Simplifying Progress

A novel defined animal component free (ACF), protein free (PF), salt base cryopreservation solution, composed of 5% DMSO designed for cell- based therapies.

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Introduction

Cryopreservation is a crucial step for long term storage of cell-based products and for "off-the-shelf" cell therapy approaches. To date, the common practice is to use cryopreservation solutions composed of 10% DMSO or other toxic permeable cryoprotective agents (CPA), such as Ethylene Glycol. Exposure of cells to these CPAs can impact the quality, safety, and efficacy of the cellular product and clinical outcome

Facing strict regulatory requirements, the development of a defined cryopreservation solution with a reduced concentration of DMSO is required and holds a unique opportunity to advance the widespread implementation of cell-based therapies.

The current study presents the performance of NutriFreez D5, a novel defined ACF, PF, salt base cryopreservation solution with a reduced concentration of DMSO (5%). Post thaw cells viability, growth recovery and cell characterizations of human mesenchymal stem cells (hMSC), pluripotent stem cells, and immune cells were evaluated after being frozen in NutriFreez D5. Continued work is invested in the development of the next generation, DMSO-free cryopreservation solution with non-toxic CPA alternatives.

1. NutriFreez D5 is optimal for hMSC

hMSC are increasingly being used in cell therapy-based applications and clinical trials. Cryopreservation is a crucial step for a scalable manufacturing process for hMSC therapies. Nutrifreez D5 was developed for optimal cryopreservation of hMSC. Figure 1 represents a comparison of the impact of different cryopreservation solutions on hMSC-BM, directly post thawing (cells viability) and after being cultured for 3 days in XF culture system (cells viability and proliferation). hMSC-BM were cryopreserved at conc. of 200-500 x10^3 cells /ml for 7 days in different cryopreservation solutions followed by growth recovery evaluation. Results show that the novel salt based freezing solution, NutriFreez D5 (composed of 5% DMSO) promotes high viability and recovery of hMSC-BM with similar performance as NutriFreez D10 (composed of 10% DMSO) and with advantage over commercially available freezing solutions composed of either 5 or 10% DMSO.

