Alternative Therapies for Orthotopic Heart Transplantation

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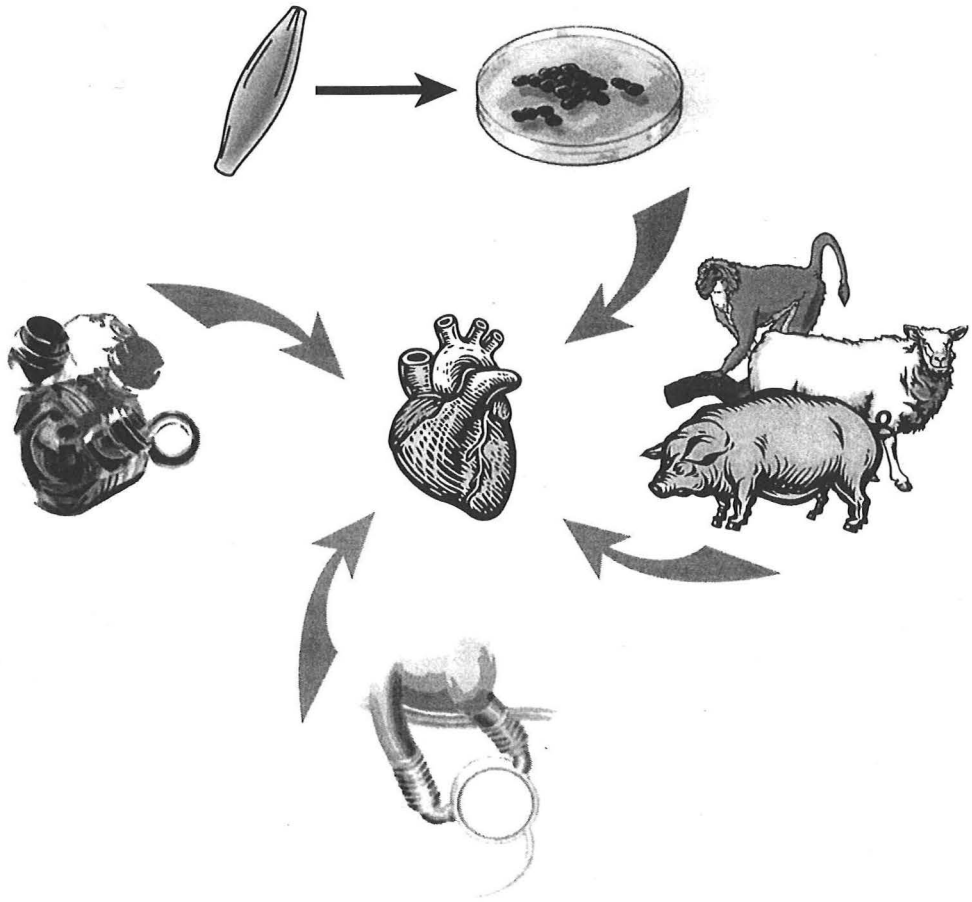

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INTERESTS:

Congestive Heart Failure/Cardiac Transplantation
Basic science mechanisms of stem cell biology & oxygen metabolism

Congestive heart failure (CHF)

Therapeutic strategies for heart failure have evolved tremendously over the past several hundred years. Treatment of congestive heart failure (CHF) or what was referred to as "dropsy" was aimed initially at restoring a balance of fundamental elements and humors. In 1683, Thomas Sydenham recommended bleeding, purges, blistering, garlic and wine. Additional treatments were attempted and abandoned after unrewarding anecdotal experiences (i.e. death). Progress regarding the treatment of heart failure was evident with the introduction of amyl nitrate, mercurial diuretics, digitalis glycosides and bed rest in the early 20th century.

Medical therapy for heart failure in the 1960's included digitalis, thiazide diuretics (introduced in 1962) and furosemide (introduced in 1965). The utilization of vasodilators for heart failure were implemented in the 1970's (nitroprusside in 1974 and hydralazine in 1977) and the first large, randomized, clinical trial for heart failure was not completed until 1986 (V-HeFT I). Since then, the design and completion of a number of large, randomized, placebo-controlled clinical trials have established angiotensin-converting enzyme inhibitors and β-adrenergic receptor antagonists as the cornerstones of therapy. The following table is provided as a summary of these trials.

Table 1: Clinical trials for heart failure management

CHF Trials	Treatment	NYHA Class	# of Pts	Outcome	Reference
ACE-I & ARB					
AIRE	Ramipril vs placebo in CHF post-MI	Not available	2006	Improved survival with ramipril	1
ATLAS	Low dose vs high dose lisinopril	II/III/IV	3164	↓ combined end-point (mortality & hospitalization) with high dose lisinopril	2
Captopril Multicenter	Captopril vs placebo	II/III	92	Captopril superior to placebo for exercise tolerance	3
CONSENSUS-1	Enalapril vs placebo	IV	253	↓ mortality and morbidity with enalapril	4
ELITE	Losartan vs captopril	II/III/IV	722	↓ mortality and hospitalization rate with losartan	5
ELITE-II	Losartan vs captopril	II/III/IV	3152	Losartan equal to captopril for mortality but better tolerated	6
Imidapril in CHF	Imidapril (high & low dose) vs placebo	II/III	244	↑ excerise capacity and ↓ ANP/BNP levels with high dose imidapril	7
Irbesartan in CHF	Irbesartan at various doses	II/III/IV	218	Sustained hemodynamic benefit and ↓ worsening of CHF at high dose of irbesartan	8
NETWORK	Enalapril at various doses	II/III/IV	1532	Similar clinical outcomes between low dose and high dose enalapril	9
RESOLVD	Candesartan vs enalapril vs combination	II/III/IV	768	Combination treatment with ↓ LV dilation but no change in mortality or morbidity	10
RESOLVD (Metoprolol Study)	Metoprolol CR vs placebo with ACEI and/or ARB	II/III/IV	426	↑ LV function and ↓ mortality with metoprolol CR in all groups	11
SAVE	Capotpril vs placebo	I	2231	↓ mortality and morbidity with captopril in pts with asymptomatic LV dysfunction post-MI	12
SOLVD-T	Enalapril vs placebo	II/III	2569	↓ mortality and morbidity with enalapril	13
SOLVD-P	Enalapril vs placebo	I/II	4228	Enalapril delayed Sx's of CHF	14
STRETCH	Candesartan vs placebo	II/III	844	↑ exercise capacity and ↓ morbidity with candesartan	15
Aldactone Receptor Antagonist					
RALES	Spironolactone vs placebo	III/IV	822	↓ mortality and morbidity with spironolactone	16

