Scale-Up and Technology Transfer of Manufactured Red Blood Cell Production to a CDMO for Clinical Manufacturing Readiness

Dr Rob Thomas PhD, CTO, Safi Biotherapeutics

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Disclosures and Learning Objectives

Disclosures

- Dr. Rob Thomas has no relevant financial or non-financial relationships with ineligible companies to disclose relating to the content of this activity.
- The views expressed in this presentation are those of the author and do not necessarily reflect the official policy or position of the Department of War, nor the U.S. Government.
- Commercial support was not received for this activity.

Learning Objectives

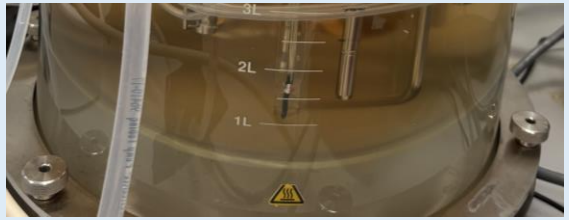
- Explain how a stirred-tank manufacturing platform produces cultured red blood cells and maintains product fidelity across 1 L to 10 L scale-up and transfer to a US-based CDMO
- Compare the in vitro and in vivo functional characteristics of manufactured red cells with donor-derived red cells
- Differentiate the novel properties of manufactured red cells — including glycerol-free cryopreservation resilience — from conventional donor red cell products

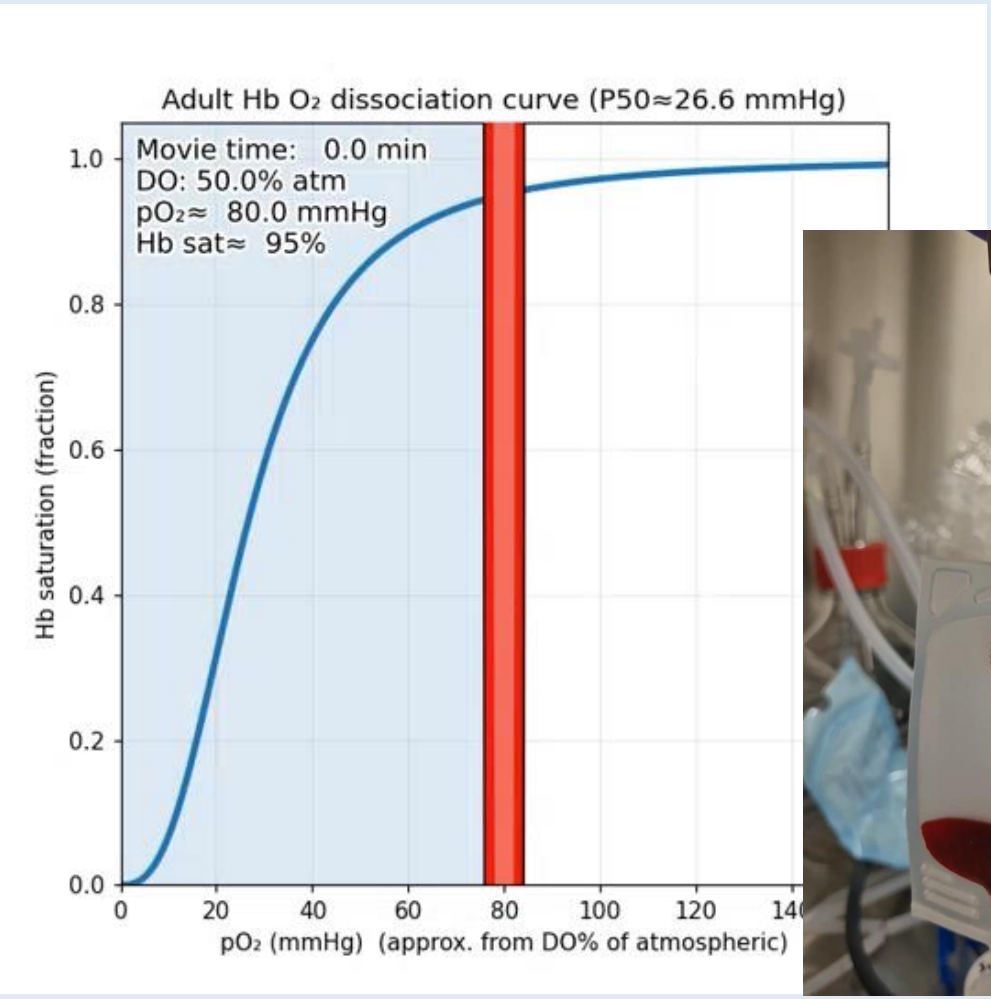
Overview

- **The Manufacturing Platform for a Cultured Red Cell Product**
- **In Vitro Data Indicating Functionality and Quality of Manufactured Red Cells**
- **In Vivo (mouse) Data Indicating Functionality and Quality of Manufactured Red Cells**
- **Novel Properties of Manufactured Red Cells**
- **Future Opportunities**

Scaled manufacturing of mRBCs

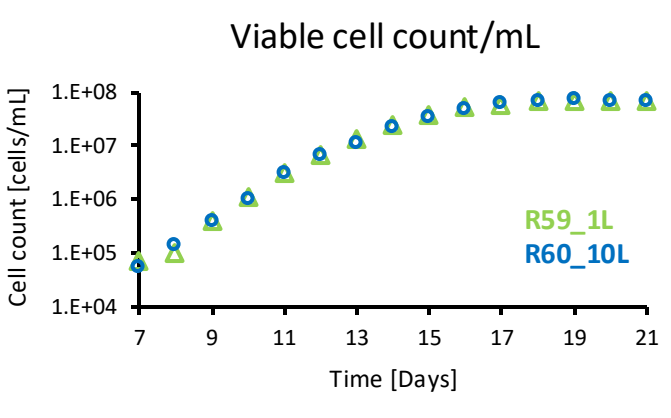
- Bioreactor platform, currently operating at up to 10 L
- Scale developed for pre-clinical and dose-escalation supply (~1 unit/batch)
- Format supports control and further scalability
- Unit operations bioreactor to bags being finalized

