JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 8, NO. 7, 2023

ORIGINAL RESEARCH - PRECLINICAL

Transvalvular Unloading Mitigates Ventricular Injury Due to Venoarterial Extracorporeal Membrane Oxygenation in Acute Myocardial Infarction

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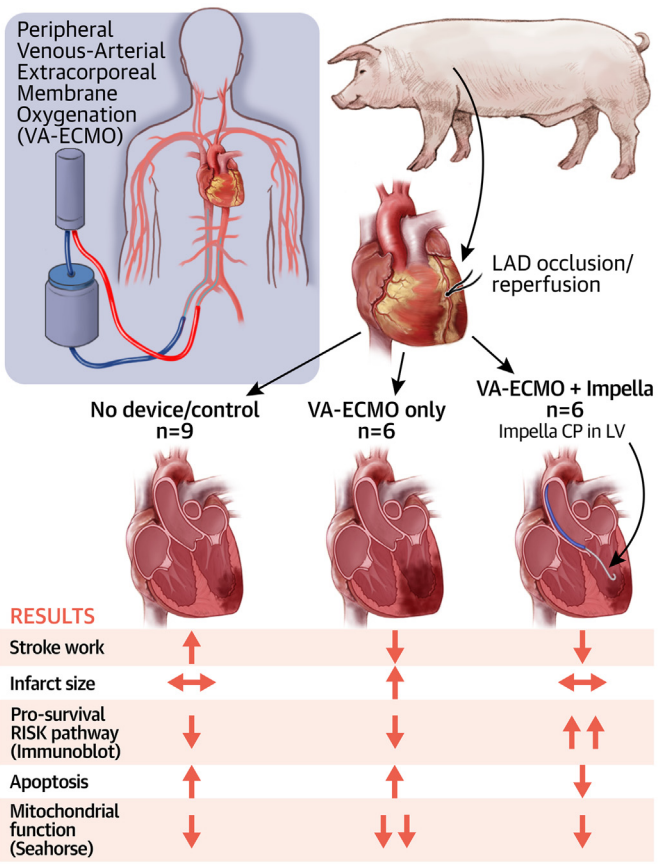
VISUAL ABSTRACT



Everett KD, et al. J Am Coll Cardiol Basic Trans Science. 2023;8(7):769-780.

HIGHLIGHTS

- **Transvalvular unloading with VA-ECMO (EC-Pella) is being used in patients with AMI.**
- **VA-ECMO increases IS despite reducing LV work while EC-Pella reduces IS and LV work.**
- **Cardioprotective signaling is activated with EC-Pella, not VA-ECMO.**
- **EC-Pella fails to rescue mitochondrial injury due to VA-ECMO.**
- **Load independent myocardial injury mechanisms due to VA-ECMO may be targets in AMI.**

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2023.01.004

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 8, NO. 7, 2023
JULY 2023:769–780

ABBREVIATIONS AND ACRONYMS

AAR = area at risk

AMI = acute myocardial infarction

Ao-LV = aortic-to-left ventricular

ECMO = extracorporeal membrane oxygenation

IRI = ischemia-reperfusion injury

IS = infarct size

LAD = left anterior descending coronary artery

LV = left ventricle/ventricular

PVA = pressure-volume area

RISK = reperfusion injury salvage kinase

TUNEL = terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick-end labeling

VA = venoarterial

SUMMARY

Whether extracorporeal membrane oxygenation (ECMO) with Impella, known as EC-Pella, limits cardiac damage in acute myocardial infarction remains unknown. The authors now report that the combination of transvalvular unloading and ECMO (EC-Pella) initiated before reperfusion reduced infarct size compared with ECMO alone before reperfusion in a preclinical model of acute myocardial infarction. EC-Pella also reduced left ventricular pressure-volume area when transvalvular unloading was applied before, not after, activation of ECMO. The authors further observed that EC-Pella increased cardioprotective signaling but failed to rescue mitochondrial dysfunction compared with ECMO alone. These findings suggest that ECMO can increase infarct size in acute myocardial infarction and that EC-Pella can mitigate this effect but also suggest that left ventricular unloading and myocardial salvage may be uncoupled in the presence of ECMO in acute myocardial infarction. These observations implicate mechanisms beyond hemodynamic load as part of the injury cascade associated with ECMO in acute myocardial infarction. (J Am Coll Cardiol Basic Trans Science 2023;8:769–780) © 2023 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Use of venoarterial (VA) extracorporeal membrane oxygenation (ECMO) has grown exponentially over the past decade, with a more than 11-fold increase for patients with acute myocardial infarction (AMI) in the United States and Europe.[1,2] VA-ECMO oxygenates and displaces venous blood into the arterial system using peripherally implanted venous drainage and arterial return cannulas, an oxygenator, and centrifugal flow pump. VA-ECMO provides full circulatory and respiratory support, is simple to implant without surgery, is widely available, and is being used more commonly in AMI and elective coronary interventions without cardiogenic shock or cardiac arrest.[3] Despite growing use, in-hospital mortality among VA-ECMO recipients remains high, about 40%, and is worse among recipients with AMI compared with those without AMI.[4,5]

Peripheral VA-ECMO displaces and oxygenates venous blood into the femoral artery, thereby driving retrograde flow in the aorta toward the left ventricle (LV), pressurizing the arterial system and increasing LV afterload and wall stress.[3] If LV contractility is impaired, as in AMI, higher LV afterload can increase LV pressure and volume.[6,7] As myocardial oxygen consumption correlates directly with the product of LV pressure and volume, known as the pressure-volume area (PVA),[8,9] VA-ECMO may increase LV PVA, thereby worsening oxygen supply-demand mismatch during AMI and increasing myocardial infarct size (IS). For every 5% increase in IS, 1-year mortality or hospitalization for heart failure increases by nearly 20%.[10] New approaches to mitigate LV injury associated with VA-ECMO could improve clinical outcomes.

Recent clinical studies suggest that LV distention after the initiation of VA-ECMO is associated with poor myocardial recovery, which has led to the use of concomitant pumps to decompress the LV in clinical practice.[11-14] However, the current paradigm of VA-ECMO-induced LV distention has not been rigorously tested, the timing of LV decompression (preemptive vs bailout) has not been studied, and the impact of peripheral VA-ECMO on ventricular hemodynamic status and reperfusion injury during AMI is poorly understood.

