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T cell dysfunction and patient age are associated with poor outcomes after mechanical circulatory support device implantation

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Abstract

Immunologic impairment may contribute to poor outcomes after implantation of mechanical circulatory support device (MCSD), with infection often as a terminal event. The study of immune dysfunction is of special relevance given the growing numbers of older patients with heart disease. The aim of the study was to define which immunologic characteristics are associated with development of adverse clinical outcomes after MCSD implantation.

We isolated peripheral blood mononuclear cells (PBMC) from patients pre- and up to 20 days post-MCSD implantation and analyzed them by multiparameter flow cytometry for T cell dysfunction, including terminal differentiation, exhaustion, and senescence. We used MELD-XI and SOFA scores measured at each time point as surrogate markers of clinical outcome.

Older patients demonstrated increased frequencies of terminally differentiated T cells as well as NKT cells. Increased frequency of terminally differentiated and immune senescent T cells were associated with worse clinical outcome as measured by MELD-XI and SOFA scores, and with progression to infection and death.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.humimm.2018.01.011.

In conclusion, our data suggest that T cell dysfunction, independently from age, is associated with poor outcomes after MCSD implantation, providing a potential immunologic mechanism behind patient vulnerability to multiorgan dysfunction and death. This noninvasive approach to PBMC evaluation holds promise for candidate evaluation and patient monitoring.

Keywords

T cell; Immune phenotype; Mechanical circulatory support; Elderly

1. Introduction

The past decade has brought substantial progress in the field of advanced heart failure and mechanical circulatory support devices (MCSD), and device implantation is increasingly the treatment of choice for patients with disease not amenable to medical therapy [1]. Given the increasing prevalence in heart failure and shortages in organ donors, the number of potential candidates for MCSD is growing [2]. However, there remains a subset of patients who undergo successful MCSD implantation and yet proceed to multisystem organ dysfunction and death, often with infection and sepsis as terminal events [1]. This observation suggests that immunologic impairment may be a contributor to poor outcomes after MCSD implantation, an important concern given the increasing number of older patients with heart failure [3], and the known associations between increased age and T cell immune cell dysfunction [4–6]. Older and physically frailer patients are at increased risk for death after MCSD implantation [7,8].

Associations between immune dysfunction and advanced heart failure have previously been described, with markers of inflammation including cytokines, C-reactive protein, and autoantibodies [9]. Older studies have shown a decrease in the numbers of CD4+ and CD8+ T cells, impaired T cell function, and inflammation after MCSD placement [10–14], postulated to be associated with increased rates of blood clotting and strokes, infection, and development of multiorgan system dysfunction. New markers of T cell dysfunction associated with impaired control of infection include T cell maturation and terminal differentiation [15,16]. In addition, immune senescence, or deficient replicative ability, and immune exhaustion, or impaired antigen response, are associated with immune dysfunction and poor infection control, worsened with in older patients as well as CMV antibody positive individuals [17–19]. These immunologic changes seen with advanced heart failure and after MCSD implantation may also contribute to the alloreactivity often seen after these patients undergo heart transplantation [1,2].

Whether T cell functional immune phenotypes are associated with poor outcomes after MCSD implantation and whether there is a mechanistic link between them has not previously been examined. We hypothesized that T cell functional immune phenotypes are associated with poor outcomes after MCS device implantation. We describe here a novel approach to immunologic evaluation of T cell phenotypes in patients undergoing MCSD implantation.

2. Materials and methods

2.1. Patients and samples

We enrolled patients from the Ronald Reagan Medical Center with advanced heart failure undergoing evaluation for MCSD. This observational study was approved by the UCLA Institutional Review Board. All patient signed informed consent. Blood was collected for peripheral blood mononuclear cell (PBMC) isolation within 24hs prior to MCSD implantation and on Days 1, 3, 5, 8, 10, 14 and 17, ± 1 day after surgery for a total of 8 planned blood draws. 28 patients were enrolled who had PBMC available for analysis, and completed at least 6 months of clinical follow-up between September 2012 and March 2015, with the last point of clinical review as of July 1, 2016. Only FDA-approved durable MCSD were included in our analysis, namely HeartMate II, HeartWare, Thoratec Paracorporeal Ventricular Assist Device, CentriMag, or Total Artificial Heart, but not percutaneously inserted devices such as extracorporeal membranous oxygenation, TandemHeart, or Impella pumps. However, patients could have temporary support devices in place prior to MCSD implantation. PBMC were isolated using previously published techniques [20], and frozen for storage until batched analysis could be performed. The majority (75%) of samples were collected between Days 0 and 8, with 57% with 5 samples and 71% with ≥5 samples available for analysis. Sensitization was defined as the presence of detectable antibodies against HLA I and II single antigens.

2.2. Flow cytometry

Viable cells were identified using a fluorescent live/dead marker (Life Technologies). T cell maturation was assessed using a cocktail of fluorochrome-conjugated antibodies against CD3, CD4, CD8, CCR7, and CD45RA to determine maturation phenotype. Naïve cells were defined as CCR7+/CD45RA+; effector memory as CCR7−/CD45RA−; and terminally differentiated as CCR7−/CD45RA+ (antibodies obtained from either BD Biosciences or Biolegend). In addition, 22 of the 28 patients were simultaneously analyzed for exhaustion, senescence, and activation of T cells using KLRG1, CD57, CD38, CD28, and PD-1 [16]. Immunophenotyping of NK T cells was performed using fluorochrome-conjugated antibodies against CD56, CD3, CD4, CD8, and KLRG1. NK cells were defined as total CD56+ KLRG1+ lymphocytes. Fluorescence from viable cells was measured by the BD LSRFortessa (BD Biosciences) with FCS Express software (DeNovo Software) for analysis. For maturation phenotype analyses, central memory (CCR7+/CD45RA−) T cells are not described given the low frequency of these cells observed in peripheral blood.

2.3. Clinical data collection

Data was collected prospectively to calculate MELD-XI (Model for End-Stage Liver Disease eXcluding INR) and SOFA (Sequential Organ Failure Assessment) scores, used as a surrogate for multiorgan dysfunction [21,22]. For MELD-XI calculation, an appropriate measure for patients on anticoagulation, serum creatinine was set to 1.0 for patients with creatinine levels of < 1.0 to prevent calculation of negative numbers, generating a minimum score of 9.44, following previously published guidelines [22]. Records were reviewed for 1 month prior to and 6 months after MCSD implantation for evidence of significant bleeding requiring transfusion or operative intervention as well as infectious episodes including sepsis

syndrome, bacteremia, driveline infection, pneumonia, and urinary tract infection following standard definitions [23,24]. Severe infection was defined as requiring intravenous antibiotic treatment and/or leading to extension of hospital stay or death. MELD-XI and SOFA measurements were evaluated at each time point for which PBMC were collected. MELD-XI values ranged from11.2 to 28.8; SOFA scores ranged from 3 to 16. Median values were used to divide patients into “High” or “Low” MELD-XI and SOFA groups either prior to or after MCSD implantation.

