CD	Cluster of differentiation
CRF	Controlled-rate freezer
DMSO	Dimethyl sulfoxide
eSOP	electronic Standard Operating Procedure
FDA	Fluorescein diacetate
FITC	Fluorescein isothiocyanate
GMP	Good Manufacturing Practice
HPC-A	Hematopoietic progenitor cells collected by apheresis
HSA	Human serum albumin
LTHT	Leeds Teaching Hospitals NHS Trust
LN_2-free	Liquid nitrogen-free
NHSBT	NHS Blood and Transplant
PE	Phycoerythrin
St James'	St. James's University Hospital

2. References

Ref. ID	Document Name	Document Reference(s)
[1]	NHSBT CAR-T vein to vein process flow	N/A
[2]	Pharmacist guide on receiving licensed CAR-T cell products in clinical areas	N/A

3. Experimental

3.1 Materials

VIA Capsule™ system:

- Dewar – catalog number: 29435427, S/N: SHP0520202
- Cryocooler – catalog number: 29435429, S/N: CRY0821202
- Thermal core for bags – catalog number: 29483894
- Sample holder for cryobags – catalog number: 29435430

2x data loggers:

- Sendum PT300D – catalog number: 29435205,
- S/N: OP4118032916212; Device identifier: 99000512112998
- S/N: OP4118032933412; Device identifier: 99000512113863

Samples transported:

- NHSBT's cryopreserved hematopoietic progenitor cells collected by apheresis (HPC-A) from 3 different donors in cryobags of different sizes and fill volumes and in cryovials
- Cytiva's cryopreserved Jurkat cell suspensions in 5% DMSO in 50mL-type cryobags, 10mL fill volume; and 2mL cryovials, 1mL fill volume. Note: these were not thawed and analysed at the time this report was written up, so they are not mentioned in the methods and results sections thereafter.

NHSBT's Chronicle™ software instance was used: pre-gmp.chronicle.bio

3. Experimental

3.2 Methods

3.2.1 Logistics

Shipment data and eSOPs were collected on Chronicle™ software. Shipment data include: temperature inside the VIA Capsule™ shipper, GPS location, tilt, light, etc. The data and graphs can be viewed directly on Chronicle™ software as a map and multiple graphs, one per shipment parameter, for each shipment (see Appendix 2); for the purpose of this report, data associated with shipments to St. James' and back to NHSBT, as well as any short-term storage period, were exported to a .csv file to draw multiple shipment parameters into a single graph.

3.2.2 Biological samples

Historic cryopreserved HPC-A cells from three different deceased donors who consented to allow samples to be used for research and development or service development work within NHSBT were used in this work. All of these samples were then cryopreserved in 10% DMSO in a LN2 controlled-rate freezer (CRF) (Planer PLC) following NHSBT's internal standard operating procedures.

3.2.3 Thawing

After completion of the field work, frozen samples (1 cryobag and 1 cryovial per run and per control) were removed from the liquid nitrogen storage vessel and placed in a water bath set at 37°C. Samples were agitated gently until the last ice crystal thaws. Thawed samples were transferred to sterile tubes and post thaw-analyses were performed immediately. Every sample was run in duplicate in the post-thaw cell analyses.

3.2.4 Post-thaw analyses

Full blood count was performed using Sysmex XS 100i haematology analyser (XS-100i Sysmex, Kobe, Japan) to determine the samples' white blood count (WBC) on a volume of 100-500 µL. Samples were then diluted with human serum albumin (HSA) to achieve WBC counts between 1-2x107 cells/mL.

The percentage of CD3+ and CD34+ cells viability was determined by flow cytometry. Briefly, for each sample to be tested, two Trucount tubes were used: 'T1' or 'T2'. A volume of 10 µL of CD3-PE or CD34-PE, CD45-FITC and 7-AAD were added to T1 and T2. A volume of 50 µL of diluted test sample was then added to T1 and T2. The tubes were mixed by gentle agitation and incubated at room temperature in the dark for 15 minutes. Freshly prepared 1 mL of lysing solution (1X diluted in deionised water) was then added into each tube and mixed by gentle agitation. The mixtures were then incubated at room temperature in the dark for 5 minutes before they were analysed using Navios flow cytometer. ISHAGE method gating strategy was used for the identification of CD3+ or CD34+ cell populations as detailed in **Figure 2.**

