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Addressing the heart failure epidemic: from mechanical circulatory support to stem cell therapy

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Thesis

**ADDRESSING THE HEART FAILURE EPIDEMIC: FROM MECHANICAL
CIRCULATORY SUPPORT TO STEM CELL THERAPY**

by

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ABSTRACT

At an annual cost of over thirty billion dollars annually, the diagnosis and management of heart failure is one of the most significant public health concerns of the twenty first century, as nearly twenty percent of Americans will develop some form of heart failure in their lifetime. The incidence of newly diagnosed heart failure has remained stable over the last several years at approximately 650,000 diagnoses per year; however, due to several contributing factors the prevalence has continued to rise despite substantial advancements in interventional therapies. The three most significant contributing factors to the rising heart failure prevalence have been identified as 1) significant advancements in technology and medical intervention have dramatically improved the survival rate of those experiencing acute coronary events. This has resulted in a greater number of patients who then progress to chronic heart failure. 2) The management of those with chronic heart failure has been dramatically improved which has allowed those with the disease to live longer and 3) heart failure is in large part a disease associated with advancing age. As the population in the United States and other developed countries continue to

grow, such a strong association will inevitably result in a rapidly increasing prevalence.

Current clinically therapies for managing heart failure can be categorized into three major groups: pharmaceutical therapy, mechanical circulatory support, or cell-based therapy. Pharmaceutical therapies are used in the earlier stages of disease progression or to manage symptoms and comorbidities of later stage heart failure. Mechanical circulatory support is often implemented when the disease progresses to a more severe state, where volume and / or pressure overload of the ventricles is present. Many modalities of mechanical circulatory support serve as a bridge to transplant, as the only long-term treatment of advanced decompensated heart failure is cardiac transplantation. The third category of treatments for HF is cell-based or stem cell therapies. These therapies are still in their infancies but hold significant potential of cardiac regeneration and reversal of the pathologic remodeling associated with heart failure.

While the management of the early stages of heart failure have improves, addressing end-stage failure remains a significant obstacle in resolving the U.S. of the heart failure epidemic. The use of ventricular assist devices (VADs) has improved the management of end-stage failure over the last few decades, but VADs serve mostly as a bridge to transplant, so eventually a donor organ and cardiac transplantation is required. As the population continues to grow, the number of patients in need of a donor heart will increase, leading to an even

larger discrepancy between the number of donor organs available and those in severe need. While advancements in VAD technology have reduced potential complications and increased the duration and effectiveness of the mechanical circulatory support, a long-term permanent treatment is still very much in need.

Cell-based cardiac therapy or cardiac stem cell therapy holds the greatest potential to solving this age-old problem. The ability to not only regenerate dead or damaged tissue in the heart but also reverse pathologic remodeling due to heart failure could cure millions of patients of heart failure, returning them to a healthy, fully functioning state. The last decade has shed much light on the potential of stem cell therapies, but also has illuminated significant barriers to creating a clinically acceptable treatment. While these barriers seem tall, it is crucial that much time and resources be invested into stem cell therapies for cardiac applications as they hold the greatest potential to being able to effectively treat, rather than manage, those with heart failure. In addition to regenerating dead or damaged myocardium, stem cell technology has the potential to grow an entire organ that is patient specific in its origin, and would fully alleviate having to wait for an available donor organ. The ability to grow an entire organ in the lab, which can later be transplanted, would forever change the way medicine is practiced, while saving millions if not billions of lives worldwide.

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Introduction

Heart failure (HF) is becoming an increasingly more prevalent public health concern, as more than 20% of Americans will develop HF in their lifetime (1). According to the Center for Disease Control and Prevention, heart failure is listed as the primary cause of 55,000 deaths and a contributing cause in another 280,000 deaths annually, representing a staggering one in nine mortalities in the United States. Despite medical advances that have improved survival over the last several years, the mortality rate for heart failure remains near fifty percent within five years of initial diagnosis (2). The cost associated with the diagnosis and management of heart failure is currently exceeding thirty billion dollars each year (3), placing further strain on an already struggling U.S. health care system.

While the incidence of heart failure has remained relatively stable at approximately 650,000 new cases diagnosed per year, the prevalence has continued to rise over at an alarming rate (2,4) in large part due to three major factors. First, as medical interventions improve, a greater number of patients are surviving acute coronary events, which subsequently result in chronic HF. Second, treatment for chronic HF has dramatically improved over the last few decades, allowing patients to live much longer with the disease. And finally, as heart disease is significantly associated with the later stages of life, the aging population will inevitably result in an increase in the prevalence of HF (5).

Although the prevalence of adult heart failure in the United States may be far greater than that of children, pediatric heart failure is also a significant public

health concern. Every year, 11,000 to 14, 000 children are hospitalized due to heart failure, more than 7% of whom die during their hospitalization. This is quite concerning when compared to 0.4% mortality for children admitted to hospitals without heart failure. The mortality for children with heart failure also exceeds that of adults with the same condition, which is between 3 and 7 percent. Additionally, the average hospital length of stay for children with HF increased over 40% from 1997 to 2006, corresponding to greater than 250,000 total hospital days per year (6).

In adults, the most common cause of heart failure is ischemic heart disease, whose etiology has been well studied and understood. The etiology of HF in pediatric patients, however, has been shown to be very different from that of their adult counterparts. Dilated cardiomyopathy (DCM) remains the leading cause of HF in children older than 1 year of age, and the leading cause of heart transplantation in pediatric patients (7,8). The most challenging aspect of treating DCM in children lies in the heterogeneity of its etiology, where approximately two-thirds of children diagnosed with DCM are idiopathic (9,10). Such a deficit in knowledge likely has contributed to the observations that, despite the significant medical advancements, modern therapy has failed to improve survival for children suffering from heart failure since the early 1970's (11). Such observations illuminate not only a gap in scientific knowledge with respect to the etiology of pediatric heart failure, but also a significant need for effective medical therapies to improve outcomes in those suffering from HF.

Current medical therapy for chronic heart failure can be categorized into three major groups: pharmaceutical therapy, mechanical circulatory support, and cell-based therapy. The specific approach to treating HF is dependent upon the severity or stage of heart failure, as well as other concurrently existing diseases. For earlier stages of HF, pharmaceutical therapy is often used to treat pathologies that are associated with HF such as hypertension, dyslipidemia, obesity, and diabetes mellitus (13).

For many patients in later stages of heart failure, mechanical circulatory support is the most appropriate treatment option, especially when pharmaceutical therapy is unsuccessful. Mechanical circulatory support is implemented to reach several different outcomes such as bridge to transplant (BTT), bridge to recovery, destination therapy, or rescue therapy.

Circulatory support is often accomplished by implantation of an Extracorporeal Membrane Oxygenation (ECMO) device, which both pumps blood for the heart, as well as provides an oxygenation function, or a ventricular assist device (VAD) (14). VADs are implantable mechanical circulatory support devices that assume the pumping function of the ventricle in which it has been implanted. Implantation of left ventricular assist devices occur in approximately 50% of patients who are eligible, compared to only 1.5% implantation for right ventricular devices. Although left heart failure predominates, there has recently been increasing interest in right heart failure and novel assist devices to treat it (15). Mechanical circulatory assist devices are currently the best treatment option for

those in late stage heart failure, but not without significant risk of adverse events. It is clear that novel technologies are required to address the complications that accompany current devices, as well as being suitable for right heart failure, especially in the pediatric population (14, 16).

A lesser developed but potentially powerful therapeutic option for the treatment of all stages of heart failure is cell-based therapy. With the discovery of cardiac stem cells came the possibilities of regenerative therapies to treat a damaged or failing heart. Stem cell therapies for heart failure focus on healing or regenerating the functional unit of the myocardium: the cardiomyocyte. By stimulating the proliferation of healthy cardiomyocytes, cell-based therapies have the potential capacity to return the myocardium to a pre-diseased state. While such capability would surely revolutionize the treatment of heart failure, there still remains several significant obstacles that researchers must overcome to make cardiac stem cell therapy a reality. These obstacles have been categorized as improving cell survival, persistence of the stem cells after intramyocardial introduction, and proliferation of the introduced cells (17).

Following an ischemic event within the myocardium, a cascade of immune cells invades the damaged area furthering the tissue damage, as well as releasing cytokines that induce native cell apoptosis. Because the stem cells are delivered into such a hostile environment, it is not surprising that many of the cells do not survive. The most recent approach to solving the survivability issues is aimed at reducing the expression of cytokines released from inflammatory cells

such as tumor necrosis factor (TNF- α), several interleukins (ILs), matrix metalloproteinases (MMPs) and transforming growth factor β (TFG- β) whose native role is to regulate cell apoptosis (18). By reducing factors that induce cell death, the therapeutic stem cells would be introduced into an environment more suitable for survival. Other investigators are proposing that survivability can be improved by preconditioning the stem cells prior to injection. While both approaches share a common goal, the most effective means to attain survivability has not been determined.

In addition to survival, the persistence of the stem cells at the delivery or injection site must also be improved. When cardiac stem cells were injected into the myocardium directly in the mouse model, only 15% of the cells remained in the myocardium after one week (19). While a proportion of this problem may be attributed to the ischemic environment, the exact cause of the cell disappearance is unknown. It is evident, however, that cell persistence is a crucial aspect of myocardial regeneration following infarction.

In correspondence with the research on improving survival and persistence is the search for new or modified stem cells types, which may elicit greater cell proliferation. Possible candidates include embryonic stem cells (ESCs), induced pluripotent stem cells, and a variety of others (17). While there exist many challenges to cell-based regenerative therapy, it promises to be the most effective treatment at regenerating functional myocardium and reversing the devastating effects of heart failure.

The Heart Failure Epidemic

To be considered an epidemic, new cases of a given disease must substantially exceed the number of expected cases over a specific time frame. Although the term epidemic was traditionally reserved for diseases that are infectious in nature, the changing public health landscape pushed modern epidemiology to also include noninfectious diseases and syndromes like heart failure. With a worldwide prevalence of more than 23 million, it is no wonder that in 1997 heart failure was specifically singled out as an epidemic requiring substantial attention both from a clinical and public health perspective (20, 21).

Definition and Diagnosis

The American Heart Association (AHA) and the American College of Cardiology Foundation (ACCF) defined heart failure in the 2013 ACCF/AHA Guideline for the Management of Heart Failure as a complex clinical syndrome that results from any structural or functional impairment of ventricular filling ejection of blood. The clinical syndrome of HF may result from disorders of the pericardium, myocardium, endocardium, heart valves, great vessels, or certain metabolic abnormalities. The most common symptom found in patients with heart failure is an impaired or failing left ventricular myocardium. However, this impairment of the left ventricular myocardial function is not synonymous with a cardiomyopathy or left ventricular dysfunction, as these pathologies would not be symptoms of heart failure. Rather, these aforementioned terms provide a

