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## Molecular- and Organelle-Based Predictive Paradigm Underlying Recovery by Left Ventricular Assist Device Support

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### History of Mechanical Circulatory Support Devices

Advanced heart failure (HF) is a major cause of morbidity and mortality in the United States, and heart transplantation remains as the gold standard therapy. Because of scarcity of donor organs, the application of mechanical circulatory support devices has become a crucial approach in HF therapy as a bridge to transplantation. Therefore, mechanical circulatory support devices have existed both conceptually and experimentally for >40 years, along which an exponential evolution of mechanical circulatory support device technology has occurred. To mimic human physiology, the first generation of left ventricular assist devices (LVADs) were pulsatile volume displacement pumps (HeartMate XVE LVAS, Thoratec PVAD, and Novacor, etc). Insights demonstrating the inessentiality of the pulsatile nature of LVADs for survival from a physiological standpoint propelled the design of second-generation continuous flow devices (HeartMate II [Thoratec Inc], the Micromed DeBakey VAD, Berlin Heart Incor [Berlin Heart AG], and the Jarvik 2000 [Jarvik Heart Inc]), which emerged with superior safety and durability. Consequently, the HeartMate II was approved for bridge to transplantation and destination therapy in the United States.<sup>1</sup> Recent reports<sup>2,3</sup> have demonstrated a 2-year survival rate of 87% in destination therapy patients under intense surveillance, comparable with open heart transplantation survival statistics. In parallel, these promising outcomes of LVADs in HF therapy have spawned the translational research field of LV reverse remodeling, which has already shown great promise for elucidating underlying molecular and cellular mechanisms.

### Clinical Insights Into Bridge to Recovery

HF is a highly complex clinical syndrome marked by a multitude of derangements, both in adult and pediatric populations.<sup>4</sup>

The clinical phenotype of HF begins with an injury or supra-normal stressor on the heart and, through prolonged dys-homeostasis, eventuates in cellular and organ failure. The compensatory adaptive mechanisms including neurohormonal activation (eg, the adrenergic system along with aldosterone, renin, and angiotensin) may initially maintain cardiac output, but the inability to sustain this eventually results in HF together with the release of proinflammatory cytokines. The response to neurohormonal activation, direct mechanical stretch, and LV volume overload results in progressive maladaptive remodeling (Figure 1). These derangements are potentially reversed during LVAD mechanical unloading.<sup>5-7</sup>

The current gold standard therapy for HF is open heart transplantation, yet it carries a 50% 10-year survival along with all complications related to long-term immunosuppression and increased medical costs. With the evolution of LVAD technology and reliability, there has been anecdotal evidence of sufficient LV recovery by LVAD support to a point allowing device removal (ie, bridge to recovery [BTR]).<sup>8</sup> The Harefield group was the first to study a homogenous population of patients with HF, in which they applied a defined pharmacological protocol along with LVAD unloading to induce recovery. This involved high-intensity neurohormonal blockade followed by induction of physiological hypertrophy with a high-dose clenbuterol, a direct  $\beta$ -agonist, to optimize LV recovery. The procedure resulted in 63.2% of patients successfully recovering after LVAD explantation.<sup>9</sup>

Reliable and robust predictive parameters and patient selection criteria for successful BTR have not yet been established. Patients with nonischemic cardiomyopathy (NICM) have been targeted in studies<sup>10,11</sup> as the group most likely to undergo successful BTR. However, this NICM population itself has heterogeneous underlying causes, such as viral myocarditis,