2.4. Statistical analysis

Statistical analysis was performed using JMP Pro 11 (SAS Software). Differences between continuous values (SOFA or MELD-XI, chronologic age, frequencies of immunologic subtypes) were compared by non-parametric 2-sample test (Mann-Whitney U-Test), while differences between categorical variables were compared by Fisher exact test. Standard least squares regression was used to compare numeric variables.

To correct for the issue of repeated measures and patient-to-patient variability, linear mixed effects models were used to evaluate the association between immune phenotype and clinical outcomes including MELD-XI and SOFA, correcting for random patient effects. Time to infection and death were analyzed using Cox proportional hazards models, with immune phenotypes included as time-varying covariates. Bridge to transplantation (BTT) was analyzed using logistic regression, with immune phenotypes averaged across repeated measures. Variables reaching a statistical significance of $p < 0.05$ were further analyzed in a multivariable model adjusted for patient age. These analyses were performed using R 3.3.2 (http://www.r-project.org/).

3. Results

3.1. Patient characteristics at time of MCSD implantation

Of the twenty-eight patients with advanced heart failure ranging in age from 24 to 80 years old enrolled in this study, 53.6% of patients were older than age 60 at the time of MCSD implantation (Table 1). Most patients underwent HeartMate II device implantation (71.4%), with several receiving implantation of other devices: Paracorporeal Ventricular Assist Device (PVAD) (10.7%), HeartWare (7.1%), Total Artificial Heart (TAH) (7.1%), and left-sided Centrimag (3.6%). Five of the 20 patients receiving left sided HeartMate II implantation required additional right-sided MCSD implantation. Prior to MCSD implantation, 10 patients (35.7%) had temporary device in place, 5 with intra-aortic balloon pump (IABP) and 5 with extracorporal membrane oxygenation (ECMO). There was no significant association between need for temporary device and age ($p = 0.698$). There was also no association between temporary device and heart failure type, INTERMACS score, MELD-XI, SOFA score, or death at 3 months.

Dividing patients into high (≥18) versus low (< 18) MELD-XI group at the time of implant revealed increased patient age (median 63 compared with 43 years) and severity of heart failure (83% INTERMACS 1 or 2 compared with 20%) in patients with higher compared with lower MELD-XI scores (Table 2). Older patients had a higher median MELD-XI score,

with a median of 20.1 in older compared with 14.0 in younger patients (Table 3). The association between age and increased MELD-XI and SOFA scores continued after MCSD implantation ($p < 0.001$ for both).

3.2. Patient outcomes after MCSD implantation by clinical characteristics

MELD-XI and SOFA scores decreased overall by post-operative Day 5 compared with pre-implant levels (Supplemental Table 1). The median follow-up time was 474 days (IQR 91 to 779 days).

The median number of days to death was 50 (Table 4). 25.0% of all patients (n = 7) died by 3 months after implant. Of the 10 patients who died by one year post implant, the majority (n = 9) died due to multiorgan failure, often in the setting of sepsis, while one patient died outside of the hospital due to unknown causes.

7 patients had infections prior to implantation, which included bacterial sepsis (n = 2), bacterial pneumonia (n = 2), urinary tract infection (n = 1), Candidal infection (n = 1), and viral pneumonia (n = 1). Post implantation, 15 patients experienced infections including bacterial sepsis (n = 7), bacterial pneumonia (n = 4), Candidal infection (n = 2), viral pneumonia (n = 1), and colitis (n = 1). Infections post-implant occurred at a median of 28 days after surgery. There were 6 significant bleeding complications requiring transfusion or surgical intervention. MELD-XI score at time of implantation was not significantly associated with clinical outcomes, as incidence of subsequent infection and death were similar in the low and high MELD-XI groups (Supplemental Table 2). Analyzing older compared with younger patients showed a trend towards, increased incidence of infection and death in older patients, with 40.0% incidence death at 3 months in the older as compared with 7.7% in the younger patients ($p = 0.084$) (Supplemental Table 3). Treating age as a continuous variable demonstrated an association with death at 3 months ($p = 0.028$).

3.3. T cell phenotype and patient age

Patients underwent longitudinal sampling of PBMC with one pre-implant blood draw and up to 7 assessments after MCSD implantation. Limiting analysis to PBMC collected prior to MCSD implant, older patients had fewer naïve CD8+ T cells compared with younger patients, with a median frequency of 9.8% compared with 40.1% ($p = 0.002$). Older patients displayed an increased frequency of terminally differentiated effector memory cells (TEMRA) CD8+ T cells, cells with a median frequency of 46.4% as compared with 26.0% in younger patients ($p = 0.038$) prior to MCSD implantation. Effector memory (EM) CD8+ T cells did not vary by patient age at Day 0. No significant associations were found for CD4+ maturation.

After MCSD implantation (excluding pre-implant values), analysis of patient age showed decreased median frequency of naïve CD8+ T cells (9.7%) in older as compared with younger patients (53.0%) ($p < 0.001$). Older patients displayed an increased frequency of EM (29.9% compared with 19.8%, $p = 0.002$) and TEMRA CD8+ T cells (48.2% compared with 17.0%, $p < 0.001$) (Fig. 1a). These differences between older and younger patients did not change significantly over time after MCSD implantation (Supplemental Fig. 1). A similar pattern was observed for CD4+ T cells by maturation subtype, with the most striking

difference between older and younger patients in the frequency of naïve CD4+ T cells (35.2% in older compared with 52.2% in younger patients, p = 0.001). There was an increased frequency of exhausted CD8+ CD57+PD-1+ T cells in older (11.6%) compared with younger (6.6%) patients (p < 0.001) but no significant association between age and T cells senescence. Interestingly, younger patients with a decreased frequency of naïve CD8+ T cells and increased frequency of TEMRA CD8+ T cells were more likely to experience infections and progression to death. Conversely, the older patients who had increased frequency of naïve and fewer TEMRA CD8+ T cells were more likely to successfully bridge to transplant without adverse clinical outcomes.

