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.1 Recent reports2–3 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.4 The clinical phenotype of HF begins with an injury or supranormal stressor on the heart and, through prolonged dyshomeostasis, 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.5–7

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]).3 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 β-agonist, to optimize LV recovery. The procedure resulted in 63.2% of patients successfully recovering after LVAD explantation.8 Reliable and robust predictive parameters and patient selection criteria for successful BTR have not yet been established. Patients with nonchimeric cardiomyopathy (NICM) have been targeted in studies9,10 as the group most likely to undergo successful BTR. However, this NICM population itself has heterogeneous underlying causes, such as viral myocarditis,
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.12 In the HeartMate II bridge-to-transplant and destination therapy trials enrolling 1108 patients with NICM, HF of <1 year and age of ≤45 years were predictors of successful BTR and 2-year transplant-free survival.13

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 al12 found that EF >50% in patients with NICM before LVAD explantation correlated with a 91.7% chance of cardiac stability lasting ≥5 years after LVAD removal. In contrast, EF ≤50% correlated with a 1.5-fold increase in the recurrence of HF at 5 years.12 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.14

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.15,16 MVO₂ 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,10 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.11,12

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).5–7,17 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.7 With regard to morphological changes, several early histological studies described a conclusive decrease in cell volume and morphological restructuring to a prediseased state.5,6,17 Accordingly, there are reports demonstrating the normalization of cardiac sarcomeric proteins, including vinculin, desmin, tubulin, tropomyosin, titin, actinin, and α-spectrin.5,6,17 LVAD support also led to enhancements in cardiomyocyte force in conjunct with accelerated contraction and relaxation times.9 Nevertheless, whether interstitial connective tissue remodeling can be reversed after LVAD support remains controversial.5,6

As a governing determinant of cardiac function, calcium (Ca²⁺) 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 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.]
Na\(^{+}\)Ca\(^{2+}\) exchanger and sarco-endoplasmic reticular Ca\(^{2+}\) ATPase subtype 2a after LVAD implantation, but corresponding protein expression remains unclear. Furthermore, levels of the Ca\(^{2+}\) regulatory protein phospholamban seems to be unchanged after LVAD implantation, whereas L-type Ca\(^{2+}\) channel function and ryanodine receptor function are improved through post-translational modifications after LVAD implantation.\(^5,6\)

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.\(^5,6,17\) Other key hormonal players in maintaining arterial blood pressure and volume homeostasis are cardiac atrial natriuretic peptide and B-type natriuretic peptide.\(^5,6,17\) 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, \(\beta\)-adrenergic receptor density and response to stimulation can be restored after LVAD support, most likely through alterations in intracellular rather than hemodynamic factors,\(^5,6\) and also reversed phosphoinositide 3-kinase-\(\gamma\) 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.\(^5,6,17\)

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

**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.\(^5\) 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), \(\beta\)-myosin heavy chain, and skeletal \(\alpha\)-actin. One of the first studies measuring the expression of cardiac genes in HF samples found that sarco-endoplasmic reticular Ca\(^{2+}\) ATPase subtype 2a, the ryanodine receptor, and the sarcolemmal Na\(^{+}\)Ca\(^{2+}\) exchanger were all upregulated after LVAD support.\(^18\) A similar study by Hall et al\(^19\) 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

