ISHLT JOURNAL WATCH
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Lauren East, MD
University of Alabama at Birmingham, Birmingham, AL, USA

Rupa Kanchi, PhD
ISSUE EDITOR
ISHLT.ORG

Plasma-supplemented Red Cell Concentrates as Alternatives to Whole Blood in Porcine Ex Vivo Heart Perfusion

Durand K, Phan C, Hatami S, Wagner M, et al. *JHLT* 2025 Nov;44(11):1811-1820. | DOI: 10.1016/j.healun.2025.06.002

Study Highlights

Objective: Normothermic Ex Vivo Heart Perfusion (EVHP) uses autologous whole blood (WB) perfusate, presenting challenges with storage, volume, and availability. This study evaluated several perfusate compositions and assessed impact on the red blood cell (RBC) quality and myocardial function during EVHP.

Methods: Utilizing a porcine model for organ procurement and blood collection, six different perfusate compositions were evaluated. EVHP was performed using an established protocol for 4 hours. Coronary blood flow (CBF), cardiac index (CI) and metabolomic changes (lactate and glucose) were monitored and measured at the start and four hours after perfusion. RBC quality testing was also performed at 0 and 4 hours.

Results:

- Myocardial hemodynamic function: 1) Perfusates with plasma (D14P, RD14P, RD21P) had similar CBF as WB at 0 or 4 hours, with a significant rise across time; and had higher CI at 0 and 4 hours than WB with differences most pronounced at 4 hours.
- Metabolic changes: Lactate remained stable over time except in rejuvenated 21-day stored RCCs with plasma. Glucose declined over time in all groups besides the WB perfusate, which had lower overall glucose concentrations.
- RBC quality: Deformability was higher and fragility was reduced in the WB perfusates at all time points, indicative of superior shear stress tolerance.

Conclusions: The addition of plasma to red cell concentrates offers a more manageable alternative perfusate to WB, with results largely demonstrating near-comparable outcomes.

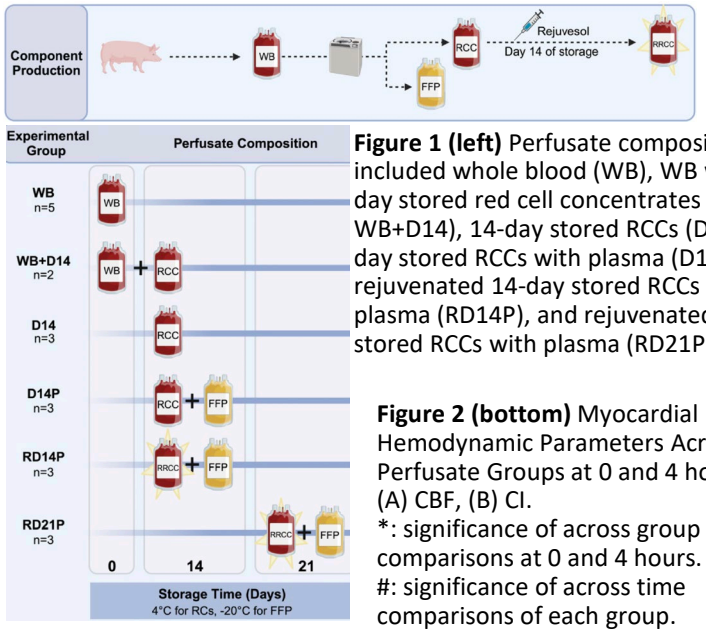

Figure 1 (left) Perfusate compositions included whole blood (WB), WB with 14-day stored red cell concentrates (RCCs; WB+D14), 14-day stored RCCs (D14), 14-day stored RCCs with plasma (D14P), rejuvenated 14-day stored RCCs with plasma (RD14P), and rejuvenated 21-day stored RCCs with plasma (RD21P).