Anti-Arrhthymic Drugs					
Amiodarone in CHF & PVCs	Amiodarone vs placebo	Not available	674	↓ mortality and hospitalization rate with amiodarone in non-ischemic cardiomyopathy	17
Diamond-CHF	Dofetilide vs placebo	III/IV	1518	No change in mortality and ↓ hospitalization with dofetilide; Also better at converting AF and maintaining sinus	18
GESICA	Amiodarone vs placebo	II/III/IV	516	↓ mortality and morbidity with amiodarone	19
β-Blockers					
ANZ Trial	Carvedilol vs placebo	II/III	415	↓ morbidity and mortality with carvedilol	20
BEST	Bucindolol vs placebo	III/IV	2708	Similar mortality rates with both bucindolol and placebo	21
CAPRICORN	Carvedilol vs placebo	Not available	1959	↓ all cause mortality with carvedilol in post-MI pts with LV dysfunction	22
COPERNICUS	Carvedilol vs placebo	III/IV	2289	↓ morbidity and mortality with carvedilol	23
CIBIS-1	Bisoprolol vs placebo	III/IV	641	↓ morbidity with bisoprolol but no change on mortality	24
CIBIS-II	Bisoprolol vs placebo	III/IV	2647	↓ mortality and hospitalization rate with bisoprolol	25
Carvedilol CHF Study	Carvedilol vs placebo	II/III	1094	↓ morbidity and mortality with carvedilol	26
MDC	Metoprolol vs placebo	II/III	383	↓ morbidity with metoprolol but no difference in mortality	27
MERIT-HF	Metoprolol CR/XL vs placebo	II/III/IV	3991	↓ morbidity with metoprolol CR/XL	28
MEXIS	Metoprolol vs xamoterol	II/III	210	↑ LV dysfunction with xamoterol	29
MOCHA	Carvedilol vs placebo	II/III	345	↓ mortality and hospitalization rate with carvedilol	30
PRECISE	Carvedilol vs placebo	II/III	278	↓ morbidity with carvedilol	31
US Carvedilol CHF Study	Carvedilol vs placebo	II	366	↓ morbidity and mortality with carvedilol in mild CHF	32
Positive Inotropic Drugs					
DIG Trial	Digoxin vs placebo	I/II/III	7788	↓ morbidity but no change in mortality with digoxin	33
Milrinone-Digoxin Trial	Milrinone vs digoxin vs combination vs placebo	II/III	230	↑ exercise capacity and clinical status with digoxin and ↑ ventricular arrhythmias with milrinone	34
PICO	Pimobendan vs placebo	II/III	317	↑ exercise capacity but also ↑ mortality with pimobendan	35
PRIME-II	Ibopamine vs placebo	III/IV	1906	↑ mortality with ibopamine	36
PROMISE	Milrinone vs placebo	III/IV	1088	↑ mortality and morbidity with milrinone	37
PROVED	Digoxin vs placebo	II/III	88	Worsening CHF with digoxin withdrawal	38
RADIANCE	Digoxin vs placebo	II/III	178	Worsening CHF with digoxin withdrawal	39
VEST	Vesnarinone vs placebo	III/IV	3833	Dose dependent ↑ mortality with vesnarinone	40
Xamoterol Trial	Xamoterol vs placebo	III/IV	516	↑ mortality with xamoterol	41
Vasodilators					
DiDi	Diltiazem vs placebo	Not available	186	↓ morbidity with diltiazem but no change in mortality	42
MDPT	Diltiazem vs placebo	Not available	623	New onset or worsening CHF with diltiazem in post-MI pts	43
PRAISE	Amlodipine vs placebo	III/IV	1153	Equal mortality and morbidity rates with amlodipine and placebo	44
REFLECT	Flosequinan vs placebo	II/III	193	↓ morbidity with flosequinan + ACEI	45
FACET	Flosequinan + ACEI vs ACEI+ placebo	Not Available	322	↓ morbidity with flosequinan + ACEI	46
V-HeFT-I	H+I vs prazosin vs placebo	II/III	642	↓ mortality with H+I	47
V-HeFT-II	H+I vs enalapril	II/III	804	Enalapril superior to H+I for survival	48
V-HeFT-III	Felodipine + enalapril vs enalapril + placebo	Not available	450	No difference in mortality between treatment groups	49

Therefore, the goals for effective management of cardiomyopathy include the treatment to prevent or delay the progression of left ventricular dysfunction (i.e. congestive heart failure) and are focussed on the relief of symptoms as well as the cellular and molecular modulation of the cardiomyocyte (remodeling). The current treatment strategy (2001) includes the following pharmacological agents (50-53):

- ACE inhibitors (enalopril, captopril, etc.)
- β-blocker therapy (carvedilol, metoprolol)
- Digoxin
- Diuretics (lasix and spironolactone)
- Vasodilator agents (hydralazine and nitrates)

Establishing a hierarchy of advanced heart failure

Heart failure is a deadly disease and accounts for more than 250,000 deaths in the U.S. each year. In advanced heart failure, the estimated survival is frequently used to influence a specific therapeutic decision for a patient. As in many other disease processes this estimation is difficult as no single observation or variable is precise in predicting mortality. Therefore, one may use a combination of factors to determine the probability of survival.

Utilizing the New York Heart Association (NYHA) functional classification, patients with left ventricular dysfunction are assigned to one of the groups based on their symptoms. The classification of patients by their symptomatology has prognostic value (i.e. Class I > Class IV survival).

CLASS I	No limitation, ordinary physical activity does not produce symptoms of heart failure.
CLASS II	Slight limitation of physical activity with no symptoms at rest; symptoms provoked by ordinary physical activity.
CLASS III	Marked limitation of physical activity with no symptoms at rest; symptoms provoked by less-than-ordinary physical activity.
CLASS IV	Inability to carry on any physical activity without symptoms. Symptoms present at rest.

Left ventricular function. Left ventricular (LV) ejection fraction is a prognostic factor for patient populations such as the survivors of myocardial infarction, but is less useful once heart failure has progressed to NYHA Class III or IV symptoms. For example, an ejection fraction over 30% is associated with better survival, but once below 30%, there is little additional prognostic information. Some studies have shown that a LV ejection fraction below 15% is associated with a particularly poor outcome. Moreover, the degree of LV dilatation, using echocardiography, has been reported to be a consistent predictor of poor outcome (Class IV patients having a LV diastolic dimension > 72 mm had a 36% 2-year survival compared to a 58% 2-year survival in Class IV patients with a LVDD < 72 mm) (54,55).

Functional capacity. Peak oxygen consumption measured during maximal exercise testing provides an objective assessment of functional capacity in advanced heart failure. Sedentary individuals have a maximal oxygen consumption ranging between 25-35 ml/kg/min, moderately active individuals have a VO2 ranging between 35-45 ml/kg/min and trained athletes greater than 50 ml/kg/min. In contrast, patients with severe CHF who have a measured maximal oxygen consumption less than 14 ml/kg/min (and < 50% predicted for gender, age and height) identifies patients with a 1-year survival less than 50% (reviewed in 55,56).

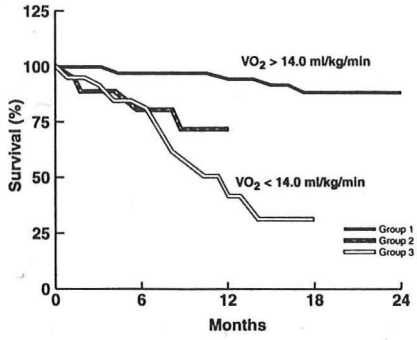

Fig. 1. Survival curves for patients with cardiomyopathy. Candidates (n = 122) were assigned to one of three groups (group 1 too well for transplant; group 2 accepted for transplantation and group 3 rejected for cardiac transplantation based on non-cardiac reasons [age, IDDM, or psychosocial reasons]). All candidates were comparable with respect to age, sex and ejection fraction (< 20%). Patients (group 1) with preserved exercise capacity (VO2 > 14 ml/kg/min) had a one and two year survival rate of 94% and 84%, respectively. Patients accepted (group 2 but prior to transplantation) and rejected (group 3) for transplant had similar survival rates and a peak oxygen consumption < 14 ml/kg/min (56).