We and others have reported that LV unloading, defined by reduced LV PVA, using left atrial-to-femoral artery bypass or transvalvular Impella (Abiomed) pumps prior to reperfusion increases myocardial collateral blood flow, activates the reperfusion injury salvage kinase (RISK) pathway, limits mitochondrial damage, and reduces IS in preclinical models of AMI.[15-19] Whether the combination of ECMO and Impella, known as EC-Pella, promotes cardioprotective signaling and reduces myocardial IS has not been studied.

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

Manuscript received November 8, 2022; revised manuscript received January 6, 2023, accepted January 6, 2023.

We hypothesized that compared with initiation of ECMO alone, mechanically unloading the LV with a transvalvular Impella pump at the time of ECMO activation limits myocardial damage by promoting cardioprotective signaling in AMI.

METHODS

ANIMAL MODEL OF ISCHEMIA-REPERFUSION INJURY. Preclinical study protocols were approved by the Institutional Animal Care and Use Committee at Tufts Medical Center. All experiments were performed in accordance with the committee's guidelines. Adult male Yorkshire swine were anesthetized, mechanically ventilated, and prepared for angiography and hemodynamic assessment. Ischemia-reperfusion injury (IRI) was induced via percutaneous occlusion of the mid left anterior descending coronary artery (LAD) for 120 minutes, followed by 180 minutes of reperfusion. The mid-LAD was selected to avoid the development of cardiogenic shock. Angiography performed at the end of the study protocol confirmed LAD patency, as described in the Supplemental Methods.

To determine the impact of EC-Pella on IS during prolonged LAD occlusion, after 90 minutes of LAD occlusion, activation of either ECMO alone for 30 minutes or EC-Pella for 45 minutes was performed with persistent LAD occlusion (**Figures 1A and 1B**). In the control arm, LAD occlusion for 120 minutes was followed by 180 minutes of reperfusion alone (n = 9). To explore whether the sequence of device activation during EC-Pella affects IS, ECMO was activated at maximum flow levels without suction events for 30 minutes, followed by concomitant Impella CP with activation at the maximum tolerated power level without suction events for 15 minutes (ECMO-Impella; n = 6). In a separate group, Impella CP activation at maximum power level for 30 minutes was followed by concomitant ECMO activation at maximum flow without suction events for 15 minutes (Impella-ECMO) before reperfusion (n = 6). The relative timing of device activation was chosen on the basis of: 1) prior reports identifying 30 minutes of unloading prior to reperfusion as required for myocardial protection[15-19]; and 2) an expected procedural delay for second pump insertion and activation prior to reperfusion in clinical practice. Both EC-Pella groups underwent 45 minutes of support prior to reperfusion.

VA-ECMO cannulation was performed using a 19-F arterial cannula and a 25-F multistage venous cannula in the left femoral artery and vein, respectively. ECMO was activated at 3,500 rpm and increased to 6,500 rpm over 5 minutes using a centrifugal pump (Cardiac Assist, LivaNova) and membrane oxygenator (Maquet, Getinge). The Impella CP was positioned in the LV via the right internal carotid artery and activated at maximal support (44,000 rpm).

HEMODYNAMIC ASSESSMENT. Invasive hemodynamic assessment included continuous acquisition of arterial and pulmonary artery waveforms and LV pressure-volume loop recordings (CD Leycom). Deviations in pressure-volume relationships and hemodynamic calculations were tabulated from a minimum of 5 consecutive beats. Volumetric calibration was performed using 10 mL hypertonic (6%) saline injections into the pulmonary artery. Pressure-volume indexes were automatically tabulated in Conduct NT software (CD Leycom). PVA was estimated as the product of end-systolic pressure and end-diastolic volume. Ventriculoarterial coupling was quantified as the difference between peak-to-peak aortic-to-LV (Ao-LV) systolic pressures from simultaneous recordings.

IS QUANTIFICATION. Upon study protocol completion, repeat LAD balloon occlusion was performed, and 0.5% Evans blue was injected into both coronary vessels to define the area at risk (AAR). The LV was removed and sectioned into 4 1-cm slices at and distal to the site of balloon occlusion. Slices were counterstained with 2,3,5-triphenyltetrazolium chloride to identify viable myocardium. The total myocardial area, AAR, and infarct zone were quantified by 3 blinded investigators. Tissue samples were obtained from the infarct and noninfarct zones for analysis.

APOPTOSIS PATHWAY AND CARDIOPROTECTIVE SIGNALING QUANTIFICATION. Total protein was extracted from tissue homogenates and isolated as previously described.[20] Immunoblot analysis was performed using antibodies against porcine B-cell lymphoma (Bcl)-2, Bcl extra-large (Bcl-XL), Bax, caspase-3, Akt, glycogen synthase kinase 3-β (GSK-3β), and extracellular regulated kinase (Erk) (Cell Signaling Technology). Protein levels were normalized to vinculin. To assess activated relative to total protein levels, phosphorylated vs total Akt, Erk, and GSK-3β; cleaved vs pro-caspase-3; and activated vs degraded Bax levels were also quantified.

Terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick-end labeling (TUNEL) was performed on peri-infarct tissue samples sectioned to 10 μm and fixed in 4% paraformaldehyde/phosphate-buffered saline for 20 minutes. Slides were assembled and tissue permeabilized on ice with 0.1% Triton