3.4. T cell phenotype and CMV antibody status

CMV antibody (Ab) positivity was associated with decreased frequency of naïve CD8+ T cells, with median frequency 11.4% in CMV Ab positive as compared with 49.3% in CMV Ab negative patients (p < 0.001) (Fig. 1b). CMV Ab positive patients had increased frequency CD8+ EM (29.9% compared with 18.9%, p = 0.001) and CD8+ TEMRA T cells (46.8% compared with 20.3%, p < 0.001). A similar association was seen for CD4+ T cells (data not shown). In addition, there was increased frequency of CD8+ exhausted (CD57+ PD-1+) (10.1% compared with 6.3%, p = 0.012) T cells in the CMV Ab positive compared with CMV Ab negative patients. CMV Ab positive patients displayed increased frequency of senescent CD8+ T cells, de-fined as CD8+ CD28− (36.1% compared with 10.7%, p < 0.001), CD8+ CD57+KLRG1+ (11.6% compared with 3.2%, p < 0.001), or CD8+ KLRG1+CD28− (10.0% compared with 2.0%, p = 0.002).

3.5. T cell phenotype and INTERMACS score

After MCSD implantation an association was seen with the frequency of CD8+ naïve T cells (18.6% in INTERMACS 1/2 compared with 39.8% in INTERMACS 3/4, p = 0.004) and CD8+ EM T cells (31.6% in INTERMACS 1/2 compared with 19.0% in INTERMACS 3/4, p < 0.001). No association was seen with CD8+ TEMRA or CD4+ T cells. After MCSD implantation, INTERMACS 1/2 patients had increased frequency of exhausted CD8+ T cells (CD57+ PD-1+) compared with INTERMACS 3/4 patients (18.5% compared with 3.0%, p < 0.001), however, no difference was seen in frequency of senescent CD8+ T cells. Frequency of CD8+ T cells expressing the activation marker CD38+ were significantly increased in INTERMACS 1/2 patients (46.8%) as compared with INTERMACS 3/4 patients (16.2%) (p < 0.001). This difference in activated CD8+ T cells was also detectable prior to MCSD implantation in INTERMACS 1/2 patients(45.3%) as compared with INTERMACS 3/4 patients (13.3%) (p = 0.019).

3.6. T cell phenotype and pre-MCSD temporary device

There was no significant association with the presence or absence of pre-MCSD temporary assist devices (IABP or ECMO) and T cell maturation, senescence, or exhaustion prior to MCSD implant. After MCSD implantation, however, patients with history of temporary device displayed increased frequency of exhausted and senescent T cells: There was increased frequency of KLRG1+ CD8+ T cells in the patients with history of temporary support device (60.9%) as compared with those without history of temporary device (14.7%) group (p < 0.001). CD8+ KLRG1+ PD-1+ analysis revealed a similar association, with

increased frequency in the temporary device (40.8%) as compared with the no temporary device group (8.0%) (p = 0.001). KLRG1+ CD28− T cells and differences in T cell maturation, however, did not show any significant differences.

3.7. T cell phenotype and MCSD pulsatility

For the 18.8% of patients receiving a pulsatile MCSD device, increased frequency of KLRG1+ CD8+ T cells was observed after implantation in patients receiving pulsatile devices (64.4%) compared with those receiving non-pulsatile devices (23.6%) (p = 0.002). Analysis of CD8+ KLRG1+ PD1+ exhausted T cells also revealed increased frequency in those receiving pulsatile (31.3%) compared with non-pulsatile MCSD (9.7%) (p = 0.006). CD8+ KLRG1+ CD28− senescent T cells were also increased in those receiving pulsatile (8.8%) compared with non-pulsatile devices (1.9%) (p = 0.006). There were no differences, however, in markers of T cell maturation.

3.8. T cell phenotype analysis and MELD-XI/SOFA score

CD8+ and CD4+ maturation phenotypes were not significantly associated with MELD-XI or SOFA scores prior to implantation. After MCSD implantation, an association between numerical MELD-XI scores and CD8+ maturation phenotype was observed, with a statistically significant association between frequency of naïve CD8+ T cells (p < 0.001), EM T cells (p = 0.26), and TEMRA T cells (p = 0.027). This association was also seen between naïve CD4+ T cells (p = 0.031) and TEMRA T cells (p = 0.005), as well as for SOFA scores (data not shown). Categorizing each MELD-XI score as ‘high’ (≥16) or ‘low’ (< 16) (divided by level post-implant) revealed a similar association: After MCSD implantation, lower frequencies of CD8+ naïve T cells were seen with high MELD-XI (11.7%) as compared with low MELD-XI (40.2%) (p < 0.001), and increased frequency of EM and TEMRA CD8+ T cells (p = 0.002 and p = 0.009, respectively) (Fig. 1c).

After MCSD implantation, lower frequencies of CD8+ naïve T cells were seen with high SOFA (≥6) (18.8%) as compared with low SOFA scores (< 6) (34.0%) (p = 0.034), and increased frequency of EM CD8+ T cells were seen in high as compared with low SOFA scores (p = 0.012). A similar pattern was seen for the CD4+ T cells (data not shown). There was increased frequency of KLRG1+ CD8+ T cells in the high MELD-XI (50.5%) as compared with the low MELD-XI (17.4%) group (p = 0.027) (Fig. 1d). CD8+ KLRG1+ PD-1+ analysis revealed a similar association, with increased median frequency in the high (27.0%) as compared with the low MELD-XI group (8.8%) (p = 0.001) (Fig. 1d). KLRG1+ CD28− T cells also demonstrated significant difference between the high MELD-XI and low MELD-XI groups (8.6% compared with 1.8%, p = 0.006). No differences in exhaustion or immune senescence was seen by SOFA score (data not shown).

3.9. T Cell phenotype analysis and clinical outcome of infection

CD8+ maturation subtypes after MCSD implantation were associated with post-implant infection, with lower frequency of naïve CD8+ T cells in patients with infection (18.5%) compared with those without infection (35.1%) (p = 0.007), increased frequency of EM (30.3% compared with 18.7%, p = 0.003), and a trend towards increased TEMRA CD8+ T

cells (14.8% compared with 30.7%, p = 0.051) (Fig. 2a). A similar finding was observed for CD4+ naïve (p = 0.003) and EM (p = 0.003) T cells.

There was an increased frequency of CD8+ KLRG1+ T cells in patients with infection (53.3%) as compared with those without (16.0%) (p = 0.004) (Fig. 2b). Analysis of exhaustion (KLRG1+ PD-1+) and senescence (KLRG1+ CD28−) showed association with infection after MCSD implantation (p = 0.008 and 0.003, respectively) (Fig. 2b). Increased frequency of activated and exhausted or senescent CD57+ PD-1+ and CD57+ KLRG1+ CD8+ T cells was also observed in patients with infection (p = 0.003 and p = 0.0004, respectively).

3.10. T Cell phenotype analysis and clinical outcome of BTT and death

Patients who were successfully bridged to transplant showed a decreased frequency of terminally differentiated TEMRA CD8+ T cells(31.2%) as compared with those who were not (55.2%) (p < 0.001). A similar pattern was seen for CD4+ T cells (data not shown). Analysis of senescence showed CD8+ CD57+KLRG1+ of 14.4% in those who did undergo transplantation as compared with 31.0% in those who did not (p = 0.018). CD8+ CD57+PD-1 frequency was 7.9% in those who were bridged to transplant as compared with 13.0% in those who did not (p = 0.004).