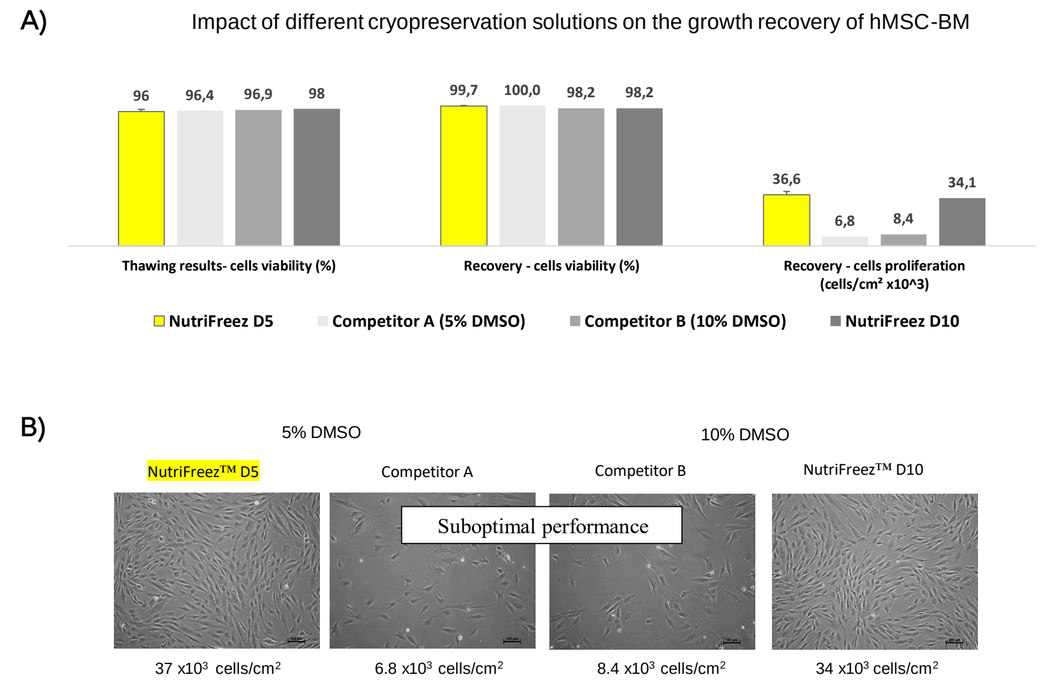

Figure 1: Growth recovery results of hMSC-BM post cryopreservation with NutriFreez D5 vs. NutriFreez D10 as well as other commercial cryopreservation solutions composed of either 5 or 10% DMSO. (A) Post thaw cell viability (%), recovery cell viability (%) and proliferation (viable cells count). (B) Representative images (x100) taken 3 days post seeding (cells recovery). The numbers represent the proliferation (viable cells count)

2. Maintaining of hMSC features

In order to evaluate the maintenance of hMSC unique features post cryopreservation, hMSC-BM were frozen in NutriFreez D5 followed by thawing (after being frozen for 7 days) and seeding in XF culture system (MSC NutriStem XF) for evaluation of hMSC features (tri-lineage differentiation potential, self- renewal potential, marker expression and genomic stability). The differentiation assays were done using MSCgo™ differentiation media.

The results indicate that hMSC after being frozen in NutriFreez™ D5 maintain tri-lineage differentiation potential, self-renewal potential, classical profile of MSC markers with low percentage of hematopoietic contamination and genomic stability.

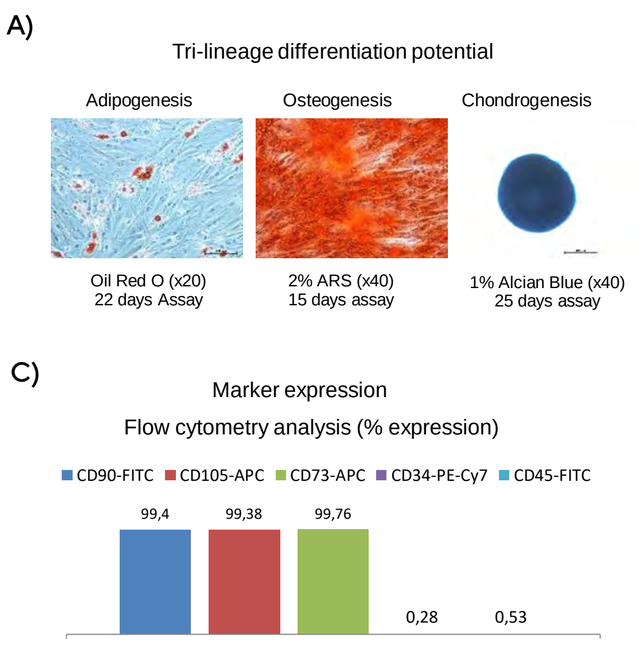

Figure 2: (A) Representative images of stained adipocytes (x20) after 22 days assay, stained osteocytes (2% Alizarin Red, x40) after 15 days assay, and stained chondrocytes (Alcian Blue, x40) after 25 days assay. (B) Representative image of mature colonies (>50 cells /colony) stained with 0.5% Crystal violet after 10 days of CFU-F assay. (C) Immunophenotyping results (summary of marker expression) of hMSC-BM using flow cytometry analysis. (D) G-banding karyotyping analysis of hMSC-BM.

3. Human embryonic stem cells cryopreservation

Safe and stable cryopreservation is critical for research involving human Pluripotent Stem Cells. The suitability of NutriFreez D5 to cryopreserve human embryonic stem cells was tested utilizing hESC cultured as single cells in XF culture condition (NutriStem hPSC XF) on laminin matrix (LN521). The assay was done in comparison to NutriFreez D10. hESC were cryopreserved (in triplicates) at conc. of 200 x10^3 cells /ml for 11 days in each cryopreservation solutions followed by evaluation of growth recovery and maintenance of hESC characteristics. NutriFreez D5 found applicable for hESC and with similar performance as NutriFreez D10.