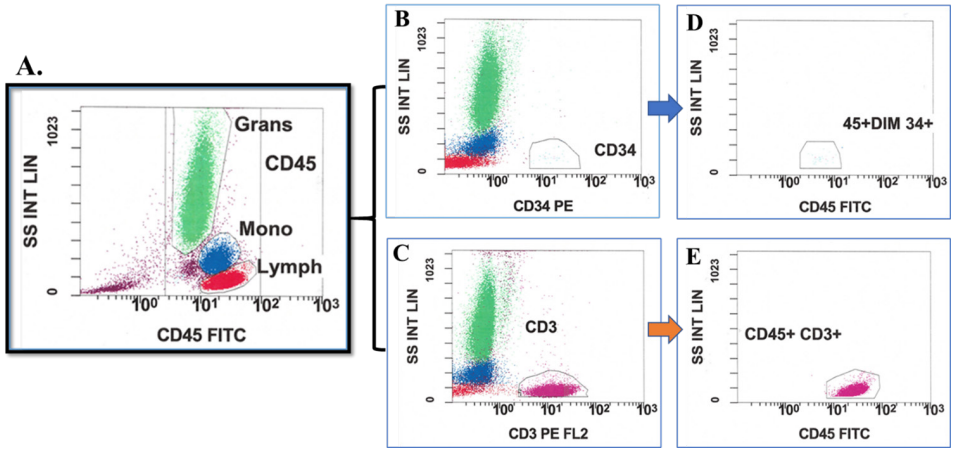


Figure 2: Gating strategy for identification of CD3+ and CD34+ cell population using ISHAGE method to identify CD45+ cells (gate CD45), Granulocytes (gate Grans), Monocytes (gate Mono) and Lymphocytes (gate Lymph) (A) the CD45+ population identified in (A) was then used to identify CD34+ (B) and CD3+ cells (C). The CD34+ and CD3+ populations identified in (B) and (C) were finally corrected by elimination of false CD34+ (D) or CD3+ cells (E), respectively.

4. Results

4.1 Mock shipping run & learnings

A mock shipping run with an empty VIA Capsule™ shipper was organised for the partners to better appreciate the device in conditions close to reality, to also familiarize NHSBT's courier service with it and check whether and how to load it into their van. It was decided the VIA Capsule™ shipper should be transported on wheels, rather than in its transport box. This makes it easier to manoeuvre in the loading bays of both NHSBT and St. James', especially if a single courier driver is on duty and couldn't carry it by himself (pallets aren't available in the van as NHSBT's courier service would only typically carry small 'ice boxes'), and to secure it in a corner of the van with straps after folding 2 of its wheels, as shown in **Figure 3.**

At the NHSBT site, it was decided samples would be inserted into a bubble wrap pouch, placed back into the LN2 storage tank for a few seconds to 'freeze-close' the pouch – which is the internal practice when shipping out cryopreserved samples – and then into the sample holder and inside the VIA Capsule™ shipper.

At the Pharmacy site, a deeper look at the VIA Capsule™ shipper and particularly at how to reach out to samples inside it revealed that long thermal gloves would be required for the pharmacist to wear for the real shipping runs. This is to protect their arms, exposed with the typical short-sleeved clinician's gown, as opposed to cell therapists' lab coat.

This mock run also allowed discussion about the eSOPs content for loading and receiving of the VIA Capsule™ shipper, which will be adapted accordingly for the real shipping runs.

Figure 3: Picture of the VIA Capsule™ shipper loaded and strapped inside NHSBT's courier van for the mock run.

4. Results

4.2 Shipping runs

4.2.1 Run #1

Shipping run #1 took place without major issues. Participants were able to ship samples out and back, to connect to Chronicle™ software and follow eSOPs, although some scanning tasks could not be completed due to missing labels. A number of comments for improvements to the upcoming runs were also made:

- The bubble wrap pouch used to fit samples into was too thick which made it difficult to load into and remove from the sample holder; an alternative solution using thin biohazard plastic bags for the upcoming runs was suggested and implemented
- Changes to eSOPs to make them shorter and better fit for purpose.

Figure 4 shows some VIA Capsule™ shipper parameters measured throughout run #1: the temperature inside the chamber, the device's tilt angle and light recorded by the data logger. In particular, the temperature inside the VIA Capsule™ shipper comfortably remained below the -120˚C threshold to ensure stability of cryopreserved cell suspensions1, 2; samples having reached a maximum of approx. -160˚C before being transferred back to cryogenic storage upon return from St. James'. The VIA Capsule™ shipper also remained upright throughout with tilt values oscillating around 0˚ in shipping mode (i.e. when transport cap is fitted), meaning that it was not laid on its side; more noisy tilt values were recorded when on charge (between 0 and up to approx. 80˚) however this is due to the vibrations caused by the working cryocooler. Finally, the 'light' parameter showed low values throughout the run, except at a couple of timepoints. These timepoints correspond to the start of the shipment from NHSBT to St. James' and when unloading/receiving it at St' James', possibly when the compartment located at the back of the VIA Capsule™ shipper where the data logger sits was opened (for instance when checking the serial number of the data logger used), and could therefore be used as a remote indicator of preparation of the VIA Capsule™ shipper ahead of a shipment and its receipt and handling at the destination site.