CD8+ maturation subtypes were associated with death at 3 months, with a lower frequency of naïve CD8+ T cells in patients who died(10.3%) compared with those who survived (27.0%) and an increased frequency of TEMRA CD8+ T cells in patients who died (63.9%) as compared with those who survived (32.1%) (p < 0.001) (Fig. 2c). Increased frequency of senescent CD8+ KRLG1+CD28− T cells after implantation was seen in those who died at 3 months (9.7%) as compared with those who survived (5.2%) (p = 0.041). There was increased frequency of CD8+ KRLG1+PD-1+ (29.9% versus 3.9%, p = 0.015) (Fig. 2d) and CD8+ CD57+PD-1+ (13.9% versus 7.7%, p = 0.001) in patients who died as compared with those who survived.

3.11. NK and NK T cells

Older patients demonstrated an increased frequency of NK T cells(5.0%) as compared with younger patients (1.5%) (p < 0.001) after MCSD implantation, and increased frequency of NK cells (49.0%) compared with younger patients (12.3%) (p < 0.001), but no difference in NKT KLRG1+ cells by age (Fig. 3a). Increased numbers of NK T cells was associated with increased MELD-XI score, with 4.6% in the high MELD-XI as compared with 2.0% in the low MELD-XI group (p = 0.004). A similar trend was seen for high versus low SOFA, and NKT+ KLRG1+ cell frequency was positively associated with MELD-XI and SOFA scores (data not shown). NKT cell frequency was associated with death, with median frequency of 5.1% in those who died compared with 2.2% in those who survived at 3 months (p = 0.002). NKT+ KLRG1+ cells were also associated with death (Fig. 3b).

3.12. Multivariable analysis combining demographic and immunologic factors

To address the issue of repeat measures analysis and patient effects on the association between immune phenotype and clinical outcomes, linear mixed effects models were used.

Increased age was positively associated with MELD-XI and SOFA scores (Table 5a). Variables reaching statistical significance or trending towards significance (defined as $p < 0.10$) are highlighted in bold. Maturation subtype, senescence (CD8+ CC28−), and NKT+ KLRG1+ cells were associated with MELD-XI or SOFA scores; maturation subtype and exhaustion (CD4+ PD1+CD57+) was associated with infection; and activation (CD4+ CD38+) and NKT cells expressing KLRG1 were associated with death. As discussed above, age is an important clinical factor associated with adverse outcomes after MCSD implantation. We corrected for age to determine whether age was a confounder impacting the observed association between T cell senescence, exhaustion and maturation, however, a statistically significant association remained between markers of immune dysfunction and clinical outcomes, indicating that these immune markers were important independent of patient age (Table 5b). Correction for CMV antibody status led to similar results as correction for age (data not shown). Analysis of these post-operative values were more predictive of clinical outcomes compared with pre-implantation analysis.

4. Discussion

Despite years of experience with MCSD implantation, it remains unclear why a sizeable minority of patients develop post-operative complications including infection, multiorgan dysfunction and death. In this analysis of MCSD recipients, we found that T cell maturation phenotypes correlated with patient outcomes, as measured by infection, death, MELD-XI and SOFA scores. We also observed increased frequencies of senescent and exhausted CD8+ T cells in patients with increased age, increased MELD-XI scores, and death. The association between T cell dysfunction and increased patient age has been previously described; however, this finding appears to more pronounced in our population of patients with advanced heart disease. While the findings reported in Table 5a. demonstrate the importance of patient age in association with T cell dysfunction in predicting patient outcomes, we have shown that T cell dysfunction has predictive value independent of patient age, as mixed effect analysis correcting for repeated measures further supported the association between T cell immune dysfunction as demonstrated by increased frequency of maturation subtypes, exhaustion, and senescence and the development of adverse clinical outcomes (Table 5b). Somewhat surprisingly, pre-MCSD characteristics including need for temporary assist devices also had impact on post-implant T cell phenotypes. Patients receiving pulsatile devices (PVAD or TAH) also demonstrated increased frequency of senescent and exhausted T cells, although this observation is limited by the relatively small numbers of patients receiving these devices in our cohort.

The important findings of our study are two-fold. First, we provide evidence that age and disease severity as measured by INTERMACS score are independently associated with differences in immune function as assessed by multiparameter immune phenotyping, shedding light into possible mechanisms associated with the development of multi-organ system dysfunction. Second, we provide data from this pilot study suggesting that measurement of these immunologic factors, in combination with clinical data, may provide important prognostic information, to predict outcomes among the highest risk patients, before and after MCSD implantation (Tables 5a, 5b, 6). These immunologic measurements are dominant even when age is included in the analysis, suggesting that immune phenotypes

may be predictive of clinical outcomes independent of patient age. Noninvasive measurement of immunologic parameters holds the potential for creation of a composite model for outcome prediction in a manner analogous to other cardiac risk models such as the AlloMap test for determination of risk for rejection after heart transplantation [25,26]. This could be applied during initial evaluation of patients with advanced heart failure to provide a mechanism-based measure of risk and allow for adequate decision-making regarding utility vs. futility of MCSD placement, and would also allow for identification of high risk patients for closer monitoring after implantation.

Another intriguing finding of our study was the potential utility of pre-implantation immune cell phenotyping to predict post-implantation outcomes up to 3 months post-surgery. Future studies will be necessary to explore whether patients who survive the early post-implantation phase despite measurable immune dysfunction may ultimately undergo ‘immunologic remodeling’ or whether their senescent or exhausted T cell phenotype will persist. This hypothetical ability for immunologic rehabilitation may be age-limited, with younger but not older patients having the potential for improvement.

Limitations to this study include the relatively small sample size and the heterogeneity of the patients included, in terms of MCSD type. Given the small number of adverse clinical outcomes, it was difficult to link immune phenotype to infrequent events such as death in the multivariate analysis. As a pilot study, however, the number of patients included is similar to other studies of immune dysfunction after MCSD implantation [10,11,13], and we observed a stability of immunophenotype after MCSD implantation regardless of device type (Supplemental Fig. 1). An advantage of the single center design for a pilot study is homogeneity in patient medical management and waiting time for heart transplantation. Despite these potential limitations, the findings from this pilot study suggest the potential for noninvasive monitoring of immunologic cells in prediction of clinical outcomes. Future multicenter studies including a more homogenous population in terms of MCSD type will be key to validate the findings from this study. In addition, future studies will follow those patients successfully bridged to heart transplantation to determine whether the immunologic changes observed during the period of MCSD implantation influence alloreactivity, development of donor-specific antibodies, and frequency of cellular or antibody-mediated rejection after transplantation.