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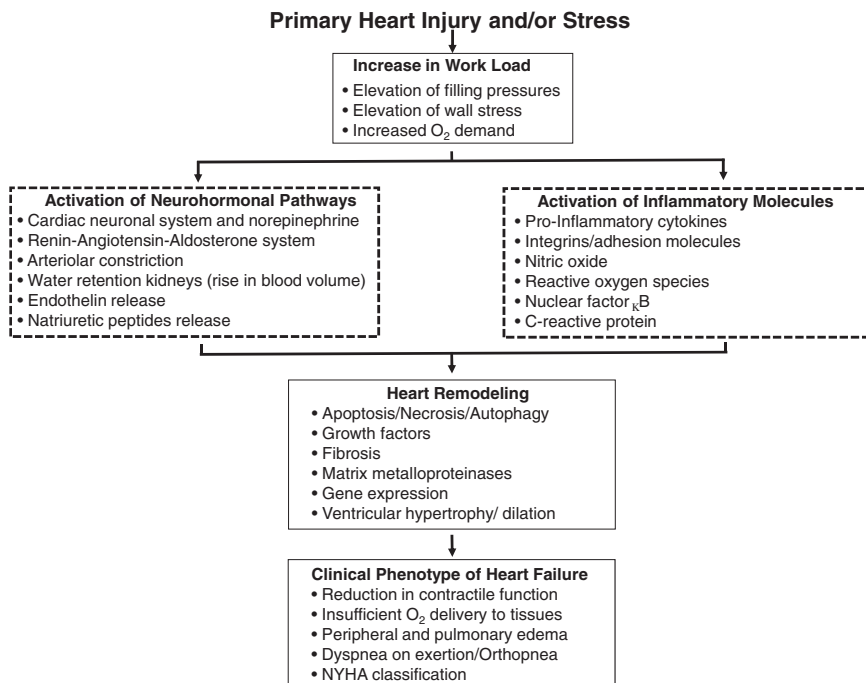
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**Figure 1.** Pathophysiology of heart failure. Compensatory mechanisms to maintain cardiac output eventually result in negative remodeling of the ventricles. NYHA indicates New York Heart Association.

postpartum cardiomyopathy, direct toxin or radiation exposure, and congenital cardiomyopathies. Pre-existing clinical determinants, such as older age, larger LV diameters before LVAD implantation, higher degree of myocardial fibrosis, and a prolonged duration of HF >6 months, have been predictive of decreased success of recovery in patients with NICM and higher risk of developing HF after LVAD explantation.<sup>12</sup> In the HeartMate II bridge-to-transplant and destination therapy trials enrolling 1108 patients with NICM, HF of <1 year and age of  $\leq 45$  years were predictors of successful BTR and 2-year transplant-free survival.<sup>13</sup>

Furthermore, echocardiographic measurements such as ejection fraction (EF), LV end-diastolic dimension, right ventricular size and wall thickness, and degree of mitral and aortic insufficiency have been used as critical parameters to predict myocardial recovery after explantation. Dandel et al<sup>12</sup> found that EF >50% in patients with NICM before LVAD explantation correlated with a 91.7% chance of cardiac stability lasting  $\geq 5$  years after LVAD removal. In contrast, EF <50% correlated with a 1.5-fold increase in the recurrence of HF at 5 years.<sup>12</sup> The positive predictive value of EF could be enhanced if additional parameters such as pre-explantation LV size and geometry, stability of unloading-induced cardiac improvement before LVAD removal, and HF duration before LVAD implantation were also considered. The recovery rate of LVEF after LVAD implantation has been associated with prolonged HF recovery after LVAD explantation as well.<sup>14</sup>

A growing area of interest is the determination of cardiac reserves and functional capacity beyond the scope of echocardiography parameters to facilitate further prognostication of patients' recoverability after LVAD implantation.<sup>15,16</sup> MVO<sub>2</sub> max and hemodynamic data, such as cardiac output, pulmonary arterial pressures, and LV end-diastolic pressure at low-level flows of HeartMate II have been studied. Exercise capacity was assessed through 6-minute walk tests