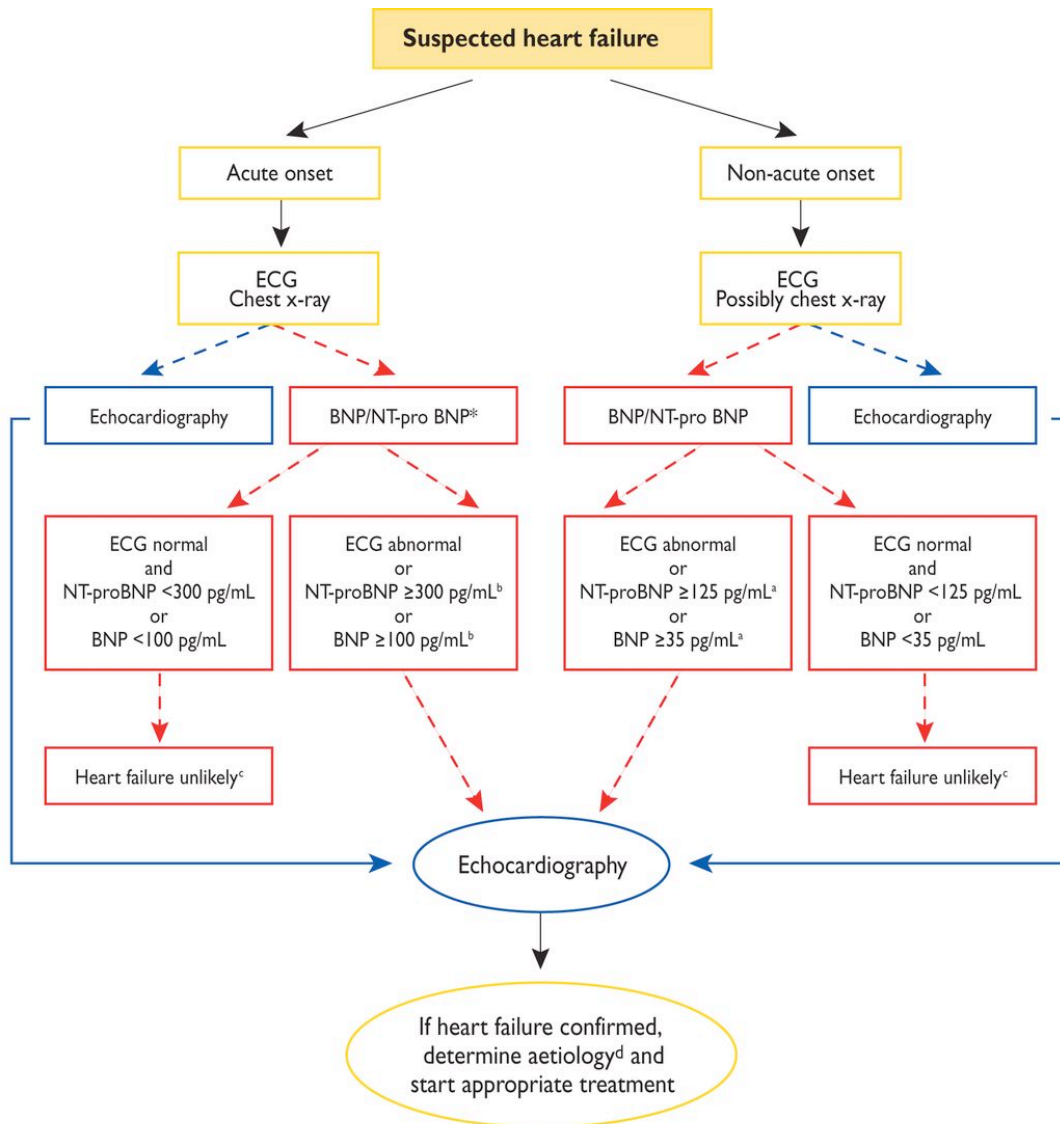
pathophysiologic cause for the development of heart failure. The cardinal manifestations of such a syndrome are dyspnea, fatigue, and fluid retention. Such retention of fluid often results in pulmonary and/or splanchnic congestion as well as peripheral edema (22). Diagnosing heart failure can also be challenging clinically as most decisions are made from a carefully acquired patient history and physical examination. Also, heart failure is not, by definition, a disease. It is more accurately a syndrome, or a compilation of observed symptoms that are indicative of a single condition, further complicating a successful diagnosis.

To make diagnosis of heart failure more manageable for clinicians, several criteria have been established. Four of these specific criteria, the Framingham criteria, the Boston criteria, the European Society of Cardiology criteria, and the Gothenburg criteria, they all share many similar indicators and symptoms and elevated filling pressures which are combined with the findings from the medical history, physical examination, and imaging (21). Of these criteria, the Boston criteria are recommended for diagnostics in older patients because of its ability to predict future adverse events (23). The Framingham criteria, which generally have a lower positive predictive value for HF diagnosis than the Boston criteria but a greater sensitivity, are well suited for secular trends. This is because of its ability to maintain a high performance irrespective to time and use of specific diagnostic tests (21). The European Society of Cardiology criteria are likely the most objective of the aforementioned criteria. Therefore, in order for these criteria to be applied in practice, cardiac dysfunction must be objectively determined to

be elevated to a predetermined threshold level or greater, which can often be challenging from a clinical perspective (24, 25).

The most useful tests for diagnosing heart failure are the electrocardiogram (ECG) and the echocardiogram. The ECG is a useful diagnostic modality to determine if there are any abnormalities in rhythm or in the normal electrical conduction of the heart. The echocardiogram is likely the most useful tool for diagnosing heart failure, as it can provide insightful information on chamber volumes, end systolic and diastolic volumes, ejection fractions, wall motion, and flow dynamics. Knowledge of these aspects of the disease is crucial in not only determining the most appropriate treatment, but also in determining the etiology of the heart failure (26).

In addition to these studies, various biochemical and hematological studies are also important in creating the most appropriate treatment plan that is tailored to the specific patient. The most important function of these exams is to determine renal function and potassium, as they are indicators of whether the renin-angiotensin-aldosterone system can be safely modulated with therapy, and also to exclude various forms of anemia. As anemia can very closely mimic the physical manifestations of heart failure, this is a very important exclusion to make before confirming heart failure as a diagnosis.



^aIn the acute setting, MR-proANP may also be used (cut-off point 120 pmol/L, i.e. <120 pmol/L = heart failure unlikely).

BNP = B-type natriuretic peptide; ECG = electrocardiogram; HF = heart failure; MR-proANP = mid-regional pro atrial natriuretic peptide; NT-proBNP = N-terminal pro B-type natriuretic peptide.

^bExclusion cut-off points for natriuretic peptides are chosen to minimize the false-negative rate while reducing unnecessary referrals for echocardiography.

^cOther causes of elevated natriuretic peptide levels in the acute setting are an acute coronary syndrome, atrial or ventricular arrhythmias, pulmonary embolism, and severe chronic obstructive pulmonary disease with elevated right heart pressures, renal failure, and sepsis. Other causes of an elevated natriuretic level in the non-acute setting are: old age (>75 years), atrial arrhythmias, left ventricular hypertrophy, chronic obstructive pulmonary disease, and chronic kidney disease.

^dTreatment may reduce natriuretic peptide concentration, and natriuretic peptide concentrations may not be markedly elevated in patients with HF-PEF.

^eSee Section 3.5 and Web Table 3.

Figure 1: A diagram illustrating the diagnosis of heart failure using various diagnostic modalities depending on specific findings (26).

In addition to these studies, a diagnosis and/or etiology is still unclear, further testing can be completed. The most common of these examinations would be studies such as perfusion imaging, angiography, or endomyocardial biopsy (26).

Classifications of Heart Failure

Patients requiring hospitalization as a result of HF can present with acute decompensated heart failure. The presentation of acute decompensated HF may be the result of new onset HF, currently existing HF that is worsening, or advanced HF. These patients are experiencing a gradual or rapid change in the signs and symptoms associated with HF resulting in the need for urgent therapeutic intervention (27). However, not all patients hospitalized for heart failure will present with acute decompensated HF, and those that do may not always be properly identified and documented. This lack of precise diagnosis and documentation places a significant barrier in the way of accurately determining the true burden of heart failure and its associated hospitalizations annually. As it has been found that many hospitals document nearly all patients admitted for heart failure as exhibiting acute decompensated HF, its true burden is likely an overestimate as many patients who present with HF as a comorbidity of another pathology (21).

The broadest classification of heart failure is based upon the left ventricular ejection fraction (LVEF). The ejection fraction is the stroke volume, or

the volume of blood ejected from the ventricle during one cardiac cycle, as a proportion of the end diastolic volume, or the volume of blood in the ventricle just before systole. Properly determining a patient's left ventricular ejection fraction is crucial because it will likely influence comorbidities, prognosis, and response to specific therapies. The most current guidelines state that if a clinical diagnosis of heart failure is made and the ejection fraction is determined to be $\leq 40\%$, the patient is classified as having heart failure with reduced ejection fraction (HFrEF). Of these patients, nearly half will also be found to have some degree of left ventricular enlargement (22,28,29).

Conversely, if a clinical diagnosis of heart failure is made but there is no significant reduction in ejection volume (EF is greater than 40%) the patient is classified as having heart failure with preserved ejection fraction (HFpEF). These patients represent about half of all those clinically diagnosed with heart failure (30). Compared to HFrEF, the diagnosis of HFpEF is much more difficult. A positive diagnosis would include signs and symptoms of heart failure, evidence of a preserved or normal ejection fraction, and evidence of abnormal left ventricular diastolic dysfunction, which is determined by Doppler echocardiography and/or cardiac catheterization (31).

Framingham	Boston	European Society of Cardiology	Gothenburg Score	
			Item and Method of Assessment	
Major criteria	Category I: History	1. Symptoms of heart failure (at rest or during exercise)	Cardiac score	
Paroxysmal nocturnal dyspnea or orthopnea	Rest dyspnea (4 pts) Orthopnea (4 pts)	and	History of heart disease (1–2 pts)	Self-report
Neck vein distension	Paroxysmal nocturnal dyspnea (3 pts)		2. Objective evidence of cardiac dysfunction (at rest)	Angina (1–2 pts)
Rales	Dyspnea on walking on level (2 pts)	and	Edema (1 pt)	Self-report
Cardiomegaly	Dyspnea on climbing (1 pt)		3. Response to treatment directed toward heart failure (in cases where diagnosis is in doubt)	Nocturnal dyspnea (1 pt)
Acute pulmonary edema	Category II: Physical examination	Criteria 1 and 2 should be fulfilled in all cases	Rales (1 pt)	Physical examination
S3 gallop	Heart rate abnormality (1–2 pts)		Atrial fibrillation (1 pt)	ECG
Increased venous pressure ≥ 16 cm water Circ. time ≥ 25 s Hepatojugular reflux	Jugular venous pressure elevation (1–2 pts) Lung crackles (1–2 pts) Wheezing (3 pts)		Pulmonary score	
Minor criteria			History of chronic bronchitis/asthma (1–2 pts)	Self-report
Ankle edema	Third heart sound (3 pts)		Cough, phlegm, or wheezing (1 pt)	Self-report
Night cough	Category III: Chest radiography		Rhonchi (2 pts)	Physical examination
Dyspnea on exertion			Cardiac and pulmonary score are calculated and used to differentiate cardiac from pulmonary dyspnea	
Hepatomegaly	Alveolar pulmonary edema (4 pts)			
Pleural effusion	Interstitial pulmonary edema (3 pts)			
Vital capacity decreased 1/3 from maximum	Bilateral pleural effusions (3 pts)			
Tachycardia rate of ≥ 120 /min	Cardiothoracic ratio ≥ 0.50 (3 pts)			
Major or minor criterion	Upper zone flow redistribution (2 pts)			
Weight loss ≥ 4.5 kg in 5 d in response to treatment	Definite heart failure 8–12 pts, possible 5–7 pts, unlikely ≤ 4 pts			
Heart failure present with 2 major or 1 major and 2 minor criteria				

Circ. indicates circulation; and pts, points.

Figure 2: A comparison of the Heart Failure Classification Methods (21)

The most significant cause of HFpEF is hypertension, which is present in 60-89% of cases (32). Other factors that have been associated with HFpEF are obesity, coronary artery disease (CAD), diabetes mellitus, atrial fibrillation, and hyperlipidemia (30,33). Ultimately, heart failure with preserved ejection fraction is

less understood than its reduced ejection fraction counterpart, thus further investigation is necessary better treat this subset of patients.

The ACCF and AHA provide further classification by providing criteria for stages of heart failure, and the New York Heart Association provides a mechanism for functional classification. The ACCF/AHA stages of HF are meant to classify the development and progression of disease within an individual or population, while the New York Heart Association functional classifiers are used to determine exercise capacity and symptomatic status of the disease (22).

Epidemiology

It has been estimated that by the year 2050, nearly one in five Americans will be older than 65 years of age (13). Because heart disease is most prevalent among this age group, the aging population signifies an even greater rise in the prevalence of heart failure well into the future. This comes with great concern, as the current prevalence of heart failure in the United States alone is already more than 5.8 million cases (34,35). In America, the current lifetime risk of developing heart failure for those age 40 or greater is 20%, as each year more than 650,000 new cases of heart failure are diagnosed (36-38).

ACCF/AHA Stages of HF ³⁸		NYHA Functional Classification ⁴⁶	
A	At high risk for HF but without structural heart disease or symptoms of HF	None	
B	Structural heart disease but without signs or symptoms of HF	I	No limitation of physical activity. Ordinary physical activity does not cause symptoms of HF.
C	Structural heart disease with prior or current symptoms of HF	I	No limitation of physical activity. Ordinary physical activity does not cause symptoms of HF.
		II	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in symptoms of HF.
		III	Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity causes symptoms of HF.
		IV	Unable to carry on any physical activity without symptoms of HF, or symptoms of HF at rest.
D	Refractory HF requiring specialized interventions	IV	Unable to carry on any physical activity without symptoms of HF, or symptoms of HF at rest.