Hemodynamic assessment. Following a thorough history and physical examination, one can usually anticipate the hemodynamic profile of the patient. In advanced heart failure the physical examination may often be misleading. Typically, the physical exam findings consistent with volume overload secondary to CHF includes: a diffuse and laterally displaced precordial apical impulse, an elevated jugular venous pulse, an S_3 heart sound (decreased myocardial compliance), pulmonary rales [observed with a pulmonary capillary wedge pressure (PCWP) > 24 mm Hg] and peripheral edema (54,55). While these physical exam findings are helpful when they are present they are lacking in sensitivity as patients with chronic decompensated heart failure may lack the physical exam findings of pulmonary rales (even when the pulmonary capillary wedge pressure is greater than 35 mm Hg), peripheral edema or an elevated jugular venous pressure (53). However, a narrow pulse pressure has been found to correlate with a low cardiac index. A predictor of outcome in the patient with advanced heart failure is the response to tailored therapy (55). Tailored therapy for advanced heart failure utilizes hemodynamic targets as determined by right heart catheterization to optimize conventional medical therapy. It is accomplished by using parenteral diuretics and vasodilators such as nitroprusside to achieve hemodynamic goals, followed by the conversion to high dose oral vasodilators in an effort to maintain those hemodynamic goals (see Table 2). Stevenson et al. reported from a group of patients referred for cardiac transplantation (n = 152; EF < 20%), those patients with a final PCWP > 16 mm Hg despite tailored therapy had a 1-year survival rate of only 38%, compared with 83% when the PCWP could be reduced to less than 16 mm Hg (55,57).

Table 2: Hemodynamic goals for tailored therapy

- Pulmonary capillary wedge pressure $\leq$ 16 mm Hg
- Systemic vascular resistance $\leq$ 1200 dynes-sec-cm^{-5}
- Right atrial pressure $\leq$ 8 mm Hg
- Systolic blood pressure $\geq$ 80 mm Hg

Neurohormonal response to heart failure. In the setting of CHF, decreased serum sodium reflects increased serum renin-angiotensin II, and predicts mortality principally from heart failure rather than sudden death. For example, patients with Class IV symptoms and depressed left ventricular function (EF < 25%) with a serum sodium greater than 136 mEq/L have a 1-year survival rate of 70% compared to those with a serum sodium < 130 mEq/L which have a 1-year survival rate of 40% (53-55).

Patients with advanced heart failure that is refractory to pharmacological therapy and lack other co-morbidities (i.e. active infection, malignancy, obesity, intrinsic renal, hepatic or pulmonary disease, peripheral vascular disease or diabetes mellitus with secondary complications) are referred for orthotopic heart transplantation (58,59).

Cardiac Transplantation: a historical perspective

Many of the principles of organ transplantation were introduced by the French surgeon, Dr. Alexis Carrel. Together with his colleague, Dr. Guthrie, Carrel transplanted arteries, veins, kidneys, thyroid glands, limbs, hearts and heart-lungs in various animal models. In 1905, Guthrie and Carrel demonstrated the feasibility of heterotopic cardiac transplantation by transplanting a neonatal canine heart into the neck of an adult dog. As a result of his experimental accomplishments, Alexis Carrel was awarded the Nobel Prize in Physiology and Medicine in 1912 (60).

Vladimir Demikhov, a Russian surgeon, developed the first artificial heart and implanted it into dogs. In 1946, Demikhov performed the first intrathoracic heterotopic heart transplant in dogs, which lived up to 32 days with the second heart. Twenty years later (1962) he reported that a heterotopic heart transplant undertaken in the dog successfully lived for 141 days (without immunosuppression therapy). In 1989, the Society for Heart Transplantation awarded him the first Pioneer Award for the advancement of intrathoracic transplantation (60).

Cardiac preservation techniques were advanced in the early 1950's with the introduction of hypothermia as well as the perfusion of donor hearts with a 4°C heparin and potassium citrate solution (i.e. cardioplegia). Modifications of surgical techniques included the introduction of cardiopulmonary bypass and the preservation of the recipient cuff of the left atrium thereby eliminating the need to individually anastomose the pulmonary veins. During the 1960's and 1970's several academic centers provided monumental advances to the field of transplant cardiology and included Stanford University (Drs. Lower and Shumway), University of Utah, Medical College of Virginia (Dr. Lower) and University of Minnesota (Dr. C. Walton Lillehei).

Among these advancements included the introduction of immunosuppressive therapy. Drs. Lower and Shumway at Stanford University utilized azathioprine and methylprednisolone to achieve long-term function (250 days) of a heterotopic heart transplant in the dog (60). The surgical and medical advancements paved the way for the first human heart transplant to be performed.

December 3, 1967. Christiaan Neethling (Chris) Barnard was born in South Africa in 1922. Following surgical training in Cape Town and Minneapolis (while training under Drs. Richard Varco and C. Walton Lillehei in Owen Wangensteen's Dept. of Surgery at the University of Minnesota), he established a successful open heart surgery program at Groote Schuur Hospital, the academic medical center at the University of Cape Town, South Africa. In preparation for the first heart transplant, Dr. Barnard spent sabbaticals working with Drs. David Hume (a pioneering kidney transplant surgeon), Thomas Starzl (University of Colorado) and Richard Lower (Medical College of Virginia) to learn the fundamentals of immunosuppressive therapy (61). Barnard performed the world's first orthotopic heart transplant on December 3, 1967 for his patient, Louis Washkansky, who lived eighteen days (expired as a result of a nosocomial pneumonia). A second orthotopic heart transplant (Philip Blaiberg) was performed by Dr. Barnard on January 2, 1968. Philip Blaiberg was the first patient to receive antilymphocyte serum, was discharged from the hospital and lived for 19 months following his transplant. Barnard went on to perform a total of ten orthotopic heart transplants, a heart and double lung transplant and forty-nine heterotopic heart transplants (60,61). The initial enthusiasm for cardiac transplantation soon waned as a result of prohibitive perioperative mortality and poor survival rates (1-year survival rate of 22% in 1968). Except for occasional clinical efforts at Stanford University, the field of cardiac transplantation remained dormant until the early 1980s when cyclosporine was introduced (1981). The advent of cyclosporine signaled the arrival of specific immunotherapy, and with it came improved survival rates, renewed interest in transplantation and an increase in patient recruitment.

Table 3: Advances in cardiothoracic surgery

Surgeon	Year	Procedure
Carrel and Guthrie	1905	Organ transplantation in dog
Mann	1933	Heterotropic heart transplantation in dog
Demikhov	1937	First artificial heart transplantation in dog
Demikhov	1939-1946	Intrathoracic heterotopic heart and heart-lung transplantation in dog
Demikhov	1947	First lung transplantation in dog
Neptune	1953	Orthotopic heart-lung transplantation using hypothermia
	1954	Development of first cardiopulmonary-bypass machine
Webb and Howard	1957	Orthotopic heart transplantation using cardioplegia and cardiopulmonary bypass
Lower and Shumway	1960	Orthotopic heart transplantation using recipient atrial cuffs
Hardy	1964	First cardiac xenotransplantation
Barnard	1967	First human-human heart transplantation
Shumway	1968-1981	Orthotopic heart transplantation with immunosuppressive therapy and endomyocardial biopsy
Cooley	1969	First human total artificial heart for bridge transplantation
DeVries	1982	First premanent total artificial heart
Bailey	1984	Neonatal xenotransplantation

Current status of orthotopic heart transplantation. Orthotopic heart transplantation is no longer considered an experimental or heroic procedure but rather this procedure now provides the only definitive solution for the irreversible loss of cardiomyocytes in the failing heart. Current one-year survival outcomes in most established programs are approximately 85% and five and ten-year survivals are approximately 75% and 60%, respectively (62,63).