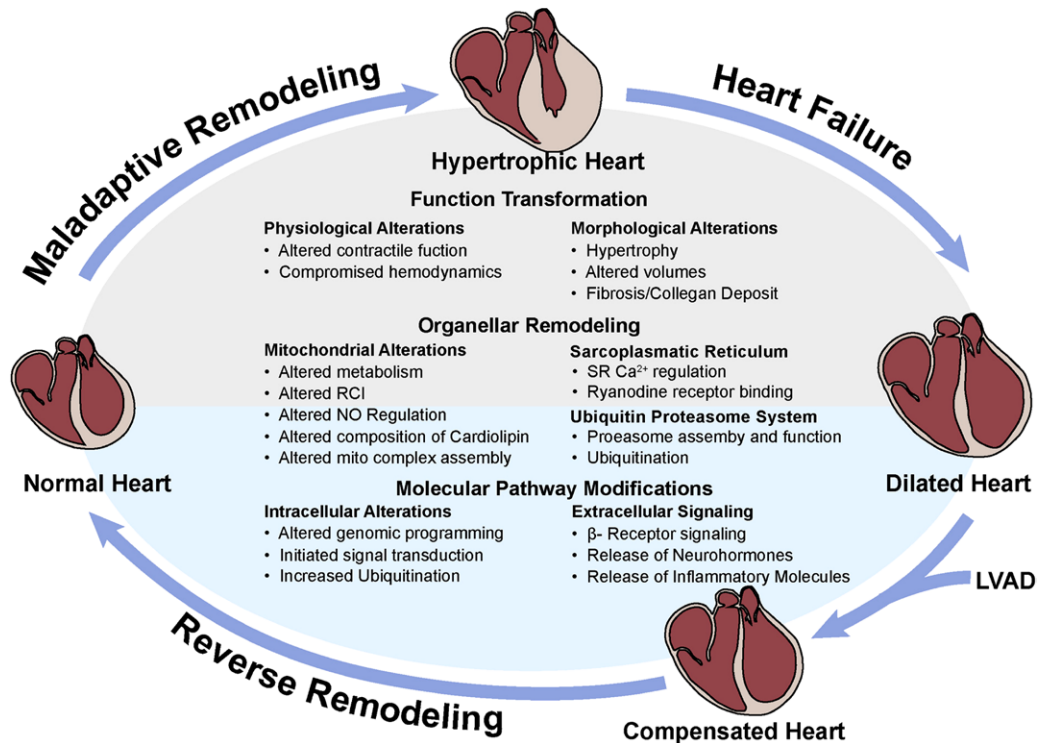
at minimal flow rates to determine inotropic reserve. In the second Harefield study,<sup>10</sup> several patients underwent explantation because of other complications, which necessitated LVAD removal before achieving certain echo parameters. These patients performed remarkably well; however, detailed molecular assessments of the cardiac recovery in these successful cases of BTR were not performed.<sup>11,12</sup>

## Morphological, Molecular, and Cellular Insights of Reverse Remodeling After LVAD Support

### Morphological and Molecular Insights

During the past 2 decades, molecular and cellular recovery after LVAD mechanical unloading (Figure 2) has been extensively investigated in an effort to understand the pathophysiology and critical pathways for potential therapeutic applications (Table).<sup>5-7,17</sup> The current consensus is that morphological, cellular, and molecular reversal after LVAD implantation precedes clinical recovery. However, these features alone have not been able to predict clinical recovery reliably.<sup>7</sup> With regard to morphological changes, several early histological studies described a conclusive decrease in cell volume and morphological restructuring to a prediseased state.<sup>5,6,17</sup> Accordingly, there are reports demonstrating the normalization of cardiac sarcomeric proteins, including vinculin, desmin, tubulin, tropomyosin, titin, actinin, and  $\alpha$ -spectrin.<sup>5,6,17</sup> LVAD support also led to enhancements in cardiomyocyte force in conjunction with accelerated contraction and relaxation times.<sup>6</sup> Nevertheless, whether interstitial connective tissue remodeling can be reversed after LVAD support remains controversial.<sup>5,6</sup>

As a governing determinant of cardiac function, calcium (Ca<sup>2+</sup>) handling of the cell is disturbed during HF, which may contribute to the decreased contractility. In this regard, there seems to be increased gene expression of the sarcolemmal



**Figure 2.** Main levels of reverse remodeling after left ventricular assist device (LVAD) implantation. Functional, organellar, and molecular recovery in reversed ventricular remodeling. RCI indicates respiratory control index; and SR, sarcoplasmic reticulum.

Na<sup>+</sup>Ca<sup>2+</sup> exchanger and sarco-endoplasmic reticular Ca<sup>2+</sup> ATPase subtype 2a after LVAD implantation, but corresponding protein expression remains unclear. Furthermore, levels of the Ca<sup>2+</sup> regulatory protein phospholamban seems to be unchanged after LVAD implantation, whereas L-type Ca<sup>2+</sup> channel function and ryanodine receptor function are improved through post-translational modifications after LVAD implantation.<sup>5,6</sup>

Cardiac function is also orchestrated by the neurohormonal system, and there is clear evidence that increased levels of neurohormones during HF (such as epinephrine, norepinephrine, renin, angiotensin II, aldosterone, and arginine vasopressin) are decreased with successful LVAD support.<sup>5,6,17</sup> Other key hormonal players in maintaining arterial blood pressure and volume homeostasis are cardiac atrial natriuretic peptide and B-type natriuretic peptide.<sup>5,6,17</sup> Both atrial natriuretic peptide and B-type natriuretic peptide are important HF biomarkers that are released by the atrium and LV, respectively, on pressure overload. Because LVAD therapy immediately relieves the burden on the overloaded heart, both atrial natriuretic peptide and B-type natriuretic peptide levels show a downward trend. In addition, β-adrenergic receptor density and response to stimulation can be restored after LVAD support, most likely through alterations in intracellular rather than hemodynamic factors,<sup>5-7</sup> and also reversed phosphoinositide 3-kinase-γ activation and alterations in adenylyl cyclase.