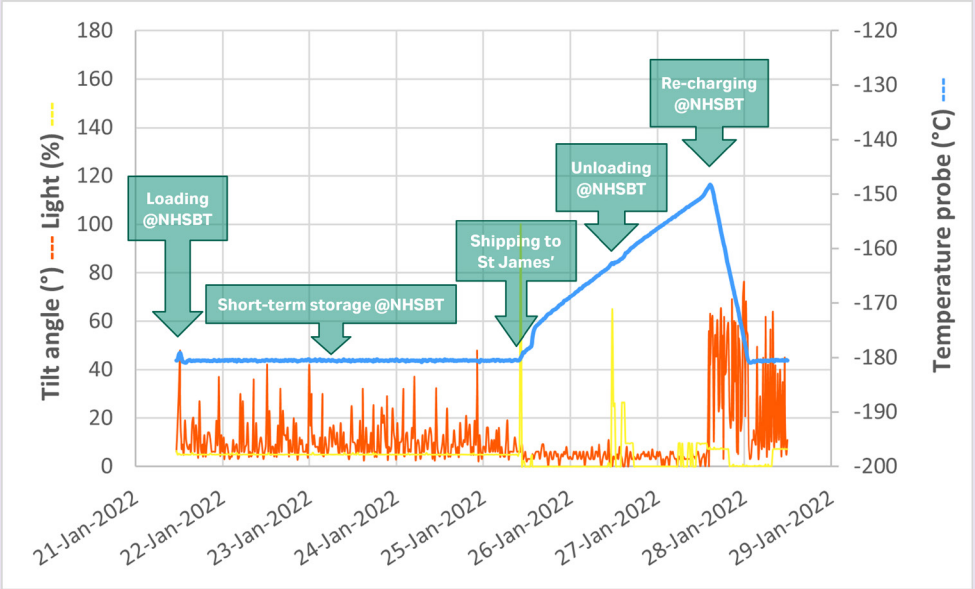


Figure 4: Temperature inside the VIA Capsule™ shipper and tilt angle and light measured by the data logger throughout shipping run #1 including short-term storage of sampes at NHSBT and return shipment to St. James' and back to NHSBT.

[1] Meneghel J, Kilbride P, Morris JG, Fonseca F. Physical events occurring during the cryopreservation of immortalized human T cells. *PLOS ONE*. 2019;14(5):e0217304. doi:10.1371/journal.pone.0217304

[2] Kilbride P, Meneghel J, Fonseca F, Morris J. The transfer temperature from slow cooling to cryogenic storage is critical for optimal recovery of cryopreserved mammalian cells. *PLOS ONE*. 2021;16(11):e0259571. doi:10.1371/journal.pone.0259571

4. Results

4.2.2 Run #2

Shipping run #2 proceeded more smoothly with the changes made (thin plastic pouch for samples and eSOPs) and **Figure 5** shows some VIA Capsule™ shipper parameters measured throughout run #2: the temperature inside the chamber, the device's tilt angle and light recorded by the data logger. Screenshots presented in **Appendix 3** show part of the updated receipt eSOP 'Receiving at Pharmacy (product receipt checklist)' that the pharmacist was tasked to follow upon receipt of the VIA Capsule™ shipper.

Again, the temperature of the samples remained comfortably below -120˚C, having been transferred back to cryostorage at approx -160˚C; tilt values remained very low while in shipping mode, and the light parameter peaked when preparing the device ahead of the shipment, when received at St. James' and once back at NHSBT when unloading the samples.