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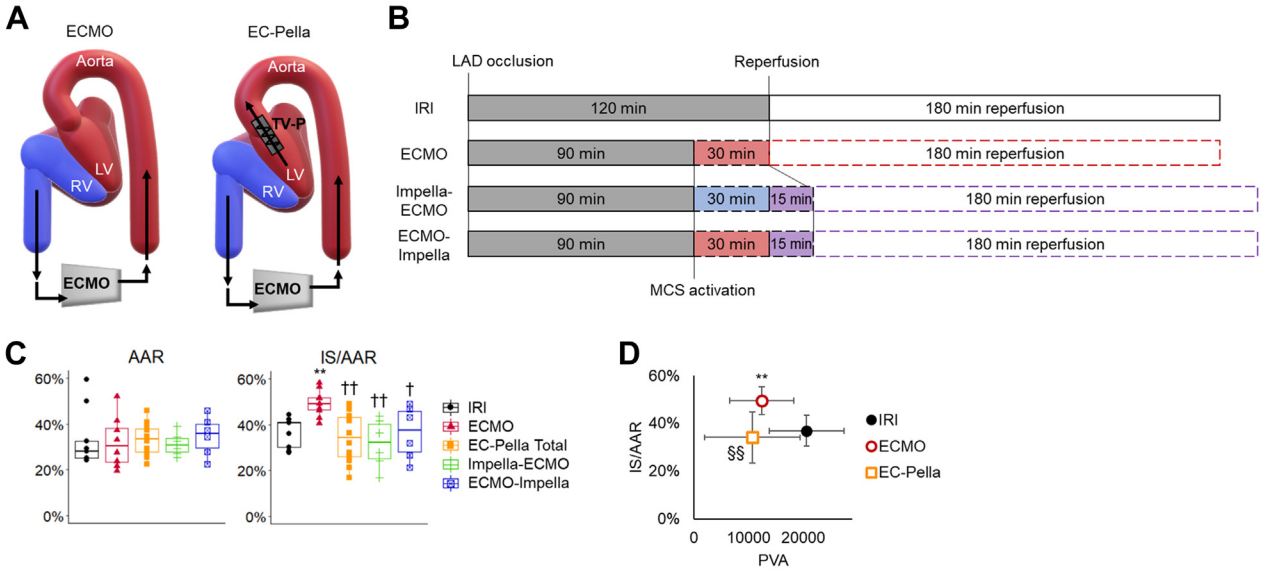
FIGURE 1 Study Protocol and Myocardial Infarct Size Among Groups



Schematic of mechanical circulatory support application in situ **(A)**, study timeline **(B)**, infarct size **(C)**, and relationship to pressure-volume area (PVA) **(D)** among groups. Values are individual data points or mean ± SD. $^{**}P < 0.01$ vs ischemia-reperfusion injury (IRI), †$P < 0.05$ vs extracorporeal membrane oxygenation (ECMO), ††$P < 0.01$ vs ECMO, and §§$P < 0.01$ for infarct size (IS)/area at risk (AAR) vs ECMO and $P < 0.05$ for PVA vs IRI. EC-Pella = extracorporeal membrane oxygenation plus Impella; LAD = left anterior descending coronary artery; LV = left ventricle; RV = right ventricle.

X-100 in 0.1% sodium citrate prior to rinsing and labeling with ProLong Gold Antifade with 4′,6-diamidino-2-phenylindole (Life Technologies) at 37 °C for 60 minutes in the dark. Images were acquired using an Eclipse E800 fluorescence microscope (Nikon) at 10× magnification and analyzed using ImageJ version 1.53k software (National Institutes of Health). Quantification of TUNEL-positive cells was performed by a blinded investigator and expressed as a percentage of all nuclei.

MITOCHONDRIAL ISOLATION, OXYGEN CONSUMPTION RATE AND CARDIOLIPIN ASSAY. Mitochondria were isolated from tissue sampled from the infarct and noninfarct zones. Oxygen consumption rate was analyzed using the Seahorse XF96 Extracellular Flux Analyzer (Seahorse Bioscience) (Supplemental Methods). Cardiolipin and monolysocardiolipin content was quantified using liquid chromatography/mass spectrometry (Supplemental Methods). Electron microscopic images were obtained on sections from the infarct zone.

STATISTICAL ANALYSIS. Continuous data are presented as mean ± SD. Comparisons between 2 groups used Student's *t*-test with unequal variance, while comparisons among >2 groups were performed using 1-way analysis of variance. A 2-sided *P* value <0.05 was considered to indicate statistical significance. Simple linear regression was used to test if PVA significantly predicted Ao-LV pressure. Analysis of covariance was used to compare regression lines by group, and results are presented using Pearson's correlation coefficient (*r*) and regression coefficient (β). All analyses were performed using RStudio Statistical Software version 2021.9.0.351 (RStudio). Every calculation is based on 6 to 9 animals from each group, as indicated in the figure legends.

RESULTS

EC-PELLA REDUCES IS COMPARED WITH ECMO, BUT NOT REPERFUSION ALONE. To evaluate whether transvalvular unloading before or after initiation of ECMO affects IS, we compared activation of ECMO alone to EC-Pella prior to reperfusion. Consistent with expected procedural mortality, 2 animals died after reperfusion in each EC-Pella group as well as in the IRI group, and 1 animal died prior to initiation of

any mechanical support. Compared with IRI, ECMO significantly increased IS relative to AAR (IS/AAR), but EC-Pella did not (Figure 1C) (36.9% ± 7% vs 49.5% ± 6% vs 34.1% ± 11%, IRI vs ECMO vs EC-Pella; $P = 0.002$, IRI vs ECMO; $P = 0.477$, IRI vs EC-Pella). Compared with ECMO, both EC-Pella configurations significantly reduced IS/AAR (31.8% ± 11% and 36.5% ± 11%, Impella-ECMO and ECMO-Impella; $P = 0.007$ and $P = 0.041$ vs ECMO). No difference in IS/AAR was observed with Impella-ECMO compared with ECMO-Impella. No difference in AAR relative to total LV area was observed between groups. These findings demonstrate that compared with ECMO alone, but not reperfusion alone, transvalvular unloading initiated before or after ECMO may limit myocardial damage attributable to ECMO.

IMPELLA-ECMO REDUCES PVA AND UNCOUPLES VENTRICULAR AND SYSTEMIC PRESSURES WITHIN MINUTES. Next, we studied whether the sequence of device activation with EC-Pella acutely influences ventricular hemodynamic status before reperfusion. For the ECMO-Impella group, mean ECMO flow was 3.8 ± 0.3 and 3.8 ± 0.4 L/min before and after initiation of Impella support, respectively. Impella flow was 2.8 ± 0.9 L/min in this group (Table 1). For the Impella-ECMO group, mean Impella flow was 3.4 ± 0.2 and 3.8 ± 0.4 L/min before and after initiation of ECMO, respectively. ECMO flow was 2.9 ± 0.5 L/min in this group. Compared with reperfusion alone, ECMO alone did not acutely change right ventricular or LV filling pressures but decreased LV end-diastolic and end-systolic volumes (Table 1, Figures 2A, 2B, and 3), with a significant decrease in PVA. Compared with reperfusion alone, both EC-Pella groups acutely reduced pulmonary capillary wedge pressure, but only Impella-ECMO significantly reduced pulmonary capillary wedge pressure compared with ECMO alone. Impella-ECMO but not ECMO-Impella significantly reduced end-diastolic volume and end-systolic volume compared with reperfusion alone or ECMO alone. Impella-ECMO also significantly reduced PVA compared with reperfusion alone, ECMO alone, or ECMO-Impella (Figures 2C and 3).