Certain aspects of mitochondrial structure and function have been investigated. Metabolism is disrupted during HF, yet studies on the effects of LVAD support on metabolic pathways are limited. Decreased creatine kinase activity during HF is restored after LVAD support, indicating improved energy production. Moreover, the composition of cardiolipin,

an integral component of the mitochondrial inner membrane that facilitates the function of numerous energetic enzymes, is normalized, after LVAD support in ischemic cardiomyopathy, but not in dilated cardiomyopathy.<sup>5,6,17</sup>

The inflammatory response characteristic of HF progression, including the upregulation of proinflammatory cytokines such as tumor necrosis factor-α, interleukin-6, interleukin-8, and several heat shock proteins, was reversed after LVAD implantation.<sup>6</sup> In addition, apoptotic cell death during HF can be attenuated by LVAD support, though controversial results from different studies have been reported.<sup>5,6,17</sup>

### Transcriptional Modifications

Because gene expression and protein expression are not always synchronized, maladaptive hypertrophy and HF are also characterized on a transcriptional and epigenetic level targeting the activation of gene expression.<sup>5</sup> Hypertrophy and HF are both correlated to the activation of genes encoding transcriptional factors (c-jun, c-fos, c-myc), hormonal passengers (atrial natriuretic peptide, B-type natriuretic peptide), β-myosin heavy chain, and skeletal α-actin. One of the first studies measuring the expression of cardiac genes in HF samples found that sarco-endoplasmic reticular Ca<sup>2+</sup> ATPase subtype 2a, the ryanodine receptor, and the sarcolemmal Na<sup>+</sup>Ca<sup>2+</sup> exchanger were all upregulated after LVAD support.<sup>18</sup> A similar study by Hall et al<sup>19</sup> using microarray analysis of mRNA transcripts in patients with HF after LVAD support in combination with pharmacological therapy revealed a significant association of both the integrin and cAMP pathways with the functional recovery in cardiac contractility and metabolism. Nevertheless, some studies have failed to demonstrate

**Table. Summary of Molecular/Cellular Constituents in Reverse Ventricular Remodeling After LVAD Implantation**

Cardiac Feature	Cellular/Molecular Constituent	Effect of LVAD Therapy	Relevance
Extracellular matrix, cytoskeleton, and sarcomere biogenesis	Collagen/fibrosis	Decreased or increased deposit	Extracellular matrix remodeling is key in HF and myocardial recovery, contradicting reports
	Metalloproteinases/matrix metalloproteinases	Alterations	Enzymes involved in breakdown of collagen in HF and reverse remodeling
	Dystrophin	Disruption is reversed	Role of reversal is still unclear
	Myosin heavy chain	Increased	Important for functional contractile recovery
	Troponin T, troponin C, actin, smooth muscle $\alpha$ -actin	Increased	Important for functional contractile recovery
	Vinculin, desmin, tubulin	Abundant changes after PTM	Indicates a restored contractile apparatus
	Tropomyosin, titin	Recovered	Only partially recovered despite improvement of the myocardial size, important during cardiac contraction
Inflammatory response	Actinin, $\alpha$ -spectrin	Increased	Indicates restored shape and structure of cardiomyocytes
	TNF- $\alpha$ , IL-8, complement C3a, glycoprotein 130	Decreased	Both in circulating and LV tissue, indicates a significant role of inflammation in HF progression
Neurohormonal release	IL-6	Increased	IL-6 increased in HF and contributes to myocardial injury
	Epinephrine and norepinephrine	Decreased	Significant in myocardial recovery attributable to deleterious effect on myocardium if chronically elevated as in HF
	Renin, AT-II, aldosterone, arginine vasopressin	Decreased	Significant in myocardial recovery attributable to deleterious effect on myocardium if chronically elevated as in HF
Calcium handling	ANP, BNP	Decreased in both circulating and cardiac tissue	Prominent HF biomarker, both act through guanyl cyclase-A
	Sarcolemmal calcium flux through sarcoplasmic channel	Increased calcium pumping function	Restored after LVAD implantation but requires optimal timing of LV unloading
	Sarcoplasmic reticulum calcium content	Increased	Leading to shorter action potential durations
	SERCA2a	Increased gene expression in LV (not in RV)	Protein abundance of SERCA is unclear (different studies show discrepancy)
	Na <sup>+</sup> /Ca <sup>2+</sup> exchanger	Increased expression and function	Leads to better contractility and inotropy
	Phospholamban	Unchanged	Ratio of SERCA/phospholamban may improve, which is important in functional recovery and contractility
	L-type calcium channel	Flux improved after PTM	Indicates improved contractility
$\beta$ -Adrenergic signaling	Ryanodine receptor	Function in LV and RV improved after PTM	Indicates improved contractility
	$\beta$ -Receptor density and location	Reversal of receptor downregulation and improved density	Correlated to reverse PI3K- $\gamma$ activation and an increase of adenylyl cyclase
	$\beta$ -Receptors location	Normalized in plasma membranes	Depletion of receptors in endosomes
Metabolism	Adenylyl cyclase	Increased after LVAD implantation	Significant component in $\beta$ -adrenergic signal transduction
	Creatine kinase	Increased back to normal levels	Indicates reversed cell damage
Signal transduction	Cardiolipin	Normalized composition in ischemic cardiomyopathy	Indicates a significant role in ischemic heart disease
	PI3K/Akt/GSK-3 $\beta$ pathway, IGF, FGF, RTKs, Her2/neu	Upregulated or no changes, Her2/neu upregulated	Potential targets for pharmacotherapy, IGF is correlated to stem cell recruitment
Apoptosis	MAP/Erk/JNK/P38 pathway	Erk downregulated, Erk no change	Potential targets for pharmacotherapy
	B-cell lymphoma 2	Normalized	Discrepancy whether apoptosis is increased or decreased
Myocyte nucleus	Cell cycle	Possible reactivation	May allow cardiac regeneration
	Cardiomyocyte DNA content and number of polyploid cells	Decline after LVAD implantation	May allow cardiac regeneration