As the numbers of patients with advanced heart disease continue to increase, especially in the older population, the need for MCSD implantation will continue to grow. The finding that older patients demonstrate increased frequency of markers of T cell dysfunction suggests that impaired T cell function including exhaustion and immunosenescence may be the mechanism by which older MCSD recipients experience increased rates of infection and death. Immunologic characterization of peripheral blood is a powerful and noninvasive technique that may improve patient risk stratification before and after surgical intervention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

MCSD	mechanical circulatory support
PBMC	peripheral blood mononuclear cell

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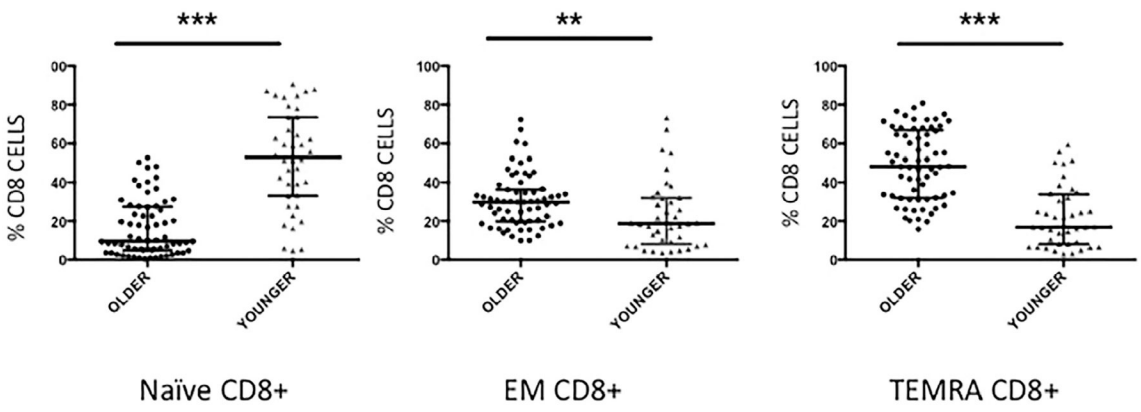

Fig. 1a.

Frequency of CD8+ maturation subtypes by patient age. PBMC from time points after MCSD implantation were analyzed for naive (CCR7+/CD45RA+), effector memory (EM) (CCR7−/CD45RA−), and terminally differentiated effector memory RA+ (TEMRA) (CCR7−/CD45RA+) CD8+ T cell content, expressed as a percentage of the total number of CD8+ T cells. Each dot corresponds to a sample; bars indicate median. *** indicates $p < 0.001$ and ** indicates $p < 0.01$, by nonparametric test.

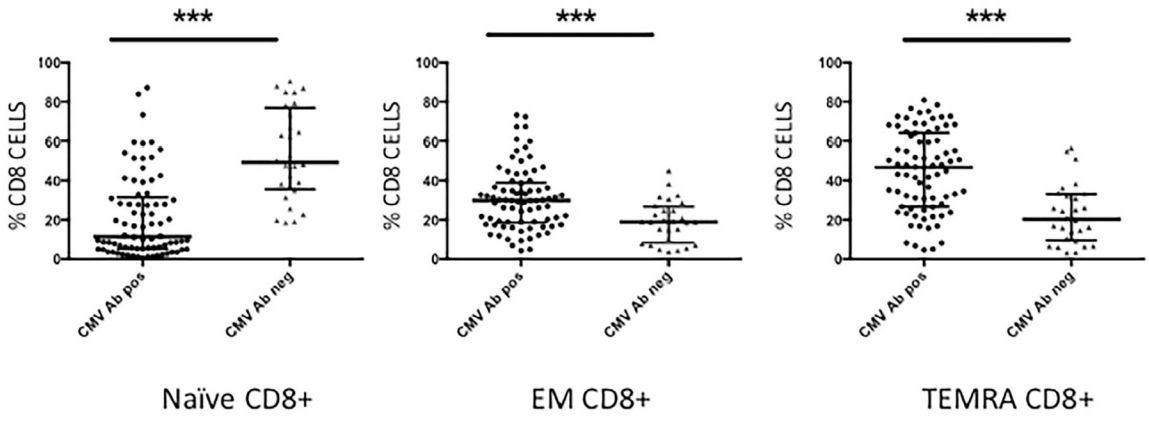

Fig. 1b.

Frequency of CD8+ maturation subtypes by CMV antibody status. PBMC from time points after MCSD implantation were analyzed for naive (CCR7+/CD45RA+), effector memory (EM) (CCR7−/CD45RA−), and terminally differentiated effector memory RA+ (TEMRA) (CCR7−/CD45RA+) CD8+ T cell content, expressed as a percentage of the total number of CD8+ T cells. Each dot corresponds to a sample; bars indicate median. *** indicates $p < 0.001$ by nonparametric test.

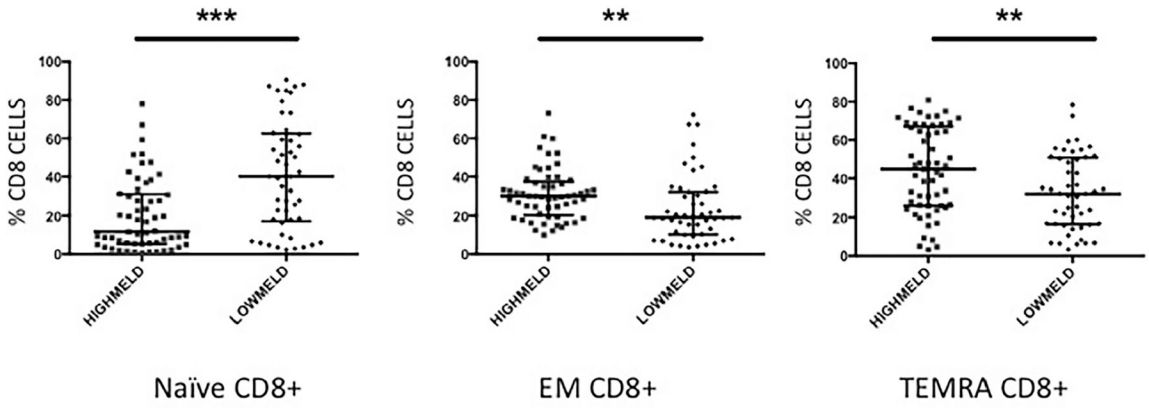

Fig. 1c.