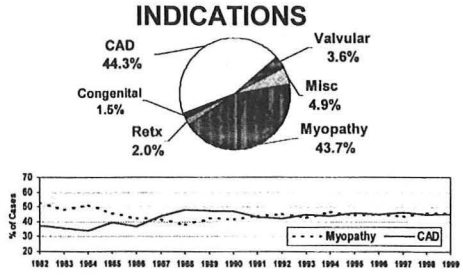

Fig. 2. Diagnosis of patients listed for cardiac transplantation. Note that coronary artery disease and myopathy constitute the majority of patients. CAD, coronary artery disease; Misc, miscellaneous; Retx, retransplantation. Data obtained from the Registry of the International Society for Heart and Lung Transplantation (62).

Donor availability. Inadequate organ availability remains the major factor that limits the number of cardiac transplantation procedures performed each year. For example, 2,292 heart transplant procedures were performed in the U.S. in 1997 but estimates suggest that more than 50,000 patients would benefit from the procedure each year (66). Despite a gradual increase in both donor awareness and willingness to donate one's or a relative's organs, families frequently do not consider organ donation or refuse to donate the heart due to inadequate knowledge of transplantation (54,62,63). It is estimated that only 10-20% of brain-dead patients with suitable hearts become actual donors. The failure of medical professionals to pursue organ donation with a brain-dead patient's next of kin remains a major obstacle to transplantation. Donor selection criteria are based on age and an absence of infection, malignancy or cardiac dysfunction (see Tables 4 and 5) (58). Donor-recipient matching is further based on ABO blood group compatibility, donor-recipient weight match and the severity of recipient illness (58,62).

CTRD: Risk Stratification, 1990 – 1999, n=7,283

Low risk patients (n=1,156)	High risk patients (n=462)
All of the following conditions must be true	At least one of the following conditions must be true
recipient age: < 65 years	donor age: > 55 years
donor age: < 40 years	ischemic time > 360 minutes
ischemic time: < 240 minutes	on ventilator at time of transplant
other risk factors*: 0	history of cocaine use
(year of transplant ≥ 1994)	other risk factors*: ≥ 4

Average risk patients (n=5,665)
Everyone else

*Other risk factors

History of insulin dependent diabetes	congenital etiology
history of alcohol abuse	history of cigarette smoking (within 6 mos)
history of peripheral vascular disease	abnormal septal wall motion (donor)
history of pulmonary disease	gender mismatch

CTRD: Risk Stratification, 1990 – 1999, n=7,283

	Percent Survival		
Years Post Transplant	Low Risk (n=1,156)	Average Risk (n=5,665)	High Risk (n=462)
1/12	95%	93%	85%
1/2	91%	88%	76%
1	89%	85%	72%
3	84%	78%	65%
6	80%	70%	55%
10	---	55%	44%

Table 4: Donor-Recipient Risk Stratification, 1990-1999. Data obtained from the CTRD (Cardiac Transplant Research Database; n = 7,283).

Table 5: Suggested Criteria for cardiac donors and guidelines for recipient matching.

- Age less than 40 years (may be extended by certain centers under certain circumstances)
- Negative serologies for HIV, hepatitis C and hepatitis B
- No active severe infection or malignancy with possibility of metastases
- No evidence of significant cardiac disease or trauma
- Very low probability of coronary artery disease (coronary angiogram may be required)
- Normal or acceptable ventricular function after intravascular volume normalization
- Blood type (ABO) compatibility with recipient
- Donor body weight usually between 80% and 120% of recipient's body weight
- If required, negative prospective cytotoxic T cell crossmatch. A retrospective crossmatch is performed in most centers
- Anticipated allograft ischemic time less than 4-5 hours.

The donor and recipient criteria are important predictors of outcome (see Tables 4,5) (64) and the utilization of these criteria has resulted in an increase in patient survival (Fig. 3).

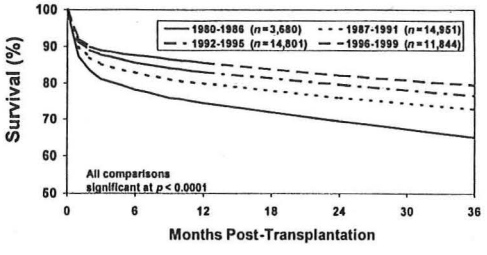

Fig. 3. Adult heart transplant survival by era. Data obtained from the Registry of the International Society for Heart and Lung Transplantation (62).

Post-transplantation course

Immunosuppression. Insufficient immunosuppression may result in rejection that threatens allograft and patient survival whereas excessive immunosuppression increases the risk of infection and malignancy (62,63). The variability of patients' responses, due to individual characteristics, multiple environmental influences, drug-induced complications, drug interactions, and patient compliance requires vigilant monitoring and adjustments of the immunosuppressive regimen.

Corticosteroids. Steroids suppress leukocyte activity by inhibiting the release of interleukin-1. Therefore, the recruitment of leukocytes to an area of foreign antigen is decreased and is effective in preventing rejection. Most centers use high-dose methylprednisolone intravenously in the immediate perioperative period followed by a tapered schedule of oral prednisone (62,63). Typically, transplant patients with two or more episodes of acute rejection receive lifetime steroid therapy.

Cyclosporine. Introduced in 1981, cyclosporine is a cyclic endecapeptide that inhibits immune responses by inhibiting the production and release of interleukin-1 and -2 and the activation of T-lymphocytes. Cyclosporine can cause a number of adverse effects including nephrotoxicity, hypertension, paresthesia, gingival hypertrophy, malignancy and others (58,63).

Mycophenolate mofetil. Recently, mycophenolate has replaced azathioprine in the standard immunosuppression regimen. Mycophenolate selectively impairs T-cell proliferation by the noncompetitive inhibition of the enzyme required for purine biosynthesis and does not affect B-lymphocytes. In animal models it has been associated with less vasculopathy.

Azathioprine. One of the original immunosuppressive agents used in transplant biology which is a competitive inhibitor of purine biosythesis and a potent but nonspecific immunosuppressive agent that inhibits lymphocyte proliferation.

Additional immunosuppressive agents that are less commonly used include Tacrolimus (FK-506) which suppresses T-lymphocytes, rappimune, methotrexate, antithymocyte globulin (ATG) and OKT3 (58,63).

Complications related to immunosuppression. Transplant recipients have an increased risk of developing skin cancer and lymphoproliferative disorders. Malignancy may be one of the greatest risks for long-term survival of the transplant patient. Skin cancer is the most common post-transplant malignancy and requires dermatological evaluation and protection from solar injury. Post-transplantation lymphoproliferative disorders (PTLDs) represent a spectrum of abnormal B-lymphocyte growths that develop after organ transplantation and immunosuppression (58,63,64). PTLDs are often associated with Epstein-Barr Virus infection. Ranging in severity from atypical lymphoid hyperplasia associated with a mononucleosis-like syndrome to malignant lymphoma with metastatic potential, PTLDs have been shown to respond to reductions in immunosuppression and systemic chemotherapy. Lymphoproliferative disorders require constant surveillance and aggressive intervention.

Infections are one of the most serious complications for the transplant recipient and are the most common cause of death early following the transplant (58,63-65). Therefore, antibiotic prophylaxis is administered to all cardiac transplant recipients. Antifungal (nystatin) and antiprotozoal (i.e. bactrim) prophylaxis are administered to each patient. Common viral pathogens, in the immunosuppressed patient population, include Herpes Simplex Virus, Epstein-Barr Virus, Varicella Zoster Virus and Cytomegalovirus.

Allograft rejection. Three types of rejection are observed. Hyperacute rejections are life threatening antigen-antibody reactions (i.e. ABO incompatibility of donor/recipient) and occurs immediately (while in the operating room). The treatment of a hyperacute rejection is retransplantation (62,63).