(Continued)



Table. Continued

Cardiac Feature	Cellular/Molecular Constituent	Effect of LVAD Therapy	Relevance
	Number of diploid and binucleated myocytes	Increased after LVAD implantation	May allow cardiac regeneration
Gene expression	Many altered genes during HF	Only small percentage of transcripts show reversal	Reverse remodeling may occur without normalization of abnormal gene expression
MicroRNA expression	Many microRNAs in HF are up- or downregulated	Many microRNAs show normalization	May be more sensitive to study reverse remodeling than genes

Akt indicates protein kinase B; ANP, atrial natriuretic peptide; AT, angiotensin; BNP, brain natriuretic peptide; Erk, extracellular signal-regulated kinases; FGF, fibroblast growth factor; GSK-3 $\beta$ , glycogen synthase kinase-3 $\beta$ ; Her2/neu, human epidermal growth factor receptor 2; HF, heart failure; IGF, insulin-like growth factor; IL, interleukin; JNK, c-Jun N-terminal kinase; LVAD, left ventricular assist device; MAP, mitogen-activated protein; PI3K, phosphoinositide 3-kinase; PTM, post translational modification; RTK, receptor tyrosine kinase; RV, right ventricle; SERCA2a, sarco-endoplasmic reticular Ca<sup>2+</sup> ATPase subtype 2a; and TNF, tumor necrosis factor.

a normalized gene expression during mechanical unloading. Margulies et al<sup>20</sup> analyzed 199 human myocardial specimens and found that the abundant changes in transcripts during HF did not respond to LVAD support.

### Intracellular Transduction Pathways

Several signal transduction pathways implicated in the progression of HF and LVAD-induced recovery are summarized in the Table.<sup>5-7,17</sup> One of the first signaling pathways that provided insight as an underlying mechanism of HF is the Ca<sup>2+</sup>/calmodulin-activated protein kinase and phosphatase (calcineurin).<sup>5</sup> Another prominent signaling pathway is the phosphoinositide 3-kinase/Akt (also known as protein kinase B) pathway, which stimulates several tyrosine kinase receptors such as insulin-like growth factor, fibroblast growth factor, transforming growth factor, as well as G-protein-coupled receptors. Finally, the mitogen-activated protein kinase cascade, which includes extracellular signal-related kinases, c-Jun N-terminal protein kinases, and p38 mitogen-activated protein kinase subfamilies, is a highly conserved signal transduction pathway.<sup>5</sup> Moreover, receptor tyrosine kinases are involved in the transmission of hypertrophic and survival signals in the cardiomyocyte.

