

CRYOPROTECTANT ADDITIVES, INCLUDING rHSA®, IMPROVE iNK AND iT CELL HEALTH & VIABILITY DURING CRYOPRESERVATION

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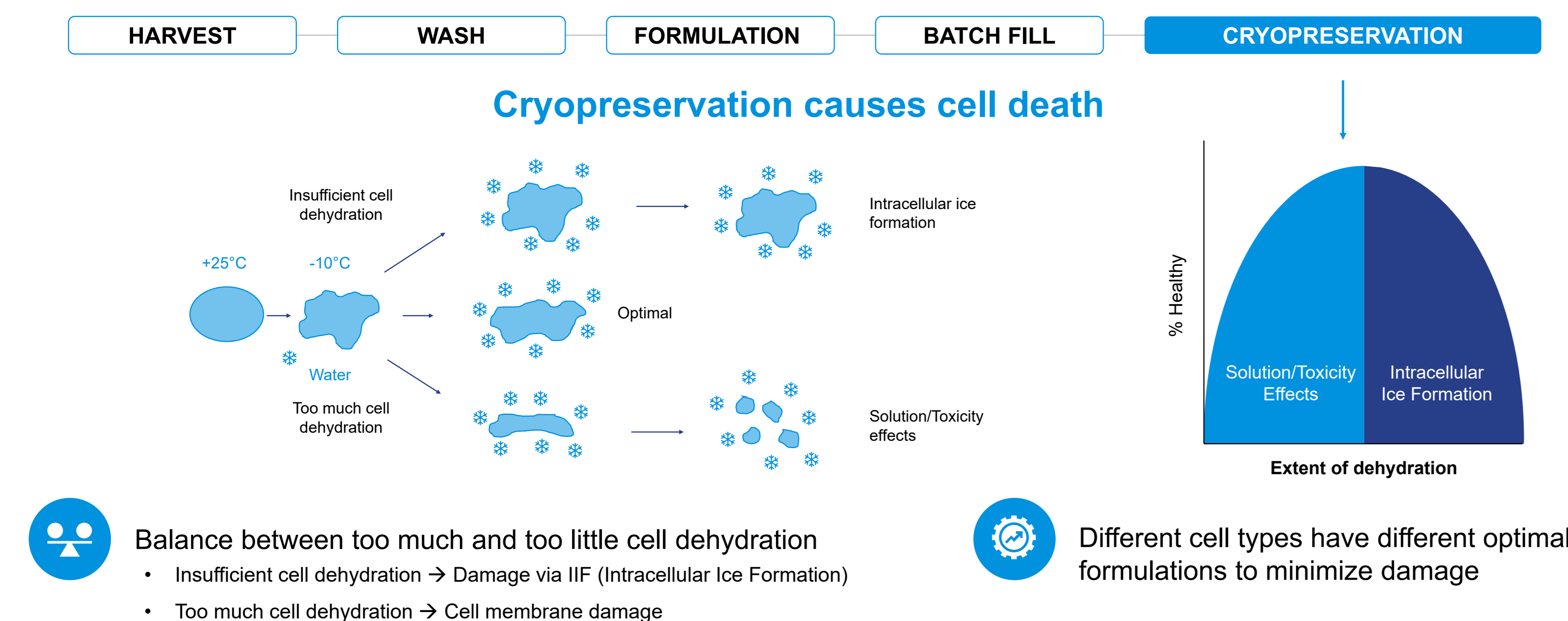
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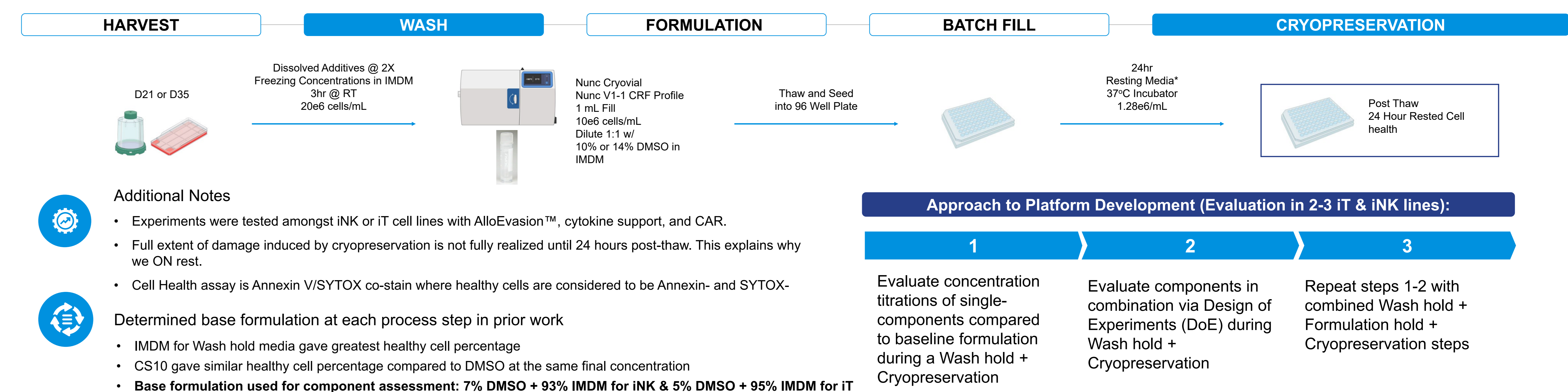
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INTRODUCTION