Acute rejections occur most frequently in the first three to six months following transplant although they may occur at any time. Acute rejection is diagnosed and monitored by endomyocardial biopsy and/or the new onset of clinical symptoms or

cardiac dysfunction. Treatment of the acute rejection episode includes an increase or expansion of the immunosuppression regimen.

Chronic rejection is more commonly referred to as cardiac allograft vasculopathy (CAV). This mode of rejection is a unique form of coronary artery disease and is the major factor limiting long-term survival of heart transplant recipients (62,63). The prevalence of this disease increases from 10-15% at 1-year to 35-50% by 5 years following transplant. Although the immunopathogenesis of CAV is unclear, evidence suggests that the lesion results from an immune-mediated injury, which diffusely affects the epicardial, myocardial and microscopic vessels. Coronary angiography is the only technique for establishing the diagnosis of CAV and should be performed when cardiac symptoms occur in the absence of histopathological evidence of rejection. The only definitive treatment for CAV is retransplantation (58,59,63,64).

CTRD: CAV Study, 1990 – 1999, n=7,283

	Interval to Death						
	< 1 year		2 to 5 yrs		5 to 10 yrs		
Primary Cause of Death	n	%	n	%	n	%	Total
Early Graft Failure	221	21%	0	0%	0	0%	221
CAD	47	4%	113	20%	45	22%	205
Infection	265	25%	56	10%	18	9%	339
Rejection	153	14%	64	11%	6	3%	223
Malignancy	27	3%	98	17%	63	31%	188
Other	315	29%	193	34%	58	28%	566
Unknown	41	4%	41	7%	16	8%	98
Total	1,069	100%	565	100%	206	100%	1,840

Fig. 4. Survival of patients post-transplantation. Data obtained from the CTRD (Cardiac Transplant Research Database; n = 7,283).

UTSW/SPMC Cardiac Transplant Program. The UTSW/SPMC heart transplant program was established in 1988 under the leadership of W. Steves, Ring, M.D. who continues to serve as program director. Clyde W. Yancy, Jr., M.D. is the medical director of the Heart Transplant Program and the Cardiovascular Heart Failure Institute (which now follows more than 700 patients). The first adult patient transplanted in the UTSW/SPMC program underwent the procedure in 1988. During that first year (1988) more than 30 additional patients underwent successful orthotopic heart transplantation and to date more than 270 heart transplants have been performed in the UTSW/St. Paul Transplant Program. Since its inception, this program has emerged as one of the premier programs in the U.S. and has the best patient survival rate among Texas Hospitals (64). The UTSW/SPMC program has utilized alternative therapies and technologies primarily as a bridge to transplant including the use of ventricular assist devices.

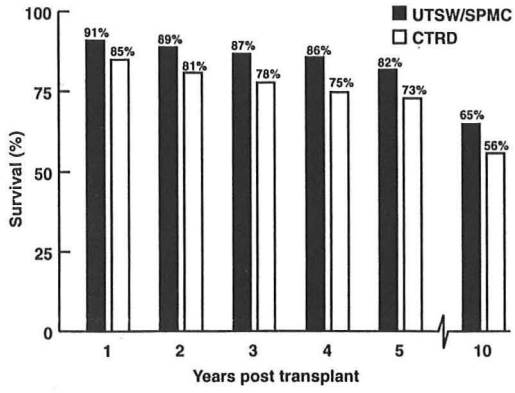

Fig. 5. Heart transplant patient survival. UTSW/SPMC (n = 271) vs. the Cardiac Transplant Research Database (CTRD) (n = 7,283).

Alternatives to Orthotopic heart transplantation

Transplantation is the most effective treatment of end-stage heart failure. However, therapy is limited to relatively few patients due the shortage of donor organs. The lack of donor organs drives the development of alternative therapeutic strategies including xenotransplantation, mechanical support and cell transfer and tissue engineering protocols.

Xenotransplantation. An unlimited supply of hearts (and other organs) is theoretically possible through the utilization of xenotransplantation. A number of xenotranplants have been previously performed in humans and despite the utilization of an immunosuppression regimen these efforts have been associated with limited success (66,67). In 1963, six chimpanzee-to-human renal transplants were performed with graft survival for a period up to nine months with the utilization of immunosuppression agents. Over the course of the next several years Drs. James Hardy (chimpanzee heart to human recipient), Cooley and colleagues (sheep heart to human) and Ross and colleagues (porcine heart to human) independently performed xenotransplants in dying recipients with immediate failure following reperfusion (hyperacute rejection) (66). In 1984, Dr. Bailey performed a baboon-to-human heart transplant in a female infant (Baby Fae) with survival for 20 days despite ABO incompatibility (66). Immunological barriers remain the most significant hurdle for xenotransplantation. Hyperacute rejection is mediated by preformed antibodies directed against xenogeneic antigens on donor endothelium (i.e. galactose α1,3-galactose). Current strategies are aimed at the genetic modification of donor endothelium to eliminate or reduce xenoreactive antigen expression. Additionally, efforts are being directed toward reducing the risk of transferring an infection associated with the xenotransplanted organ to the human recipient (referred to as xenoses) and furthermore, the risk of the recipient transferring this novel infection to other members of the community (66,67).

TABLE 6 World experience in clinical heart xenotransplantation

Case	Year	Surgeon	Institution	Donor	Type of transplant	Outcome
1	1964	Hardy	University of Mississippi, Jackson, Mississippi, USA	Chimpanzee	OHT	Functioned 2 hours (heart too small to support circulation)
2	1968	Ross	National Heart Hospital, London, UK	Pig	HHT	Cessation of function within 4 minutes (? vascular rejection)
3	1968	Ross	National Heart Hospital, London, UK	Pig	Perfused with human blood but not transplanted	Immediate cessation of function (? vascular rejection)
4	1968	Cooley	Texas Heart Institute, Houston, Texas, USA	Sheep	OHT	Immediate cessation of function (? vascular rejection)
5	1969	Marion	Lyon, France	Chimpanzee	? OHT	Rapid failure (? raised pulmonary vascular resistance)
6	1977	Barnard	University of Cape Town, Cape Town, South Africa	Baboon	HHT	Functioned 5 hours (heart too small to support circulation)
7	1977	Barnard	University of Cape Town, Cape Town, South Africa	Chimpanzee	HHT	Functioned 4 days (failed from probable vascular rejection)
8	1984	Bailey	Loma Linda University, Loma Linda, California, USA	Baboon	OHT	Functioned 20 days (failed from vascular rejection)
9	1991	Religa	Silesian Academy of Medicine, Sosnowiec, Poland	Pig	OHT	Functioned , 24 hours
10	1996	Baruah	India	Pig	OHT	Functioned , 24 hours

Source: reference #66
OHT 5 orthotopic heart transplantation
HHT 5 heterotopic heart transplantation

Artificial heart. In 1964 the National Heart, Lung and Blood Institute instituted a program aimed at the development of a fully implantable artificial heart (68). The artificial heart was first successfully utilized as a bridge to transplantation by Dr. Denton Cooley at the Texas Heart Institute in 1969 (68,70). In 1982, Dr. DeVries implanted the Jarvik-7-100 heart in Dr. Barney Clark who survived 112 days demonstrating that circulation could be supported with the total artificial heart (70). Further use of the total artificial heart by Dr. DeVries was associated with hemorrhagic, infectious, and thromboembolic complications and ultimately culminated in 1991 with a moratorium on the use of the Jarvik heart in the U.S. (70). Nevertheless, these efforts ultimately resulted in the utilization of the left ventricular assist device (LVAD). Studies revealed that use of the LVAD as a bridge to transplantation had a 65% survival rate to transplantation compared to 50% for patients receiving medical therapy (68,69). In 1994, the FDA approved the use of the LVAD as a bridge for transplantation. Currently, bridge to transplant with an LVAD is a relatively common procedure, and its use will likely continue to increase in the future. Furthermore, a prospective randomized trial (REMATCH; Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure) is in progress to evaluate the effectiveness of the LVAD as a

terminal therapy in patients (Class IV) that are not transplant candidates (ages 65-72 years) (68). Complications associated with use of the LVAD include bleeding necessitating surgical reexploration (5-30%), device-related infections (5-30%), thromboembolism (5-25%) and immunomodulation (elevated panel reactive antibodies due to anti-HLA antibodies induced by blood products) (68,69). Future efforts are aimed towards the development of a new generation of pneumatic, physiologically responsive, direct mechanical assist devices that are wrapped around the heart and do not directly contact the blood while providing the recipient with a good quality of life.