The peak-to-peak Ao-LV pressure gradient increased with EC-Pella compared with reperfusion alone or ECMO alone (Figure 2D). Regression of PVA to the difference between Ao-LV pressure demonstrated that as PVA decreased, Ao-LV pressure increased, reflecting VA uncoupling associated with ventricular unloading (Figures 2E and 3). The overall regression for each group was statistically significant. PVA significantly correlated with Ao-LV pressure, and this relationship was statistically different between groups ($P < 0.001$, interaction test). These findings support that ECMO alone can reduce PVA before reperfusion and further that Impella activation before ECMO provided superior LV unloading compared with Impella activation after ECMO.

EC-PELLA INCREASES CARDIOPROTECTIVE SIGNALING. To explore mechanisms governing the cardioprotective effect of EC-Pella, we quantified signaling effectors of the canonical RISK pathway and apoptosis. Compared with IRI, ECMO failed to activate the RISK pathway or alter apoptotic signaling (Figures 4A and 4B). Compared with IRI or ECMO alone, both EC-Pella configurations significantly increased levels of phosphorylated Akt, Erk, and GSK-3β. Both EC-Pella configurations also increased antiapoptotic signaling with higher levels of Bcl-2 and Bcl-XL, reduced levels of active Bax, and lower levels of cleaved caspase-3 (Figures 4A and 4B). Compared with ECMO alone, TUNEL staining was significantly reduced by Impella activation before but not after ECMO (Figures 4C and 4D).

EC-PELLA IMPROVES MITOCHONDRIAL COMPLEX I ACTIVITY. To explore the functional integrity of mitochondria, we tested various components of the electron transport chain in mitochondria isolated from the infarct and noninfarct zones (Figure 5). Compared with the noninfarct zone, reperfusion alone reduced the rate of basal oxygen consumption in the presence of complex I substrates, whereas function of complexes II, III, and IV was similar between the noninfarct and infarct zones. Compared with noninfarct zones, ECMO initiation significantly decreased oxygen consumption rate in complexes I, II, and III. EC-Pella failed to rescue complexes I, II, and III irrespective of whether Impella was initiated before or after ECMO. Compared with IRI and ECMO alone, EC-Pella increased oxygen consumption rate in complex I in the infarct zone when Impella was initiated before but not after ECMO. Compared with IRI, cardiolipin levels were reduced with EC-Pella (Figure 5D).

DISCUSSION

ECMO use in the setting of AMI is growing. We now provide new mechanistic insight into the effect of ECMO on myocardial IRI with and without concomitant transvalvular unloading. We first observed that compared with reperfusion alone, ECMO initiation before reperfusion increases IS, fails to activate canonical cardioprotective signaling via the RISK

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TABLE 1 Hemodynamic Indexes Prior to Reperfusion

	IRI (n = 9)	ECMO (n = 6)	EC-Pella Total (n = 12)	Impella-ECMO (n = 6)	ECMO-Impella (n = 6)
RAP, mm Hg	6.2 ± 3.0	4.0 ± 1.4	3.7 ± 3.3	2.2 ± 2.1	5.2 ± 3.8
PCWP, mm Hg	11.3 ± 3.1	8.8 ± 4.1	4.8 ± 3.1[a]	3.5 ± 3.0[a,b]	6.2 ± 2.9[c]
MAP, mm Hg	95.7 ± 19.3	90.8 ± 21.3	83.7 ± 14.7	76.0 ± 11.7	91.3 ± 14.1
Ao SBP, mm Hg	111.3 ± 20.7	104.7 ± 21.3	89.8 ± 18.8	82.8 ± 17.9	96.8 ± 18.5
Ao DBP, mm Hg	82.2 ± 17.2	83.8 ± 19.7	79.1 ± 13.2	75.0 ± 11.2	83.2 ± 14.9
CO, L/min	4.2 ± 1.8	2.6 ± 0.8[d]	2.0 ± 0.9[c]	2.0 ± 1.1[c]	2.0 ± 0.7[c]
ECMO flow, L/min	NA	3.5 ± 0.36	3.3 ± 0.6	2.9 ± 0.5[b,e]	3.8 ± 0.4
Impella flow, L/min	NA	NA	2.5 ± 0.8	2.2 ± 0.7	2.8 ± 0.9
EDP, mm Hg	15.3 ± 6.8	6.4 ± 8.3	2.3 ± 8.0[a]	−1.9 ± 8.4[c]	6.4 ± 5.2[d]
ESP, mm Hg	99.7 ± 24.0	85.2 ± 13.9	60.2 ± 37.0[c]	43.4 ± 38.0[b,d]	77.0 ± 30.0
EDV, mL	210.0 ± 53.1	140.8 ± 40.8[d]	144.0 ± 87.6[d]	88.8 ± 63.1[c,f]	199.1 ± 74.8
ESV, mL	162.1 ± 58.8	107.8 ± 26.3[d]	117.8 ± 81.6	61.8 ± 48.7[c,e]	173.8 ± 68.9
SW, mm Hg · mL	3,905 ± 1,598	2,450 ± 1,438	1,190 ± 1,146[a]	1,022 ± 1,206[c]	1,358 ± 1,170[c]
PVA, mm Hg · mL	20,652 ± 6,802	12,456 ± 5,912[d]	10,729 ± 8,697[c]	5,137 ± 5,166[a,b,f]	16,322 ± 8,041
dP/dt max, mm Hg/s	916.9 ± 283.6	634.8 ± 111.2[d]	371.0 ± 172.7[a,g]	289.6 ± 175.5[a,g]	452.4 ± 137.6[b,c]
Tau, ms	33.1 ± 5.4	34.8 ± 4.9	30.8 ± 13.7	23.1 ± 15.7	38.5 ± 4.9
EDPVR, mm Hg/mL	0.08 ± 0.05	0.04 ± 0.04	0.11 ± 0.35	0.20 ± 0.50	0.03 ± 0.03
ESPVR, mm Hg/mL	0.73 ± 0.37	0.81 ± 0.11	0.45 ± 0.41	0.46 ± 0.57	0.49 ± 0.20