Frequency of CD8+ maturation subtypes by MELD-XI score. PBMC from time points after MCSD implantation were analyzed for naive (CCR7+/CD45RA+), effector memory (EM) (CCR7−/CD45RA−), and terminally differentiated effector memory RA+ (TEMRA) (CCR7−/CD45RA+) CD8+ T cell content, expressed as a percentage of the total number of CD8+ T cells. Each sample was evaluated for MELD-XI score at the corresponding timepoint. 'HighMELD' defined as ≥16, and 'LowMELD as < 16′. Each dot corresponds to a sample; bars indicate median. *** indicates $p < 0.001$; ** indicates $p < 0.01$, by nonparametric test.

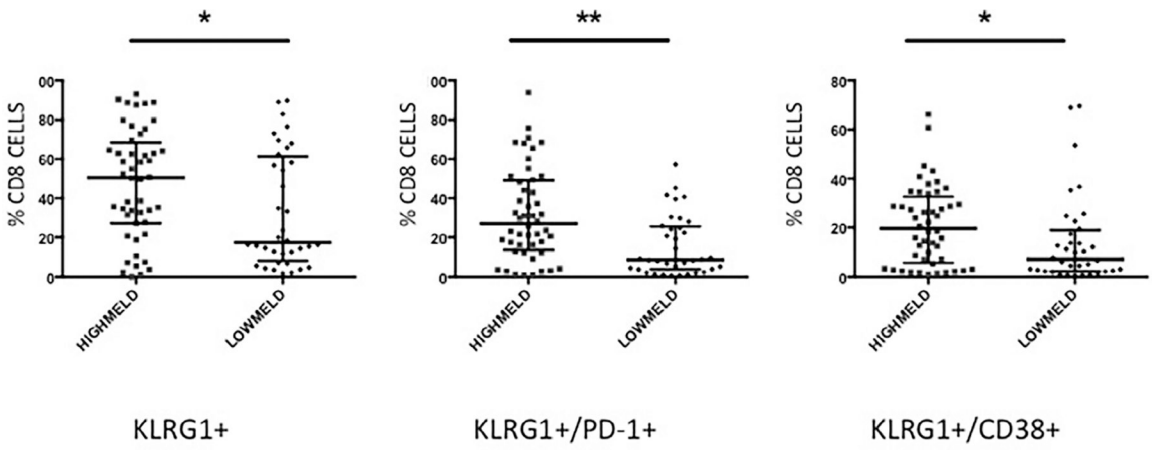

Fig. 1d.

Frequency of senescent, exhausted, and activated CD8+ T cells by MELD-XI score. PBMC from time points post MCSD implant were analyzed for senescent (KLRG1+), exhausted, (KLRG1+/PD-1+), or activated (KRLG1+/CD38+) CD8+ T cells, expressed as a percentage of the total number of CD8+ T cells. Each sample was evaluated for MELD-XI score at the corresponding timepoint. 'HighMELD' defined as ≥16, and 'LowMELD' as < 16'. Each dot corresponds to a sample; bars indicate median. ** indicates $p < 0.01$, and * indicates $p < 0.05$, by nonparametric testing.

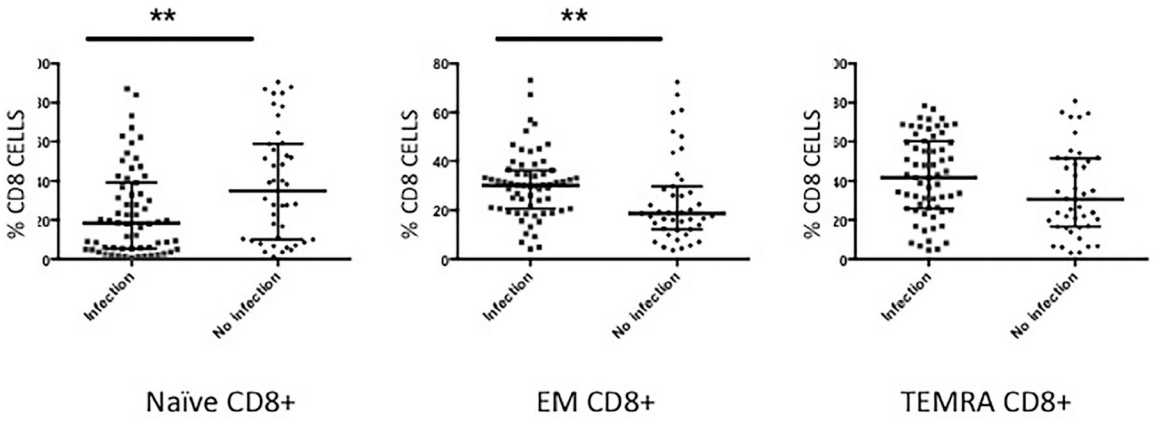

Fig. 2a.

Frequency of CD8+ maturation subtypes by post-implant infection. PBMC from time points post MCSD implant were analyzed for naive (CCR7+/CD45RA+), effector memory (EM) (CCR7−/CD45RA−), and terminally differentiated effector memory RA+ (TEMRA) (CCR7−/CD45RA+) CD8+ T cell content, expressed as a percentage of the total number of CD8+ T cells. Each dot corresponds to a sample; bars indicate median. ** indicates $p < 0.01$ by nonparametric testing.

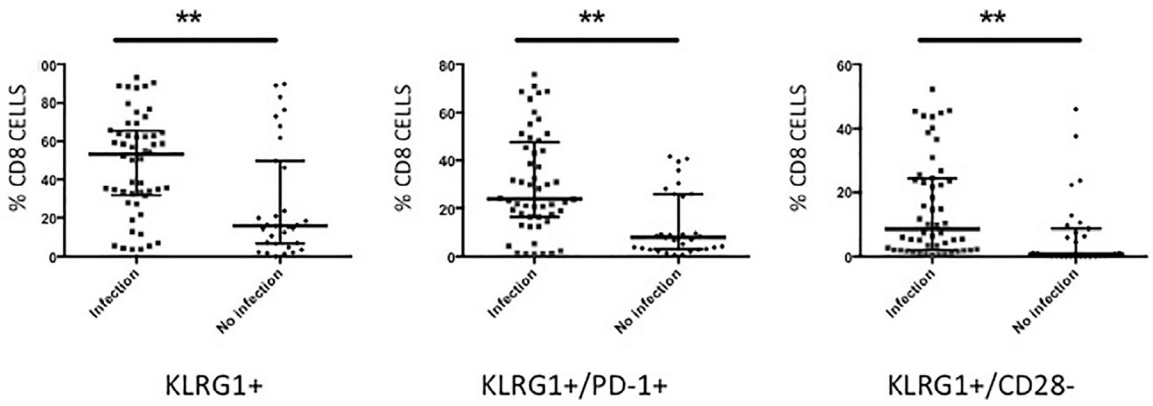

Fig. 2b.