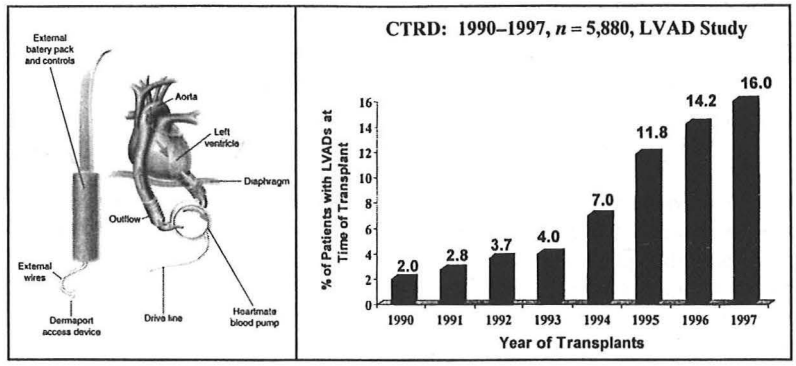

Fig. 6. Utilization of Left Ventricular Assist Device (LVAD) as a bridge to transplant. Left panel is a schematic outlining the placement of the LVAD with the inflow cannula inserted into the apex of the left ventricle and the outflow cannula anastomosed to the ascending aorta. Right panel reveals the percentage of patients with LVADs at the time of transplant. Since 1990, the percentage of patients with LVADs at the time of transplant has increased each year. CTRD, Cardiac Transplant Research Database (64,68,69).

Strategies for cardiac regeneration. Unlike other adult tissues, a stem cell population has not been identified in the adult heart. Preliminary data suggests that such a stem cell population may exist but may masquerade behind morphological and biochemical features normally attributed to differentiated cardiomyocytes. Nevertheless, several strategies are being pursued by a number of laboratories utilizing cell transfer technology and includes the following cell sources:

- fetal cardiomyocytes
- genetic modification of cardiomyocytes
- hematopoitic stem cells
- myogenic stem cells

Each strategy that utilizes the respective sources of cells is supported by preliminary experiments that establish biological plausibility but also includes ethical or biological obstacles or considerations. Recent cell transfer studies have been reported using various animal models.

Jackson et al. have observed the ability of purified stem cells derived from adult bone marrow to participate in cardiac muscle regeneration following the induction of ischemia by coronary artery occlusion and reperfusion. Moreover, Orlic et al. have shown that a subpopulation of hematopoietic stem cells can successfully repopulate the injured myocardium and results in functional improvement. The results of these studies provide a proof of concept supporting the feasibility of using stem cell technology to repopulate the failing heart.

Cellular augmentation and tissue engineering. Stem cells are capable of self-renewal and multilineage differentiation along a specified molecular pathway (71-73). While diversification of cell types is largely complete at or shortly after birth, many tissues in the adult undergo self-renewal and, accordingly, must establish a life-long population of stem cells for maintenance and regeneration of tissues (71,72). A number of adult tissue-specific stem cells have been identified including hematopoietic, neural, intestinal, pancreatic, hepatic and myogenic stem cells (i.e. satellite stem cells) (71-77).

The molecular pathways that regulate the hematopoietic lineage have been elegantly and extensively characterized compared to other stem cell systems (reviewed in references 71-73). These studies have identified extracellular matrix ligands (i.e. integrins), cytokine requirements (LIF, EGF, FGF, PDGF, HGF, etc.), transcriptional regulators and cell surface proteins necessary for hematopoietic stem cells to interact within their niches as well as the molecular distinctions between hematopoietic stem cells and their progenitors (71-73). These studies have resulted in the development of a linear model or regulatory hierarchy wherein totipotent stem cells (i.e. embryonic stem cells contained within the inner cell mass of the blastocyst) are progressively regulated by the combinatorial interaction of transcription factors and cytokines resulting in the differentiation and generation of tissue or organ specific stem cells (72,73).

Tissue-specific adult stem cells are important in the maintenance and regeneration of the respective organs (71-73). However, recent findings challenge such a stem cell-hierarchy for lineage specification and underscore the importance of niche or milieu on stem cells and their ability to adopt different lineages (71). Transplantation of hematopoietic stem cells has been used to successfully repopulate regenerating skeletal muscle as well as the muscle of dystrophin-deficient mice (78-80). Furthermore, a recent report by Clarke et al., revealed that neural stem cells isolated from the adult mouse brain had a very broad developmental capacity and could contribute to virtually all tissues when delivered into the developing embryo (76). These results suggest that adult stem cells or progenitor cells are capable of dedifferentiation or dedetermination to adopt alternative lineages in a permissive environment (71-73). *The underlying mechanisms that control differentiation and self-renewal are poorly understood primarily because stem cells are rare and difficult to identify due to the lack of markers.*

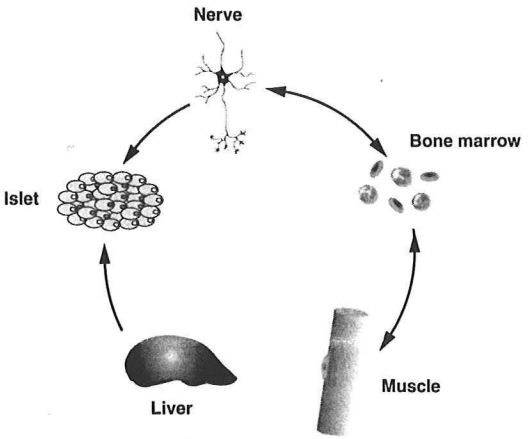

Fig. 7. Plasticity of tissue specific stem cells in the adult. Almost all adult tissues have a subpopulation of stem cells or progenitor cells that function in maintenance and regeneration of the respective tissues. Recent studies challenge previously held paradigms regarding organogenesis and cell specification. Myogenic stem cells have considerable plasticity and are capable of dedetermination to contribute to alternative lineages when exposed to a permissive environment.

Myogenic stem cells: Unlike the heart, skeletal muscle has the capability of self-repair (81-83). Resident within adult skeletal muscle is a pool of undifferentiated myogenic stem cells termed satellite cells because of their anatomical location at the periphery of mature skeletal myofibers, which were first described by Mauro in 1961 using ultrastructural techniques (81). These small mononuclear cells share a common basal lamina with the larger multinucleated myocytes (84). In the adult mouse, myogenic stem cells (satellite cells) account for 1-5% of all nuclei in hindlimb skeletal muscle.