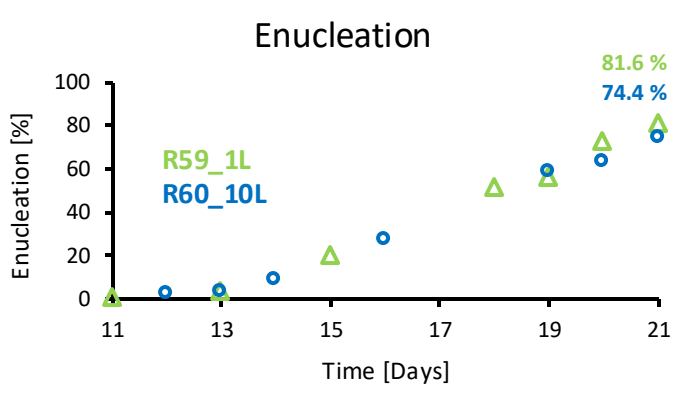


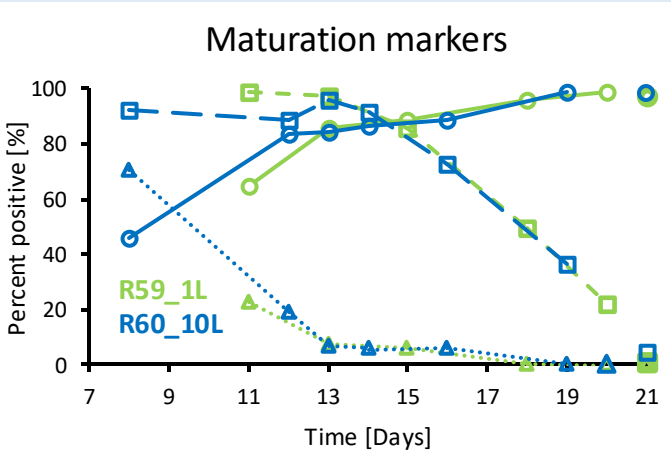


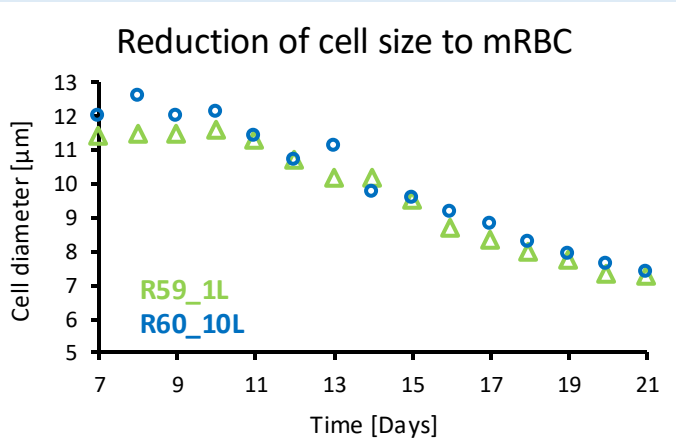
Initial clinical scale-up achieved: mRBC production

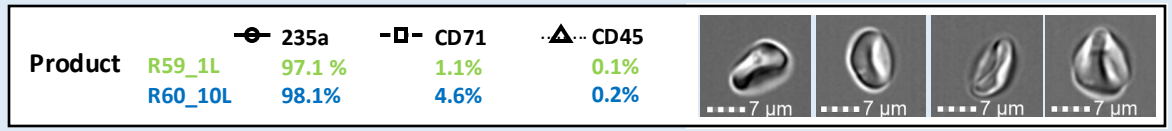
Expanded from 1L to 10L bioreactor production systems with high process and product fidelity