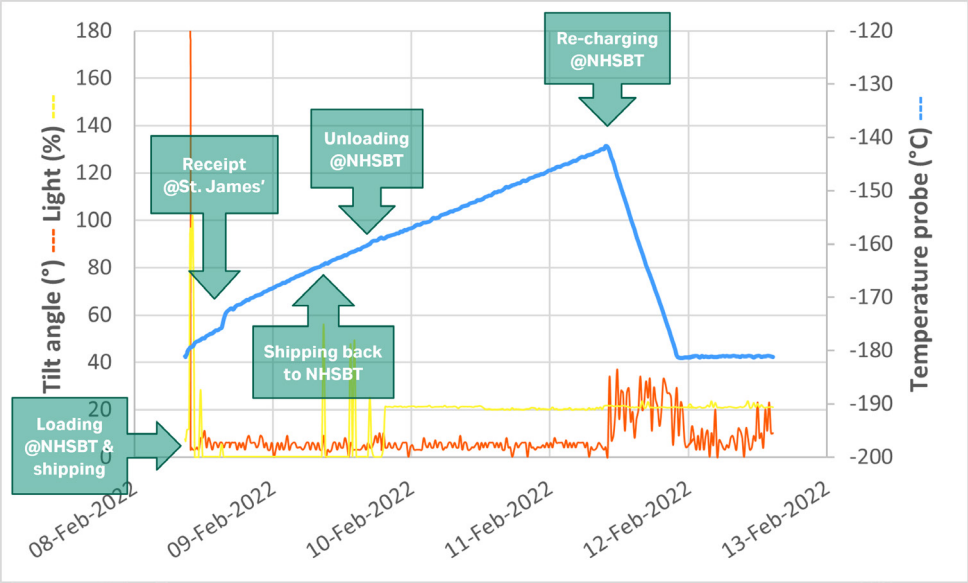


Figure 5: Temperature inside the VIA Capsule™ shipper and tilt angle and light measured by the data logger throughout shipping run #2 from NHSBT to St. James' and back.

4. Results

4.2.2 Run #3

Shipping run #3 was initiated on the 14th February with samples loaded at NHSBT site. However, it had to be put on hold shortly afterwards for SARS CoV-2 related reason, therefore the samples remained inside the VIA Capsule™ shipper for short-term storage until the run could resume, as shown in **Figure 6**. The run resumed two weeks later and the VIA Capsule™ shipper was prepared for transport on the 28th February and shipped to St. James' the following day (see **Figure 6**).

Upon arrival at St. James', the samples unfortunately happened to be stuck inside the VIA Capsule™ shipper chamber and the Pharmacists weren't able to take them out and go through the product receipt checklist. The receipt eSOP was completed, though, mentioning this issue as comments. The VIA Capsule™ shipper was returned to NHSBT where staff just about managed to get the samples out. The samples were transferred to cryostorage, and the VIA Capsule™ shipper put on charge. The temperature remained again comfortably within limits, samples having been transferred as their temperature reached again approx. -160°C.

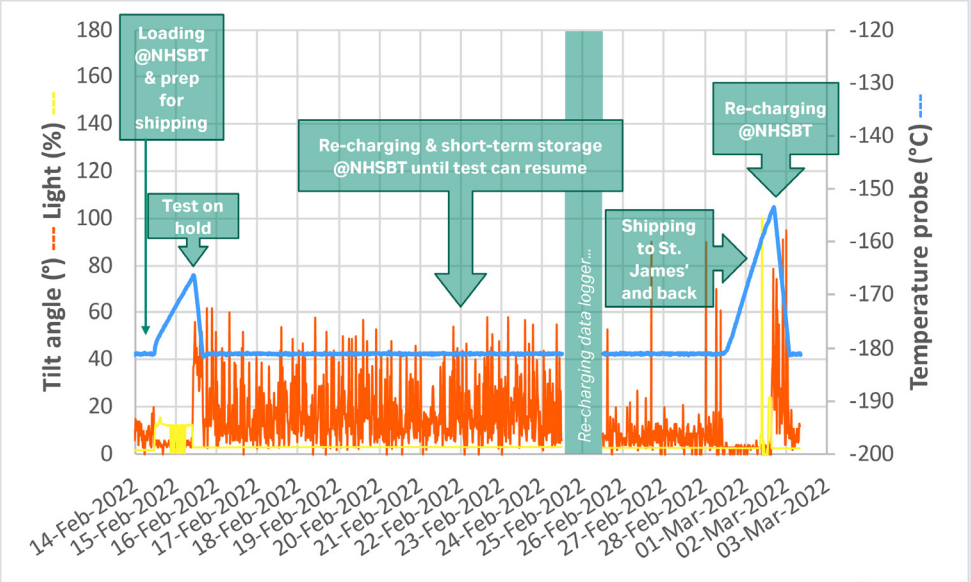


Figure 6: Temperature inside the VIA Capsule™ shipper and tilt angle and light measured by the data logger throughout shipping run #3 from NHSBT to St. James' and back.

4. Results

4.3 Biological results

For each shipping run, a set of HPC-A samples from a donor, consisting of a cryobag and a cryovial was shipped in the VIA Capsule™ shipper, while another, matching set of samples from the same donor donation was kept in LN2 cryostorage. These were all thawed and analysed for CD3+ and CD34+ cell viability, and the results are shown in **Figure 7** below (the raw data is presented in **Appendix 4**). Note: similar sets of cryopreserved Jurkat cell suspensions were also prepared by Cytiva and shipped or kept untransported alongside NHSBT's HPC-A samples, but these haven't been thawed and analysed at the time this report is being written.