LVAD induces significant perturbations of these key signal transduction pathways.<sup>21</sup> For example, the activation of Akt, glycogen synthase kinase-3 $\beta$ , and phosphorylation of P70S6K (as part of a prohypertrophy signaling pathway) in patients with HF are downregulated after LVAD, whereas both c-Jun N-terminal protein kinase- and p38-mediated signaling remained unchanged by LVAD implantation. Extracellular signal-related kinase is downregulated by mechanical unloading, whereas c-Jun N-terminal protein kinase signal transduction exhibited no change.<sup>5-7,17</sup> Lastly, unloading of the heart resulted in an upregulation of Her2/neu and Her4, particularly in patients with ischemic cardiomyopathy. At the same time, glycoprotein 130, the common signal transducer of interleukin-6 cytokines,<sup>22,23</sup> which plays an important role in receptor tyrosine kinase signal transduction, was decreased after LVAD implantation.

In conclusion, a comprehensive profile of the pivotal elements in signal transduction pathways (and their post-translational modifications) in maladaptive hypertrophy and HF progression are only beginning to be discovered, and future investigations in this area will likely unveil many potential therapeutic targets.

## Organelle-Specific Insights in HF and Reverse Remodeling

### Mitochondria in HF and Reverse Remodeling

Many reports from both animal and human clinical studies strongly indicate that mitochondrial dysfunction is crucial in the pathophysiology of HF.<sup>24</sup> Next to supplying energy in the form of ATP, mitochondria are also involved in the regulation of intracellular Ca<sup>2+</sup> fluxes, redox potential, biosynthesis, reactive oxygen species (ROS) generation/signaling, cell death pathways, and protection against stressors, such as ischemia reperfusion injury.<sup>25</sup> Mitochondrial energy supply involves the coupling of electron transfer and oxygen consumption through the mitochondrial electron transport chain complexes I, II, III, and IV with the phosphorylation of ADP to ATP by F<sub>0</sub>F<sub>1</sub>-ATP synthase, also known as complex V. In the process of oxidative phosphorylation, the coupling efficiency between respiration and phosphorylation is often measured as the respiratory control index (RCI). In isolated mitochondria, the active respiring state is often referred to as state 3 respiration, whereas the slowest rate when all the ADP has been phosphorylated to ATP is referred to as state 4. The RCI can be determined as a ratio of state 3/state 4. A lower RCI indicates a disturbed coupling of oxidation and phosphorylation, causing an inefficient energy production during which oxygen is prematurely reduced as ROS. Under basal conditions, the catabolism of fatty acids through  $\beta$ -oxidation provides  $\approx$ 90% of the total ATP used in the heart. In addition to fatty acids, other substrates used for oxidative phosphorylation include glucose, pyruvate, lactate, and ketone bodies.<sup>24</sup> Thus, several mitochondrial energy pathways including oxidative phosphorylation, the tricarboxylic acid cycle, and fatty acid oxidation are essential for maintaining the contractile function of the heart.<sup>24</sup> Both basic research and clinical studies have reported a shift toward glucose oxidation at the expense of fatty acid oxidation in hypertrophied and failing hearts.<sup>24</sup> Consequently, ATP levels in failing hearts rapidly decline, leading to cardiac energy deficits. Concomitantly, studies involving transgenic alterations in mitochondrial proteins (eg, adenine nucleotide translocator and respiratory complex enzymes) during the progression of HF indicate a crucial role for mitochondria.<sup>24</sup>

Supranormal mitochondrial ROS production is implicated in many pathological conditions such as contractile dysfunction, calcium dysregulation, cell death, and ventricular hypertrophy and dilation. ROS, including superoxide, hydroxyl

radicals, and hydrogen peroxide, are normal byproducts during mitochondrial metabolism. The majority of reports describing pathological ROS release in HF have come from studies conducted in animal models. In agreement, it has been demonstrated that ROS levels are elevated in the failing human heart.<sup>26</sup> Contractile dysfunction can result from disturbed mitochondrial oxidative phosphorylation with inefficient energy production, where oxygen is prematurely and incompletely reduced, causing increased ROS release from electron transport chain complexes I and III. During HF, elevated ROS release by itself can further induce more ROS release.<sup>27</sup> The release of ROS from the mitochondria can lead to extensive oxidative damage to a variety of intracellular molecules such as proteins (eg, mitochondrial respiratory enzymes, matrix enzymes), DNA, and specific lipids (eg, membrane phospholipids such as cardiolipin).<sup>24</sup> Furthermore, ROS also modulate multiple overlapping signaling pathways in the progression of ventricular hypertrophy. It is evident that an excessive generation of ROS serves as an important mechanism in the pathophysiology and progression of HF.