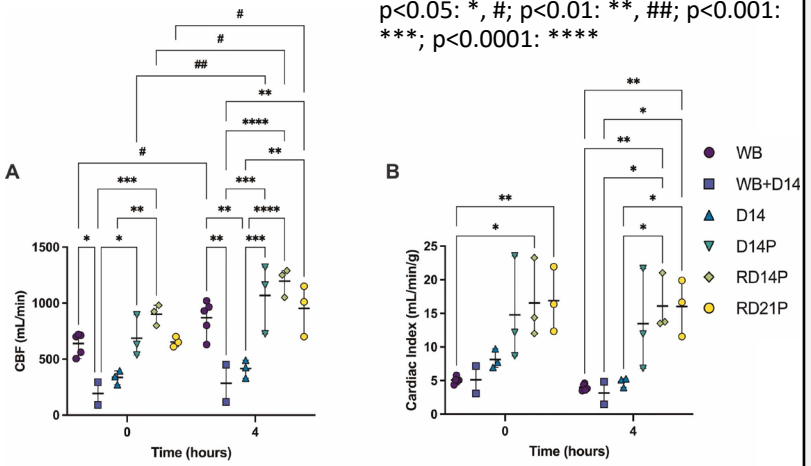

Figure 2 (bottom) Myocardial Hemodynamic Parameters Across Perfusate Groups at 0 and 4 hours. (A) CBF, (B) CI.
*: significance of across group comparisons at 0 and 4 hours.
#: significance of across time comparisons of each group.
$p<0.05$: *, #; $p<0.01$: **, ##; $p<0.001$: ***; $p<0.0001$: ****

Reviewer's Comments

- This study is an exciting advancement for normothermic EVHP as plasma-supplemented red cell concentrates appear to be contenders to replace whole blood, improving a logistical hurdle of EVHP.
- While this is progress for the field, several barriers to translation will need to be addressed, including optimization of RBC integrity.
- Additional study of other interesting findings is needed. For example, the suggestion of lactate as a poor performance marker in ex vivo settings is intriguing because this marker and its trend are clinically used.

Limitations

- The generalizability and statistical power of this study was impacted by the small sample size.
- Investigators could not blood type the animals, so there may have been some hemolysis from cross-reactivity.
- Standardization of methodologies for blood product use in EVHP is needed as optimal perfusate could be impacted by variables like temperature and flow rates.
- Further studies are required to translate these findings to human EVHP.

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Nicole Chrysler, HBSc
University of Toronto, Toronto, ON, Canada

Rupa Kanchi, PhD
ISSUE EDITOR

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IL-2 Complex Therapy Prolongs Fully MHC-Mismatched Murine Cardiac Allograft Survival

El-Ayachi I, Teodorescu RN, Azar J, Keslar K, et al. *J Immunol*. 2025 Dec 10:vkaf303. | DOI: 10.1093/jimmun/vkaf303

Study Highlights

Objective: To determine whether pretransplant IL-2/anti-IL-2 immune complex (IL-2c) therapy prolongs fully MHC-mismatch murine cardiac allograft survival and to define the immunological mechanisms underlying this effect.

Methods: C57BL/6 mice received fully allogeneic BALB/c or hemi-allogeneic F1 cardiac allografts after pretransplant IL-2c or control treatment. Graft survival days (d), donor-specific T cell priming and antibody responses were measured using high-dimensional flow cytometry, transcriptomic profiling, and histologic analyses. Effect of combining IL-2c with short-course tacrolimus was also assessed.

Results: IL-2c significantly prolonged cardiac allograft survival in both fully mismatched and hemi-allogeneic models in a Treg-dependent manner (fully mismatched median survival time (MST) 7 vs 14 d, p<0.0001; F1 MST 13 vs 22 d, p=0.0018). IL-2c reduced donor-specific T cell and antibody responses (~5-fold), expanded FOXP3$^+$ regulatory T cells (Tregs; CD4$^+$CD25$^+$FOXP3$^+$), and lowered intragraft myeloid activity (p<0.05). IL-2c also increased Treg:Teff ratios, where effector T cells (Teffs) were defined as CD3$^+$CD44^hi non-Tregs. Combined IL-2c and transient tacrolimus extended graft survival (MST 32→48 d; p=0.0018), with some recipients achieving long-term survival off immunosuppression (~100 d).

Conclusions: Pretransplant IL-2c therapy promotes regulatory immune dominance, suppresses adaptive and innate alloimmune responses, and prolongs cardiac allograft survival. These findings support IL-2c based Treg expansion as a promising adjunctive strategy to improve transplant outcomes and reduce reliance on long-term immunosuppression.

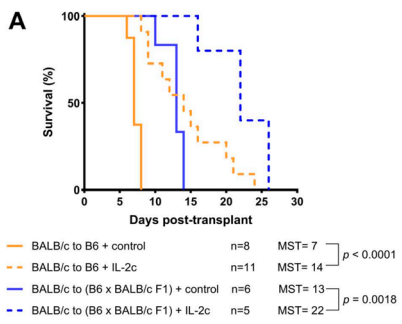

Figure 1A. Pretransplant IL-2c therapy prolongs cardiac allograft survival in both fully MHC-mismatched and hemi-allogeneic transplant models