Under unstressed conditions, these cells are quiescent. Following muscle injury or in response to increased work demand, myogenic stem cells are mobilized to proliferate, differentiate and fuse into multinucleated myofibers in a manner that recapitulates the fundamental events of muscle development in the fetus (Fig. 8) (84). The myogenic stem cell population is self-renewing, and a residual pool of stem cells, capable of supporting additional rounds of regeneration, is reestablished after each discrete episode of muscle injury (84). However, this capacity for self-renewal is finite. The exhaustion of the myogenic stem cell pool is an important factor contributing to the clinical deterioration observed with advancing age in human patients with muscular dystrophy, and perhaps to the loss of muscle mass observed in normal aging (84).

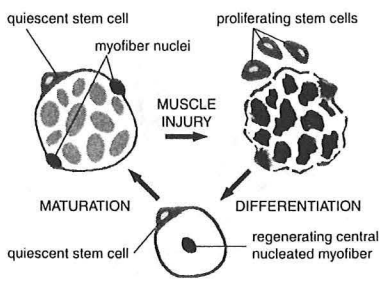

Fig. 8. Myogenic stem cell repopulation of injured muscle. Quiescent myogenic stem cells are activated within six hours of injury and have a tremendous proliferative capacity. They withdraw from the cell cycle to form differentiated myotubes and reestablish the quiescent state (i.e. self-renewal).

Myogenic stem cells are arrested at an early stage of the myogenic program, such that they do not express any of the myogenic basic helix-loop-helix (bHLH) proteins of the MyoD family (82,84). A number of anatomical studies have examined the physiological

responses of the satellite stem cells and the architectural responses to various stimuli. In contrast, the molecular events that restrict differentiation of these stem cells during muscle development, or regulate cellular proliferation in response to an injury and maintain their quiescence within adult muscles remain ill-defined (Fig. 9) (81,82,84).

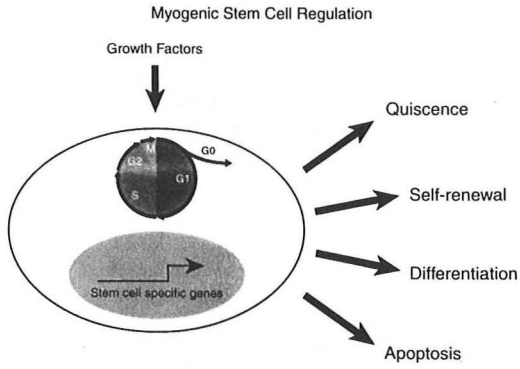

Fig. 9. Paradigm for regulation of the myogenic stem cell fate. Crosstalk between extrinsic growth promoting factors, cell cycle machinery and stem cell specific genes are potentially important determinants of stem cell fate (quiescence, self-renewal, proliferation, differentiation and apoptosis).

Recently, hematopoietic and muscle stem cells (also called side population or SP cells) have been isolated from adult tissues using dual-wavelength flow cytometric analysis of bone marrow or skeletal muscle, respectively, and stained with the fluorescent DNA-binding dye, Hoechst 33342 (79,80). This method, which relies on the differential ability of stem cells (SP cells) to efflux the Hoechst dye, defines an extremely small and homogeneous population of cells that can adopt alternative fates in permissive environments. Gussoni et al., demonstrated that SP cells isolated from adult bone marrow were able to reconstitute the irradiated dystrophin-mutant mouse bone marrow and later these cells were recruited from the bone marrow to participate in muscle repair (Fig. 10) (79). Further, Jackson et al., utilized the same protocol to isolate muscle SP cells from adult mice and observed that as few as 100 SP cells could reconstitute the entire bone marrow of a lethally irradiated mouse (80). These results demonstrate that adult skeletal muscle contains a stem cell population that can be purified based on the Hoechst exclusion and can adopt alternative fates (Fig. 10).

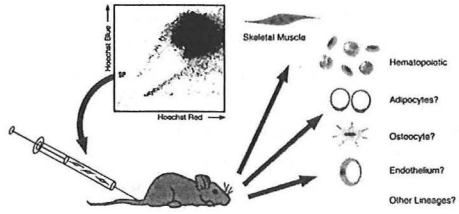

Fig. 10. Potentiality of adult skeletal muscle specific stem cells. Purified stem cells (SP cells) are gated and isolated from adult skeletal muscle based on the ability to exclude Hoechst 33342 dye. Purified muscle stem cells home and reconstitute the irradiated bone marrow in its entirety (80). These stem cells are recruited from the bone marrow to repopulate injured skeletal muscle. Stem cells isolated from adult skeletal muscle can contribute to the skeletal muscle lineage and the hematopoietic lineage. It is possible that this stem cell population may contribute to multiple lineages.

***forkhead*/winged helix transcription factor family:** This family of DNA binding proteins, which has more than 80 members, is defined by a 110 amino acid domain, that encodes the DNA binding domain, first identified in the *forkhead* gene of *Drosophila melanogaster* (67,68). Several members of the *forkhead*/winged helix family of transcription factors including HNF-3β are known to exert important regulatory functions during development in the control of cell fate, proliferation, differentiation and tissue morphogenesis (69-71). With respect to stem cells and/or tissue repair in particular, a mammalian *forkhead*/winged helix protein termed Genesis is expressed selectively in embryonic stem cells (72) and a protein related to HNF3 has been identified in regenerating hepatocytes (73). Our current data concerning MNF provide direct evidence for a specific role for members of this extended gene family in the regulation of stem cell function.

MNF is localized to myogenic stem cells in adult mouse skeletal muscle. Recent advances in our laboratory have prompted us to examine the use of stem cells to repopulate the human heart. We have identified a novel member of the *forkhead*/winged helix transcription factor family, which we termed **M**yocyte **N**uclear **F**actor or MNF. Using light and electron microscopic immunohistochemical techniques, we localized MNF to the myogenic stem cell population. Notably, MNF is the first and only molecular marker of the myogenic stem cell population (82).

The cellular localization of MNF is further supported by the observation that the 4 kb MNF promoter fragment, using transgenic technology, can confer expression to the myogenic stem cell population. Our initial results reveal a heterogeneous pattern of expression in muscle stem cells of the developing limbs. These results indicate that the 4 kb promoter of MNF contains regulatory elements that control expression in a cell lineage that are destined to be skeletal muscle (the myogenic stem cell population). Following injury to hindlimb skeletal muscle, we have shown that the myogenic stem cell population undergoes activation and a tremendous stem cell expansion occurs with evidence of newly regenerated myotubes by Day 5 (following injury). We observed that the expression of the 4 kb MNF promoter recapitulates MNF expression during muscle regeneration and identifies both quiescent and proliferating stem cells but is not expressed in differentiated myotubes. These data indicate that MNF is a selective marker of non-differentiated myogenic stem cells and that it does not mark differentiated myocytes.

MNF-α and MNF-β are reciprocally expressed during the sequential stages of muscle regeneration. We have identified two alternatively spliced isoforms for MNF (two proteins that are produced as products of a single gene) and have termed them MNF-α and MNF-β. These two alternatively spliced isoforms are reciprocally expressed during myogenesis and during muscle regeneration suggesting that they reciprocally regulate gene expression. Potential downstream target genes for MNF include the cell cycle regulatory genes p21, c-myc and cdc2. The promoters of these genes contain an evolutionarily conserved MNF binding motif, supporting the hypothesis that MNF regulates cell cycle progression of the myogenic stem cell population and thus participates in pathways that modulate cell growth during muscle development and regeneration (82-84).

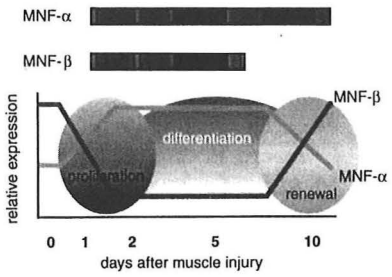

Fig. 11. Schematic structure of MNF isoforms and their reciprocal expression during sequential stages of muscle regeneration.