More recently, it has become apparent that cell death by apoptosis and necrosis are both important contributors in HF.<sup>5</sup> Multiple studies have demonstrated the significant importance and magnitude of cardiac apoptosis during HF. The intrinsic apoptotic pathway features profound interactions between mitochondria, nucleus, and other subcellular organelles. The release of specific mitochondrial proteins from the intermembrane space (eg, cytochrome-c, endonuclease G, apoptosis-inducing factor, and Smac) triggers these events that subsequently cause DNA fragmentation and the activation of apoptotic protease activating factor 1 and caspases. This chain of events ultimately culminates in cell death. In contrast to apoptosis, the exact contribution of necrosis in HF has not been extensively studied. However, evidence has shown that HF could be rescued by deletion of the pronecrotic factor cyclophilin D, but not by the antiapoptotic factor B-cell lymphoma 2, implicating that necrosis is also a major component in the progression of HF. Consequently, the mitochondrial release of these pro-cell death factors also involves the mitochondrial permeability transition pore.<sup>25</sup> Mitochondrial permeability transition is related to several detrimental mitochondrial events such as mitochondrial membrane potential ( $\Delta\Psi$ ) deterioration, ROS overproduction,  $\text{Ca}^{2+}$  overload, and impaired NO signaling.<sup>25</sup> Accordingly, mitochondrial permeability transition pore opening has previously been reported in HF resulting from  $\text{Ca}^{2+}$ -induced cardiomyopathy<sup>28</sup> and diabetic cardiomyopathy.<sup>29</sup>

Surprisingly, there are relatively few studies available that investigate the role of mitochondrial function in reverse ventricular remodeling by LVAD support. An early study by Lee et al<sup>30</sup> investigated mitochondrial respiratory function in patients with advanced HF before and after LVAD support. Progression of HF was associated with extremely low RCIs. However, the RCIs were significantly improved after LVAD support,<sup>30</sup> suggesting an important role of the device in benefiting oxidative phosphorylation and electron transport. Another study by Mital et al<sup>31</sup> examined the effects of NO on mitochondrial respiratory control during reverse ventricular remodeling, a coupling that is usually disrupted during HF progression. The study reported that chronic LVAD support potentiates

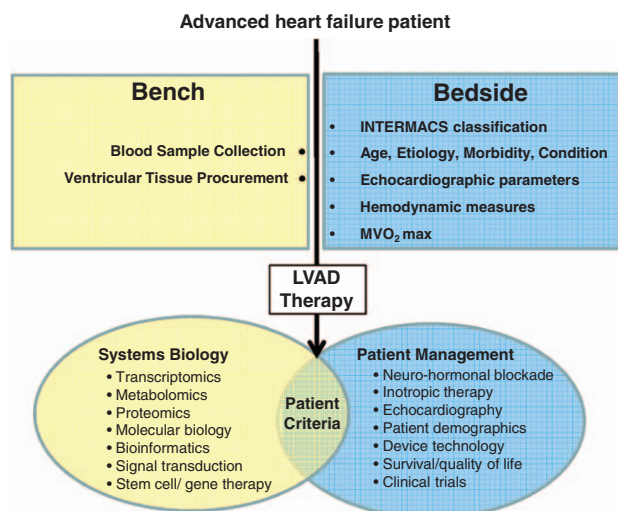
endogenous NO-mediated regulation of mitochondrial respiration as measured by improved  $\text{MVO}_2$  consumption. This effect could in turn be abrogated by NO synthase inhibition.<sup>31</sup> As aforementioned, Heerdt et al<sup>32</sup> observed that LVAD-supported hearts exhibited a normalization of cardiolipin content within the inner mitochondrial membrane. Cardiolipin is essential for normal functionality of the mitochondrial respiratory chain, as well as substrate transport.<sup>33</sup> In conclusion, it is evident that the pathological proteomic mitochondrial phenotype is reversible, which may be an important underlying mechanism in reverse ventricular remodeling by LVAD.