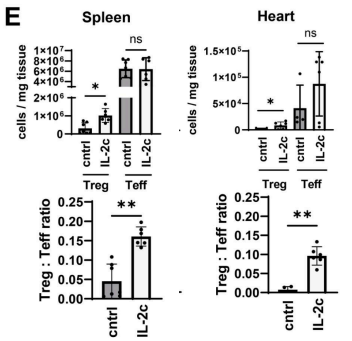

Figure 3E. IL-2c increases absolute Treg numbers and Treg:Teff ratios in spleen and heart (*p<0.05, **p<0.01; ns = not significant)

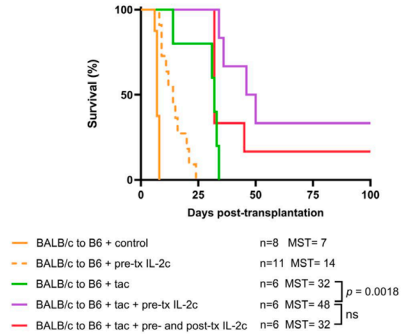

Figure 5. Pretransplant IL-2c + tacrolimus prolongs graft survival vs tacrolimus alone (p=0.0018); ns = not significant (p ≥ 0.05)

Key Findings: IL-2c therapy prolongs cardiac allograft survival, promotes regulatory T cell expansion, and shows additive benefit with transient tacrolimus, with some long-term graft survival off immunosuppression (Fig. 1A, 3E, 5).

Reviewer's Comments

- This study provides a rigorous evaluation of Treg based IL-2/anti-IL-2 complex therapy in a fully MHC-mismatched vascularized cardiac transplant model, addressing a key gap in preclinical transplant immunotherapy research.
- IL-2c therapy was associated with suppression of donor specific cellular and humoral alloimmune responses, supporting regulatory T cell expansion as an effective strategy to modulate early alloimmunity.
- Notably, IL-2c treatment was linked to reduced intragraft myeloid gene expression and macrophage infiltration, suggesting a broader immunomodulatory effect beyond adaptive immunity.

Limitations

- Immunologic effects of IL-2c, dose- and receptor-dependent, and study findings specific to anti-IL-2 mAb clone 5344.111 may limit their generalizability to other IL-2-based therapies or clinical settings.
- Potential off-target effects of IL-2 signaling, including CD25 upregulation on Teffs, raise concerns about unintended activation of alloreactive T cells or loss of Treg selectivity, and could decrease therapeutic efficacy or increase rejection risk.
- Despite prolonging graft survival, IL-2c monotherapy did not consistently achieve durable graft acceptance across full MHC mismatch; additional immunosuppression such as tacrolimus, IL-2c combination may be required.

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Alexandra Mihai, MD
Sapienza University, Rome, Italy

Rupa Kanchi, PhD
ISSUE EDITOR

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Endothelin-1 Overexpression in Pulmonary Endarterectomy Specimens of CTEPH Patients is Associated with Pulmonary Hypertension Development

Asghar U, Man HSJ, McInnis M, Ladak AM, et al. *JHLT* Jan 2026 Jan;45(1):116-124. | DOI: 10.1016/j.healun.2025.09.013

Study Highlights

Objective: To investigate if endothelin-1 (ET-1) expression in pulmonary endarterectomy (PEA) specimens is associated with development and severity of pulmonary hypertension in patients with chronic thromboembolic pulmonary hypertension (CTEPH).

Methods: PEA was studied in 75 patients including 40 with high mPAP (≥ 50 mmHg) and 35 with low mPAP (≤ 25 mmHg). PEA specimens were analyzed via immunohistochemistry (ET-1 protein) and qRT-PCR (ET-1 mRNA). Control specimens (n=20) were selected from non-CTEPH patients with mPAP > 25 mmHg who underwent lung transplantation (positive controls) and their corresponding donor lungs with normal hemodynamics (mPAP <20 mmHg, negative controls). Control tissues were processed in parallel as comparators for ET-1 expression. Preoperative CT angiograms were analyzed to assess vascular obstruction load (CTOI) and number of occluded segmental/subsegmental vessels.

Results: High mPAP group had significantly higher ET-1 expression (both protein and mRNA) in thrombotic tissue compared to the low mPAP group (p<0.0001). ET-1 expression correlated positively with total pulmonary vascular resistance. CTOI did not differ between groups, but the high mPAP group had a more blocked segmental and subsegmental vessels. Females in the high mPAP group exhibited higher ET-1 expression and more obstructed subsegmental vessels compared to males.

Conclusions: Non-resolving thromboembolic material represents a biologically active source of ET-1 in CTEPH, suggesting that mechanisms beyond mechanical obstruction contribute to PH progression and providing pathophysiological support for the use of endothelin receptor antagonists in selected CTEPH patients.