MNF mutant mice exhibit impaired muscle regeneration and dysregulation of gene expression. Using gene disruption technology, we have produced mice that lack MNFs and have observed that these mutant mice display a pronounced growth deficit and impaired muscle regeneration in response to cardiotoxin induced injury (83). Isolated myogenic stem cells from MNF-/- animals are capable of forming differentiated myotubes following the removal of serum. However, in media supplemented with high concentrations of serum (20%), MNF-/- stem cells grow more slowly than MNF+/- or MNF+/+ cells and exhibit dysregulated expression of the cyclin dependent kinase inhibitor, p21 (83).

MNF regulates the myogenic stem cell population (SP cells). A recently described strategy for isolation of stem cells from adult skeletal muscle employs Hoechst 33342 staining and dual-wavelength FACS analysis. This isolation protocol defines a homogeneous subset of cells in skeletal muscle that are capable of adopting alternative fates including the ability to completely reconstitute the hematopoietic lineage in a lethally irradiated mouse (79,80,84). This latter finding suggests that the myogenic stem cell population (SP cells) may be capable of commitment to alternative lineages, given the right environmental cues (84). Furthermore, the stem cells isolated from adult skeletal muscle have been shown to express stem cell antigens (i.e. Sca-1^+, lin^-) and be enriched at least 1,000-fold for in vivo reconstitution activity (79,80).

Studies recently undertaken in our laboratory, further verified the presence of the side population (SP cell population) of cells isolated from adult mouse skeletal muscle (Fig. 14). The ability of the SP cells to efflux Hoechst dye is blocked when staining is performed in the presence of verapamil (indicating that the distinctly low staining pattern of the SP cells is due to a multidrug resistance protein or a mdr-like mediated efflux of the dye from the myogenic stem cells as previously reported) (Fig. 12A & B) (80,84).

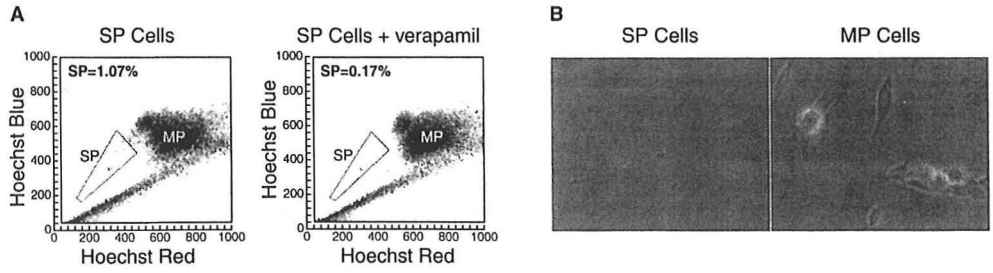

Fig. 12. Isolation and characterization of muscle SP cells. A) Using Hoechst 33342 staining and FACS analysis a side population (SP) of cells was identified (see gated box). In the presence of verapamil this population was not visible. The non-gated cells that are isolated from skeletal muscle are referred to as the main population (MP). The percent of SP cells relative to that of the MP population is presented in the upper left corner. B) Muscle SP cells appear small and spherical. They have been cultured without differentiation for a three-week period in our laboratory. In contrast, the non-gated cell population, or MP cells, readily differentiate after 4-5 days in culture forming myoblasts and myotubes.

Following skeletal muscle injury in wild-type adult mice, we observed more than a 50-fold increase in the SP cell population consistent with the hypothesis that this cell population participates in muscle repair (Fig. 13A). SP cells are not associated with the inflammatory response as they lack inflammatory markers (lin$^-$, Thy1.1$^-$ and Mac1$^-$) (18,19). Moreover, muscle SP cells isolated from the ROSA26 mouse (a transgenic mouse that constitutively expresses β-galactosidase in all cells; engineered by P. Soriano and colleagues) are able to engraft and participate in skeletal muscle repair as 250 myogenic stem cells (SP cells), delivered intramuscularly, into an injured skeletal muscle were able to compete with the endogenous stem cells to contribute to myofiber repair (Fig. 13B).

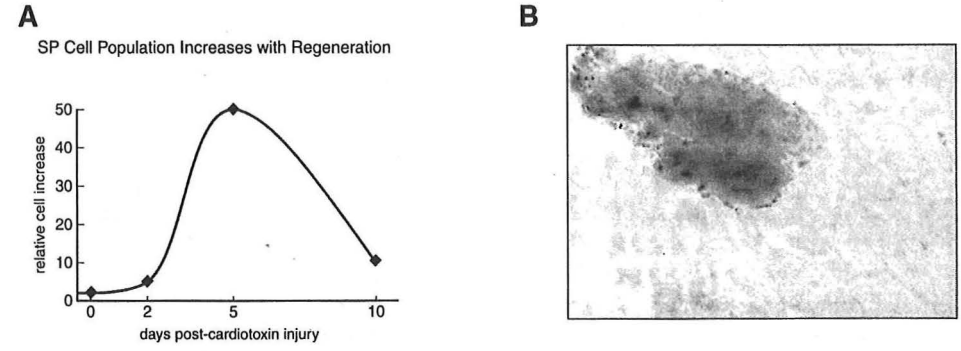

Fig. 13. Muscle SP cells have a tremendous proliferative capacity in vivo and retain their engraftment potential. (A) Relative increase in the number of SP cells determined at specified intervals following cardiotoxin-induced muscle injury. (B) Engraftment of isolated muscle SP cells from the ROSA26 mouse into a cardiotoxin injured TA muscle of a syngeneic SCID mouse.

Using this purification strategy, we have observed that the MNF mutant mice have a severe decrease in the number of SP stem cells isolated from skeletal muscle compared to age and gender-matched controls (Fig. 14). *These results support the hypothesis that MNF is critical for the maintenance of the myogenic stem cell population (SP cells) in skeletal muscle.* Furthermore, we have utilized this strategy to isolate SP cells (stem cells) in the wild-type adult mouse heart.

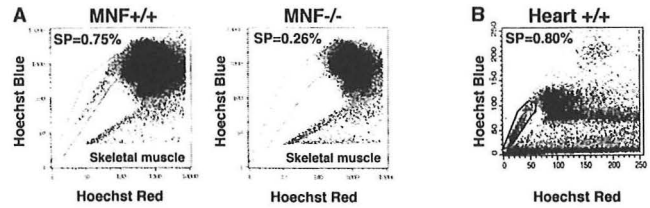

Fig. 14. Flow cytometric analysis of MNF+/+ and MNF-/- adult skeletal muscle. A) The percentage of SP cells (stem cells) is severely decreased in MNF-/- skeletal muscle compared to age and sex-matched controls. The percentage of cells in each quadrant is indicated in the upper left corner. B) SP cell (stem cell) isolation from the wild type mouse heart using Hoechst 33342 staining and FACS analysis.

Future studies will continue to focus on the genetic regulation of the myogenic stem cell population as well as the plasticity of this cell population for the utilization in cellular augmentation therapy. Use of human stem cells as a source to repopulate the injured heart is an achievable goal based on data from our laboratory and others. We have shown the MNFs are critical regulators of this stem cell population and these cells are capable of adopting alternative fates. Moreover, studies have shown that as few as 100 muscle stem cells are capable of repopulating the entire bone marrow of an irradiated mouse emphasizing the tremendous proliferative capacity and multipotency of this cell population. Utilization of the individual patient's stem cells for use as a cell source will further obviate the need for immunosuppression therapy as the recipient would also serve as the donor of the skeletal muscle stem cells.

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