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ADVERSE IMPACT OF NH_4CL-BASED ERYTHROREDUCTION ON T CELLS

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BACKGROUND

- Typical QbD study evaluations of new cell processing platforms assess cell yield, viability, purity and proliferation but do not evaluate relevant functional characteristics of the cells that relate to their efficacy.
- Excellos has developed a cell functional characterization platform that evaluated cell effector potential, metabolic fitness and memory potential termed ***E360*** or ***Escore*** (Fig. 1).
- Isolation and enrichment of PBMCs from leukopaks of healthy donors frequently utilizes NH4Cl to remove contaminating RBCs by lysing them and the functional impact of NH4Cl on T cells in these isolates exposed to this type of procedure has not been evaluated.
- As part of our QbD process to evaluate various PBMC enrichment platforms and procedures, we evaluated 3 methods of PBMC enrichment from healthy donor leukopaks.
- Pre- and Post-processing cell samples from each of these processes were assessed for yield, viability, purity and various functional characterization parameters.

MATERIALS AND METHODS

- The general study design is shown in Fig. 2.
- 3 methods of PBMC enrichment were evaluated using matched healthy donor leukopaks.
  - Manual ficoll (F), an automated microfluidics-based system (MF) and an automated differential centrifugation-based system (DC).
- Pre- and post-processing analyses for cell yield, viability and post-cryopreservation proliferation were carried out.
- Additional T cell functional characterization using Excellos' proprietary ***Escore*** platform, which assesses effector potential and metabolic fitness was carried out.
- Tyrosine phosphorylation and mitochondrial mass assessments were carried out.

RESULTS

- Cell yield and viability were acceptable for all 3 isolation methods (Fig. 3).
- Post-thaw viability was acceptable for all 3 isolation methods (Fig. 4).
- Differences were noted in post thaw proliferation, with the DC product having less PBMC and T cell proliferation than F or MF-based isolation methods (Fig. 5).
- Both MF and DC systems had similar effects on T cell effector function and were superior to manual Ficoll (F) (Fig. 6, left panel).
- However, the DC system decreased metabolic fitness compared to F and MF systems (Fig. 6, right panel)
- NH_4CL-treated T cells also had decreased Tyrosine phosphorylation in T cells compared to control or Ficoll treated cells (Fig. 7).
- NH_4CL-treated T cells also had decreased mitochondrial mass compared to control or Ficoll treated cells (Fig. 8).

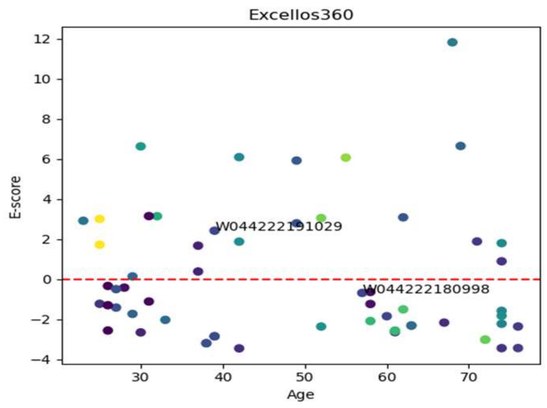
FIGURE 1. *Escore* Donor characterization

A Proprietary ***Escore*** Algorithm has been developed

Multiple weighted donor factors are incorporated into the ***Escore*** platform

- Donor demographics (Age, BMI)
- Effector potential (PSI)
- Metabolic fitness (Glycolysis, PER)
- Memory potential (Proteomic subset analysis/FACs)

High ***Escore*** and low ***Escore*** donors can be identified



Enables identification of preferred donors for starting material

Variable within healthy young donor cohorts

Donor ***Escore*** >0: Acceptable

Donor ***Escore*** <0: Unacceptable

FIGURE 2. Study Design – 3 Replicates

PBMC Isolation Process Metrics

- Manufacturability
- Cell viability and yield

T Cell Product Quality

- T Cell purity and yield
- Cell proliferation
- Cell metabolism/Effector potential
- Mitochondrial mass, Tyrosine phosphorylation

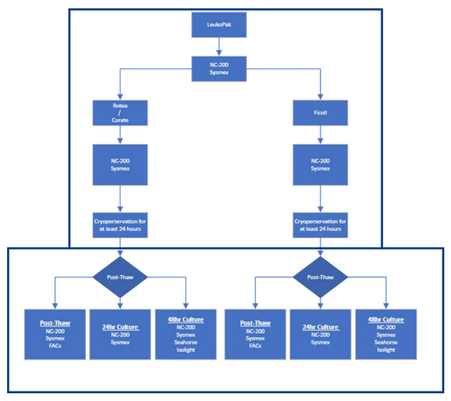


FIGURE 3. PBMC Isolation Data

QC following PBMC isolation

- Cell viability after processing was comparable between all systems
- MF and DC methods showed superior WBC purity
- MF showed superior overall WBC recovery to other systems