CD3+ cell viability post-thaw was comprised between 86 and 93%, and CD34+ cell viability between 65 and 97%. The release criteria on cell viability for cryopreserved products where tested should be ≥70% (for both CD3+ and CD34+ cells), meaning that HPC-A sample cryobags corresponding to Run #2 may not have been released, should they have been intended to treat a patient, as CD34+ cell viability from this run's thawed cryovials was: 65.80% for the control, and 65.63% for the transported counterpart – although concessionary issues on samples that do not meet release criteria can be done on request of the Principal Investigator. We do not have access to pre-processing data to these donations, but it would have been interesting to see if this data was low for this sample as well, the release criteria on fresh products both pre- and post-processing being ≥ 90%, for both CD3+ and CD34+ cells.

It is worth noting that, regardless of having been shipped or not, viability of both CD3+ and CD34+ cells measured from cryobags samples (with 90.8% ± 2.6% and 86.1% ± 8.3%, respectively) was consistently higher than from cryovial samples (with 89.8% ± 2.5% and 79.8% ± 11.3%, respectively). This observation is not statistically significant though (T test p-values: 0.5 for CD3+ cell viability and 0.3 for CD34+ cell viability), but this may be due to the small sample size (n=6).

This highlights the fact that the cryopreservation protocol applied may be more adapted to cryopreserve HPC-A samples in cryobags rather than in cryovials. This phenomenon has been observed previously in cord blood samples, where quality control tubing segments of lower volume than the bulk cryobags, and nucleating at higher subzero temperature, were therefore subjected to a higher degree of supercooling, resulting in poorer cell outcome post-thaw3. This could be the case here as well where cryovials – typically contain approx. 1mL of cell suspension – despite having been cryopreserved alongside cryobags in a CRF, would have nucleated at a later timepoint of the protocol than cryobags - typically containing a volume of 10 to 100mL (or more). Cryovials would therefore experience more extensive temperature fluctuations as latent heat is released upon ice nucleation and bulk crystallisation of the sample, and subsequently as catching up again with the CRF's temperature, as compared to cryobags.

When comparing samples having been shipped versus the controls, it therefore makes more sense to distinguish cryobags and cryovials in light of the above observation. Overall, the samples used in each run showed very similar cell viability results post-thaw, both in cryobags (T-test p-values: 0.6 and 0.9 for CD3+ cell and CD34+ cell viability, respectively) and cryovials (T-test p-values: 0.8 and 0.8 for CD3+ and CD34+ viability, respectively). Some fluctuations can be observed, in some cases the transported and control samples were virtually equal (e.g. CD3+ cells of run #1 in cryobags as well as CD34+ cells of run #2), in other cases transported samples showed higher cell viability post-thaw than the control samples (e.g. for CD3+ cells of run #1 in cryovials and of run #3 in cryobags and cryovials, as well as CD34+ cells of run #1) or the opposite (e.g. CD3+ cells of run #2 as well as CD34+ cells of run #3), but this is likely to be attributed to biological and/or technical variability, rather than being due to the shipping process.