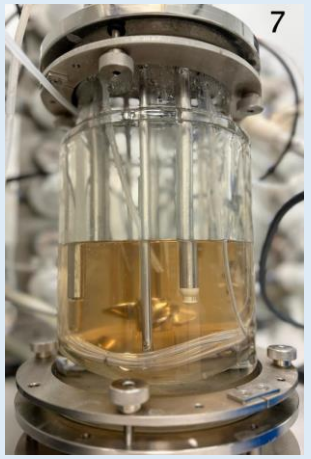








Process Development clinical process enabling



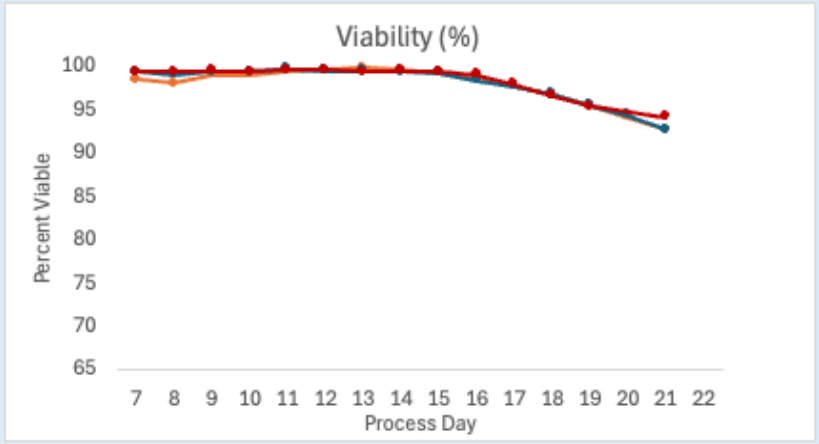
1L mRBCs

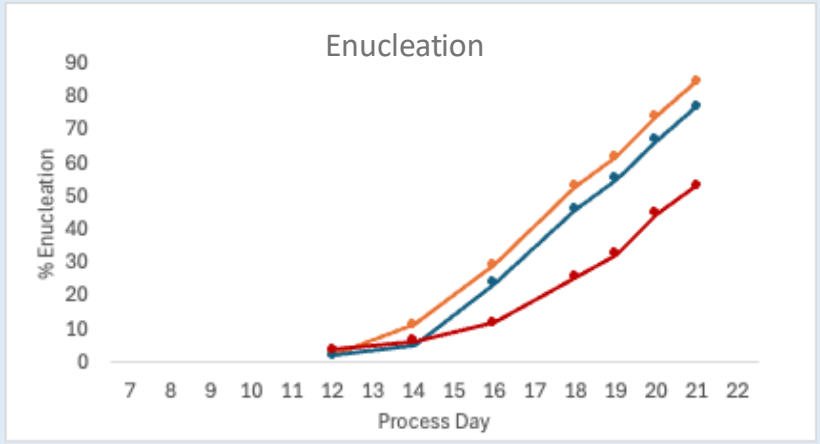
Scaled for clinical production

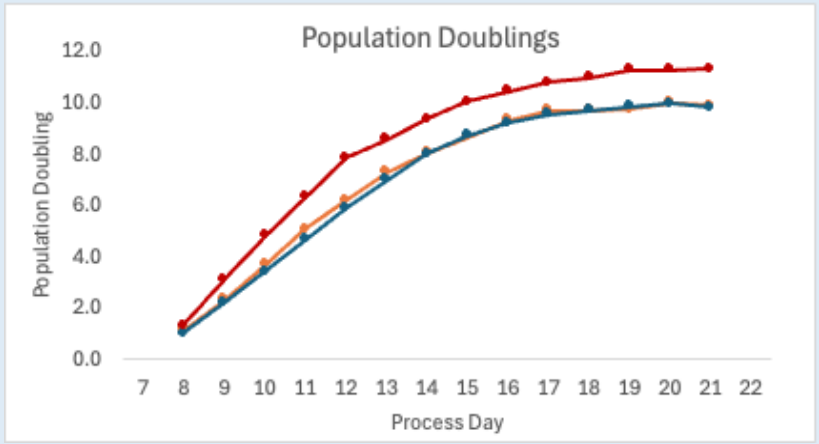
10L mRBCs 2025
1 mRBC unit

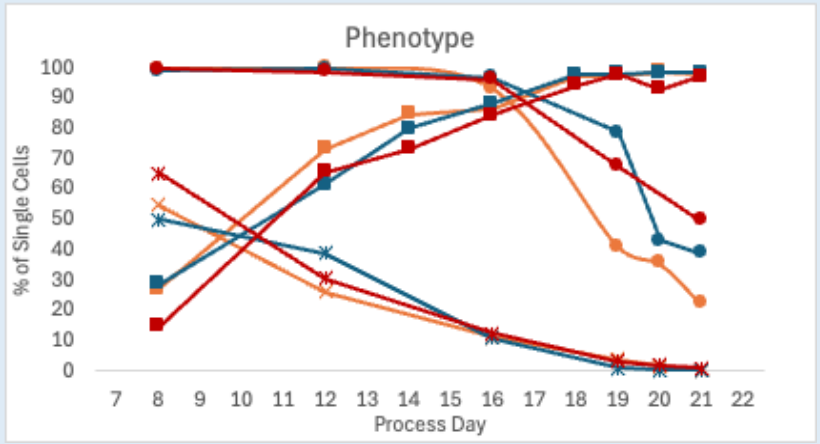
Safi Biotherapeutics Inc

Manufacture Ready: Scaled batch-to-batch consistency at US-based GMP-ready CDMO facility (1L, 10L)









Key

Tech Transfer Run 1L
Confirmation Run 1L
Tech Transfer Run 10L

CD235a
CD71
CD45

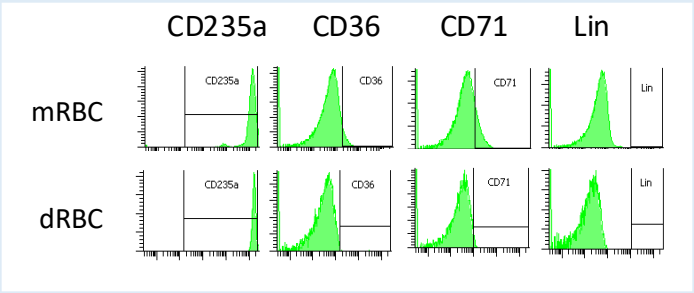
- Site transfer completed with success in growth, yield and quality via tech transfer runs at the 1L and 10L bioreactor scale
- US-based supply chain and material GMP compatibility

Safi Biotherapeutics Inc; Sciperio Inc; ARMI | BioFAB USA

In vitro analysis across scales and tech transfer

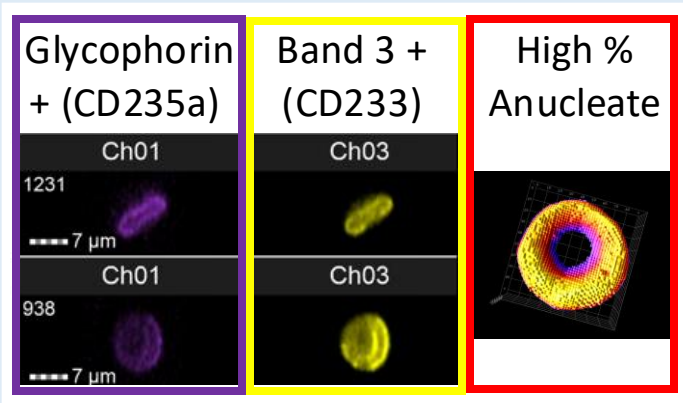
Red cell assays demonstrate specific identity and functionality key to clinical application

Identity Phenotype: Manufactured(m) vs Donor (d)



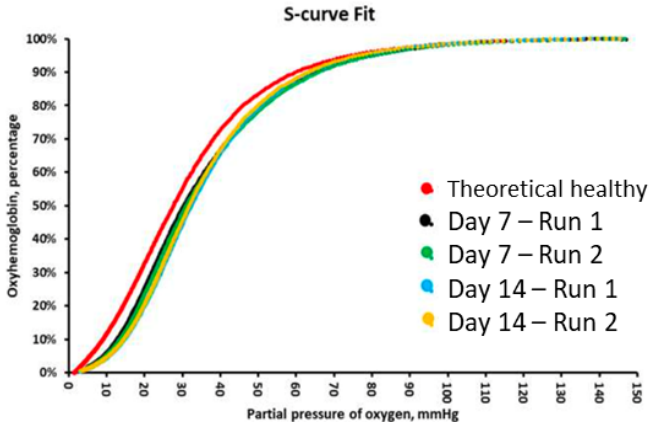
Phenotype Drug Product Release parameters:

- >97% CD235a positive; CD45 negative
- >90% Draq5 negative (enucleation)

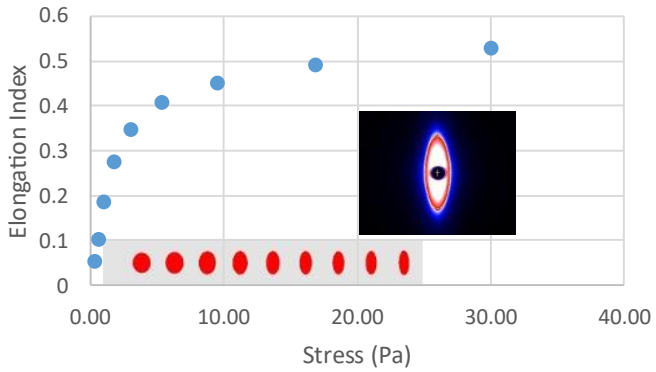


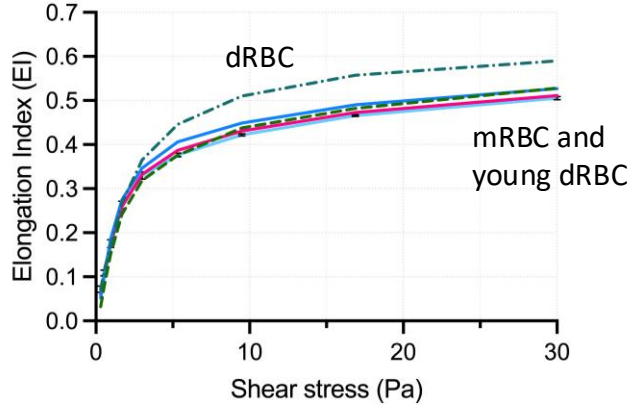
Representative In Vitro Functional Analysis at Clinical Scale Manufacture

mRBC O2 dissociation curves during storage: Normal Hb oxygen affinity demonstrated via p50 saturation point and calculated Hill coefficient: Target range: >2.4



mRBC Deformability: Provides evidence of capacity to pass microcapillaries (Target >0.4 EI at 30 Pa)





Functional CQA data generated at Functional Fluidics, Dr. Patrick Hines

Development of enhanced in vitro analysis

Example of enhanced specification tailored for manufactured cell attributes

Batch	Day	EImax (def)	*SS½	**Ohyper	***Δ Def–Osmo	****n_50	*****P50 (µ)	Verdict
10L Reference Run (Safi)	0	0.477	1.75	463	0.026	3.31	31.7	Pass
10L Reference Run (Safi)	7	0.465	2.01	514	0.003	4.00	33.5	High p50
1L Reference Run (Safi)	0	0.467	1.63	446	0.022	2.30	22.4	Low n_50
1L Reference Run (Safi)	7	0.458	1.85	492	0.009	2.42	20.1	Pass
▲1L Negative Control Run	0	0.211	1.06	290	-0.169	1.86	26.4	Fail (multi-assay)
Donor 1	0	0.554	2.19	404	0.015	3.15	16.5	Reference
Donor 1	7	0.575	2.79	375	0.047	3.44	15.8	Reference
Donor 1	0	0.584	2.40	400	0.044	3.12	17.1	Reference
Donor 1	7	0.604	3.82	374	0.082	3.29	15.6	Reference
CDMO 1L (ARMI)	0	0.451	1.62	431	0.018	3.02		
CDMO 1L (ARMI)	7	0.450	1.59	496	-0.001	2.86		
CDMO 10L (ARMI)	0	In progress	In progress	In progress	In progress	In progress		
CDMO 10L (ARMI)	7	In progress	In progress	In progress	In progress	In progress		

*SS1/2 shows low shear deformability is better in manufactured cells (relative to EImax) compared to donor cells

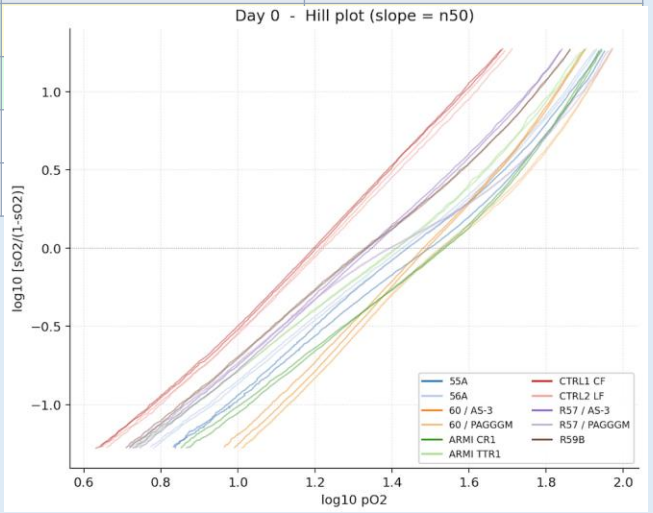
**Ohyper shows osmolarity of max deformability; lower = more dehydration