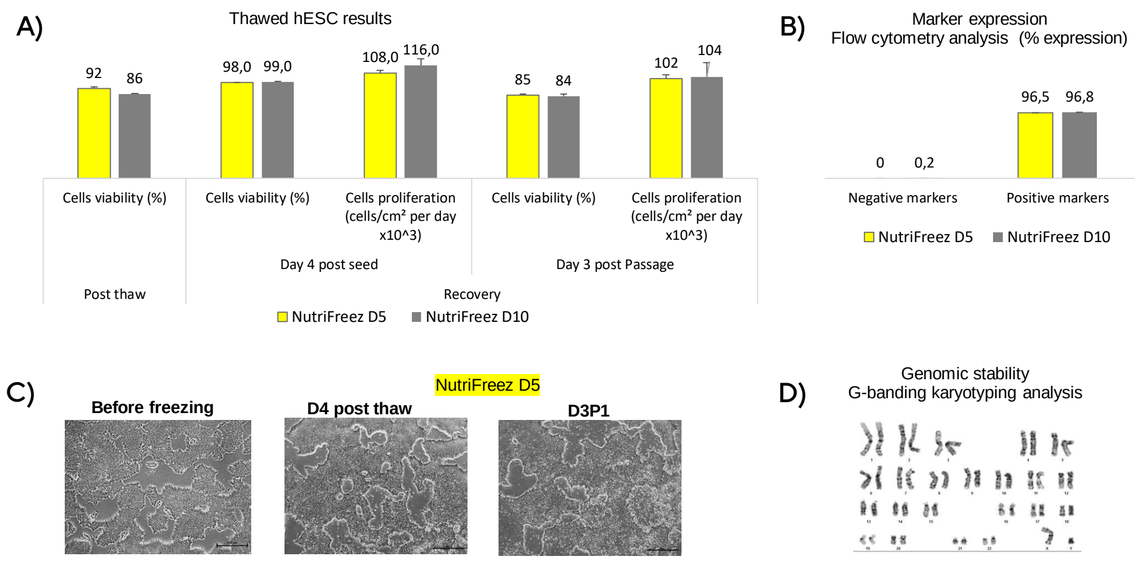

Figure 3: Growth recovery results of hESC post cryopreservation with NutriFreez D5 and NutriFreez D10. (A) Post thaw cell viability (%), recovery cell viability (%) and proliferation rate (viable cells count). (B) Flow cytometry analysis of hESC after 2P. Average of pluripotent markers expression. (positive: SSEA-4,Tra-1-60, Oct-4,Nanog and Sox-2; Negative: SSEA-1). (C) Representative images (x40) taken before and after freezing in NutriFreez D5, during 2 passages (cells recovery). (D) G-banding karyotyping analysis of hESC (D3P1).

4. Immune cells cryopreservation (patient's CAR-T cells)

Chimeric Antigen Receptor T (CAR-T) is an essential tool for cancer treatment. Cryopreservation is a necessary step for CAR- T cell manufacturing processes. NutriFreez D5 was tested to verify its suitability to CAR-T in comparison to NutriFreez D10. Patient's CAR-T (transduces PBMCs at day 6 of culture) from 2 donors were cryopreserved in NutriFreez D5 vs. NutriFreez D10 at conc. of 25x10^6 cells/ml per vial for 3 days, followed by thawing and recovery evaluation. The cells were re-seeded in G-Rex 24 well-plate at conc of 5x10^6 cells/well in 8ml of Nutri-T XF medium supplemented with OKT-3 (50ng/ml) and IL2 (300IU/ml) up to day 10 of culture followed by evaluation of fold expansion (FE), transduction efficiency and T-cell subsets shown as CD4+/CD8+. Results reveal similar performance using NutriFreez D5 and NutriFreez D10.