4. Results

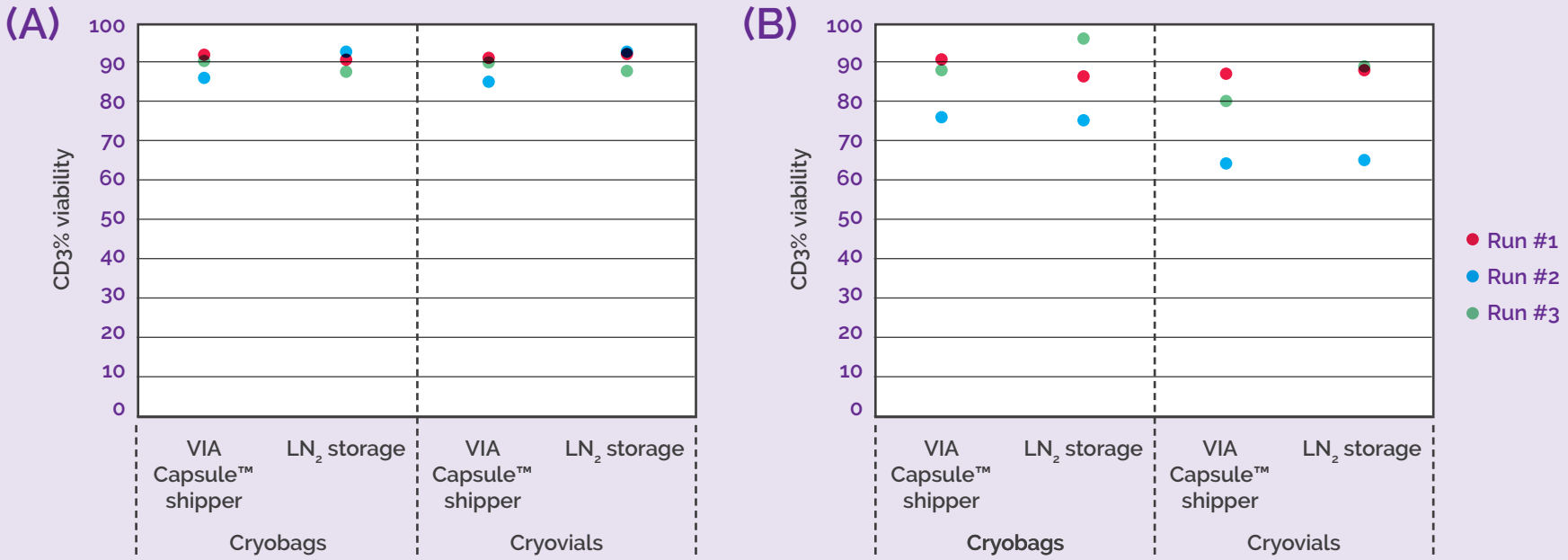


Figure 7: CD3+ (A) and CD34+ (B) cell viability post-thaw from matched pairs of hematopoietic progenitor cell samples collected by apheresis (HPC-A) in cryobags and cryovials; one cryovial and one cryobag were transported in the VIA Capsule™ shipper from NHSBT, Barnsley to St. James' Hospital Pharmacy, Leeds and back to NHSBT ('VIA Capsule™ shipper' condition), while the other cryobag and cryovial were kept at NHSBT in cryogenic storage ('LN_2' condition) as controls.

5. Discussion / comments

One of the major challenges faced on 2 out of the 3 shipping runs was related to the sample holder meant to make loading and unloading of samples in and out of the VIA Capsule™ shipper easier. This is made of cardboard, and its handle has been ripped off when trying to pull it out as it was stuck. Under intended use of the VIA Capsule™ shipper, only 1x large cryobag sample in cassette should be fitted in the sample holder, or up to 3x small 50mL-type cryobags in cassettes. Here, to maximise this field work, cryobags and cryovials were loaded and shipped during each shipping run, causing the sample holder to be overloaded and so difficult to lift out of the VIA Capsule™ chamber.

Even though the use that was made of the device during this field work does not comply with its intended use, this issue is important and will be taken into consideration for future improvement of the VIA Capsule™ system, by e.g. changing the material of the sample holder to something more resistant to tear, and/or developing a system – like a metallic platform sitting at the bottom of the VIA Capsule™ chamber connected to a rod – to pull sample(s) out.

Nevertheless, benefits from using this LN2-free cryogenic shipper over a dry shipper were identified. Dry shippers being usually delivered 'just in time' to the clinical area, this practice is therefore subject to a number of risks.

First, there is the risk of delays in transport from the stem cell laboratory in this just-in-time practice, and shipping the therapy in a VIA Capsule™ shipper a day prior to infusion for instance rather than on the day would minimise this risk. Second, upon delivery of a therapy in a dry shipper, the pharmacist is called to go to the ward and check the product and paperwork there and then. This can cause delays for the ward staff and patient.

The patient is already prepared for infusion so if there are any issues e.g. missing paperwork, then there is an urgency to resolve this in order to release the treatment as soon as possible. In this context, using the VIA Capsule™ shipper could ease some of that pressure by being shipped in advance to Pharmacy and allowing the pharmacist to perform their checks at a time and place more convenient than a busy ward environment. In the event where an issue is detected then, more time would be available to rectify it, therefore reducing both the risk of delaying the nursing staff and anxiety for the patient.

In the event where patient treatment has to be delayed at the last minute, the product can stay at Pharmacy in the VIA Capsule™ system for a short period of time (within the device's standby time), and then subsequently administered, or returned to the stem cell laboratory.

Third, Pharmacies generally do not have capability for storing dry shippers and/or pharmacists generally wouldn't have received training on handling them, or have expertise in cell therapy as not equipped with liquid nitrogen infrastructure. Being inherently less hazardous than a dry shipper, the VIA Capsule™ would make it possible. Requirements include a suitable location in pharmacy to securely store the device, appropriate space to unload and check the contents, iPad or similar device to complete the eSOPs, and training on using the device including handling cryogenics products.

Fourth, this solution would free up some storage space in the stem cell laboratory and reduce their staff work burden by making it possible to consider shipping straight from the manufacturer to the hospital pharmacy, especially as the cell therapy market grows.