***Delta of max deformability from repeated stress (Def) vs single pass (Osmo) identifies fragile cells

****Hill coefficient calculated to account for p50 heterogeneity in manufactured product; expect >2.4

*****p50 highly variable due to sensitivity to allosteric modifiers and their loss dependent on temperature

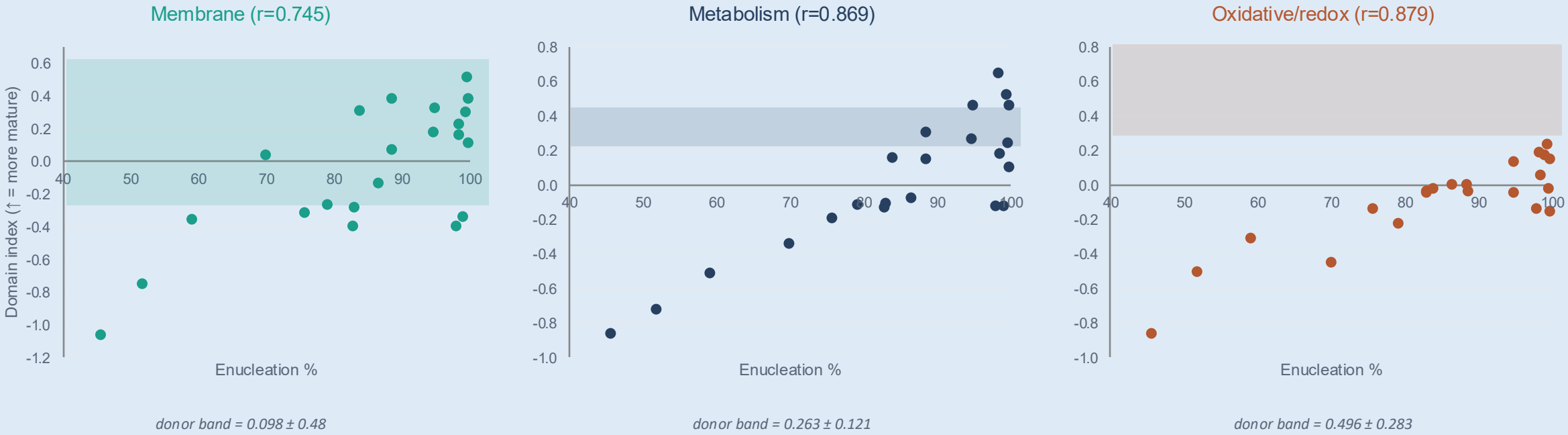
▲ Negative control generated by overexposure to stress inducing reagents during culture to show clear failure



Hill plot: Exclude MetHb then distribution of p50 (spread, skew, or bimodality) × intrinsic n × metabolic ΔpO_2

*Omics-based understanding driving continual improvement

As mRBC mature (enucleation) they become donor like across key biological domains