Figure 3: Classification of HFrEF and HFpEF (22)

Because heart disease is most prevalent among this age group, the aging population signifies an even greater rise in the prevalence of heart failure well into the future. This comes with great concern, as the current prevalence of heart failure in the United States alone is already more than 5.8 million cases (34,35). In America, the current lifetime risk of developing heart failure for those age 40 or greater is 20%, as each year more than 650,000 new cases of heart failure are diagnosed (36-38).

The heart failure incidence stratified by age shows significant increases as the age group also increases. The incidence rate for individuals between the ages of 29 and 79 years is between 1.4 and 2.3 new HF cases per 1000 people in the at risk population per year (21,39). However, when looking at individuals ages 65 to 69 years of age, the incidence rises approximately 20 new HF cases per 1000 people per year.

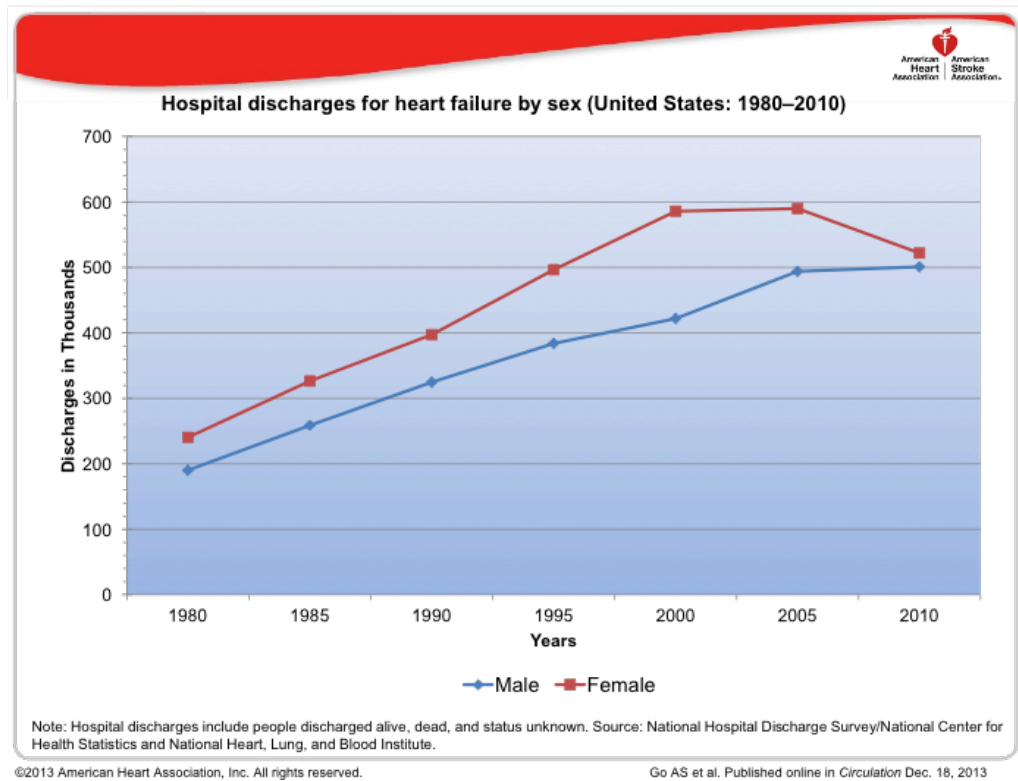


Figure 4: Hospital Discharges for Heart Failure from 1980-2010. The figure clearly illustrates a significant increase in the number of discharges for heart failure over the last several decades. (https://www.heart.org/idc/groups/heart-public/@wcm/@sop/@smd/documents/downloadable/ucm_449852.pdf).

Finally, and most alarming when considering the ever increasing life expectancy and aging population, the incidence for those greater than 85 years of age is more than 80 new heart failure cases diagnosed per 1000 people per year (40). While these national incidence rates have remained relatively stable over the last few decades, advancements in the medical technology and treatment has attributed to the rapidly increasing prevalence of heart failure both in the U.S. and abroad. While these national incidence rates have remained relatively stable over the last few decades, advancements in the medical

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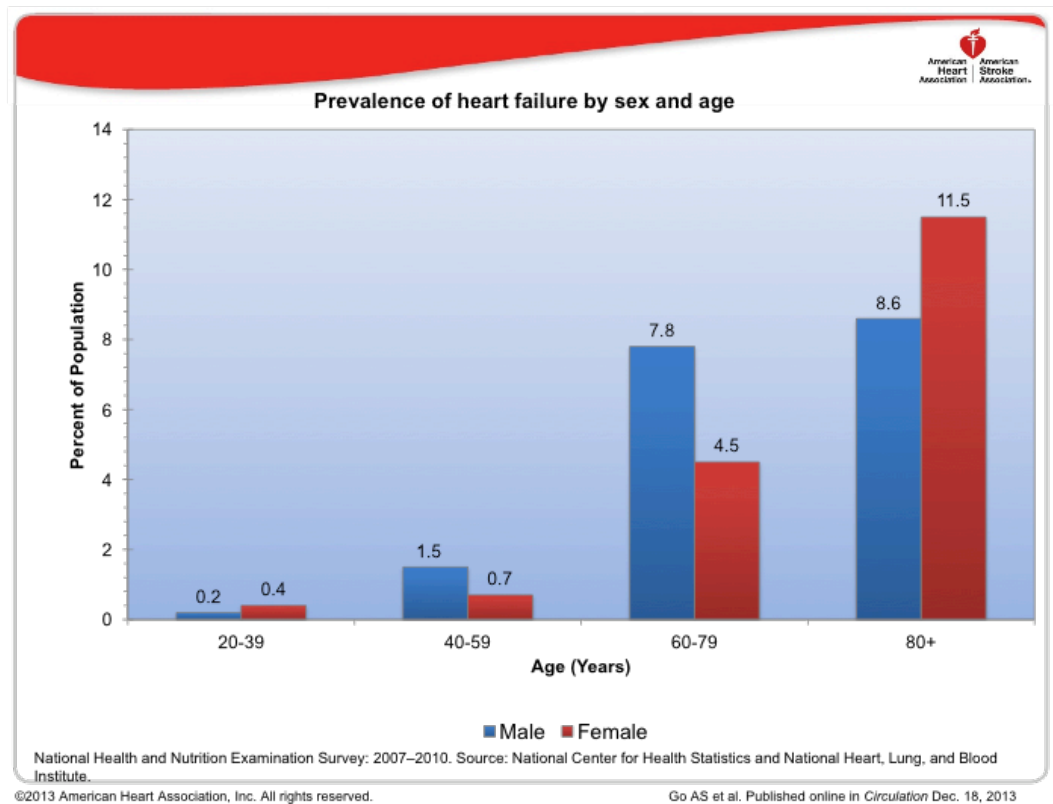


Figure 5: The age and gender stratified prevalence of heart failure, as a percent of the population at risk (https://www.heart.org/idc/groups/heart-public/@wcm/@sop/@smd/documents/downloadable/ucm_449852.pdf).

With respect to mortality rates, the five and ten year survival following the initial diagnosis of heart failure is 50% and 10%, respectively (21). While a five-year survival rate of 50% is considered poor at best, it is a marked improvement in comparison to the 30-40% 5 year survival rates observed before the 1990s (26). Like mortality rates, the hospitalization rates as a result of heart failure have

also declined over the past decade. The largest study to date examining temporal trends in hospitalization rates from HF using the Medicare database indicated that from 1998 to 2008 there was a relative decline of 29.5% in risk-adjusted HF hospitalization rate (41). This decline has been attributed to fewer unique individuals hospitalized for heart failure, yet considering that advancements in HF care have improved survival, a decrease in hospitalizations is quite surprising.

One would expect longer survivals to result in a greater number of rehospitalizations and an overall increase in hospitalization rates compared to those in 1998, but this was not the case. This lack of an increase, when considering the average hospitalization cost for heart failure of \$18,000 per patient, represents a saving solely due to heart failure hospitalization in 2008 of 4.1 billion dollars in fee-for-service Medicare. It should also be noted that this study also demonstrated substantial geographic variation for both heart failure hospitalization and one-year mortality rates. Such a variation represents significant and undeniable differences in outcomes that cannot be explained by insurance status (41).

While the evidence supports the notion that we are moving in a positive direction with respect to addressing the heart failure epidemic, there are many significant hurdles that must be overcome in order to truly make a significant difference. One of those hurdles is addressing those that are already in advanced end-stage heart failure. The majority of these patients will no longer

benefit from pharmacological therapy. The gold standard for patients at this stage of HF is undoubtedly heart transplantation, which has been proven to elicit good long-term survival post-operatively. However, as the number of patients with advanced heart failure continues to rise, the limited number of hearts available for transplant falls vastly short of demand, leaving thousands of patients searching for viable alternatives.

The most effective alternative to transplantation is mechanical circulatory support (MCS) therapy, and more specifically, ventricular assist devices or VADs (McMurray). Looking into the future, it is likely that patients in end-stage heart failure will have two options if transplants are not available. The first being more highly developed mechanical circulatory support devices, and the second being a cell-based therapy. While cell-based therapies are merely in their infancies today, they possess the potential to replace injured or dead cells, even organs, with new, properly functioning ones. The future looks bright as the potential of both technologies promise to ease the burden of HF and improve the lives of millions of people around the world plagued by heart failure.

Current Technology: Treating Heart Failure

The ultimate goal when treating patients who have been diagnosed with established heart failure is to improve survival while also bettering their quality of life by relieving as many of the resultant symptoms as possible. Such an approach has additional benefits of reducing hospital admissions and

readmissions due to heart failure, which is beneficial both for the patient and the healthcare system (42). The initial treatment plan for those suffering from HF is usually pharmaceutical therapy. Many of these therapies have been shown to effectively relieve many of the symptoms of HF, which then lead to a greater quality of life, higher functional capacity, and better survival. Cardiac Resynchronization Therapy (CRT) is also a more primary treatment option for HF, and often has the same many of the same benefits as pharmaceutical therapies. In addition to treating HF and its symptoms, therapies aimed at treating the various comorbidities of heart failure are also a crucial aspect in providing the best possible care for patients. Comorbidities are important to consider because they may influence the pharmaceutical therapies available to specific patients, and their existence is strongly associated with poorer prognoses. Clinicians must, therefore, closely monitor and manage the various comorbidities associated with heart failure for each patient, and strongly consider him or her when making decisions with respect to the best possible treatment plan (26). However, when the aforementioned treatment options fail to be effective and the patient's status continues to decline, many clinicians will turn to mechanical circulatory support or transplantation. While these treatment options may accompany greater risk, as at this point in the disease pathway, there are few other options.

Non-Surgical Device Treatments:

The most common treatment modalities, which are much less invasive than mechanical circulatory support devices, are the implantable cardioverter defibrillators (ICD) and Cardiac Resynchronization Therapy (CRT). Both of these treatment options act by regulating and modulating the electrical signals of the cardiac cycle to either prevent or treat arrhythmias. As nearly half of all deaths in patients diagnosed with heart failure are due to a ventricular arrhythmia, preventing such deadly arrhythmias is a principal goal in improving survival in these patients. In addition to ICD and CRT, several new technologies are being developed using similar principles such as implantable cardiac monitors and wearable defibrillator vests (26,43).

An implantable cardioverter defibrillator (ICD) is a small device that is implanted in the body and has leads that are placed near the patient's heart. The leads of the ICD carry electrical current that acts to both provide a normal pacing of the heart as to maintain normal sinus rhythm, as well as having the capability of high energy cardioverting/defibrillation discharges. These allow the heart to be resynchronized in the event of dysrhythmia to restore normal cardiac rhythm (44). The original ICD devices developed were quite large in size, and required a pocket for implantation of the body of the ICD into the abdomen, as well as a thoracotomy to place the leads onto the surface of the heart. Technological advances have now allowed for the production of much smaller transvenous pectoral devices. These advances have markedly reduced the likelihood of

complications as a result of device implantation, as well as improving functionality and monitoring capabilities, all of which have improved patient survival by dramatically reducing the incidence of sudden cardiac death in patients with heart failure. (45).