Frequency of senescent and exhausted CD8+ T cells by post-implant infection. PBMC from time points after MCSD implant were analyzed for KLRG1+, exhausted (KLRG1+/PD-1+), and senescent (KLRG1+/CD28−) CD8+ T cells, expressed as a percentage of the total number of CD8+ T cells. Each dot corresponds to a sample; bars indicate median. ** indicates $p < 0.01$ by nonparametric testing.

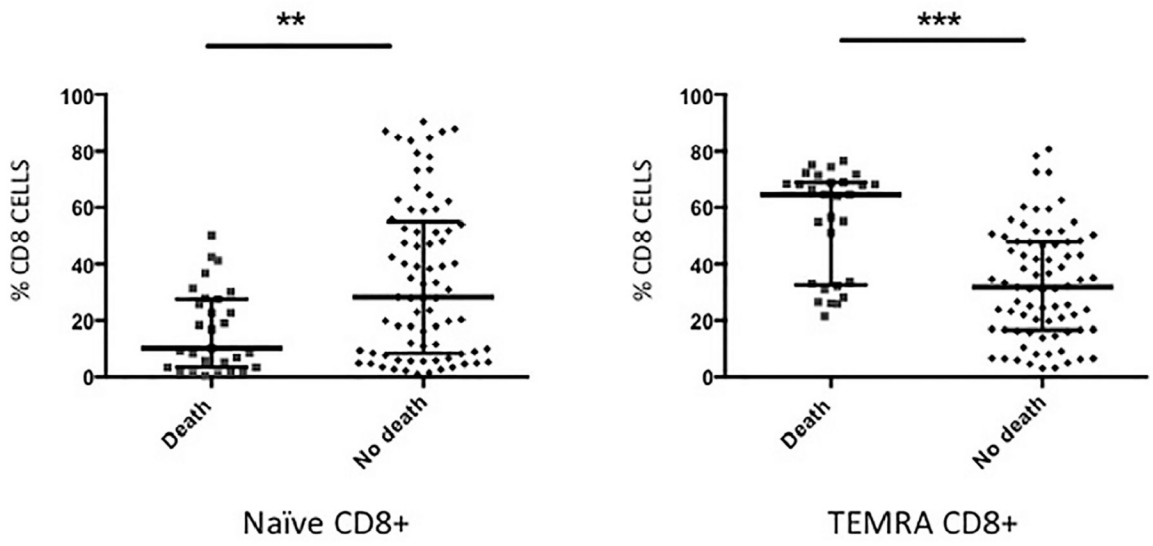

Fig. 2c.

Frequency of CD8+ maturation subtypes by patient survival at 3 months post-implantation. PBMC from time points after MCSD implant were analyzed for naive (CCR7+/CD45RA+) and terminally differentiated effector memory RA+ (TEMRA) (CCR7−/CD45RA+) CD8+ T cell content, expressed as a percentage of the total number of CD8+ T cells. Each dot corresponds to a sample; bars indicate median. *** indicates $p < 0.001$, and ** indicates $p < 0.01$ by nonparametric testing.

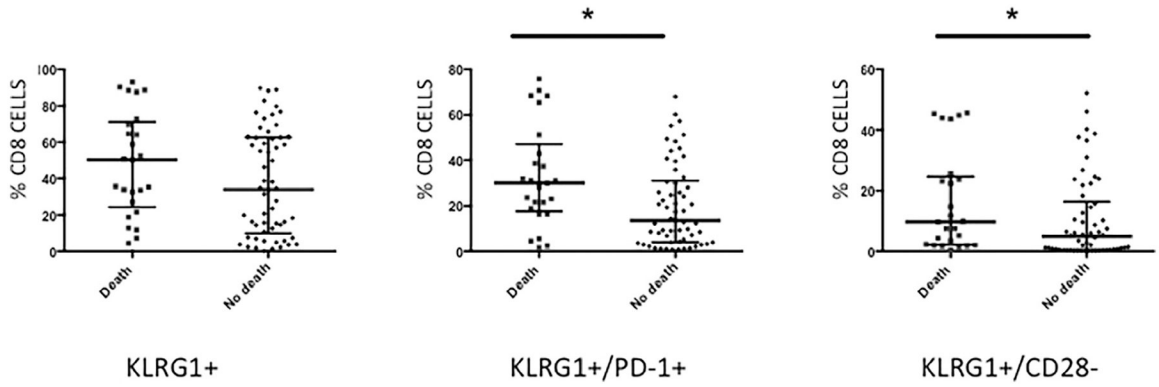

Fig. 2d.

Frequency of senescent and exhausted CD8+ T cells by patient survival at 3 months post-implantation. PBMC from time points after MCSD implant were analyzed for senescent (KLRG1+) and exhausted (KLRG1+/PD-1+) CD8+ T cells, expressed as a percentage of the total number of CD8+ T cells. Each dot corresponds to a sample; bars indicate median. * indicates $p < 0.05$ by nonparametric testing.

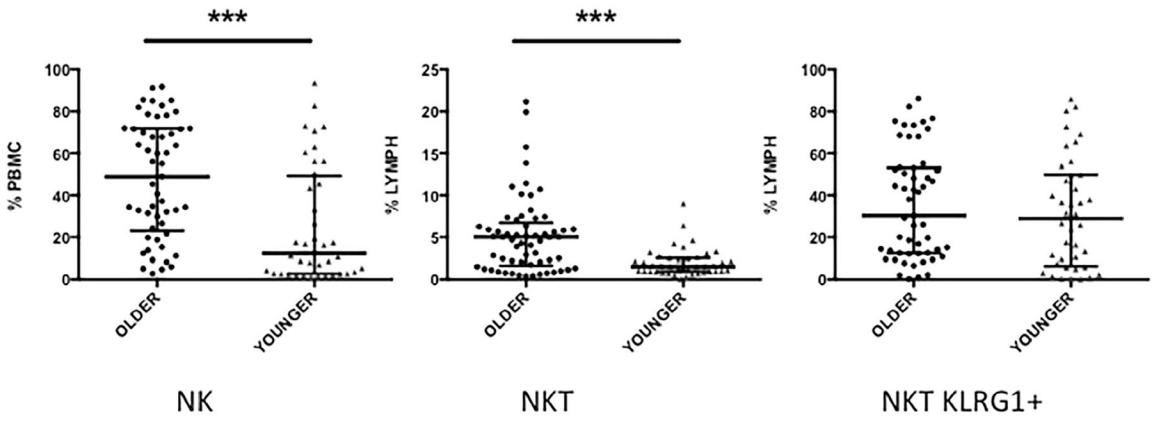

Fig. 3a.