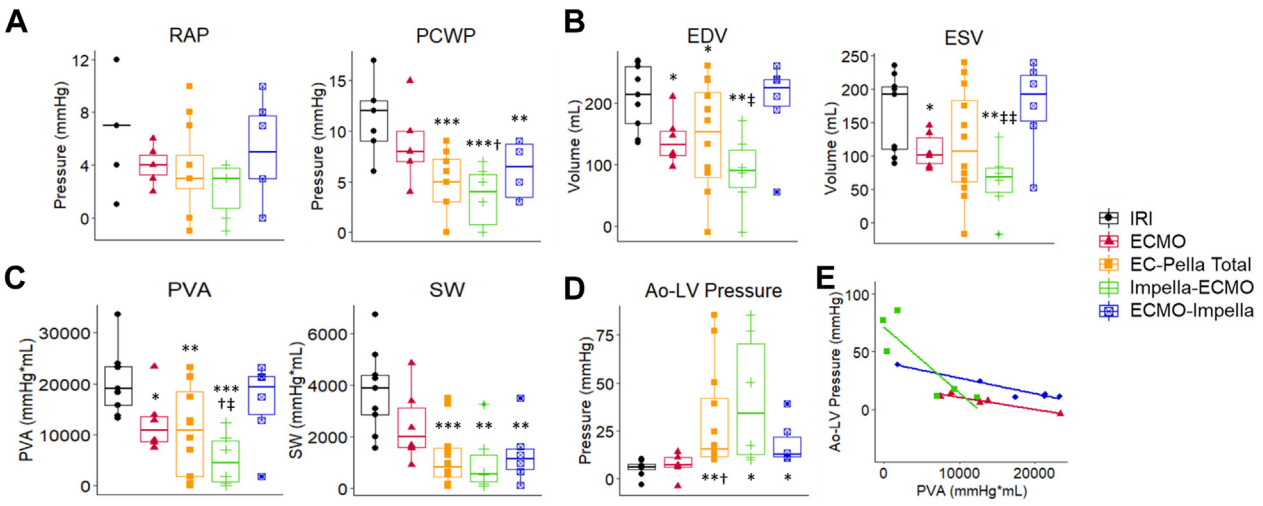
Values are mean ± SD. [a]$P < 0.001$ vs IRI. [b]$P < 0.05$ vs ECMO. [c]$P < 0.01$ vs IRI. [d]$P < 0.05$ vs IRI. [e]$P < 0.01$ vs ECMO-Impella. [f]$P < 0.05$ vs ECMO-Impella. [g]$P < 0.01$ vs ECMO.
Ao = aortic; CO = cardiac output; DBP = diastolic blood pressure; dP/dt max = maximum rate of change in pressure vs time; ECMO = extracorporeal membrane oxygenation; EDP = end-diastolic pressure; EDPVR = end-diastolic pressure-volume relationship; EDV = end-diastolic volume; ESP = end-systolic pressure; ESPVR = end-systolic pressure-volume relationship; ESV = end-systolic volume; IRI = ischemia-reperfusion injury; MAP = mean arterial pressure; NA = not applicable; PCWP = pulmonary capillary wedge pressure; PVA = pressure-volume area; RAP = right atrial pressure; SBP = systolic blood pressure; SW = stroke work.

pathway or reduce apoptosis, and disrupts the function of multiple complexes within the mitochondrial electron transport chain. We further observed that EC-Pella, when initiated before reperfusion, limits IS compared with ECMO alone but not reperfusion alone. Given that the devices are not commonly inserted and activated simultaneously in clinical practice, we further tested whether the sequence of device activation influences myocardial salvage. Impella activation before, but not after, ECMO significantly reduced LV PVA. Both EC-Pella configurations increased cardioprotective signaling and reduced apoptosis but failed to rescue mitochondrial function compared with ECMO alone.

These observations introduce new concepts with important considerations for future clinical evaluation and management. We demonstrate for the first time that ECMO fails to promote cardioprotective signaling or limit IS in the setting of AMI with reperfusion alone. Second, we illustrate that to achieve optimal LV unloading with ECMO, Impella activation should be performed before, not after, ECMO activation. Third, we introduce the concept that LV unloading mitigates LV injury due to ECMO but is not sufficient to rescue reperfusion injury. These findings suggest that mechanisms independent of hemodynamic load may be responsible for increased myocardial injury associated with ECMO. Further investigation to identify novel targets of therapy to reduce reperfusion injury in the presence of ECMO is required.

Several clinical trials are currently evaluating the utility of ECMO in AMI.[21-23] The premise behind these studies is that ECMO replaces cardiac output and provides systemic circulatory support. However, our current understanding of ECMO suggests that the cost of pressurizing the arterial circulation is an increase in LV afterload, which in turn increases ventricular wall stress and myocardial oxygen consumption, all factors that would potentially increase IS. Preclinical and clinical studies identified that reducing LV wall stress, stroke work, or PVA when initiated before reperfusion limits myocardial IS and may improve survival.[15-19,24,25] We now introduce new data showing that in a preclinical model of AMI with ECMO initiated prior to reperfusion, LV unloading is uncoupled from myocardial salvage. In this model, ECMO significantly reduced LV PVA and stroke work by reducing LV volume, but despite this hemodynamic effect, myocardial IS increased compared with