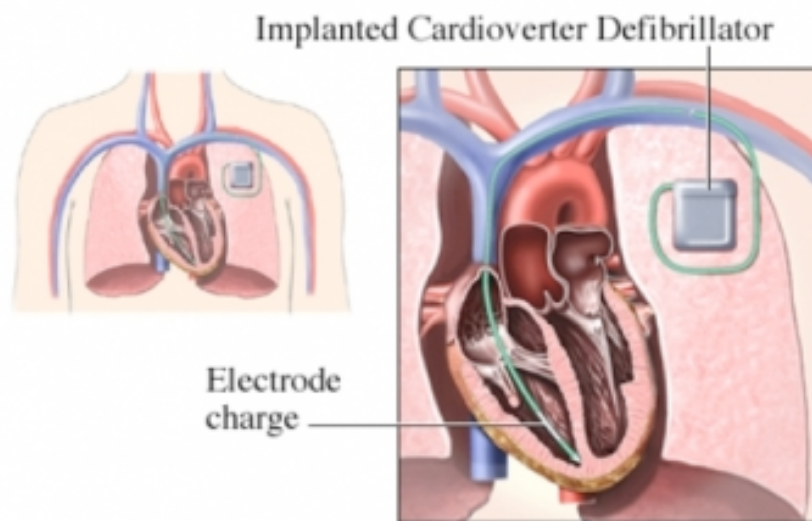


Figure 6: Depiction of the placement of an implantable cardioverter defibrillator (ICD). Image courtesy of Munson Medical Center. (<http://www.munsonhealthcare.org/?id=1321&sid=2>)

A second treatment modality that utilizes electrical stimulation is cardiac resynchronization therapy. In patients that have discoordinate wall motion due to delays in the native conduction system of the heart, CRT can be highly effective. These patients with discoordinate contraction of the lateral walls are a different subset of heart failure patients than those with fixed functional defects. Identifying this subset of patients can be done using electrocardiography (ECG). Because

these patients have a delay in the native electrical conduction through the ventricles causing the discoordinate contraction, a widened QRS interval would be present, particularly an LBBB-type morphology (46). Of all patients presenting with heart failure, approximately one quarter of them will have a widened QRS interval. Compared to patients who don't have widening, patients who do are 1.7 times more likely so experience worsening heart failure and sudden cardiac death (47), giving these patients with discoordinate contraction among the worst prognosis and underlying left ventricular dysfunction of all heart failure patients.

Recently it has been shown that the discoordinate wall motion alone is an independent predictor for worsening heart failure regardless of QRS interval duration. In a study done by Bader et al., ventricular free wall dyssynchrony that was diagnosed using tissue Doppler imaging conferred a significant reduction in event free survival and experienced much worse outcomes (48). The intuitive link between an electrical delay in the conduction system and a resultant discoordinate mechanical wall motion is relatively simple to explain in a healthy heart. However, in a diseased heart, especially a failing one, this link becomes substantially blurred.

Effective cardiac resynchronization therapy can very quickly resynchronize a previously dyssynchronous ventricular contraction, which subsequently enhances systolic cardiac function (46). Many of the therapies aimed at treating end-stage heart failure, like ventricular assist devices, do so by improving the ventricular ejection fraction. Not only does an increase in ejection fraction provide

a greater cardiac output for the rest of the body, it also dramatically reduces the burden of a pressure or volume overload of the ventricles.

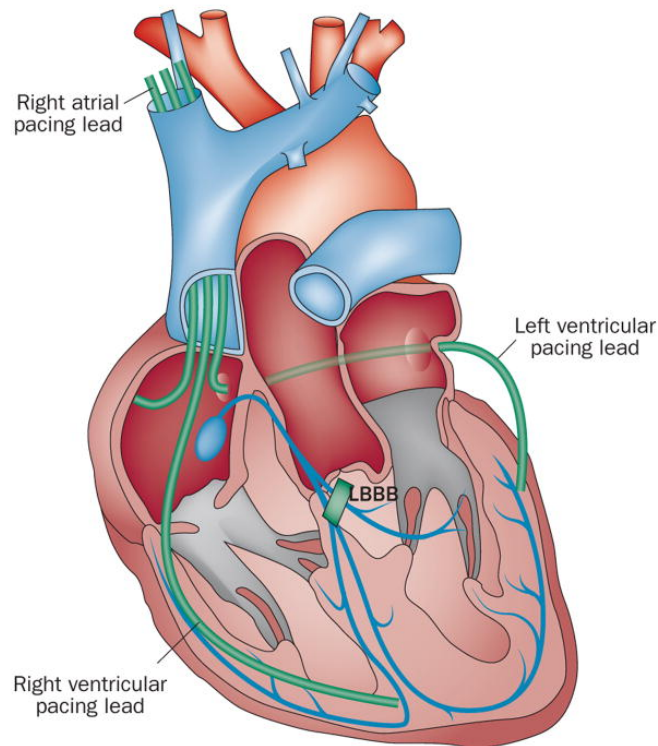


Figure 7: A Depiction of the lead placements for a Cardiac Resynchronization Device (51)

CRT is able to improve the ventricular ejection fraction, doing so without a marked increase in the metabolic demand of the myocardium, by significantly improving the efficiency of the chamber (49). In addition to an improved ejection fraction and cardiac output, patients with longer-term use of CRT also experience a reduction in both end-diastolic volume (EDV) and end-systolic volume (ESV).

The ultimate effect of these changes is creation of a cardiac environment that is conducive to a reversal of the pathogenic cardiac remodeling (46,50).

Cardiac resynchronization has been shown to be highly effective at reducing clinical symptoms and improving survival in patients with dyssynchronous contraction and electrical conduction delays as well as allowing for a reversal of deleterious cardiac remodeling from chronic failure. By combining CRT with an implantable cardioverter defibrillator, an even greater improvement in survival is observed. This is likely due to the prevalence of arrhythmia in patients with dyssynchronous wall motion and heart failure, allowing the effective treatment of both life-threatening events.

Mechanical Circulatory Support

When patients fail to respond to pharmaceutical interventions, cardiac resynchronization therapy (CRT) has been shown to improve symptoms, reduce hospitalizations, promote deleterious remodeling, and decrease mortality. However, there still exist many challenges in identifying the ideal candidates for CRT, and it has not proven to be effective for patients in end-stage refractory heart failure (51). End stage refractory HF is defined as exhibiting symptoms at rest or upon minimal exertion of profound fatigue, inability to perform most activities of daily living, requirement of repeated or prolonged hospitalizations for intensive management, or evidence of refractory cardiogenic shock (52).

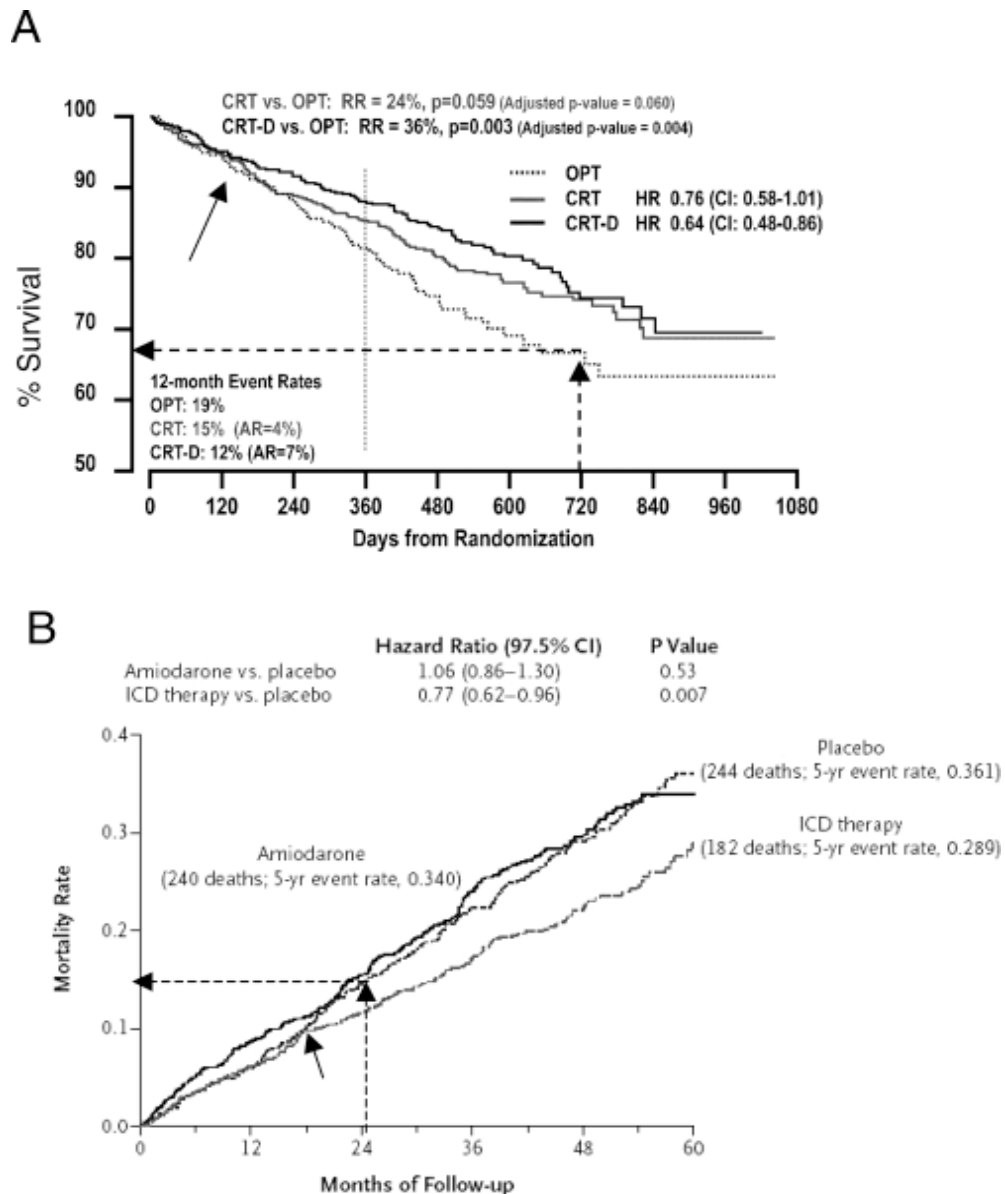


Figure 8: Implantable Cardioverter Defibrillator and Cardiac Resynchronization Therapy Combination (46)

(A): A comparison of all-cause mortality between patients who received cardiac resynchronization therapy (CRT) alone, CRT in concert with defibrillator, and no treatment. The data shows a significant decrease in mortality for those who received CRT + defibrillation compared to controls, and a borderline improvement for CRT alone. (B): A comparison of outcomes in patients receiving Implantable Cardioverter Defibrillator (ICD) vs placebo and those taking Amiodarone, a pharmaceutical treatment used for treating cardiac arrhythmias, versus placebo based on mortality rate.

For these patients, cardiac transplantation remains the most effective treatment, however, a significant shortage of donor organs, and the fact that many patients are poor candidates for transplantation, leaves many patients looking for alternative treatments (53).

Ventricular Assist Devices (VADS)

For these patients who find themselves unfit for cardiac transplantation, mechanical circulatory support can be a viable option as a bridge to transplantation for those on a wait list, destination therapy for those unsuitable for transplantation, or bridge to recovery. Mechanical circulatory support is defined as using a mechanical device to assist or replace the hearts ability to adequately circulate blood throughout the body (54). The most extensive of these devices are the ventricular replacement devices, which act as total artificial hearts (TAHs), who do not act to assist the natural heart, but to perform the task of pumping blood unassisted by native heart contraction (55). More common, and more developed are the ventricular assist devices (VADs). There currently exist VADs, which can be implemented for the left ventricle, known as LVADs, the right ventricle, known as RVADs, or both ventricles, known as BiVADs.

The first developed mechanical circulatory support device became available in the 1960's, which was an extracorporeal left heart bypass pump. While its success may not have been ideal, it inspired successful implantation of a pneumatically driven total artificial heart by the mid 1980's (56,57). Throughout

the following decade, much interest was sparked in the development of ventricular assist devices, rather than total artificial hearts, as they could serve as a viable bridge to transplant for many severely ill patients. By 1992, it had been shown that even early LVAD technology increased survival to transplant by at least 15% compared to patients receiving other medical therapies (58,59), and two years later, the first LVAD, which used an external power source, was approved by the FDA for bridge to transplant applications (60).