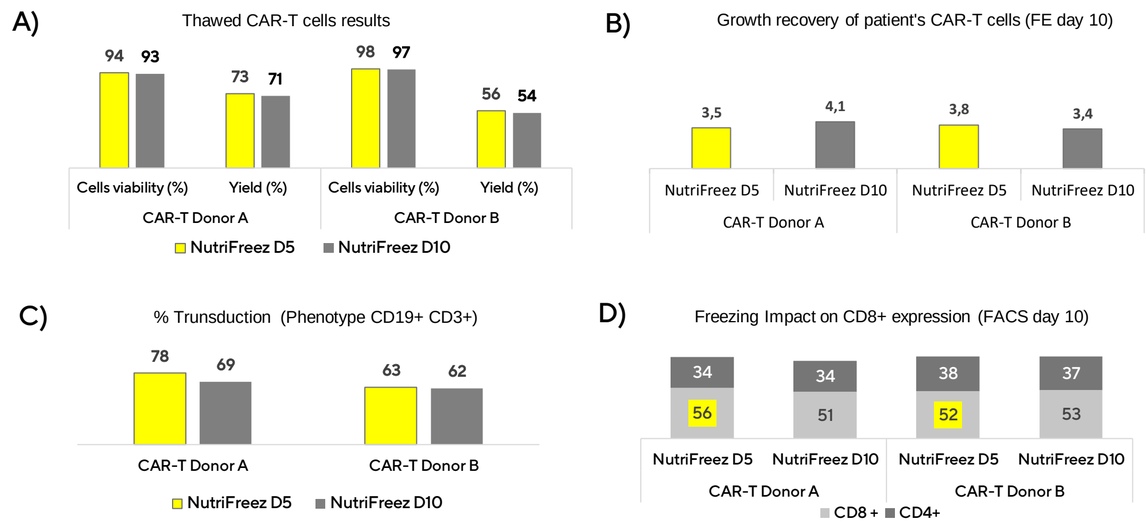

Figure 4: Post thaw and growth recovery results of 2 patients CAR-T cells that were cryopreserved during ex vivo expansion. (A) Post thaw cell viability and yield (%). (B) Fold expansion at day 10 . (C) Transduction efficiency by flow cytometer (anti CD19+) . (D) T-cell subsets shown as CD4+/CD8+ (flow cytometry analysis)

5. Immune cells cryopreservation (NK cells)

As part of the qualification of NutriFreez D5 for immune cells, Natural Killer cells (NK) cryopreservation was evaluated as well. NK-92 cell-line were cryopreserved in NutriFreez D5 vs. NutriFreez D10 at conc. of 3x10^6 cells/ml per vial for 17 days, followed by thawing and recovery evaluation. Results indicate an advantage to NutriFreez D5.

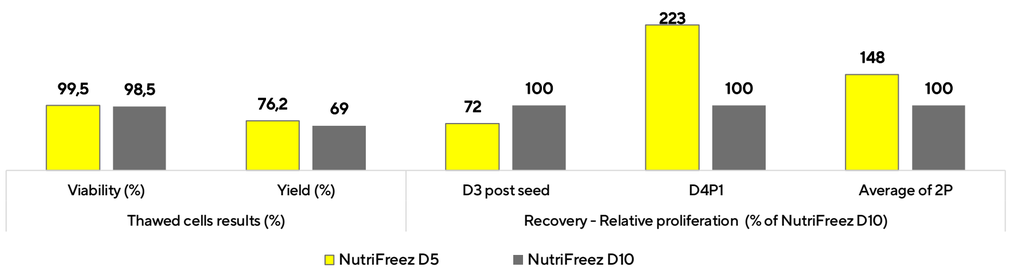

Figure 5: Post thaw cell viability (%), cell yield (%) and proliferation rate (% of NutriFreez D10).

6. Conclusion

The data presented, demonstrates that novel salt base cryopreservation solution, NutriFreez D5, which was initially designed for hMSC, enables optimal cryopreservation of different cell's, including hESC and immune cells (CAR-T, NK), with similar or advantage performance in comparison to cryopreservation solution composed of 10% DMSO (NutriFreez D10).

The novel salt base cryopreservation solution, NutriFreez D5 :

- Defined, serum- free, ACF, PF and composed of 5% DMSO
- Enables optimal cryopreservation of hMSC while marinating of hMSC features
- Suitable for different type of cells, such as hESC and immune cells (CAR-T, NK)
- Shows advantage over commercially available freezing solutions composed of either 5% or 10% DMSO