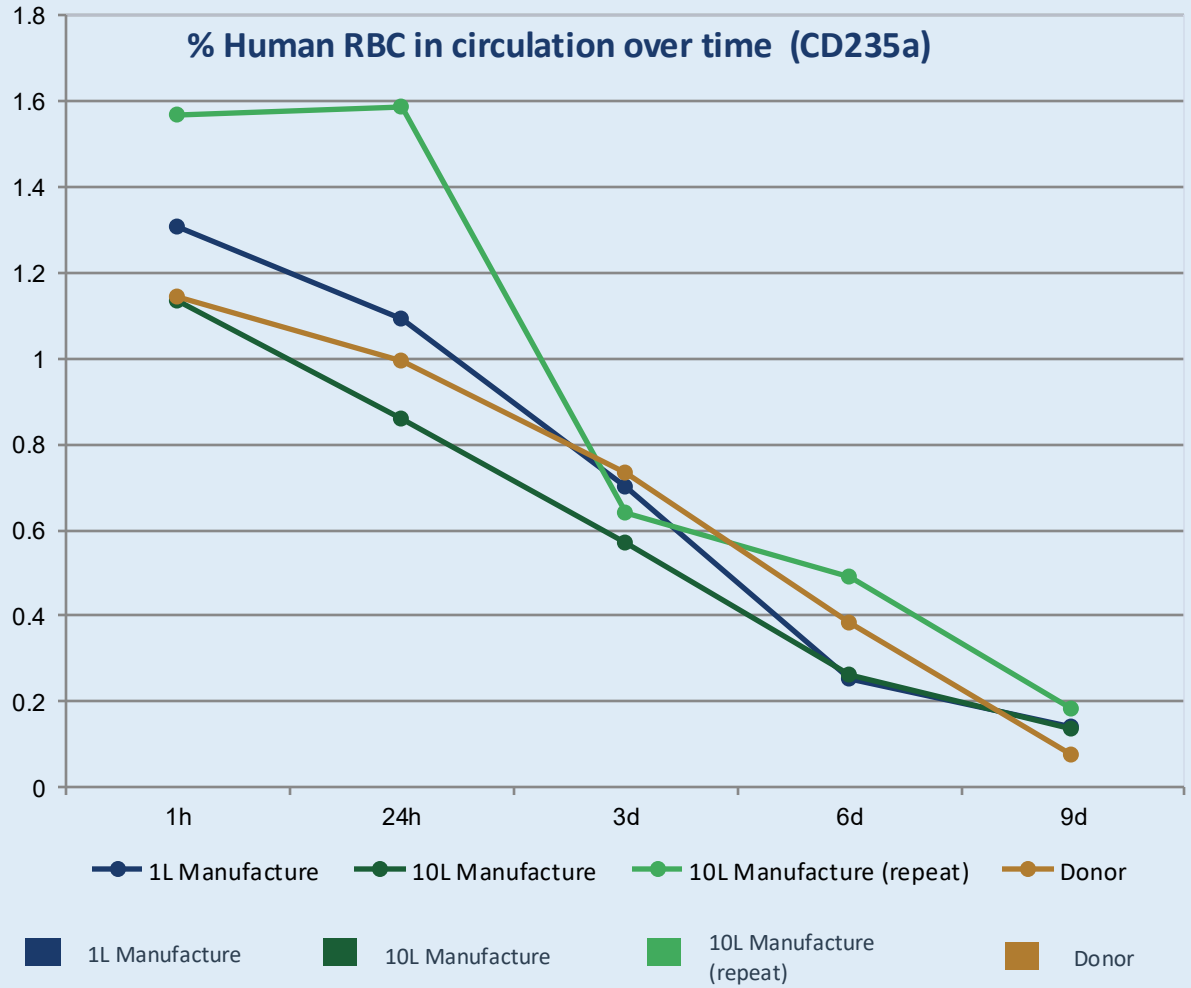


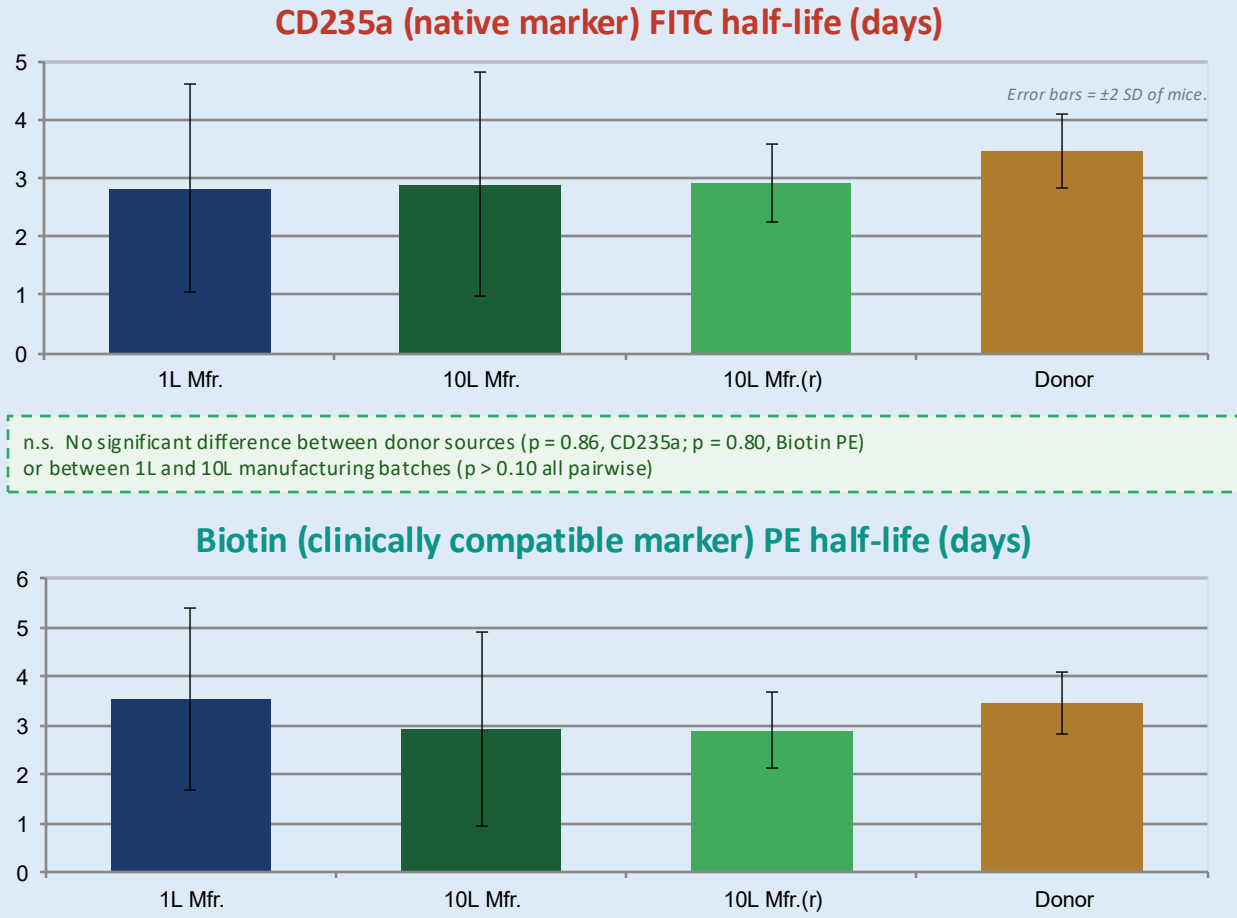
- Indices created to functionally group metabolic attributes show expected trajectory of canonical red cell properties in maturing manufactured product towards donor reference
- Deconstruction of indices enables in depth comparison to donor product, mechanistic insight into storage related changes, and data driven process/product improvement

*Collaboration with Angelo D'Alessandro, CU

*Refrigerated mRBC in vivo circulation model

Equivalent circulatory half-life of donor vs 1L/10L *manufactured RBCs in Mouse Model