Fifth, the eSOP system was also considered as an improvement to paper-based SOPs, by potentially reducing the risk of human error, particularly in a busy context where staff would have to prepare and handle multiple ATMPs a day.

6. Conclusions

A small number of biological samples were used in this LN2-free shipping project, however no difference in the post-thaw viability of CD3+ and CD34+ cells from HPC-A samples could be observed between those having been transported in the VIA Capsule™ shipper from NHSBT to St. James' and back, as compared to control samples that remained in cryogenic storage. Another conclusion from this work is that cells from these HPC-A samples, particularly CD34+ cells, cryopreserved in cryobags survived the cryopreservation procedure better than when cryopreserved in cryovials, in line with other works on cord blood samples.

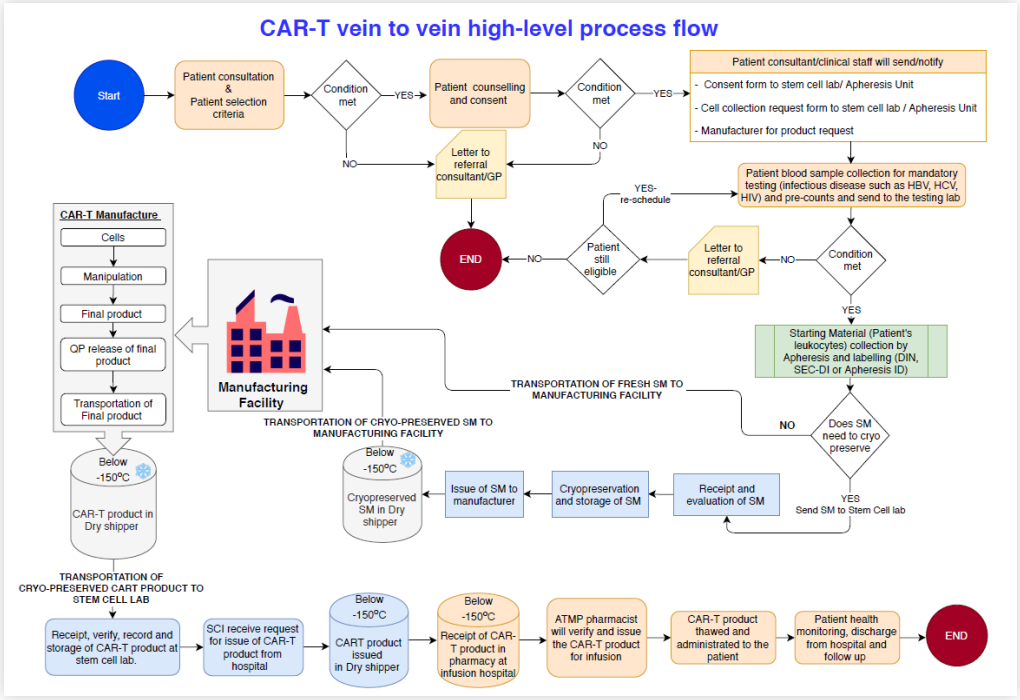
From this work, the VIA Capsule™ shipper with eSOPs was found suitable at transporting cryopreserved cell therapy products to a hospital pharmacy prior to infusion. Some improvements to this solution are needed, in particular a more robust and fit-for-purpose sample holder to make sample loading and unloading easier. After staff training and hands-on practice, the VIA Capsule™ system with eSOPs from the Chronicle™ software would potentially alleviate many of the logistics and safety challenges associated with dry shipper just-in-time delivery of cryopreserved cell therapies to clinics that do not have an on-site stem cell laboratory service and liquid nitrogen infrastructure.

Repeating a similar shipping work using more HPC-A samples, and/or different sample types, would be necessary to confirm the biological validation of this solution, though. Including a second shipping condition in such a future work using a dry shipper alongside the VIA Capsule™ shipper to transport cryopreserved cell samples, would be ideal.

7. Appendix

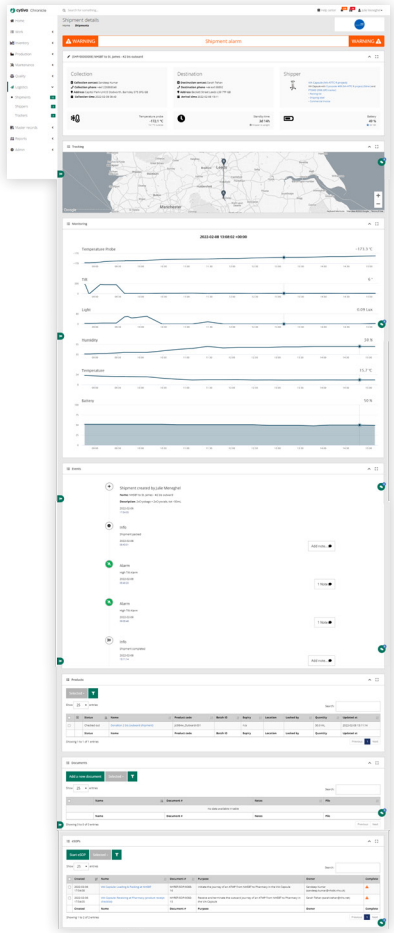
Appendix 1:

CAR-T vein-to-vein process flow [1]. Process step background colours refer to various locations: Hospital/Infusion centre in orange, Therapeutic Apheresis Services (TAS) in green, Stem Cells and Immunotherapies (SCI) in light blue and the Manufacturer in light grey.



Appendix 2:

Screenshot of the 2nd 'NHSBT to St. James' shipment taken from Chronicle™ software showing the shipment details (origin & destination), map, graphs of the evolution of key shipment parameters, shipment events and any alarms, the product(s) shipped, any document(s) and eSOPs for loading/unloading associated with the shipment.



7. Appendix

Appendix 3:

Screenshot of the Pharmacy receipt eSOP taken from Chronicle™software, showing some tasks the pharmacist of the field work had to complete in order to receive the samples shipped from NHSBT. Full eSOPs are available upon request.

VIA Capsule: Receiving at Pharmacy (product receipt checklist)

1 Purpose

Receive and terminate the outward journey of an ATMP from NHSBT to Pharmacy in the VIA Capsule

2 Description

This eSOP describes the tasks to follow to:

- Receive the VIA Capsule sent from NHSBT with the ATMP at the Pharmacy department of St James' Hospital.
- Perform the product receipt checklist
- Check that all required documentation has been received.

This eSOP assumes the starting point is a VIA Capsule in shipping mode at the Pharmacy department of St James' Hospital, i.e. transport cap on.

Before starting this eSOP, please ensure that you:

- Have the appropriate PPE at hand and wear it when necessary (in particular: thermal gloves with long sleeves to protect your arms).
- The QR codes labels corresponding to the VIA Capsule and the outward ATMP sample transported in the VIA Capsule.
- Bring your laptop along to follow the tasks of this eSOP!

3 Instruments

Manufacturer	Model	Additional notes
Cytiva	VIA Capsule	

4 Procedure

Task 1 — Select shipment. (Shipment automatically selected - no further action required from operator) — Incomplete

Task 2 — Take the VIA Capsule somewhere convenient and lock its wheels. — Incomplete

Task 3 — Scan the VIA Capsule QR code label. — Incomplete

Task 4 — *Starting NHS' 'CAR-T product receipt checklist' here to verify the integrity of the shipper and the documentation.* These checks are mixed with other tasks, and highlighted in *italics* for ease of differentiation. — Incomplete

Task 5 — *Tamper-evident ties intact?* — Incomplete

Task 6 — Cut, remove the tamper-evident tie(s), and indicate the seal number(s) here. — Incomplete

Task 7 — *Transit temperature logger checked on receipt?* The data logger (4) is located in the compartment at the back of the VIA Capsule (1), and secured with straps (2); it should be connected to the VIA Capsule temperature probe (3) as shown below: — Incomplete

Task 8 — Press on the control panel button and ensure that the current temperature inside the VIA Capsule is low enough. When in transport mode, the VIA Capsule should remain below -120°C. Is the temperature indeed lower than -120°C? — Incomplete

Task 9 — *All required documentation received?(1/4)* • *Temperature log* — Incomplete

Appendix 4:

Post-thaw CD3+ and CD34+ cell viability data for donor lymphocyte infusion samples in cryobags and cryovials having been transported in the VIA Capsule™ shipper from NHSBT, Barnsley to St. James' Hospital, Leeds, and back, versus untransported controls having remained in cryogenic storage (LN_2 storage).

	CD3+ cell viability (%)				CD34+ cell viability (%)			
	Cryobags		Cryovials		Cryobags		Cryovials	
	VIA Capsule™ shipper	LN_2 storage	VIA Capsule™ shipper	LN_2 storage	VIA Capsule™ shipper	LN_2 storage	VIA Capsule™ shipper	LN_2 storage
Run #1	92.62	92.51	92.09	88.77	90.68	87.22	87.94	88.61
Run #2	86.68	93.26	86.13	93.02	76.34	76.10	65.63	65.80
Run #2	91.12	88.58	90.15	88.39	89.19	97.06	81.29	89.47
Average	90.14	91.45	89.46	90.06	85.40	86.79	78.29	81.29
stdev	3.09	2.51	3.04	2.57	7.88	10.49	11.45	13.42