FIGURE 2 **Hemodynamic Indexes Among Groups Prior to Reperfusion**



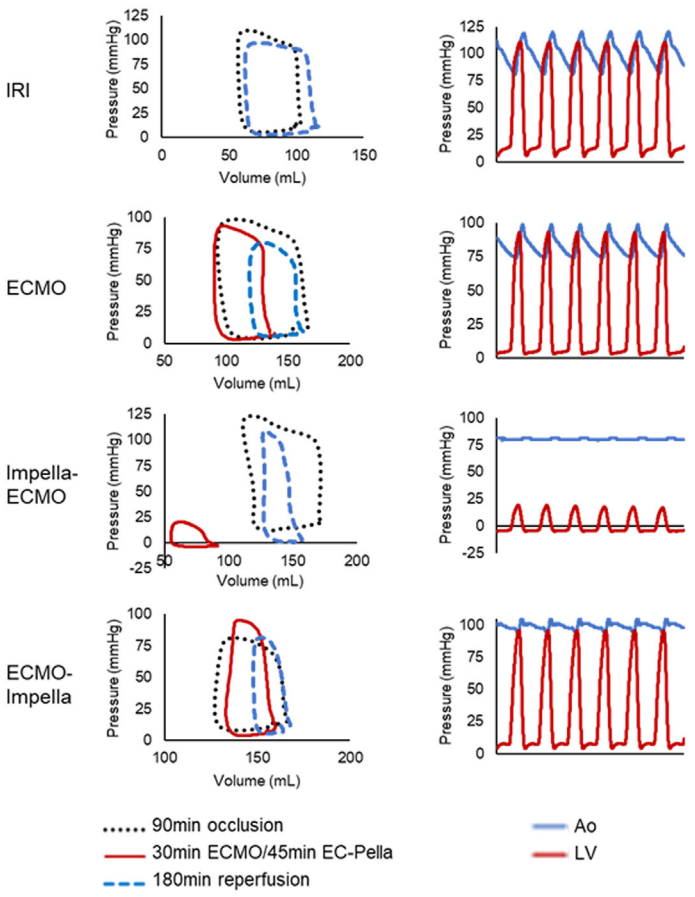
Key metrics demonstrating RV and LV filling pressures **(A)**, LV volumes **(B)**, LV work **(C)**, ventriculoarterial coupling **(D)**, and linear regression of PVA to aortic (Ao)-LV pressure **(E)** among groups. Values are individual data points. *$P < 0.05$ vs IRI, **$P < 0.01$ vs IRI, ***$P < 0.001$ vs IRI, †$P < 0.05$ vs ECMO, ‡$P < 0.05$ vs ECMO-Impella, and ‡‡$P < 0.01$ vs ECMO-Impella. For the regression, $r = -1.06E\text{-}3$ ($P = 0.009$), $r = -1.35E\text{-}3$ ($P = 0.002$), and $r = -5.68E\text{-}3$ ($P = 0.026$) for ECMO, ECMO-Impella, and Impella-ECMO, respectively; regression coefficients: $\beta = -0.0011$, $\beta = -0.0014$, and $\beta = -0.0057$ for ECMO, ECMO-Impella, and Impella-ECMO, respectively (P for interaction = 0.0002). EDV = end-diastolic volume; ESV = end-systolic volume; PCWP = pulmonary capillary wedge pressure; RAP = right atrial pressure; SW = stroke work; other abbreviations as in Figure 1.

reperfusion alone. In contrast to prior preclinical studies using transvalvular pumps or left atrial bypass, reduced LV PVA with ECMO was not associated with increased cardioprotective signaling. These finding suggest that ECMO may promote LV injury via mechanisms that are not load dependent. One possible explanation for increased myocardial damage with ECMO may be related to impaired coronary perfusion. We previously reported that compared with a transvalvular pump, ECMO may decrease coronary blood flow and thereby increase IS.[26] Decreased coronary blood flow may reflect microvascular vasoconstriction due to exposure to hyperoxemia and requires further investigation.

Apoptosis is a vital process for programmed cell death of damaged cells and is a central mediator of myocardial IRI.[20] The intrinsic apoptotic pathway is regulated by antiapoptotic proteins known as Bcl-2 and Bcl-XL and proapoptotic proteins including Bax and Bak. During coronary occlusion, reduced myocardial oxygen delivery disrupts mitochondrial oxidative phosphorylation and increases anaerobic glycolysis, which in turn reduces intracellular pH and promotes mitochondrial calcium influx and opening of mitochondrial transpermeability pores in the inner mitochondrial membrane that accelerates mitochondrial damage.[27] As a result, loss of Bcl-2 during IRI allows Bax and Bak to create a second pore in the outer mitochondrial membrane that releases cytochrome C into the cytoplasm and activates caspase-mediated cellular degradation via caspase-3.[28,29] Recent reports illustrate that stabilizing mitochondrial electron transport chain function during ischemia preserves Bcl-2 content and limits myocardial cell death after reperfusion.[30] Mitochondrial complex I, the reduced form of nicotinamide adenine dinucleotide (nicotinamide adenine dinucleotide + hydrogen) ubiquinone oxidoreductase, is deactivated during myocardial ischemia and is a particularly important target of therapy to stabilize electron transport chain function.[31]

We now introduce for the first time that ECMO activation alone prior to reperfusion fails to stabilize mitochondrial electron transport chain function, does not improve Bcl-2/Bax ratio, and is associated with increased IS compared with reperfusion alone. We further observed that LV unloading with Impella before or after ECMO initiation was sufficient to increase RISK pathway activity and Bcl-2/Bax ratio. However, despite activation of cardioprotective