Adapted from University of Minnesota BioCoR Cryopreservation Course slides and Tamarin, S. (2022)

METHODS



AIM

Cryoprotectant additives can tailor extent of cell dehydration and reduce damage thereby improving post-thaw outcome

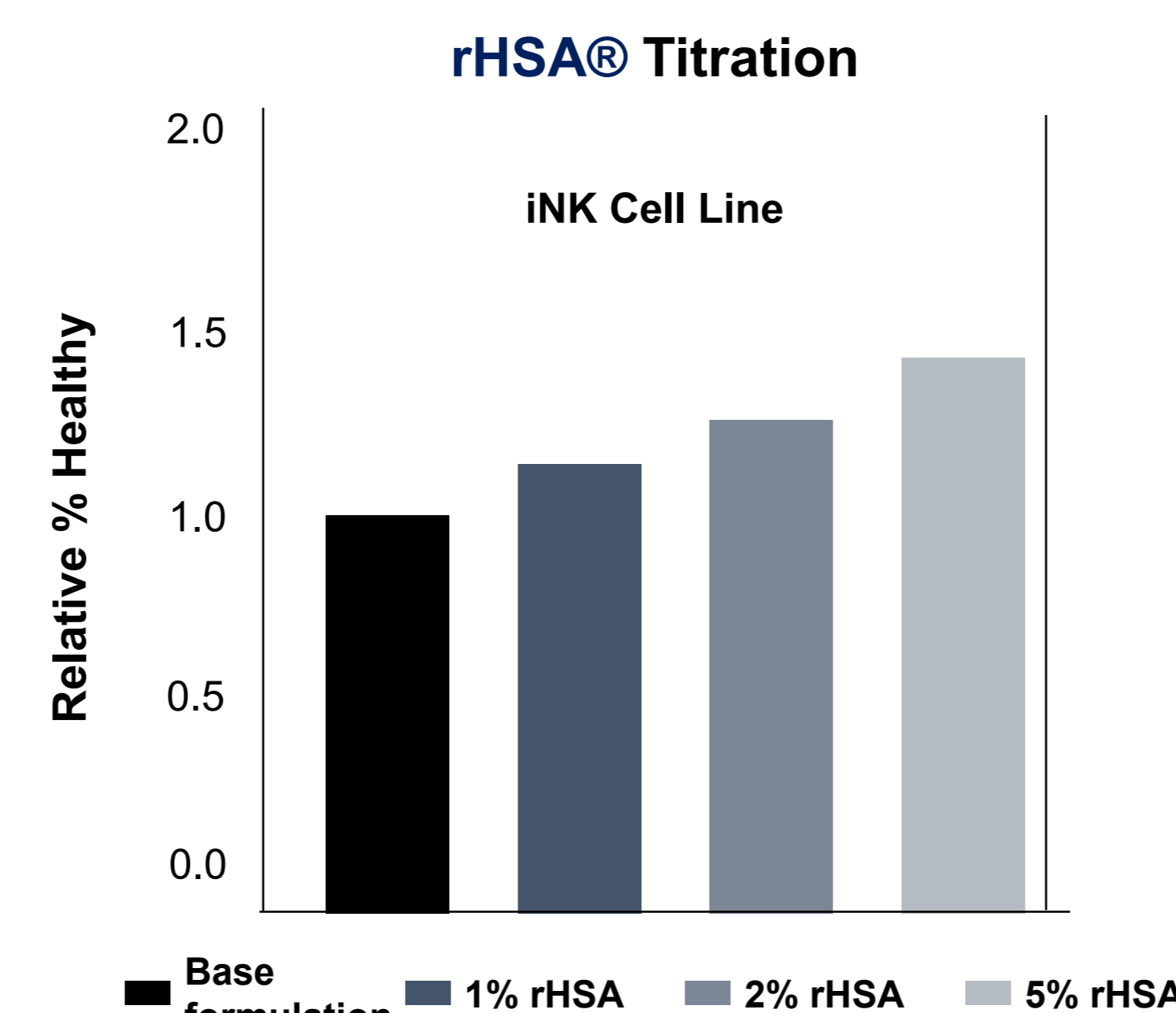
| | Harvest | Wash | Formulation | Fill | Cryopreservation | Thaw |
|----------------------------------|-------------------------------------|--|---|--|-----------------------------------|---------------------------------|
| Point of Stress | Time cells spend out of 37°C | IMDM hold at high conc. (length of wash process) | DMSO hold time; Osmotic Shock (~300 mOsm → ~1200 mOsm); Temperature Shock (4°C CPA to 20°C Cells) | DMSO hold time | Freezing process/CRF Cycle | In-use DMSO hold time |
| Typical mfg. of full-scale batch | ~10e6 cells/mL ~1.5 hours ~RT | ~250 e6 cells/mL ~2.5 hours ~RT | ~50e6 cells/mL 1.5 hours ~4°C/RT | ~50e6 cells/mL 1.5 hours ~4°C/RT | Room. Temp → -100°C in 2 hours | 2-3 hours from thaw to infusion |