### Proteasome in HF and Reverse Remodeling

The proteasome is usually referred to as the 26S complex, which consists of a 20S catalytic core particle and 1 or 2 19S regulatory particles.<sup>34</sup> The main function of the 26S proteasome is to degrade proteins that are damaged or have reached the end of their functional lifetime.<sup>35</sup> As a part of the ubiquitin-proteasome system (UPS), the proteasome maintains cardiac protein homeostasis and thus plays a significant role in LV remodeling and HF.<sup>35-37</sup>

During the years, clinical studies have observed detrimental side effects in the heart when proteasome inhibition was used for the treatment of cancer. For example, a large clinical trial of 315 patients using the proteasome inhibitor bortezomib to treat multiple myeloma revealed significant cardiotoxicity and occurrence of HF.<sup>38</sup> During ventricular remodeling and HF, ubiquitinated proteins accumulated, suggesting impaired protein degradation capacity of the UPS.<sup>39</sup> Further investigation on human pressure-overloaded hearts also revealed a correlation between ventricular hypertrophy and a depressed proteasomal activity.<sup>36</sup> In a rodent model, our group recently found that ventricular hypertrophy by prolonged  $\beta$ -adrenergic stimulation is concomitant with decreased 20S caspase-like and trypsin-like activities, with an unchanged chymotrypsin-like activity. This functional alteration may be attributed to an increased incorporation of inducible subunits in 20S proteasomes.<sup>40</sup> Besides removal of damaged proteins, proteasomes also play critical regulatory roles by degrading pivotal components of biological pathways, such as prohypertrophic signals (eg, Akt and extracellular signal-related kinase 1/2).<sup>41</sup> Inhibition of the proteasome pathway in sympathetically stimulated mice resulted in the prevention<sup>42</sup> or regression of maladaptive ventricular hypertrophy.<sup>43,44</sup>

Although there is increasing evidence that the UPS plays a significant role in ventricular remodeling and HF, there are still relatively few studies describing whether and how the UPS is affected by ventricular unloading after LVAD implantation. Kassiotis et al<sup>45</sup> reported that the progression of autophagy in the failing heart is reversed after LVAD support. In this study, the investigators further observed that 20S proteasome activity was increased by mechanical unloading.<sup>45</sup> Subsequently, Wohlschlaeger et al<sup>46</sup> reported a depression of the UPS during HF, which was reversed after LVAD therapy.<sup>46</sup> Both studies strongly suggest a significant role for the UPS in ventricular hypertrophy and HF, as well as in reverse cardiac remodeling. Although the UPS plays a crucial role in the pathophysiology of maladaptive ventricular remodeling and HF, further studies are necessary to define its causative<sup>42</sup> or compensatory<sup>38</sup> role.



**Figure 3.** Translational heart failure research. A bidirectional bench-to-bedside approach combining a systems biology strategy with clinical patient management offers new possibilities for the development of novel therapeutics and clinical parameters. INTERMACS indicates Interagency Registry for Mechanically Assisted Circulatory Support; and LVAD, left ventricular assist device.

LVAD therapy offers a platform to increase our understanding of the UPS in maladaptive remodeling and HF.

### Future Perspectives and Directions

Standard HF therapy has shifted from traditional heart transplantation to LVAD implantation with a focus on destination therapy and BTR. Nevertheless, clinical parameters to predict LV recovery after LVAD support are still ill defined. Hence, we think that the development of novel patient selection criteria requires that HF is targeted by a systems biology approach incorporating transcriptomics, proteomics, metabolomics, cell biology, and bioinformatics (Figure 3). A plausible initial approach would be the exploration of molecular or organellar assays in combination with pre-existing clinical parameters as predictive measures. Collection of blood and ventricular tissue samples during LVAD implantation are suitable means for accomplishing this (Figure 3). In addition to molecular and cellular alterations (Table), there is growing evidence that mitochondria and the UPS have important roles in LV remodeling, HF progression, and reverse cardiac remodeling. A plausible initial method for integrating organellar-based profiles would be to implement mitochondrial functional assays (eg, mitochondrial  $\Delta\Psi$ , susceptibility to mitochondrial permeability transition, RCI, and electron transport chain activities)<sup>47</sup> and proteasome activity assays<sup>47</sup> into the current panel of clinical parameters. In line with mitochondrial function, metabolism is also an important determinant of HF. Accordingly, we propose a comprehensive comparison of essential metabolites using blood samples<sup>48</sup> before and after LVAD implantation. These omics data can be further interrogated using state-of-the-art bioinformatics tools to provide insights into disease.<sup>49</sup> This bidirectional translational medicine approach will enable us to study HF and identify patients who would benefit from BTR, destination therapy, or bridge to transplantation. This molecular- and organelle-based predictive paradigm may advance personalized medicine through individual patient profiling.

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### Disclosures

None.

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