

CELL HEALTH & VIABILITY DURING CRYOPRESERVATION

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Performance. Defined.

Balance between too much and too little cell dehydration

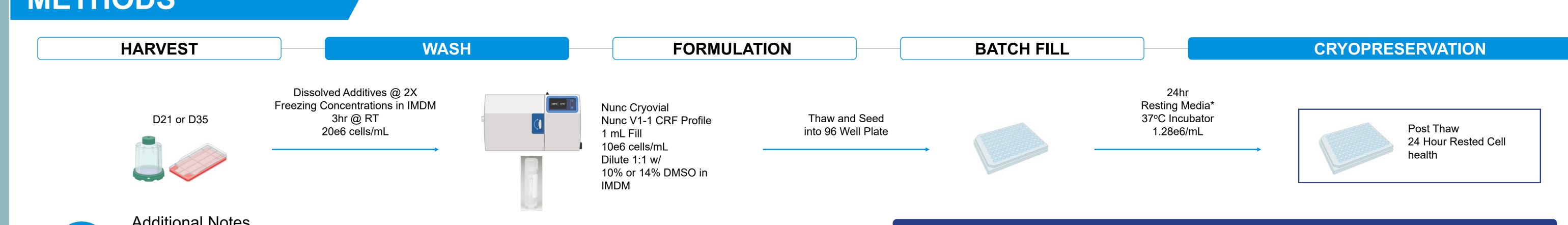
• Too much cell dehydration → Cell membrane damage

Insufficient cell dehydration → Damage via IIF (Intracellular Ice Formation)

INTRODUCTION **WASH BATCH FILL CRYOPRESERVATION Cryopreservation causes cell death** Solution/Toxicit Intracellular Effects Ice Formation dehydration **Extent of dehydration**

Different cell types have different optimal formulations to minimize damage Adapted from University of Minnesota BioCoR Cryopreservation Course slides and Tamarin, S. (2022)

METHODS





Additional Notes

- Experiments were tested amongst iNK or iT cell lines with AlloEvasion™, cytokine support, and CAR.
- Full extent of damage induced by cryopreservation is not fully realized until 24 hours post-thaw. This explains why
- Cell Health assay is Annexin V/SYTOX co-stain where healthy cells are considered to be Annexin- and SYTOX-



Determined base formulation at each process step in prior work

- IMDM for Wash hold media gave greatest healthy cell percentage
- CS10 gave similar healthy cell percentage compared to DMSO at the same final concentration
- Base formulation used for component assessment: 7% DMSO + 93% IMDM for iNK & 5% DMSO + 95% IMDM for iT

Approach to Platform Development (Evaluation in 2-3 iT & iNK lines):

Evaluate components in combination via Design of Experiments (DoE) during

Wash hold + Cryopreservation

Repeat steps 1-2 with combined Wash hold + Formulation hold + Cryopreservation steps

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	

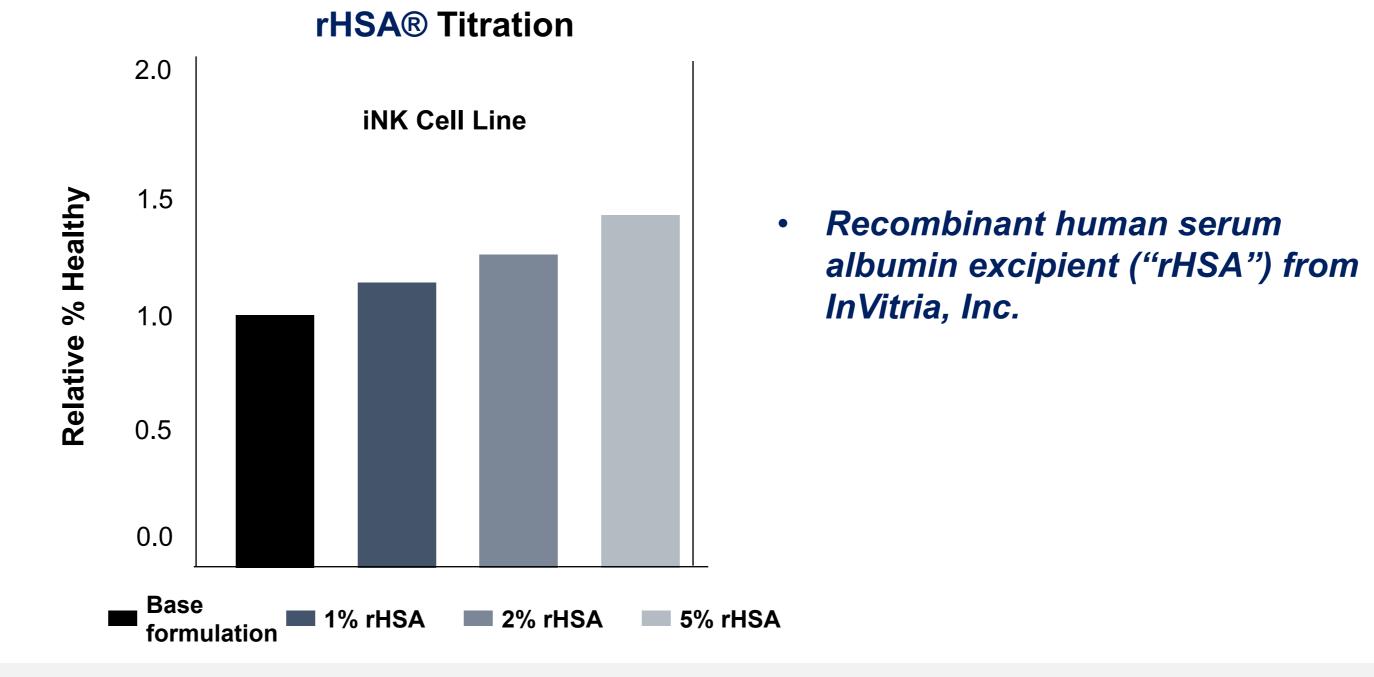


Additional components can be added during Wash, Formulation, and/or during Cryopreservation to rescue cells and protect them from damage induced by these process steps

RESULTS PART 1

Cryoprotectant additives improve iNK & iT cell health during Wash hold

CRYOPROTECTANT ADDITIVES, INCLUDING rHSA®, IMPROVE INK AND IT



- - 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

RESULTS PART 2

Evaluate concentration

components compared

to baseline formulation

during a Wash hold +

Cryopreservation

titrations of single-

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

						groate	
	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	



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

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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