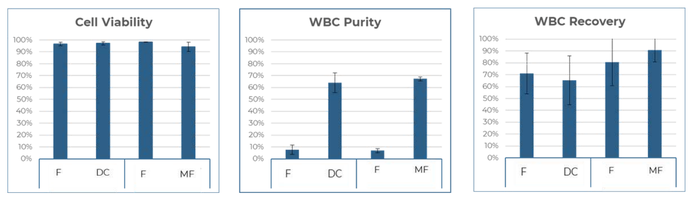


FIGURE 4. Post-Thaw Viability

After thawing, PBMC samples were cultured for 24 and 48 hours. Pan-T cells were isolated from the cultured PBMC samples

- Cell viability was comparable between systems and time points

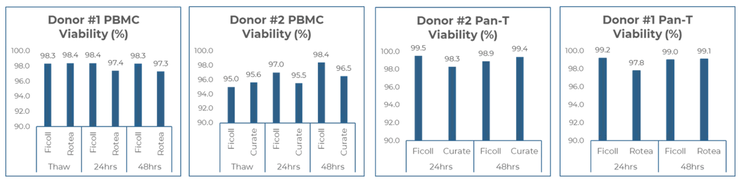


FIGURE 5. Culture Expansion Cell Yields

- The DC process had a lower PBMC yield while the MF process had higher PBMC yields after culturing
- The DC process showed lower CD3 Pan-T cell yields than the MF-based process

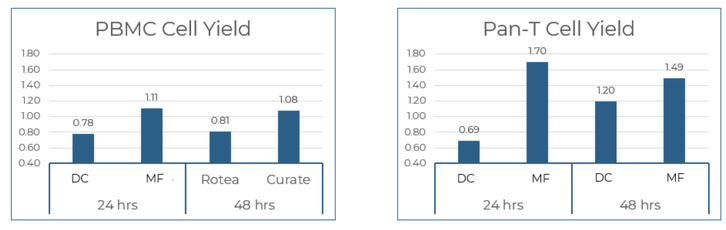


FIGURE 6. Pan-T Cell Phenotype Analysis

- Both MF and DC systems showed slight improvements in PSI values compared to manual Ficoll(F)
- The DC system showed a significant decrease in glycolysis potential

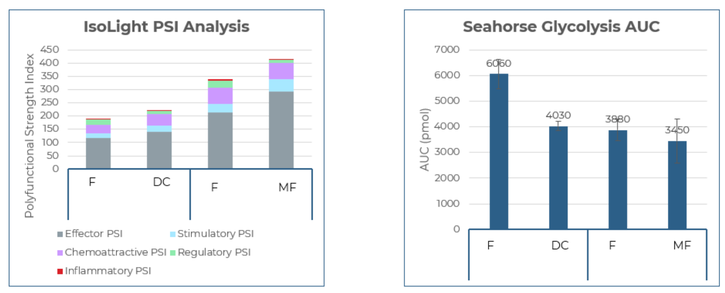


FIGURE 7. NH_4Cl effect on T Cell Tyrosine Phosphorylation

- NH_4Cl exposure caused a significant decrease (~30-40%) in Phosphorylation compared to control and Ficoll treated T cells

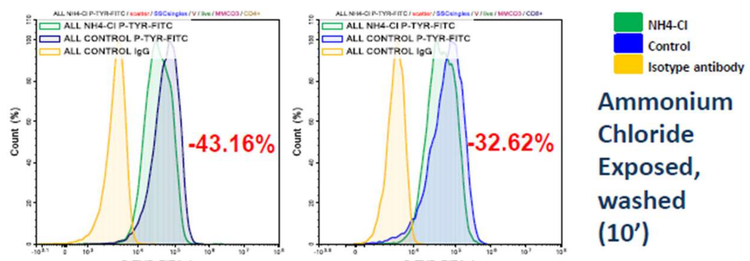
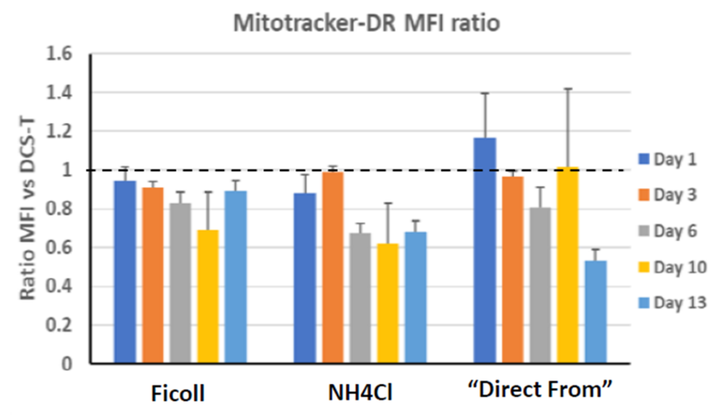


FIGURE 8. NH_4Cl Mitochondrial Mass Effects

- NH_4Cl treatment caused a decrease in mitochondrial mass compared to Ficoll or untreated T cells



DISCUSSION

- Typical QbD platform assessments for cell therapy processing focus on yield and viability and do not assess impacts on desired cell functions.
- These data show that a standard practice of removing erythrocytes from PBMC and T cell preparations can have deleterious impacts on the cells, including metabolic, proliferative, signal transduction via phosphorylation and mitochondrial mass effects that may impact manufacturability, and potency of the cell therapy final product.
- These data suggest that deeper QbD analyses that include key functional attributes may reduce the risk of manufacturing failures or decrease clinical potency of the cell therapy products.

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