At the turn of the century, VADs had yet to be approved for anything other than bridge to transplant. This is hardly surprising as the second leading cause of death for those that had undergone VAD implantation was device failure, preceded only by sepsis, as the one and two year survival with LVAD support was 52% and 23%, respectively (61). Despite the complications, the LVAD HeartMate VE was approved by the FDA for destination therapy in 2003 (51).

In the ten years following the approval of HeartMate VE, significant advancements have been made in ventricular assist device technology. The vast majority of current ventricular assist devices employ highly efficient rotary pumps. In fact, many VADs are classified by the type of flow the pump provides, as well as other technical aspects. The first generation pumps are pulsatile, thus the device acts to pump the blood in a pulsatile fashion in an attempt to mimic the hearts natural pumping mechanism. These pulsatile pumps can work either in synchrony with the native heart, or in a counterpulsation manner. The second and third generations of VADs are continuous flow rotary pumps, which operate

at a constant flow rate, and can be further classified as axial, centrifugal, or mixed flow. Separating the second and third generation of continuous flow pumps is the use of bearings in the second-generation devices, which are in constant contact with the blood. Such contact dramatically increases the risk of thrombotic events, among other complications. The most current, third generation devices, have achieved a contactless bearing system through magnetic or hydrodynamic suspension, which reduces the probability of thrombotic events, but at the cost of increased control complexity and power consumption (55).

Surgical Implantation of VADs

The surgical implantation of ventricular assist devices has not significantly deviated over the last several years. Implantation requires a median sternotomy followed by inflow and outflow cannulation. Inflow cannulation is made at the left ventricular apex, and outflow cannula is anastomosed to the ascending aorta, usually via a Dacron graft. A single driveline exits the abdomen to the power supply and controller (62).

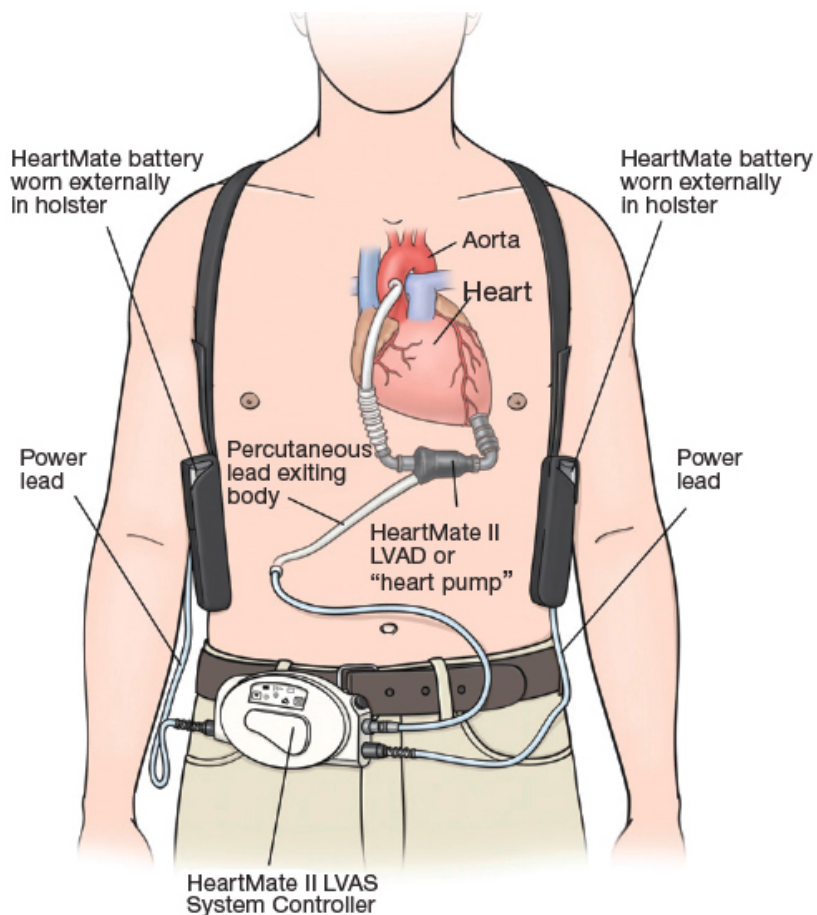


Figure 9: Depiction of an implanted HeartMate II Left Ventricular Assist Device (62)

Pulsatile Versus Continuous Flow Ventricular Assist Devices

For patients in late stage advanced heart failure, continuous flow ventricular assist devices have become the standard of care. Their small size and durability offer a number of significant advantages and reduce the likelihood of post-implantation complications (63). Many first generation pulsatile flow devices are much larger in size and weight compared to their continuous flow

counterparts, as well as lacking the ability to operate silently without vibration. Pulsatile pumps are also used most frequently for short to medium duration circulatory support, as complications have long prevented extended support. Therefore, the design objective of the continuous flow devices was to provide extended circulatory support for patients in advanced heart failure. These devices consist of an internal continuous flow rotary pump, which is percutaneously connected to an external controller and power source. While the percutaneous lead comes with a risk of infection, this risk has been reduced in comparison to previous generations of ventricular assist devices (63).

The most significant feature that accompanies the continuous flow rotary pump devices is the continuous unloading of the ventricle throughout the entirety of the cardiac cycle. In comparison, pulsatile pumps in synchrony with the native heart are only able to unload the ventricle during systole. As pressure and volume overload of the ventricles often result in the progression of a failing heart, the ability to reduce the burden of either pressure or volume overload by continuous ventricular unloading has proven beneficial. Although continuous flow pumps virtually eliminate an arterial pulse, there appears to be no significant physiologic consequence (64), apart from the difficulties of attaining a routine blood pressure measurement (63), leaving the weight of its benefits far greater than that of its flaws.

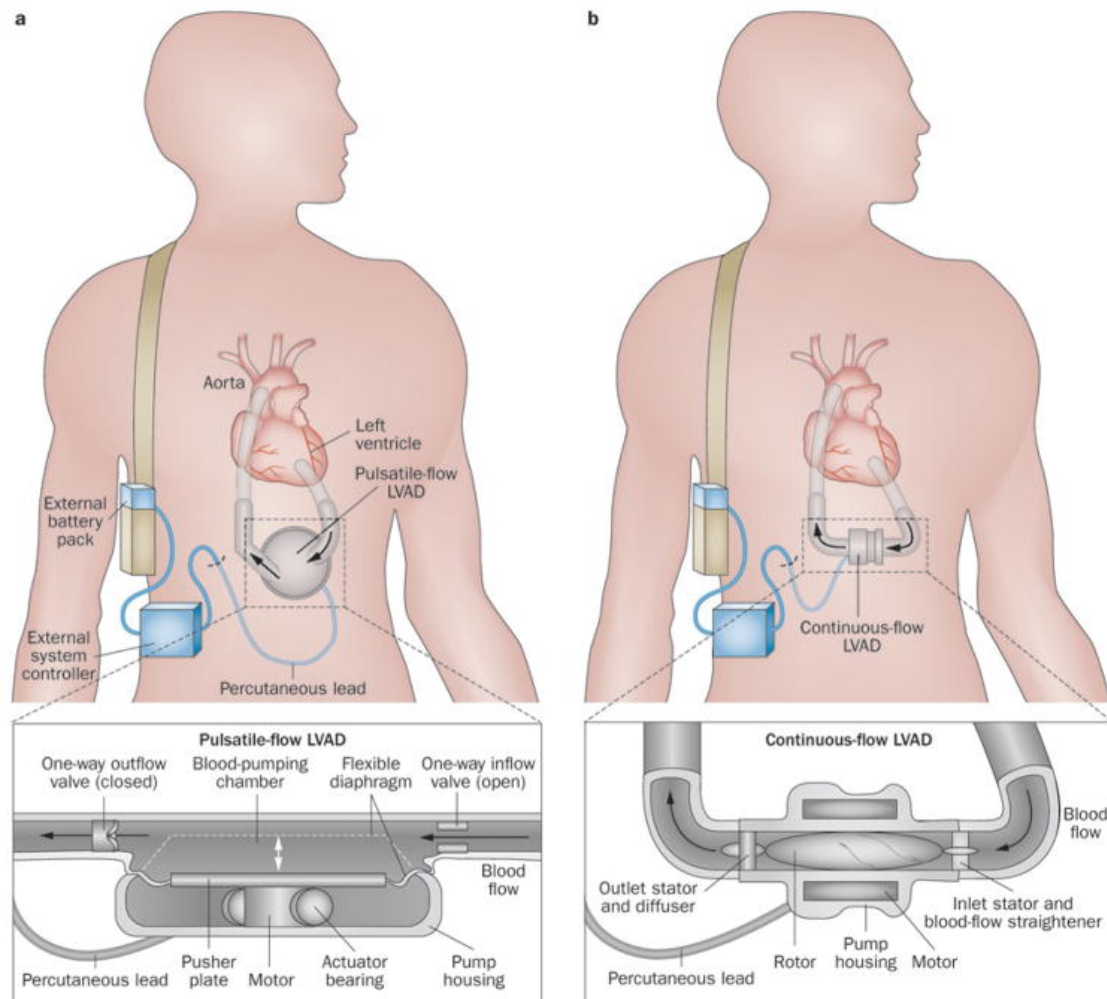


Figure 10: A comparison of the inner mechanics between the pulsatile-flow LVAD (a, left) and the continuous-flow LVAD (b, right) (51)

Both continuous centrifugal flow and axial flow devices share a common simplicity of design: a single moving component. This component, termed an impeller, rotates within the pump, propelling the blood forward. The internal bearings that allow rotation of the impeller can be blood immersed,

hydrodynamically suspended, or magnetically levitated, all three of which exhibit very little or absent friction, heat generation, and physical wear, making them ideal candidates for extended circulatory support (65-67). Efficacy of the continuous flow devices was further improved through the elimination of a reservoir chamber and inflow/outflow valves, reducing the blood contact surface area. Additionally, the blood contact surfaces that remained were designed with specialized textured titanium to further minimize the thrombogenicity of the device (68).

Attribute	Pulsatile-flow VAD	Continuous-flow VAD
Size	Large; intracorporeal devices limited to large patients; extracorporeal devices especially suited for smaller patients or for biventricular support	Smaller; accommodates most patients, excluding infants
Blood flow capacity	Up to 10 liters/min	Up to 10 liters/min
Type of pump	Sac or diaphragm	Centrifugal or axial flow by rotating impeller
Implantation	Extracorporeal or intracorporeal types: sub-diaphragmatic intraperitoneal or preperitoneal	Extracorporeal, intracardiac, pericardial, sub-diaphragmatic
Main hemodynamic characteristic	Intermittent unloading of ventricle; pulsatile arterial pressure; asynchronous with heart	Continuous unloading of ventricle
Physiologic flow variables	Pre-load dependant	Pre-load and after-load dependant
Mechanical flow variables	Automatic or fixed rate and stroke volume capacity	Set speed of the impeller rotation

Figure 11: Comparison of the attributes of pulsatile and Continuous flow VADs (63).

The Potential of Cardiac Stem Cell Therapy for Treating Severe Heart

Failure

While medical technology has taken significant strides forward over the last several decades, patients in end-stage heart failure still are quite limited in their therapeutic options. When pharmaceutical therapy begins to fail, many patients are left with little choice, mainly mechanical circulatory support by means of ventricular assist devices. While these devices have dramatically improved outcomes, they still accompany significant risk of adverse events. In the future, it is vital for researchers to improve the efficacy of these devices while making them smaller and less invasive. It is also crucial that the significant risk of adverse effects be addressed. When considering the most effective treatment for those in end-stage heart failure is transplantation, improving the ventricular assist technology will allow a greater number of people to survive long enough to receive a transplanted heart.

However, as the population continues to grow, and the prevalence of heart failure remains high, it is inevitable that the number of patients requiring transplant will significantly outweigh the number of organs available. With this in mind, it is clear that the most effective treatment would be one in which the patients own heart, and own cells, could be regenerated to a healthy state. The recent discovery of cardiac stem cells has made such a treatment a possibility, even in the near future. From an injection of stem cells that regenerate a specific infarcted area of the heart, to growing an entirely new organ in the lab that can

later be transplanted into the patient, the possibilities of cardiac stem cell therapy seem endless.