FIGURE 3 Representative Hemodynamic Tracings

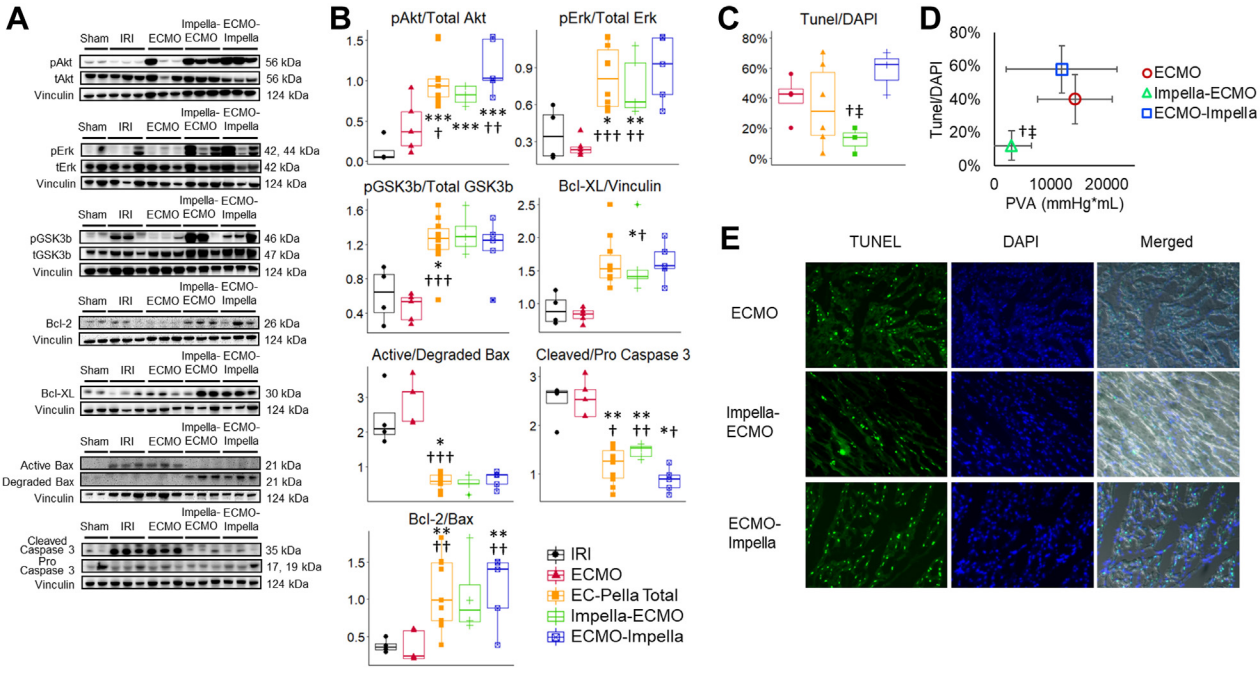


Changes in LV pressure-volume loops **(left)** and the association with simultaneous Ao-LV pressure-time data **(right)**. Pressure-volume loops are shown at 90 minutes of occlusion **(black, dashed)**, after mechanical circulatory support (MCS) activation (30 minutes ECMO, 45 minutes EC-Pella), prior to reperfusion **(red, solid)**, and 180 minutes of reperfusion **(blue, hashed)**. The **right panels** show pressure-time tracings for aorta (Ao) **(blue)** and LV **(red)** prior to reperfusion. After MCS activation and prior to reperfusion, ECMO decreased LV volumes. With Impella-ECMO, a phenomenon of maximal PVA reduction was observed, at which time simultaneous Ao-LV pressure-time recordings demonstrated maximal venoarterial uncoupling. Values are simultaneous recordings at noted time points. Abbreviations as in Figure 1.

signaling, neither EC-Pella configuration rescued mitochondrial function, and only Impella activation before ECMO reduced apoptosis. This suggests a phenomenon whereby ECMO may induce mitochondrial injury that is not recoverable with EC-Pella. One explanation for the dissociation between cardioprotective signaling and mitochondrial protection with EC-Pella may be that ECMO directly impairs mitochondrial function, and as a result upstream cardioprotective signaling is no longer effective. We recently reported that ECMO reduces mitochondrial levels of cardiolipin, a critical lipoprotein required for mitochondrial structural and functional integrity.[18] Loss of cardiolipin may account for reduced mitochondrial integrity and may not be rescued with LV unloading.

In current clinical practice, the addition of a transvalvular unloading pump with ECMO is performed to decompress the LV and limit the likelihood of worsening pulmonary congestion in the setting of impaired LV function or when a patient deteriorates with transvalvular unloading alone and requires ECMO initiation to enhance circulatory support because of right heart failure, sepsis, cardiac arrest, or worsening shock.[11-14] Recent analysis of clinical registries identified that the combination of ECMO and transvalvular unloading may be associated with improved survival despite increased risk for vascular complications.[11] However, the mechanism underlying this potential benefit remains poorly understood. We now introduce the concept that pre-emptive unloading before initiation of ECMO may be a superior strategy compared with bailout unloading after ECMO to optimally reduce cardiac filling pressures and LV PVA. Flow through a transvalvular pump is associated directly with LV preload and inversely with LV afterload. This is observed most commonly in the presence of right heart failure or bleeding, in which reduced LV preload triggers uncoupling of aortic and LV pressures followed by a suction event in which LV volumes are maximally reduced.[32] By initiating LV unloading with Impella first, we observed reduced LV PVA that was further accentuated by ECMO, which may reflect a decrease in LV preload leading to an uncoupling of aortic and LV pressures. In contrast, when initiating ECMO, any reduction in LV preload is offset by an increase in LV afterload, leading to a relative decrease in PVA. Impella activation after this has occurred is unable to further reduce LV preload or afterload, hence there is no effect on PVA. Whether the duration of Impella or ECMO support prior to activation of the second device in sequence influences changes in PVA remains unknown. We selected 30 minutes of Impella or ECMO before activating the second device to reflect clinical practice in which a period of time may be required to stabilize the first pump, then insert and activate the second pump and allow an additional 15 minutes on support to perform revascularization. Despite 15 minutes' less ischemic time required for activation of EC-Pella,

FIGURE 4 Cardioprotective Signaling



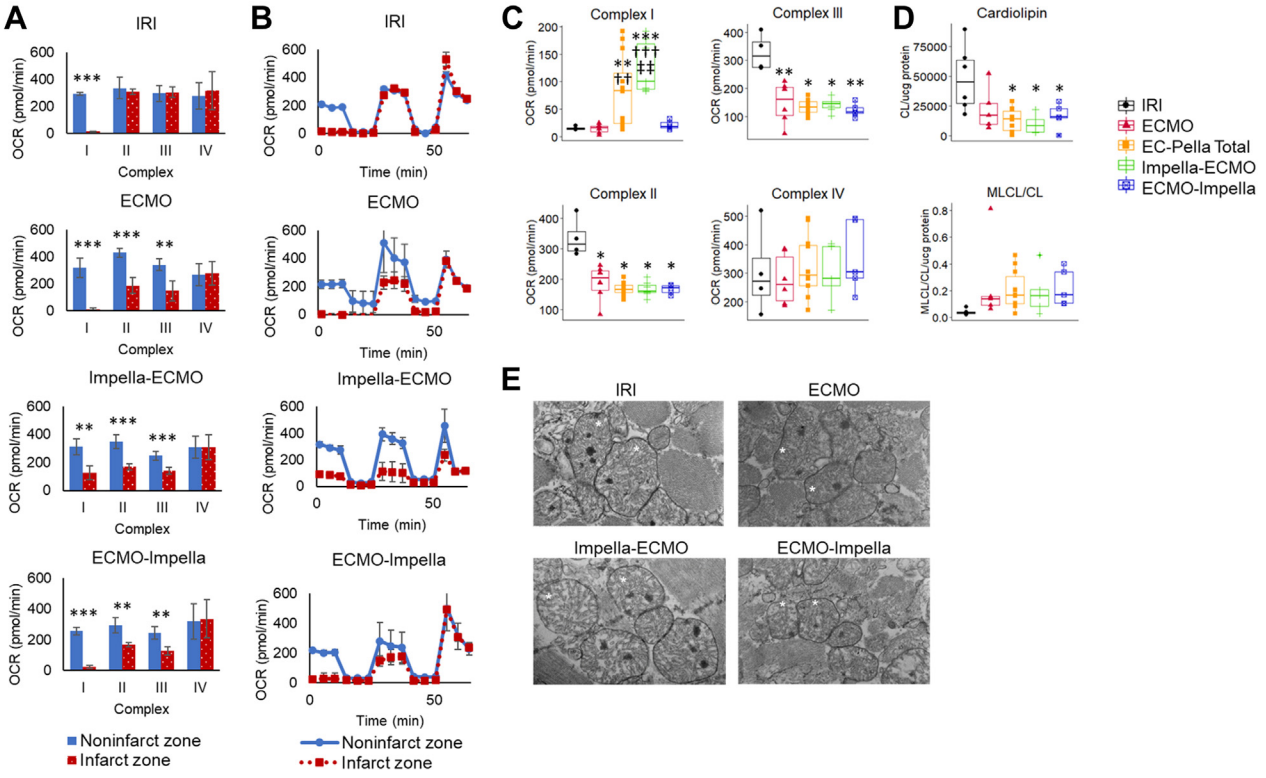
Western blots **(A)** and quantification **(B)** for cardioprotective and apoptotic signaling, terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick-end labeling (TUNEL) and 4′,6-diamidino-2-phenylindole (DAPI) staining **(C)**, representative images **(E)**, and relation to PVA **(D)** among groups. Values are individual data points or mean ± SD. *$P < 0.05$ vs IRI, **$P < 0.01$ vs IRI, ***$P < 0.001$ vs IRI, †$P < 0.05$ vs ECMO, ††$P < 0.01$ vs ECMO, †††$P < 0.001$ vs ECMO, and ‡$P < 0.05$ vs ECMO-Impella. Bcl = B-cell lymphoma; Bcl-XL = Bcl extra-large; GSK3b = glycogen synthase kinase 3; other abbreviations as in Figure 1.