Additional components can be added during Wash, Formulation, and/or during Cryopreservation to rescue cells and protect them from damage induced by these process steps

RESULTS PART 1

Cryoprotectant additives improve iNK & iT cell health during Wash hold



Recombinant human serum albumin excipient ("rHSA") from InVitria, Inc.



Recombinant HSA® (InVitria, Inc.) improved healthy cell percentage in a dose-dependent manner in an iNK cell line

- Trolox, Dextran, and Dextrose also improved healthy cell percentage in single-component titrations
- Dextran only needed for Formulation + Cryopreservation steps and not Wash step

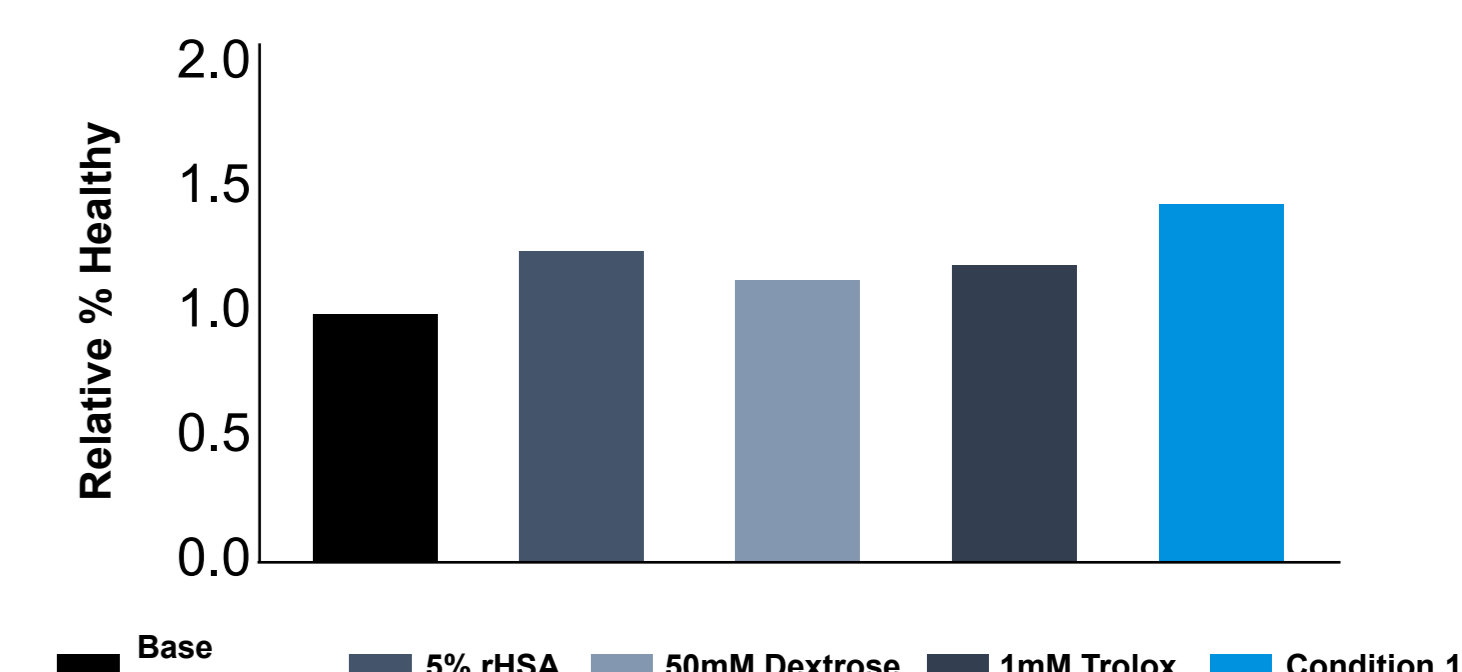


What do these components look like in combination? Can we get even greater improvement?

RESULTS PART 2

Additives improve cell health to an even greater extent when added in combination

| | Trolox (mM) | rHSA (%) | Dextrose (mM) | Time (h) | Cell Conc. (e6/mL) | Temp |
|----|-------------|----------|---------------|----------|--------------------|------|
| 1 | 0.55 | 10 | 150 | 3 | 200 | 4C |
| 2 | 0.1 | 10 | 85 | 0.5 | 20 | 4C |
| 3 | 1 | 10 | 150 | 0.5 | 200 | RT |
| 4 | 1 | 1 | 150 | 0.5 | 20 | 4C |
| 5 | 1 | 5.5 | 150 | 3 | 20 | 4C |
| 6 | 0.55 | 5.5 | 85 | 1.75 | 110 | RT |
| 7 | 1 | 1 | 85 | 3 | 200 | RT |
| 8 | 0.55 | 5.5 | 85 | 1.75 | 110 | 4C |
| 9 | 0.1 | 1 | 150 | 3 | 110 | RT |
| 10 | 0.1 | 1 | 150 | 0.5 | 200 | 4C |