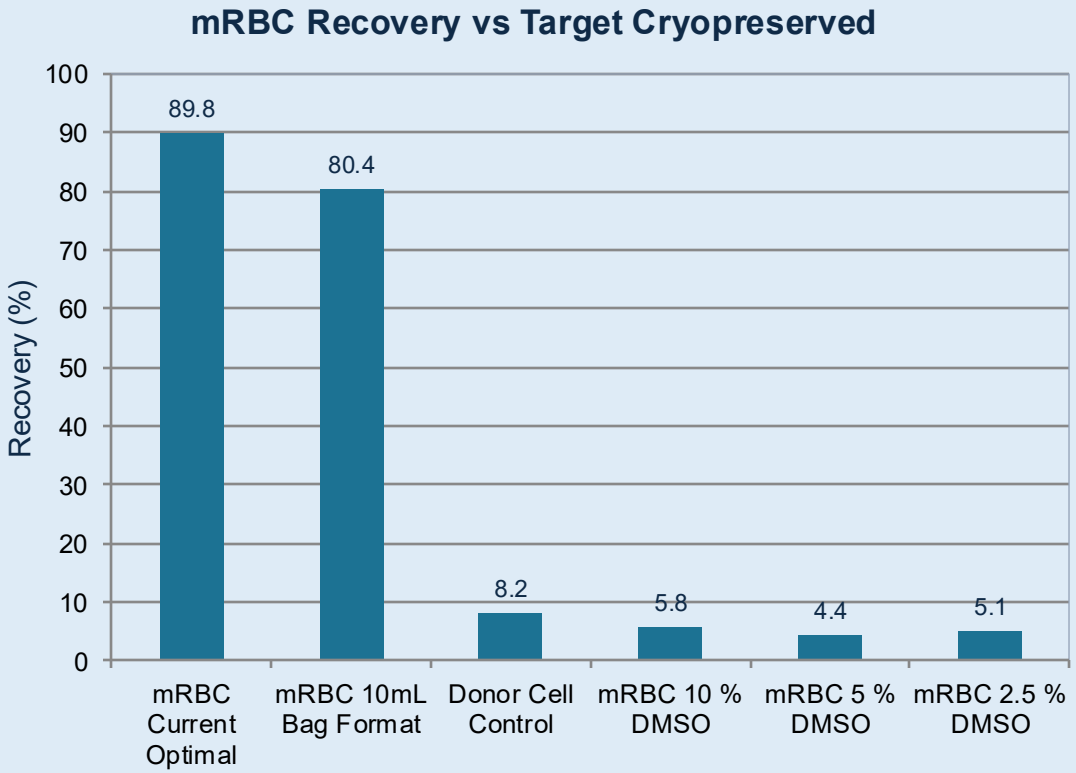


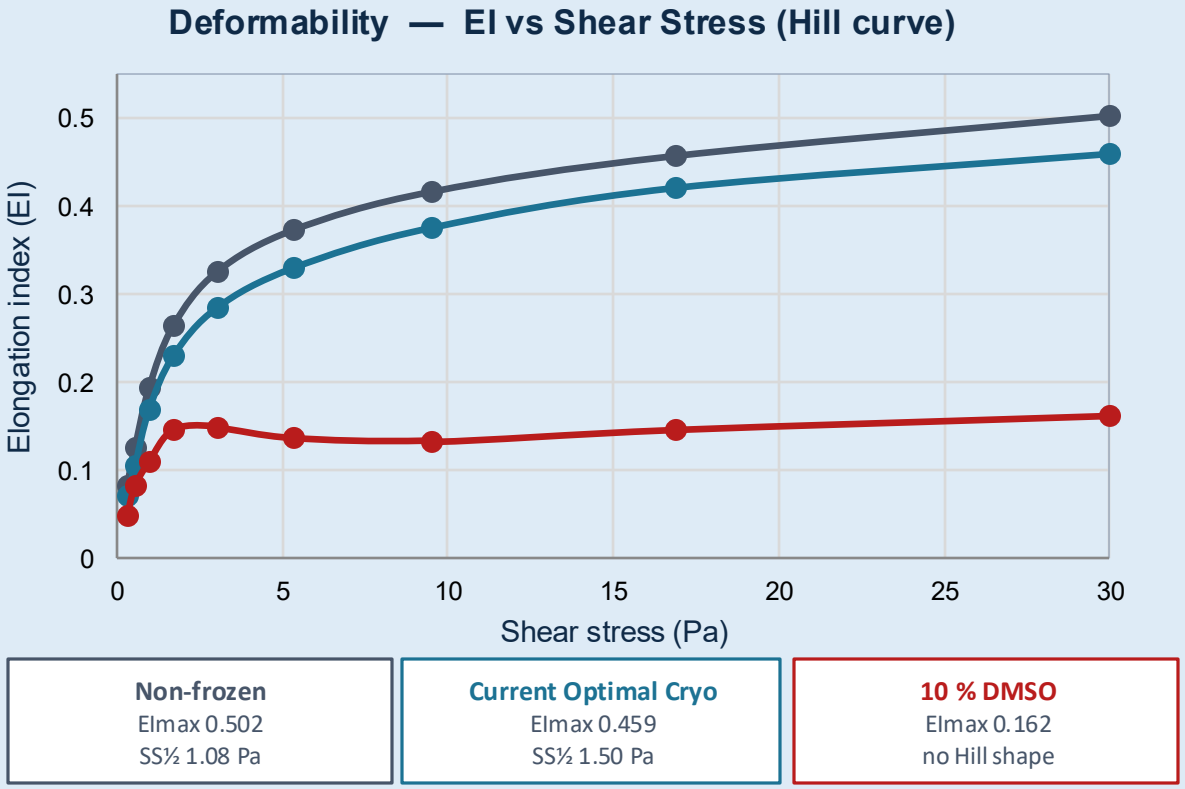
*Cells approx.. 7-10 days stored at point of transfusion after transatlantic shipment.

*Collaboration with J. Roback and M. Lancelot, Emory University

mRBC robustness and homogeneity support glycerol-free cryopreservation

- mRBCs recover ~90% with preserved deformability versus <10% for donor cells under equivalent storage media conditions or mRBC in standard DMSO cryopreservatives
- mRBCs higher glutathione/ATP content relative to donor cells is a mechanistic basis for advantages