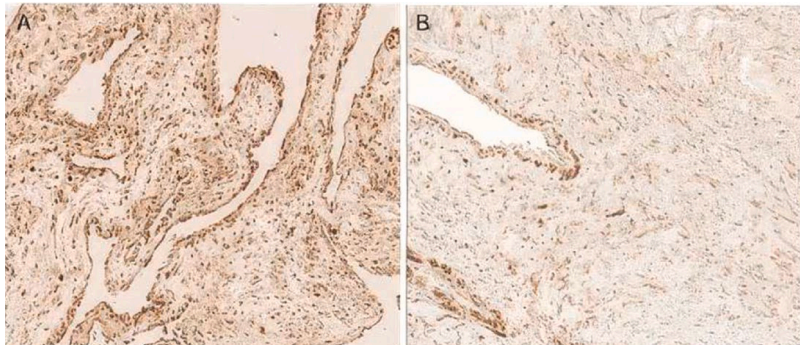

Figure 1 Immunolocalization of Endothelin-1: (A) higher mPAP group showing strong positive staining. (B) lower mPAP showing weak staining.

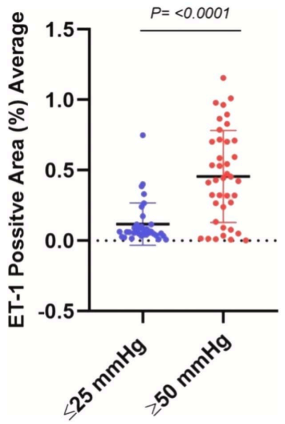

Figure 2 ET-1 expression by immunohistochemistry in High vs Low mPAP Groups.

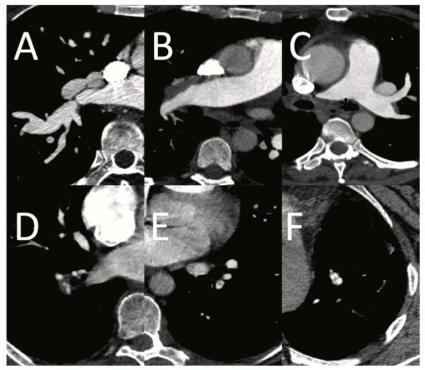

Figure 3 Spectrum of chronic thromboembolic findings on CT angiograms to assess CTOI.

Reviewer's Comments

- This study challenges the traditional mechanical paradigm of CTEPH by highlighting biological activity of chronic thromboembolic material. Despite similar CT obstruction burden, patients with higher mPAP exhibited markedly worse hemodynamics, suggesting proximal obstruction alone does not fully account for disease severity.
- Findings support a potential distal vascular involvement and molecular mechanisms, including ET-1 related pathways.
- Sex-specific differences in imaging presentation of CTEPH could be clinically relevant; future studies in subsegmental vessels may uncover role of microvascular disease in CTEPH.
- Plasma ET-1 levels did not differ between groups, likely reflecting predominantly local (paracrine) ET-1 activity rather than systemic release.

Limitations

- The retrospective, single-center design precludes causal inference; whether increased ET-1 drives higher mPAP or reflects more advanced disease remains uncertain.
- Baseline differences between high and low mPAP groups (including age and PH-targeted therapy) were not adjusted for in multivariable models, leaving potential residual confounding.
- ET-1 expression was not modeled using continuous mPAP values or formal interaction analyses (e.g., sex × mPAP), limiting assessment of independent and interaction effects.
- ET-1 was assessed only in proximal PEA specimens, and plasma levels were highly variable, limiting insight into distal microvascular involvement and systemic relevance.

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Baharan Zarrabian, DO, MPH
Duke University, Durham, NC, USA

Rupa Kanchi, PhD
ISSUE EDITOR
ISHLT.ORG

Early Post-transplant Recipient Tissue Injury Predicts Allograft Function, Rejection, and Survival in Lung Transplant Recipients, Evidence from Cell-free DNA

Alnababteh M, Keller MB, Kong H, Phipps K, et al. *Eur Respir J* 2025 Jul 31:2402537. | DOI: 10.1183/13993003.02537-2024

Study Highlights

Objective: To investigate if early post-transplant recipient tissue injury, measured by rd-cfDNA, is associated with death, rejection, and allograft dysfunction in lung transplant recipients.