Regenerative Properties of the Heart

For many years, the heart has been considered a terminally differentiated postmitotic organ without capacity for myocyte regeneration. Research dating as far back as 1850 suggested that myocardial hypertrophy was the result of hyperplasia and hypertrophy of existing cardiac myocytes (69). The increase in weight of the heart, as observed from birth to senescence or in cardiac disease, was the consequence of equivalent increases in cardiomyocyte cell size. This widely accepted paradigm, therefore, implied that cardiomyocytes persist throughout one's entire life, making them immortal in both their structure and function. However, this assumption of cell immortality seemed to contradict the modern concept of cell aging and programmed cell death by apoptosis. It has been determined that cardiac myocytes do indeed undergo apoptosis, yet for this to be plausible, myocytes must possess the ability to replace lost cells, or the organ itself would cease to exist far before what is regularly observed (70).

Evidence suggesting the presence of cardiac stem cells capable of maintaining homeostasis of the myocardium was supported by the discovery of high levels of cardiac chimerism as a result of the migration of primitive cells from a male heart transplant recipient to the grafted heart he received, whose donor had been female. Because the transplants were sex mismatched, the presence

of a Y-chromosome in cells within the cardiac allograft indicated that those cells had migrated to the female heart and given rise to cardiac cell progenies. This observation brought to light the possibility that the Y-Chromosome-positive cells within the female heart were either the progeny of primitive cells of the bone marrow, or the result of differentiated stem-cell like cells that migrated to the allograft from cardiac remnants. These cells were found to express c-kit, MDR1, and Sca-1 (71, 72) which are stem cell-related antigens found in stem cell populations of the bone marrow, brain, skeletal muscle, and heart. Specifically, cardiac stem cells are undifferentiated, lineage-negative cells that are self-renewing, clonogenic, and capable of differentiating into mature progenies both in vitro and in vivo (73).

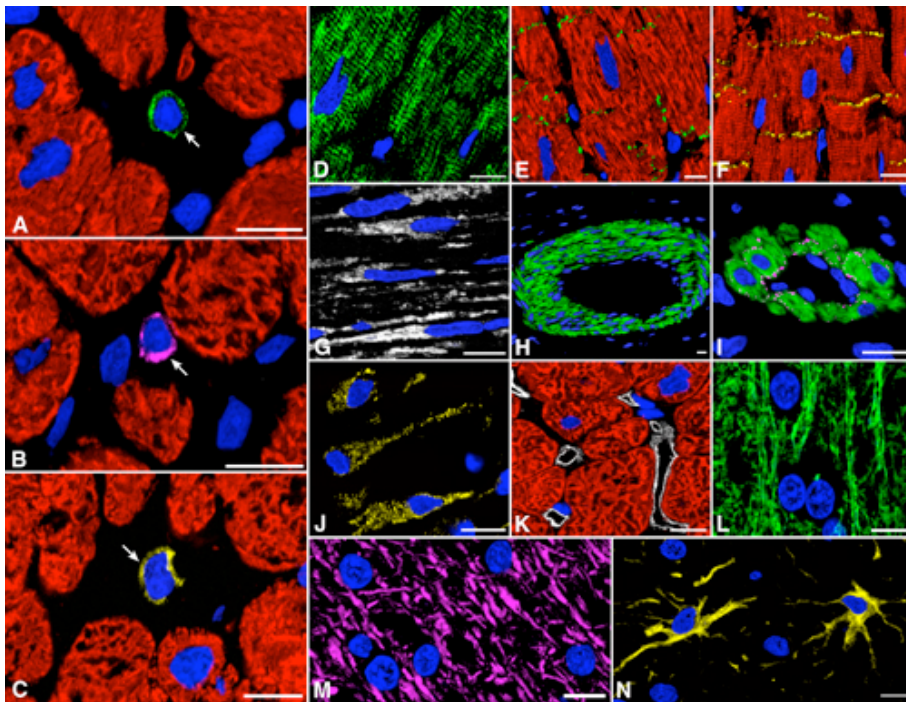


Figure 12: Histological slides of various tissues of the Heart including cardiac myocytes, smooth muscle cells, endothelial cells, neural cells, and fibroblasts. In A, the green fluorescence indicates the presence of c-kit. In B, the purple fluorescence indicates the presence of MDR1. The yellow fluorescence in C indicates Sca-1 reactive protein. The remaining cells were used as controls. (Scale bars = 10 microns.) (<http://www.pnas.org/content/suppl/2003/08/20/1832855100.DC1/2855Fig9Legend.html>)

Further supporting the hypothesis that the cells of interest were not fully differentiated were blatant discrepancies in the volume of the cells in mitosis before cytokinesis. A fully mature cardiac myocyte has an approximate volume of $25,000 \mu\text{m}^3$. If the cell were to be fully differentiated, it must expand its volume in mitosis before cytokinesis to approximately twice that of its original volume ($50,000 \mu\text{m}^3$) in order to give rise to two symmetrically divided, identical cells. Observation of the replication of human cardiomyocytes in mitosis revealed that their volumes differed markedly from expected values with volumes ranging from less than $1,000 \mu\text{m}^3$ to a maximum observed volume of $5,000 \mu\text{m}^3$. This irregular behavior of mitotic cells suggests progressive differentiation. (74-76). It was this discovery, evidence indicating that the mechanism of myocyte formation differs substantially from what would be expected of nondifferentiating cells, along with the identification of cells with male origin within the myocardium of a female heart, which provided the foundation for the identification and characterization of cardiac stem cells in the adult myocardium (73).

Myocardial Progenitor Cells

As the study of cardiac plasticity and myocardial regeneration continues to expand, so does the variety of stem, precursor, and progenitor cell lines present within the myocardium. The first of these cells to be discovered was the c-kit^{POS} stem cell. Following its discovery was the recognition of several other progenitor cells including ISL1 progenitor cells, epicardial progenitor cells, side population progenitor cells, Sca1 progenitors, and the recognition of progenitor cells forming a complex structure known as a cardiosphere. It has recently been shown that the c-kit^{POS} CSCs play the most significant role in the generation of myocytes, vascular smooth muscle cells, and endothelial cells following injury as the result of an infarcted myocardium. Cardiomyocytes that are the progeny of c-kit^{POS} CSCs have also been shown to acquire all of the appropriate mechanical and electrical properties of a fully functioning, mature Myocytes (77-80). Therefore, its emergence in a previously infarcted ventricular myocardium is associated with improvements in ventricular function (79, 80).

The LIM-homeobox transcription factor islet-1 (ISL1) marks one of two specific pools of cardiac progenitors originating from the embryonic cardiogenic plate. ISL1⁺ cells represent the second lineage of cells located more dorsal and medial in the cardiogenic plate, with a unique, delayed, migratory path.

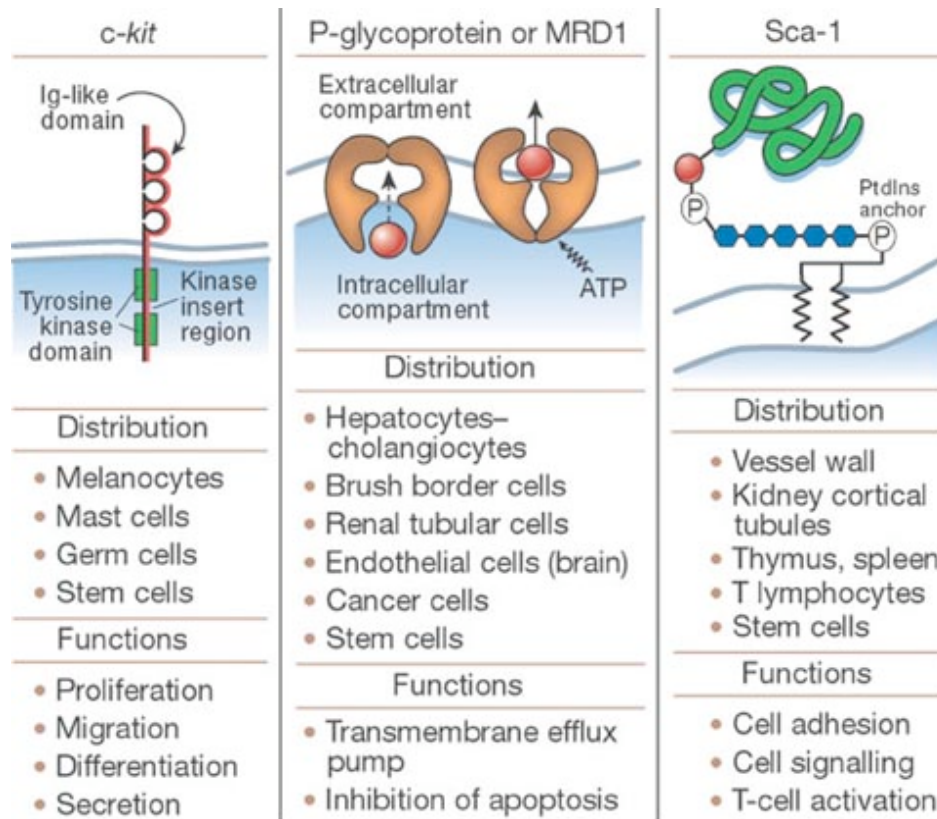


Figure 13: The Cellular Distribution and Function of Stem Cell Associated Cell Surface Markers (<http://www.hmc.org.qa/en/>)

These cells migrate into the developing heart from dorsal positions at the anterior and posterior poles (81-83). This lineage of cells will become the major source of cardiac progenitors that ultimately form the outflow tract, the right ventricle, portions of the left ventricle, and the atria (84).

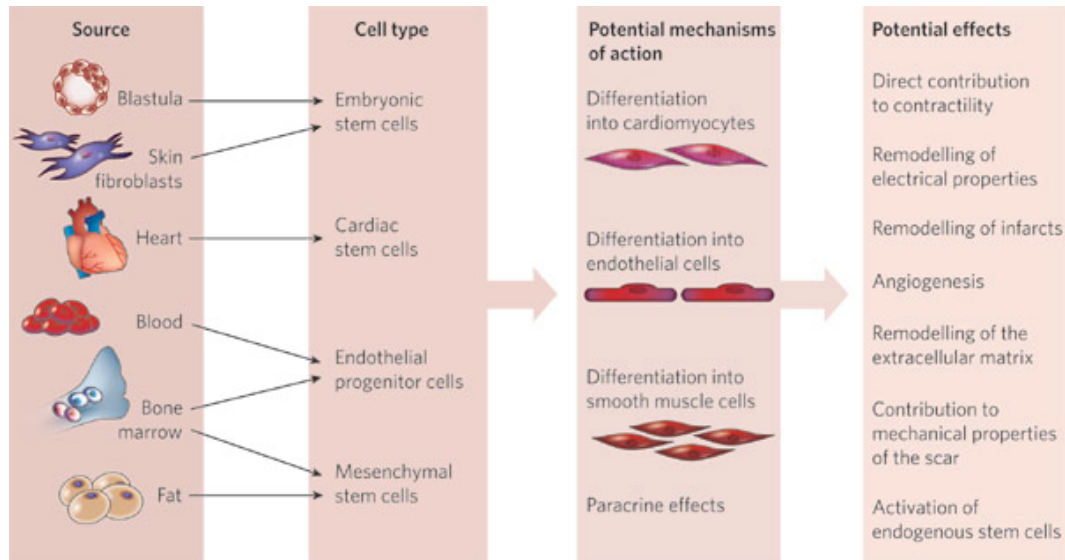


Figure 14. The Various Origins and Classes of Stem Cells for Cardiac Therapy (94)

Therefore, ISL1⁺ progenitor cells from the second heart field account for all three lineages of cells within the heart; endothelial cells, smooth muscle cells, and cardiac muscle cells. While it has been explicitly demonstrated that ISL1⁺ cells play a pivotal role in the developing heart, and do in fact account for a substantial portion of cardiac progenitors during cardiac development, their ability to serve as a postnatal stem cell appears to be negligible (80). More research must be completed to determine the effectiveness of using ISL1⁺ cells for therapeutic cardiac regeneration.

Classification of Cardiac Stem Cells and Progenitors

Piero et. al., 2006, proposed a means of classifying the various immature cells within the heart on the basis of their relative differentiation (73). These four

classes are: cardiac stem cells (CSCs), progenitors, precursors, and amplifying cells. CSCs are the most primitive, and all sequential classes are progressively more differentiated. Additionally, CSCs, progenitors, and precursors all express c-kit, MDR1, and Sca-1 antigens, while the amplifying cells fail to express any of the aforementioned cell surface antigens (73). Differences in the expression of CSC surface antigens have dramatic implication on the development of the cell, thus a thorough understanding of this concept must be attained in order to create the most beneficial cardiac stem cell therapy.