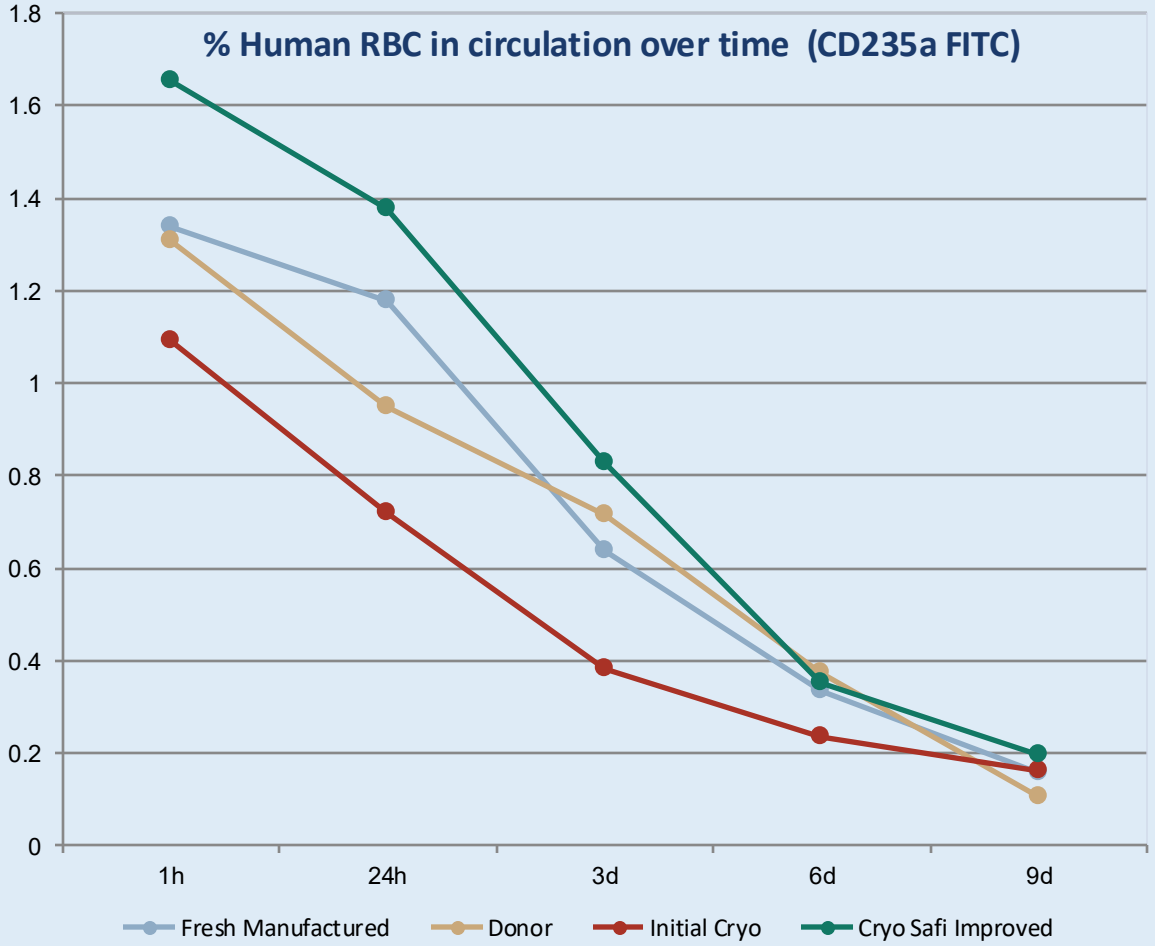


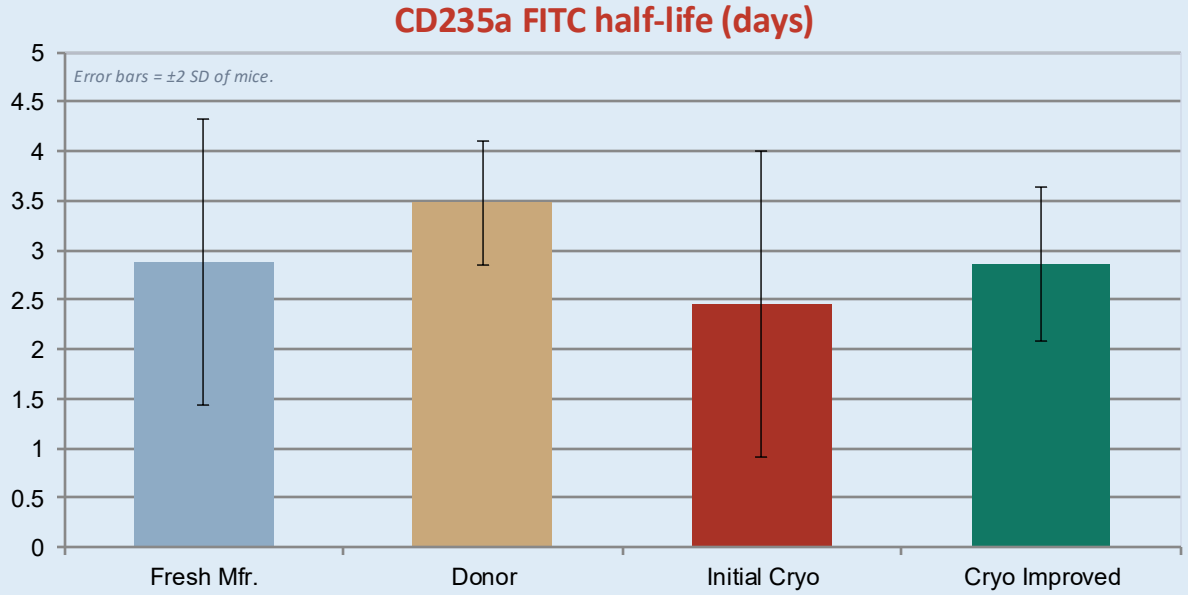


Current MTEC funded program

Cryopreserved mRBC in vivo circulation model

Equivalent circulatory half-life of cryopreserved manufactured RBCs in Mouse Model





Cryo = commercially available (DMSO free); Cryo Improved uses further Safi in house protective additives (DMSO free)

Group	CD235a HL (mean ± SD, days)	n (mice)
Fresh Manufactured	2.89 ± 0.72	15 (3 batches)
Donor	3.48 ± 0.32	10 (sourced locally at transfusion site)
Cryo	2.46 ± 0.77	5
Cryo Improved	2.86 ± 0.39	11 (2 batches)

Fresh Manufactured Donor Cryo Cryo Improved

*Collaboration with J. Roback and M. Lancelot, Emory University

Opportunities and Future Development

Near Term Clinical

- This platform has the capability to deliver manufactured red cells at a clinically meaningful scale; partnerships (ARMIBioFAb, Sciperio Inc.) are ensuring regulatory readiness of the technology and facilitating transfer for GMP manufacture

Near Term Manufacture Development

- Metabolomics data indicates opportunities to tune upstream formulation for higher production; specific targets identified in metabolism, membrane composition and oxidative resilience
- Evaluation of re-engineered gas transfer to enhance Oxygen/Carbon Dioxide flux at higher upstream production intensity and increased scale
- Downstream and formulation are specific to manufactured red cells: modifications for both cryo and refrigerated show path to enhanced recovery and storability (eg membrane active agents, metabolic/redox support)

Summary

- **A stirred-tank platform now produces cultured red cells at approximately unit scale (10 L), with unit operations defined for clinical supply.**
- **Process fidelity is maintained across scale-up and transfer to a US-based, GMP-ready CDMO.**
- **Manufactured red cells demonstrate identity, function, and in vivo circulatory survival in mouse models supporting clinical safety and efficacy**
- **Manufactured cells show novel, tunable attributes — including glycerol-free cryopreservation resilience — supported by omics-based characterization.**
- **Metabolomics-guided development and manufacturing partnerships are advancing regulatory and manufacturing readiness toward the clinic.**

Acknowledgements

- Thank you for listening

- Collaborators: ARMI BioFabUSA Team (NH); Sciperio Inc. (FL); Angelo D'Alessandro (CU); J. Roback and M. Lancelot, Emory University; Dr Patrick Hines and Functional Fluidics Team

- Funders: MTEC, DoW

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