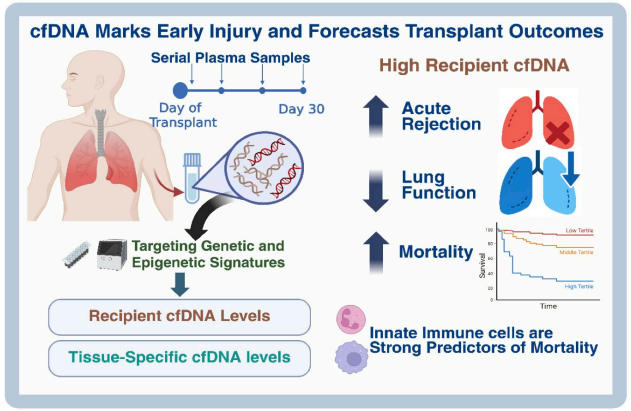
Methods:

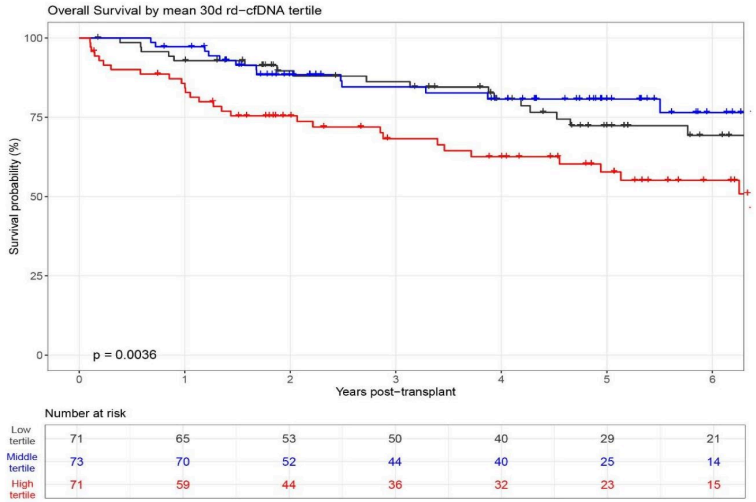
- Prospective cohort, multi-center study (GRAfT, NCT02423070)
- 215 adult lung transplant recipients with 2,530 cf-DNA measurements (at least 3 cfDNA samples per patient within first 30 days post-transplant).
- Healthy adults with no chronic disease recruited as controls
- Recipient-derived (rd) cfDNA measured by digital droplet PCR; tissue sources identified by whole-genome bisulfite sequencing.
- Associations between rd-cfDNA expression and outcomes were evaluated using Cox proportional hazards regression models.

Results:

- Median rd-cfDNA levels in first 30 days were ~16-fold higher than healthy controls.
- High tertile rd-cfDNA group (median 69,686 copies/mL, IQR 56,687-98,027) had lower lung function post-transplant (%FEV1 16.2% at 6 mo, 12.9% at 12 mo lower than low rd-cfDNA tertile), increased risk of death (HR:3.15, 95%CI:1.59-6.24) and acute rejection (HR: 2.33, 95%CI:1.33-4.08) vs. low (17,250 copies/mL, 14,028-20,033) and middle tertile (34,606 copies/mL, 30,373-40,658) groups.
- Tissue specific cfDNA sources collected at peak rd-cfDNA (n=76, day 7 post-transplant) differed between high and low tertiles, and lung transplant recipients and healthy controls. Innate immune cell cfDNA was strongest mortality predictor.

Conclusions: Early post-transplant recipient tissue injury, measured by rd-cfDNA levels, is predictive of worse outcomes.





Kaplan-Meier survival analysis of lung transplant recipients stratified by rd-cfDNA tertiles within 30 days post-transplant. Patients in the high rd-cfDNA tertile demonstrated significantly lower survival rates compared to those in the middle and low tertiles (p=0.0036).

Reviewer's Comments

- Innovative application of cfDNA technology to assess recipient (not just donor) tissue injury in lung transplantation.
- Large-prospective multi-center cohort design with large cohort size and many serial measurements
- Longitudinal sampling strategy to capture dynamic changes in tissue injury over time .
- Focuses on critical early post-transplant window when interventions could potentially prevent long-term complications.
- Strong associations demonstrated with clinically meaningful hard endpoints (mortality HR 3.15, acute rejection HR 2.33).

Limitations

- Moderate sample size and some missing outcome data (e.g. CLAD, ACR, AMR, BLAD) due to incomplete biopsies, limited follow-up or early death.
- Unclear whether rd-cfDNA provides independent predictive value beyond existing clinical assessment tools and biomarkers already used in routine practice, such as bronchoscopic biopsies, dd-cfDNA, HLA Abs, imaging etc.
- Tertile-based analysis require further validation in larger and diverse cohort studies prior to use for clinical decision-making.