It has also been found that CSCs are negative for the expression of hematopoietic and endothelial antigens CD45, CD34, CD31, and KDR. CD45 and KDR surface markers are typically present on c-kit^{POS} cells that originate in the bone marrow and migrate to the heart following myocardial injury. Therefore, the absence of CD45 and KDR surface markers in a population of hCSCs indicates that these cells do in fact originate from stem cell niches within the myocardium (72,79). Upon activation, these cells will divide symmetrically, as well as asymmetrically ultimately giving rise to fully differentiated, lineage-negative cells. This evidence strongly supports the existence of a linear relationship between human cardiac stem cells and the formation of mature, fully differentiated myocytes (79). Refuted, therefore, is a mechanism of dedifferentiation of mature myocytes resulting in the formation of a pool of cardiac stem cells.

Side Population Cells

The existence of a resident pool of myocardial progenitors was first determined due to the presence of an ATP-binding cassette transporter protein which gave the stem cells the ability to expel Hoechst dye and other toxic compounds (85). The side population hematopoietic cells were among the first class of myocardial progenitors to be identified, as a result of their P-glycoprotein Abcg2 transporter activity, as well as the later discovered Mdr1 (77,79). It was also observed that the population of side population cells residing in the mouse model was noticeably depleted following infarction, indicating that the side population cells were being committed to the myocyte lineage (85).

Although the investigators failed to identify a cardiac stem cell throughout their studies, it should be noted that they effectively documented a myocardial stem cell response following ischemic injury to the heart, introducing the concept of resident cardiac stem cells and cardiac regeneration. It was later determined that the side population cells primarily generate vimentin-positive fibroblasts and calponin-positive smooth muscle cells in response to myocardial injury, and that only a small fraction of the side population cells contribute to the myocyte or endothelial cell lineages (86).

Side population cells comprise 2% of the cardiac cells in a mouse model. They are Sca1^{high}, c-kit^{low}, CD34^{low}, and CD45^{low}. The major determinant of the side population phenotype is Bcrp1, however, there exist Bcrp1 cells that are both positive and negative for CD31. Those cells that are Bcrp1 and CD31

positive are found within the intima of the vessel wall. Bcrp1 positive cells that are negative for CD31 are very different from those that are CD31^{NEG}, and also express CD29 and N-Cadherin at the interface between the myocytes and adjacent smooth muscle cells. These cells were found to exist in the perivascular regions well as the myocardial interstitium (77). The most recent data indicates that the only class of cardiac side population cells with a high cardiomyogenic potential is the Sca1^{POS} CD31^{NEG} subset. This unique subset of cardiac side population cells possess the ability to differentiate into fully functional adult myocytes (87).

Interestingly, the expression of Abcg2 that regulates the proliferation of cardiac side population cells has also been implicated in the proliferation of cancer cells. Therefore, as new chemotherapies that specifically target Abcg2 are introduced, we must be conscious of the significant potential of resultant cardiac toxicity as a side effect (87).

Epicardial Progenitors

The epicardium, also known as the visceral pericardium, has been identified as source of cardiac progenitor cells. The embryonic fetal epicardium hosts a variety of progenitor cell classes, providing the capacity to participate in a variety of previously unknown functions. Most relevant to cardiac regeneration, a population of proepicardial Tbx18-positive progenitor cells is thought to give rise to a substantial fraction of cardiomyocytes embryologically (88).

Importantly, a pool of c-kit^{POS} cells has been discovered in the epicardium of the human heart. Following ischemic insult, the c-kit^{POS} CD117-positive cells accumulate in the subepicardial space and later migrate across the subepicardial space or myocardium to the region of the heart that has been damaged and is likely undergoing regeneration (89). After migration, the c-kit^{POS} cells differentiate into myocyte precursors and vascular cells. Therefore, it is likely that these cells, originating from the postnatal epicardium, play a role in cardiac regeneration following injury (90).

Cardiospheres

A significant challenge in producing a clinically useful cardiac stem cell therapy is the expansion of progenitor cells retrieved from the human heart. An increasingly effective approach to this problem is the direct growth of endogenous cardiac progenitor cells from percutaneous endomyocardial biopsy specimens as spherical aggregates termed cardiospheres (91,92). These cardiospheres consist of several distinct layers. The core of the sphere consists of c-kit^{POS}, Ki67+ proliferative cells that have a cardiac progenitor immunophenotype, as supported by the expression of stem cell and cardiomyocyte-related antigens (92).

More superficial to the cardiosphere core are several layers of increasingly differentiated cells expressing cardiomyocyte proteins and connexin 43. It has been shown that the expression of connexin 43 promotes various cell fates

depending on the level of differentiation of the cell. Expression on undifferentiated progenitors favors their proliferation, whereas expression on cells that have already been committed to the myocyte lineage will promote electrical coupling with cells in its direct vicinity, and subsequent acquisition of functional competence. As these cells develop, they form gap junctions with less differentiated cells, thus the connexin 43 positive differentiated cells likely play a supportive role for the undifferentiated cells within the cardiosphere. Finally, the outermost layer of the cardiosphere is composed primarily of mesenchymal stromal cells (80).

Therefore, when cardiac progenitor cells are directly cultured from cardiac tissue, the formation of cardiospheres creates a unique, niche-like environment allowing for vast proliferation of cardiac progenitors within its core, as well as surrounding layers of early committed cells that may enhance the viability of cardiosphere-derived therapy to regenerate the myocardium and improve cardiac function following infarct (91,93).

Clinical Applications of Cardiac Regeneration

While the causes of heart failure are vast in origin, each and every one ultimately results in injury or death to the cardiomyocytes, impinging on their ability to function effectively. As the cardiomyocytes begin to lose functionally,

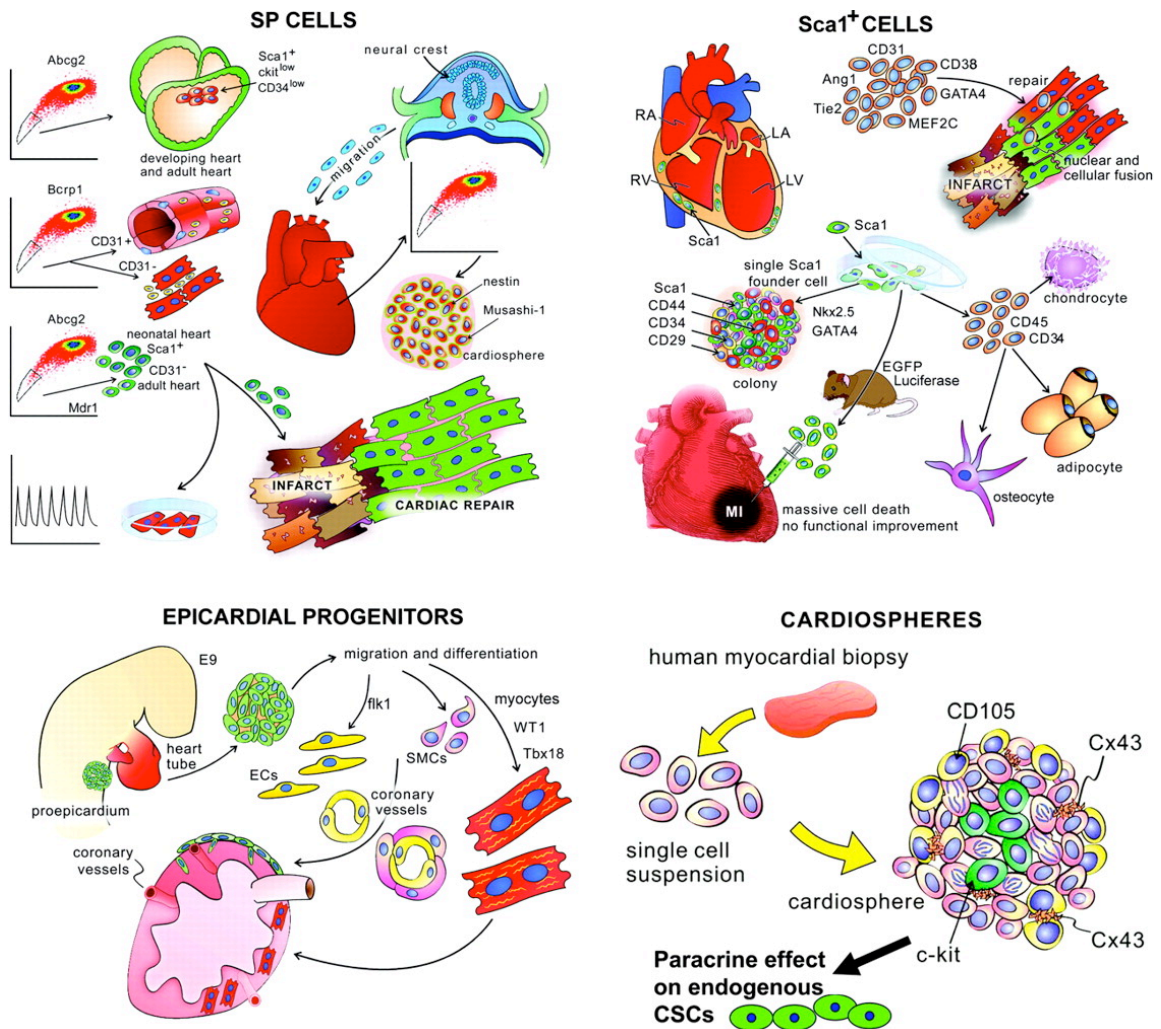


Figure 15. The classes of Cardiac stem cells and progenitor cells. Schematic representation of populations of cardiac-derived stem cells and progenitor cells. (80)

heart failure ensues on a more macro scale. This is a crucial piece of the puzzle, as all current treatment modalities for those in heart failure do not actually heal or restore the ability of the cardiomyocytes to function properly, with the exception of cardiac transplantation. Cardiac stem cell therapy would therefore be the first

therapy capable of transforming dead or deleteriously remodeled tissue into physiologically active, functioning myocardium allowing medical professionals to prevent or even reverse the pathophysiology of heart failure (94).

While there is little argument with respect to the potential that cardiac stem cell therapy may have on the field of medicine, creating a therapy that would be effective and safe clinically has proven much more challenging than originally thought. In addition to the various obstacles of creating an effective therapy, the discovery of so many different cell types that may serve as candidates for stem cell therapy expands the work load of scientists as they attempt evaluate which would prove most beneficial. Such a vast spectrum of cell types also points to the significant lack of mechanistic understanding of cell-based therapies at many levels (94). In fact, there have been very few studies that have done any sort of comparison between the various cell types to decipher which have the greatest potential for a cell based therapy, as to focus time and resources on those more qualified candidates (95).