<table>
<thead>
<tr>
<th>Cardiac Feature</th>
<th>Cellular/Molecular Constituent</th>
<th>Effect of LVAD Therapy</th>
<th>Relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracellular matrix, cytoskeleton, and sarcomere biogenesis</td>
<td>Collagen/fibrosis</td>
<td>Decreased or increased deposit</td>
<td>Extracellular matrix remodeling is key in HF and myocardial recovery, contradicting reports</td>
</tr>
<tr>
<td></td>
<td>Metalloproteinases/matrix metalloproteinases</td>
<td>Alterations</td>
<td>Enzymes involved in breakdown of collagen in HF and reverse remodeling</td>
</tr>
<tr>
<td></td>
<td>Dystrophin</td>
<td>Disruption is reversed</td>
<td>Role of reversal is still unclear</td>
</tr>
<tr>
<td></td>
<td>Myosin heavy chain</td>
<td>Increased</td>
<td>Important for functional contractile recovery</td>
</tr>
<tr>
<td></td>
<td>Troponin T, troponin C, actin, smooth muscle α-actin</td>
<td>Increased</td>
<td>Important for functional contractile recovery</td>
</tr>
<tr>
<td></td>
<td>Vinculin, desmin, tubulin</td>
<td>Abundant changes after PTM</td>
<td>Indicates a restored contractile apparatus</td>
</tr>
<tr>
<td></td>
<td>Troponymyosin, titin</td>
<td>Recovered</td>
<td>Only partially recovered despite improvement of the myocardial size, important during cardiac contraction</td>
</tr>
<tr>
<td></td>
<td>Actinin, α-spectrin</td>
<td>Increased</td>
<td>Indicates restored shape and structure of cardiomyocytes</td>
</tr>
<tr>
<td>Inflammatory response</td>
<td>TNF-α, IL-8, complement C3a, glycoprotein 130</td>
<td>Decreased</td>
<td>Both in circulating and LV tissue, indicates a significant role of inflammation in HF progression</td>
</tr>
<tr>
<td></td>
<td>IL-6</td>
<td>Increased</td>
<td>IL-6 increased in HF and contributes to myocardial injury</td>
</tr>
<tr>
<td>Neurohormonal release</td>
<td>Epinephrine and norepinephrine</td>
<td>Decreased</td>
<td>Significant in myocardial recovery attributable to deleterious effect on myocardium if chronically elevated as in HF</td>
</tr>
<tr>
<td></td>
<td>Renin, AT-Ⅱ, aldosterone, arginine vasopressin</td>
<td>Decreased</td>
<td>Significant in myocardial recovery attributable to deleterious effect on myocardium if chronically elevated as in HF</td>
</tr>
<tr>
<td></td>
<td>ANP, BNP</td>
<td>Decreased in both circulating and cardiac tissue</td>
<td>Prominent HF biomarker, both act through guanyl cyclase-Α</td>
</tr>
<tr>
<td>Calcium handling</td>
<td>Sarcolemmal calcium flux through sarcoplasmic channel</td>
<td>Increased calcium pumping function</td>
<td>Restored after LVAD implantation but requires optimal timing of LV unloading</td>
</tr>
<tr>
<td></td>
<td>SERCA2a</td>
<td>Increased</td>
<td>Leading to shorter action potential durations</td>
</tr>
<tr>
<td></td>
<td>Na+/Ca2+ exchanger</td>
<td>Increased expression and function</td>
<td>Protein abundance of SERCA is unclear (different studies show discrepancy)</td>
</tr>
<tr>
<td></td>
<td>Phospholamban</td>
<td>Unchanged</td>
<td>Ratio of SERCA/phospholamban may improve, which is important in functional recovery and contractility</td>
</tr>
<tr>
<td></td>
<td>L-type calcium channel</td>
<td>Flux improved after PTM</td>
<td>Indicates improved contractility</td>
</tr>
<tr>
<td></td>
<td>Ryanodine receptor</td>
<td>Function in LV and RV improved after PTM</td>
<td>Indicates improved contractility</td>
</tr>
<tr>
<td>β-Adrenergic signaling</td>
<td>β-Receptor density and location</td>
<td>Reversal of receptor downregulation and improved density</td>
<td>Correlated to reverse PI3K-γ activation and an increase of adenylyl cyclase</td>
</tr>
<tr>
<td></td>
<td>β-Receptors location</td>
<td>Normalized in plasma membranes</td>
<td>Depletion of receptors in endosomes</td>
</tr>
<tr>
<td></td>
<td>Adenylyl cyclase</td>
<td>Increased after LVAD implantation</td>
<td>Significant component in β-adrenergic signal transduction</td>
</tr>
<tr>
<td>Metabolism</td>
<td>Creatine kinase</td>
<td>Increased back to normal levels</td>
<td>Indicates reversed cell damage</td>
</tr>
<tr>
<td></td>
<td>Cardiolipin</td>
<td>Normalized composition in ischemic cardiomyopathy</td>
<td>Indicates a significant role in ischemic heart disease</td>
</tr>
<tr>
<td>Signal transduction</td>
<td>PI3K/Akt/GSK-3β pathway, IGF, FGF, RTKs, Her2/neu upregulated</td>
<td>Upregulated or no changes, Her2/neu upregulated</td>
<td>Potential targets for pharmacotherapy, IGF is correlated to stem cell recruitment</td>
</tr>
<tr>
<td></td>
<td>MAP/Erk/JNK/P38 pathway</td>
<td>Erk downregulated, Erk no change</td>
<td>Potential targets for pharmacotherapy</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>B-cell lymphoma 2</td>
<td>Normalized</td>
<td>Discrepancy whether apoptosis is increased or decreased</td>
</tr>
<tr>
<td>Myocyte nucleus</td>
<td>Cell cycle</td>
<td>Possible reactivation</td>
<td>May allow cardiac regeneration</td>
</tr>
<tr>
<td></td>
<td>Cardiomyocyte DNA content and number of polyploid cells</td>
<td>Decline after LVAD implantation</td>
<td>May allow cardiac regeneration</td>
</tr>
</tbody>
</table>

(Continued)
a normalized gene expression during mechanical unloading. Margulies et al 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. One of the first signaling pathways that provided insight as an underlying mechanism of HF is the Ca2+/calmodulin-activated protein kinase and phosphatase (calcineurin). 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. 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. For example, the activation of Akt, glycogen synthase kinase-3β, 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. 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, 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.
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. 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. 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). Furthermore, ROS also modulate multiple overlapping signaling pathways in the progression of ventricular hypertrophy. It is evident that an excessive generation of ROS render the intracellular environment highly reactive, 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. The main function of the 26S proteasome is to degrade proteins that are damaged or have reached the end of their functional life. 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.

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. During ventricular remodeling and HF, ubiquitinated proteins accumulated, suggesting impaired protein degradation capacity of the UPS. Further investigation on human pressure-overloaded hearts also revealed a correlation between ventricular hypertrophy and a depressed proteasomal activity. In a rodent model, our group recently found that ventricular hypertrophy by prolonged β-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. 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). Inhibition of the proteasome pathway in sympathetically stimulated mice resulted in the prevention or regression of maladaptive ventricular hypertrophy.

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 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. Subsequently, Wohlschlaeger et al reported a depression of the UPS during HF, which was reversed after LVAD therapy. 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 or compensatory role.
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 (e.g., mitochondrial ΔΨ', susceptibility to mitochondrial permeability transition, RCI, and electron transport chain activities)⁷⁷ and proteasome activity assays⁷⁷ 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 before and after LVAD implantation. These omics data can be further interrogated using state-of-the-art bioinformatics tools to provide insights into disease.⁷⁸ 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.

References


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