| 11 | 1 | 1 | 20 | 1.75 | 200 | 4C |
| 12 | 0.1 | 10 | 150 | 1.75 | 20 | RT |
| 13 | 1 | 10 | 20 | 0.5 | 110 | 4C |
| 14 | 0.1 | 5.5 | 20 | 0.5 | 200 | RT |
| 15 | 0.1 | 10 | 20 | 3 | 200 | RT |
| 16 | 0.55 | 1 | 20 | 0.5 | 20 | RT |
| 17 | 0.1 | 1 | 20 | 3 | 20 | 4C |
| 18 | 1 | 10 | 20 | 3 | 20 | RT |



Condition 18 showed the **most significant improvement** over base formulation and over each component by itself in an iNK cell line

- Validated in additional iNK cell line
- Statistically, only **rHSA®** improved cell health
- rHSA showed greater improvement than other single-components
- However, greater improvement observed with components in combination
- Follow-up studies will focus on whether all or only some components are necessary in the formulation

Notably, rHSA statistically interacts with cell concentration where, at higher cell concentrations, the extent of improvement seen with rHSA diminishes

CONCLUSIONS

- Cryoprotectant additives in Wash hold can improve post-thaw cell health, and furthermore, additives in combination can improve cell health and viability to an even greater extent
- Excipient grade rHSA®, recombinant human serum albumin supplied by InVitria Inc. significantly improved cell health, dependent on cell concentration

SOURCES

- Some images created with BioRender.com
- JMP, FlowJo

ACKNOWLEDGEMENTS

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