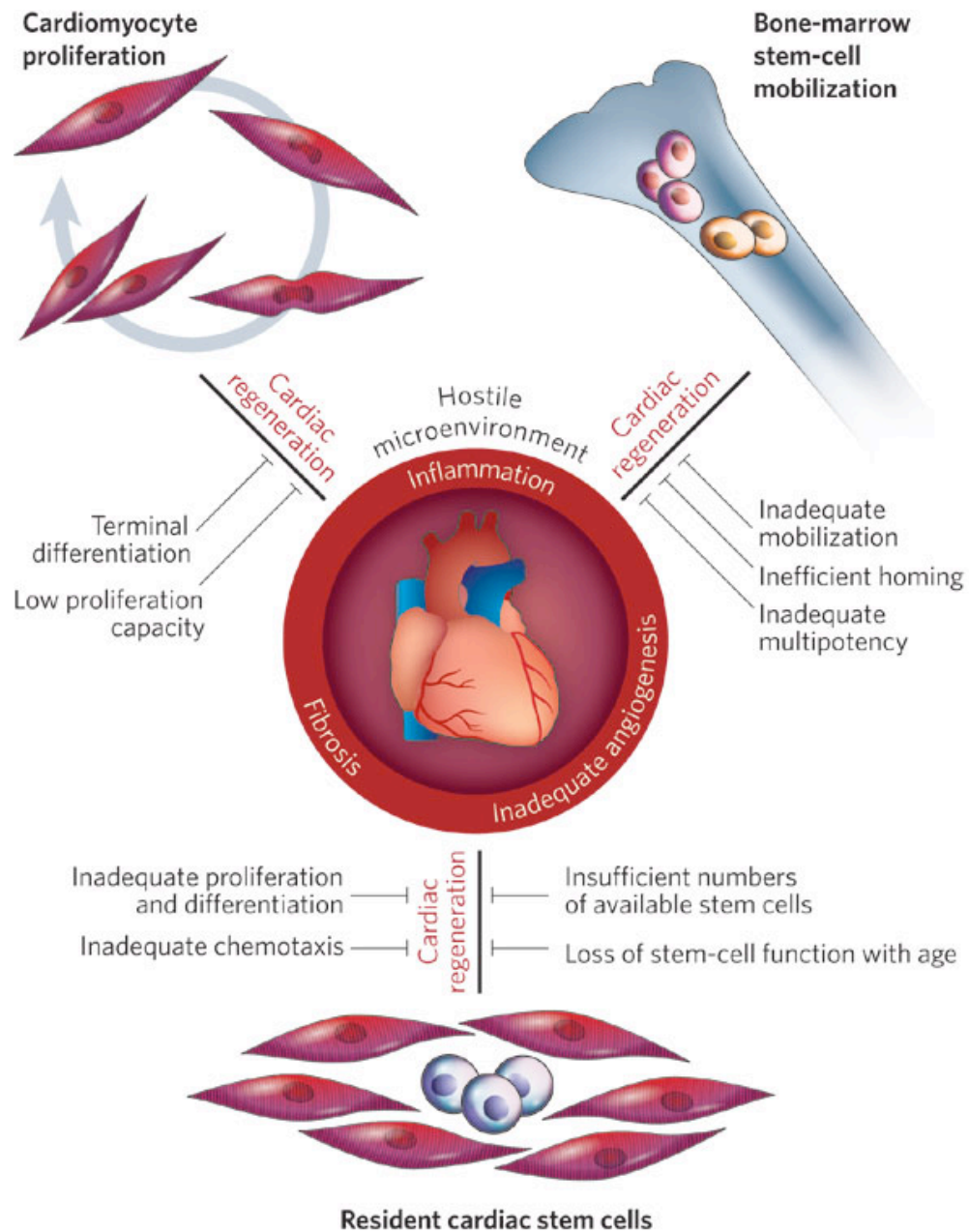


Figure 16: Depiction of the Various Mechanisms and Barriers to Stem Cell Therapy and Cardiac Regeneration (94)

The Current Status of a Cell-Based Therapy for Heart Failure

It has now been established that the human adult heart contains a population of cardiac stem cells that are self renewing and possess the capacity to differentiate into all three major cardiac cell lineages; myocytes, vascular smooth muscle cells, and endothelial cells (96). Over the last decade, research has shown that the transplantation of these cells into a failing heart of an animal model attenuates pathologic left ventricular remodeling and improves the efficiency and function of the left ventricle that had previously experienced a myocardial infarct (79). While these findings are undoubtedly encouraging, very little has been done with respect to regenerating a human heart.

In 2011, one of the first phase one human trials was undertaken, the SCIPIO (cardiac stem cells in patients with ischemic cardiomyopathy) study, evaluated the effect of intracoronary infusion of autologous cardiac stem cells in improving left ventricular systolic function and reducing the infarct size in patients with heart failure as the result of a myocardial infarction, and if further investigation in the form of a phase two trial is warranted for this therapy (97). The results of the SCIPIO study suggest that in fact cardiac stem cells can be reproducibly isolated and expanded from approximately one gram of myocardial tissue that can be harvested during cardiac surgery. They found that patients who received infusions of cardiac stem cells showed marked improvement in left ventricle systolic function at four months post-infusion compared to patients who did not receive the cardiac stem cells, and an even greater improvement in LV

systolic function one year following infusion of CSCs. After one year of follow up, it was observed that the patients who received stem cell therapy had not only a significantly improved LV systolic function, but also an increased functional capacity, improved quality of life, and reduction in the size of the scar that resulted from the myocardial infarct (97). Ultimately, the SCIPIO study provided strong rationale for further studies into the use of cardiac stem cell therapies for patients in severe heart failure with a poor prognosis.

To date, there have been several studies that have examined the effects of cardiac stem cells on improving LV systolic function and improving outcomes in patients with severe heart failure. These studies have had very small study populations and used a variety of stem cell types delivered either intracoronary transendocardial, as well as differing with respect to number and volume of stem cells delivered, timing and processing, and whether or not adjunctive therapy was used (98). Some of these studies have shown significant improvements in LV systolic function and a reversal of pathologic remodeling, while others observed no significant difference between those who received no therapy at all. Interestingly, a few studies have even shown that evidence that the control group, those who did not receive stem cell therapy, exhibited slight improvements in cardiac function over time (99,100).

While these studies did show that the use of cardiac stem cell therapy is safe and not likely to result in adverse outcomes directly related to CSC therapy, they have been unable to consistently show that the therapy has the ability to

regenerate a failing ventricle. However, they have provided valuable insight into the many challenges that stand in the way of a truly effective CSC therapy, and brought to our attention the most significant hurdles that must be overcome to attain the ultimate long-term goal of true myocardial regeneration. The two most important barriers at present are providing the right environment into which the stem cells can be infused, and the specific nature of the cells utilized. Additionally, and also noteworthy barriers that current research is aiming to address are improving the survival of the injected stem cells, increasing their persistence at the site of injection, and expanding their capacity to proliferate once introduced into the tissue (98).

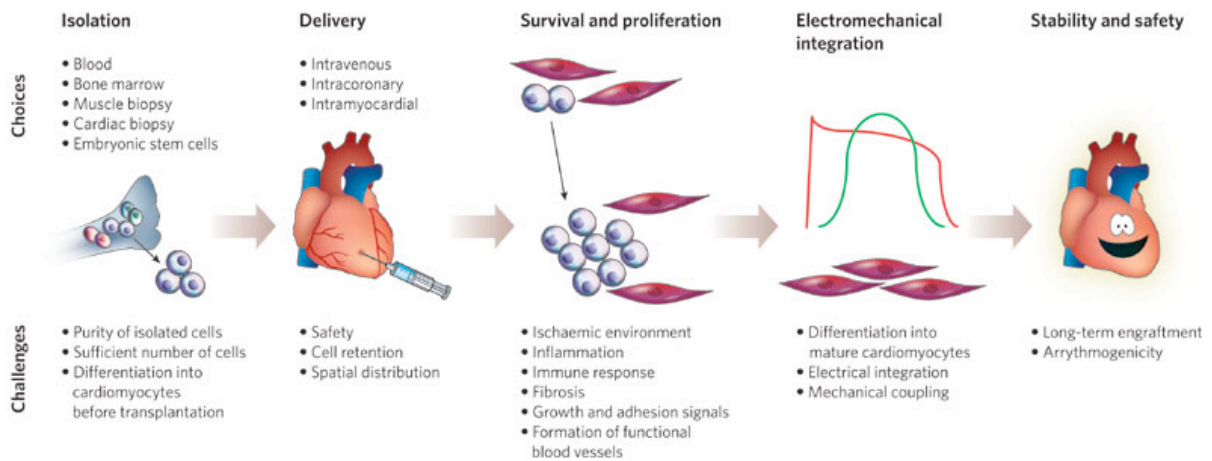


Figure 17: The Challenges of a Clinically Acceptable Stem Cell Therapy (94)

Conclusions and the Future of Treating Heart Failure

It is clear that heart failure is a significant public health concern with an ever-increasing prevalence in most developed countries worldwide. Such an increase is thought to be primarily the result of three things. First, advances in medical technology over the last few decades have allowed a far greater number of patients to survive acute coronary artery disease, myocardial infarctions, and acute decompensated heart failure. As these cardiac events often serve as the initial insult in the heart failure sequelae, these surviving patients now go on to develop chronic heart failure. Second, improvements in the treatment of those with chronic heart failure have increased the time one can live with the chronic disease. Finally, the U.S. population as a whole is not only exponentially growing, but also aging. Because advanced age is significantly associated with heart failure, the increasing aging population inevitably results in an increase in the number of people being diagnosed with HF (5). In addition, the management and treatment of heart failure costs the U.S. health care system more than 30 billion dollars a year (3), adding immense financial strain to a health care system significantly struggling to control costs. It is therefore critical that much attention be given to this growing epidemic at present and well into the future.

Because there is currently no treatment for reversing the pathologic remodeling of heart failure, once a patient develops HF, it is only a matter of time before they progress to late stage failure. For these patients, the best available treatment option is cardiac transplantation where they receive a healthy donor

heart. Unfortunately, the number of patients on the list for a donor organ far outweighs the number of hearts available for transplant. Mechanical Circulatory Support is the next best option for those that are not able to undergo transplantation. Specifically, ventricular assist devices are recommended for these patients, although they are not without significant risk (14). According to the INTERMACS database, the number of assist devices implanted has increased from 352 in 2007 to 2,217 in 2012. As the number of assist device implantations continues to rise, so will the number of patients with adverse events. It is therefore critical that much attention be allocated to treating or reducing the spectrum of adverse events that accompany current ventricular assist devices.

The most current ventricular assist devices are a significant improvement from their predecessors. Not only can they produce a significant volume output allowing them to significantly alleviate volume and pressure overload in the ventricles, but they have also reduced the likelihood of adverse outcomes as a direct result of the VAD (16). The biggest obstacle that accompanies current VAD technology is their non-biological nature. Not only is mechanical failure a significant risk with many VADs, but also the pump aspect of the device has a high propensity to form thrombi. It is crucial that future models of VADs reduce the risk of thrombus formation and infection, as well as increasing the duration that they can provide circulatory support.

No matter how advanced ventricular assist devices become, they will never possess the ability to regenerate the hearts ability to effectively function on its own. They also will always have a risk of adverse events because they will always be foreign to normal physiology. The only treatment option with the potential to regenerate the native myocardium, excluding cardiac transplantation, is cardiac stem cell therapy. While there will be a place for highly efficient assist devices in the practice of medicine, cardiac stem cell therapy is the only treatment modality with the potential to truly solve the heart failure epidemic.

The future of cardiac stem cell therapy and its ability to provide myocardial regeneration remains bright. However, there is still much work to be done before an effective therapy will be available for clinical use. Over the next several years, it is crucial that researchers determine the specific environmental characteristics that will provide the stem cells the greatest probability to persist within the intended tissue and allow them to effectively proliferate. It is likely that these modifications to the cell environment will be aimed at reducing the expression of inflammatory cytokines that are released as a result of injury to the myocardium during the infarct. A reduction in the expression of these inflammatory cytokines, such as tumor necrosis factor (TNF- α), interleukins (ILs), matrix metalloproteinases, and transforming growth factor- β (TGF- β), will reduce the pro-apoptotic influence on the infused stem cells, thus improving their survival (101). Other proposed methods of improving the survival of cardiac stem cells

are to precondition the cells prior to injection, or to genetically modify the cells to ensure improved survival (98).

More important still is the question of which type of stem cell or progenitor cell is the best candidate for a clinical therapy (95). In order to make this distinction, we must first attain a thorough understanding of the specific mechanisms by which each type of stem or progenitor cell influences the physiology of the myocardium (102). This will likely prove to be a pivotal piece to the stem cell therapy puzzle, because there is a strong possibility that different types of cardiac pathologies will require different cell types. By further expanding our knowledge of the complexities that underlie cardiac regeneration, we will move toward the possibility of creating a therapy that can be infused into a diseased heart, triggering a complete reversal of pathologic remodeling while returning the diseased heart to a healthy functioning state.

To take stem cell technology and regeneration one step further is to consider the possibility of creating or growing an entire organ, such as a heart, in vitro, from the patient's own cell lines. This organ could then be transplanted into patients who have suffered catastrophic injury to their heart, or who are so severely diseased that the "traditional" stem cell therapy would likely not work quickly enough. Because the lab grown tissue is composed of the patient's own cells, intense immunosuppressant therapy would no longer be necessary. As the population continues to grow, the number of people with heart failure is likely rise to an extraordinary level. With the number of donor hearts available for

transplantation ever dwindling, the ability to grow a complete organ in the lab that can then be transplanted into the severely ill patient would undoubtedly save millions of lives, impacting billions of people worldwide.

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