Manufacture of a Commercial Cell Therapy Product
– From Donor to Patient

10th October 2019
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ALOFISEL® (DARVADSTROCEL): OVERVIEW

- Allogeneic Stem Cell Therapy – One of a kind, first allogeneic stem cell therapy approved by EMA.
- Used to treat non-healing perianal fistulas, caused by Crohn’s Disease.
- Treatment consists of 4 Vials, each containing 6mL at a concentration of 5 Million eASCs/mL for a total of 120 million cells.
- Administered locally by a surgeon in the tissue surrounding the fistulas.
- Currently manufactured at Madrid site (previously TiGenix).
- Being tech transferred to Grange Castle for manufacturing by 2020.
eASCs/MSCs – A Quickstart Guide

• eASCs (expanded Adipose-Derived Stem Cells), more commonly known as MSCs (Mesenchymal Stem Cells) are adult stem cells derived from adipose or bone marrow tissue.

• They exhibit certain properties of therapeutic interest and are multipotent (can differentiate into fat cells, bone cells or cartilage).

• They are plastic adherent.

• They also are also considered “immuno-privileged” and will secrete anti-inflammatory cytokines (cell signals) in pro-inflammatory environments.

• This is the primary mode of action of Alofisel (Immunoregulation).

• For Alofisel, starting tissue is procured via lipo-aspiration from female donors in Madrid.
**Raw Materials**

Trypsin – porcine-derived, used to detach cells from Cell Factories (CF5s).

FBS (Foetal Bovine Serum) – bovine-derived, key raw material containing growth factors to supplement media.

DMAX – chemically defined Media in which cells are grown.

**Excipients**

DENV (DMEM no Phenol Red) – Similar to DMAX, has no red colouring, used for final centrifugations and as excipient.

HSA (Human Serum Albumin) – Stabilising agent, used as excipient, derived from blood plasma product.
Isolation and Expansion of eASCs to produce ALOFISEL® Master Cell Stock P1 + P2

- After Lipo-aspirate starting material is received, it is centrifuged and the middle phase containing adipose tissue (stromal vascular fraction) is removed.
- Collagenase is added to break down tissue and release more ASCs.
- This is then added to a T175 cell culture flask and cultured with media (DMAX, 10% FBS) and antibiotics (Pen-Strep).
- This is the isolation step, cells which adhere to the plastic of the culture flask are the ASCs, any residual cells suspended in media are not wanted.
- After a week of incubation, the cells are detached using trypsin and a cell count is performed.
- These cells are then placed at a set density of in CF5 flasks. Grown for 2 – 4 Weeks, harvested and reseeded into a higher number of CF5s, these are then grown for 2 – 4 weeks before harvesting and cryopreservation.
- Currently done at the Madrid Site, Tech Transfer to Grange Castle at a later date.
**Expansion of eASCs to produce ALOFISEL® Frozen Drug Substance (P3 + P4)**

- MCS vials are thawed quickly, centrifuged and resuspended before seeding into CF5s.
- The cells are grown in culture media (10% FBS, DMAX) for 2 – 4 weeks.
- **Week 1** - always a media change, waste culture media is removed and fresh culture media is added.
- **Week 2** - a “Witness” vessel is harvested by adding trypsin and incubating, this is considered a control CF5 representative of the entire batch.
- Trypsin is inactivated by adding the same amount of culture media and the trypsin/culture media mix containing the cells is collected into a falcon tube.
- A cell count is performed, if there are more than the target number cells, the entire batch is harvested. If there are not more than the target number, a media change is performed.
- **Week 3** – Repeat of **Week 2**
- **Week 4** – Batch is harvested regardless and re-seeded into a higher number of CF5s.
Expansion of eASCs to produce ALOFISEL® Frozen Drug Substance (P3 + P4)

- The P4 of FDS manufacturing takes the harvested cells from P3 and re-seeds into a higher number of CF5s.
- Proceed exactly as P3 of FDS manufacturing. Witness Vessel cell count criteria for harvest at this stage is population doublings.
- If criteria is met or on 4th week, harvest the entire batch.
- Cells are harvested and cryopreserved in the same way as MCS manufacturing.
- There is potential for process improvements by using previous growth trends for given donors to remove witness harvests.
Cryopreservation of ALOFISEL® Frozen Drug Substance

- All CF5s are harvested, each CF5 produces 200mL containing cells in 225mL falcon tubes. The tubes are then centrifuged, suspended and unified.

- The unified cell suspension is then centrifuged and resuspended in “Freezing Media” (90% FBS, 10% DMSO).
- The cell suspension is then filled into cryovials at a set cell density with 1mL per vial.
- These vials are then frozen in a controlled rate freezer and placed in a cryotank for long-term storage.
Recovery of FDS to produce ALOFISEL® Final Product (P5)

- FDS Vials are thawed, the number of cells in the vials can vary batch-to-batch and vial to vial.
- QC department thaw a vial of each FDS batch and perform cell count to give better idea of actual number of cells per vial.
- Cells required at thawing is higher than at harvesting for Final Product.
- Once thawed a cell count is performed.
- If the count does not meet the required number of cells, additional cryovials may be requested.
- Cells are seeded at a 30x higher density than previously.
- The aim of this stage (P5) is to allow cells to recover in culture from cryopreservation, not expand. The limit on PDs for Final Product is 16, the cells are seeded at this high density to ensure there is little to no space for them to expand.
• At 7 Days ± 1 day, the cells are ready for harvest. The CF5s are harvested in the same manner as before.
• The cells are centrifuged and resuspended in DMEM with no phenol red, from this point no animal-derived products are used.
• A cell count is performed, acceptance criteria is number of cells per treatment and population doublings with a limit of 16 PDs.
• The cells are then centrifuged.
• Using the cell count data, we can calculate how much final excipient solution to re-suspend the cells in to get a final concentration of 5M/mL cells. The final excipient solution is HSA and DMEM no phenol red.
• 6mL of the final product solution are then filled into each vial, 4 vials per treatment up to 3 treatments.
• Product has 48-Hour Shelf-Life
ALOFISEL® - Process Specific Challenges

• Largely manual, high-risk of contamination due to the nature of the process.

• Main mitigation for this is a high-level of technologist training and expertise.

• Production of one treatment is over months of manufacturing (though from FDS stock, final product can be made to order in 7 days.

• Full Cleanroom facility, scheduling of cleaning rotas, shift patterns and training.

• Requires an in-depth knowledge of MSCs (eASCs), their growth properties and morphology.

• 48-Hour shelf-life, on demand, made to order treatment.
Thank you, questions?