ECMO increased IS compared with ischemia-reperfusion injury alone, and additional ischemic time may only magnify the effect of VA-ECMO on IS.

The REVERSE trial is currently enrolling patients with cardiogenic shock due to any cause and randomizing them to ECMO or ECMO followed by Impella within 10 hours of ECMO initiation, with a primary endpoint of survival free from mechanical circulatory support, heart transplantation, or inotropic support.[21] The ECLS-Shock trial is currently enrolling and randomizing patients with AMI and cardiogenic shock referred for early revascularization to ECMO vs no circulatory support, with a primary efficacy endpoint of 30-day mortality.[22] LV venting or unloading is permitted but not part of the randomization protocol. On the basis of our findings, both trials may identify limited benefit of ECMO initiation alone on myocardial damage, and neither specifically tests the timing of LV unloading relative to ECMO initiation. For this reason, a better understanding of how ECMO and LV unloading can be used in concert to optimize myocardial salvage and recovery in AMI is required.

STUDY LIMITATIONS. Limitations of this study include the small number of large animals studied per group. Statistical analysis did not account for multiple pairwise comparisons, which could limit the interpretability of the results. Healthy animals without comorbidities were included, and this may limit the clinical applicability of the findings. Specifically, the systemic effects associated with cardiogenic shock were not encompassed in this study and were intentionally avoided to limit any potentiation of myocardial damage imparted by reperfusion injury. Finally, longer term studies evaluating myocardial recovery, hemometabolic shock, and heart failure after ECMO and EC-Pella support are needed.

FIGURE 5 Mitochondrial Function and Integrity



(A) Quantification of individual complex activity in the noninfarct **(blue)** and infarct **(red, dotted)** zones. **(B)** Representative Seahorse plots. **(C)** Comparison of complex activity. **(D)** Cardiolipin (CL) and monolysocardiolipin (MLCL) levels in mitochondria isolated from the infarct zone. **(E)** Electron microscopy demonstrating mitochondrial structure **(asterisks)**. Values are individual data points or mean ± SD. In **A**, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$. In **C** and **D**, *$P < 0.05$ vs IRI, **$P < 0.01$ vs IRI, ***$P < 0.001$ vs IRI, ††$P < 0.01$ vs ECMO, †††$P < 0.001$ vs ECMO, and ‡‡$P < 0.01$ vs ECMO-Impella. OCR = oxygen consumption rate; other abbreviations as in Figure 1.

CONCLUSIONS

ECMO may be necessary in the setting of emergent circulatory collapse. Our findings suggest that ECMO activation before reperfusion increases myocardial IS despite a reduction in LV PVA. Activation of Impella before but not after ECMO initiation significantly reduces LV PVA, but irrespective of the sequence of device activation, EC-Pella limits myocardial damage compared with ECMO alone but fails to reduce reperfusion injury. Collectively, these findings suggest that myocardial damage is uncoupled from LV unloading in the presence of ECMO, and further study to identify potential targets of therapy to reduce myocardial injury with ECMO during AMI is required.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

This work was supported by National Institutes of Health grants R01HL159089-01 and R01HL139785-01. Dr Kapur has received consulting and speaker honoraria and institutional grant support from Abbott Laboratories, Abiomed, Boston Scientific, Edwards Lifesciences, LivaNova, Getinge, and Zoll. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: EC-Pella is designed to limit LV pressure overload while increasing systemic blood pressure for patients with AMI and cardiogenic shock. Recent preclinical studies suggest that LV unloading with Impella for 30 minutes before reperfusion may limit IS. In contrast, ECMO support before reperfusion may increase IS. Whether EC-Pella reduces IS remains unknown. We now report that initiating EC-Pella before reperfusion promotes cardioprotective signaling and reduces myocardial IS compared with ECMO alone but not compared with reperfusion alone. We further observed that activating Impella before ECMO significantly reduces LV wall stress compared with ECMO before Impella. Despite unloading the LV and increasing cardioprotective signaling, EC-Pella failed to limit mitochondrial damage associated with ECMO. These findings suggest that EC-Pella may be an effective method to limit LV distention and partially mitigate LV injury associated with ECMO and highlight the need for additional studies to understand the impact of ECMO on ischemia and reperfusion injury in AMI.

TRANSLATIONAL OUTLOOK 1: Although PVA is a surrogate for myocardial oxygen consumption, myocardial damage due to IRI reflects a complex interplay among cardioprotective, pro- and antiapoptotic signaling, and mitochondrial injury, among others.

TRANSLATIONAL OUTLOOK 2: Combining transvalvular unloading with VA-ECMO (EC-Pella) mitigates LV loading compared with VA-ECMO alone but does not rescue damage due to reperfusion injury.

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KEY WORDS **cardioprotection, hemodynamic status, mechanical circulatory support, unloading**

APPENDIX **For a supplemental Methods section and tables, please see the online version of this paper.**