Frequency of NK (CD56+ KLRG1+) NKT, and NTK KRLG1+ cells by patient age. PBMC from all time points after MCSD implant were analyzed. The data are expressed as a percentage of the total number of viable PBMC or lymphocytes, respectively. Each dot corresponds to a sample; bars indicate median. *** indicates $p < 0.001$ by nonparametric test.

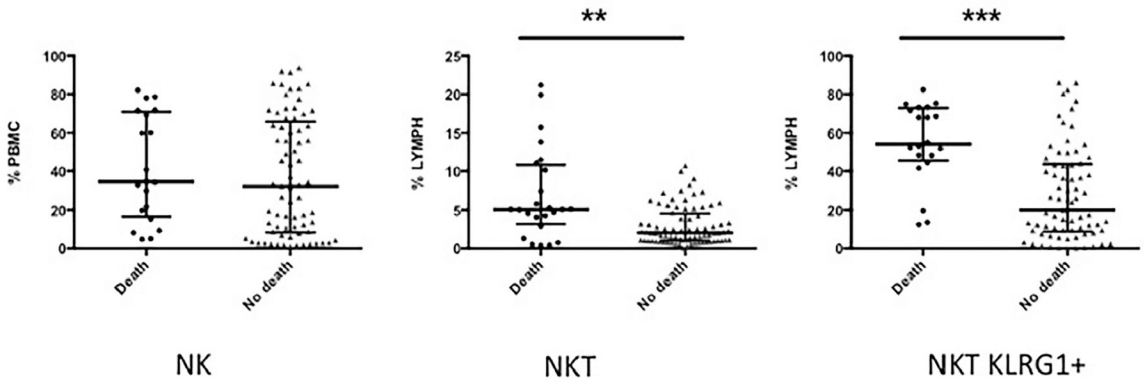

Fig. 3b.

Frequency of NK (CD56+ KLRG1+) NKT, and NTK KRLG1+ cells by death at 3 months post-implantation. PBMC from time points after MCSD implant were analyzed. The data are expressed as a percentage of the total number of viable PBMC or lymphocytes, respectively. Each dot corresponds to a sample; bars indicate median. *** indicates $p < 0.001$ by nonparametric test, and ** indicates $p < 0.01$ by nonparametric testing.

Table 1

Demographic characteristics of study participants (n = 28). % (n) for each variable.

Age (yrs) (median) (range)	61 (24–80)
Older (≥age 60)	53.6% (15)
Sex (% male)	78.6% (22)
Nonischemic CMY	71.4% (20)
Intended bridge to transplantation	85.7% (24)
HeartMate II device	71.4% (20)
RVAD *	35.7% (10)
INTERMACS 1/2	60.7% (17)

* RVAD includes right-sided Centrimag or ventricular support from PVAD or TAH.

Table 2

Demographic characteristics of study participants by high MELD-XI (≥18) (n = 18) compared with low MELD-XI group (< 18) (n = 10) at Day 0, prior to MCSD implantation. Bold font indicates $p < 0.05$.

Characteristic	High MELD-XI	Low MELD-XI	p-value
Age (yrs), median (range)	63.0 (36–74)	43.0 (24–80)	0.058
Older (≥ age 60)	72.2%	20.0%	**0.017**
Sex (% male)	88.9%	60.0%	0.147
Nonischemic CMY	72.2%	70.0%	1.000
Intended bridge to transplantation	66.7%	77.8%	0.669
HeartMate II device	72.2%	70.0%	0.921
RVAD *	27.8%	50.0	0.412
INTERMACS 1/2	83.3%	20.0%	**0.003**
SOFA score, median (range)	10 (5–16)	6 (3–10)	< **0.001**

* RVAD includes right ventricular support from PVAD or TAH.

Table 3

Demographic characteristics of study participants by Age ≥60 (Older) (n = 15) compared with those < 60 (Younger) (n = 13) at time of implantation. Bold font indicates $p < 0.05$.

Characteristic	Older	Younger	p-value
Age (yrs) (median) (range)	63.0 (36–74)	43.0 (24–80)	N/A **
Sex (% male)	93.3%	61.5%	0.069
Nonischemic CMY	53.3%	92.3%	**0.038**
Intended bridge to transplantation	58.3%	83.3%	0.371
HeartMate II device	80.0%	61.5%	0.322
RVAD *	20.0%	53.95	0.114
INTERMACS 1/2	73.3%	46.2%	0.246
MELD-XI at Day, median (range)	20.1 (13.0–28.8)	14.0 (9.4–21.5)	**0.009**
SOFA at Day 0, median (range)	8 (4–16)	5.5 (3–13)	**0.047**
CMV ANTIBODY positive	80.0%	69.2%	0.670

* RVAD includes right ventricular support from PVAD or TAH.

** Statistical analysis not performed as cohorts defined by age.

Table 4

Clinical outcomes of study participants (n = 28). % (n) for each variable.

Infection pre- or post-implantation	78.6% (22)
Severe infection post-implantation	64.3% (18)
Successful bridge to transplant *	70.8% (17) *
Death 30 days	14.3% (4)
Death 3 months	25.0% (7)
Death 1 year	35.7% (10)
Days to death (median) (range)	50 (12–736)
Infection pre-MCSD (≤ 1 month)	25.0% (7)
Infection post-MCSD (≤ 6 months)	53.6% (15)
Severe Infection post-MCSD (≤ 6 months)	46.4% (13)
Significant bleeding post-MCSD (≤ 6 months)	21.4% (6)

* Excluding 4 patients with MCS implant as destination therapy.

Table 5a

Univariate analysis of age and immune phenotype on clinical outcomes, p values < 0.100 in bold.

Effect	MELD-XI		SOFA		Time to infection		BTT*		Time to death	
	Diff. (95% CI)	p	Diff. (95% CI)	p	HR (95% CI)	p	OR (95% CI)	p	HR (95% CI)	p
Age (yrs)	0.17 (0.06, 0.29)	**0.004**	0.09 (0.01, 0.17)	**0.030**	1.00 (0.97, 1.04)	0.857	0.91 (0.83, 1.00)	**0.061**	1.01 (0.97, 1.05)	0.635
CM CD4 +§	0.11 (−0.01, 0.23)	**0.063**	0.04 (−0.05, 0.14)	0.372	1.00 (0.94, 1.06)	0.966	0.91 (0.81, 1.02)	**0.096**	1.06 (0.99, 1.13)	**0.096**
EM CD4 +§	0.01 (−0.06, 0.09)	0.762	0.03 (−0.03, 0.08)	0.392	1.02 (0.99, 1.05)	0.298	0.97 (0.91, 1.04)	0.366	1.02 (0.99, 1.05)	0.244
TMRA CD4 +§	0.25 (−0.05, 0.55)	**0.098**	0.04 (−0.20, 0.28)	0.743	1.09 (0.98, 1.22)	0.129	0.99 (0.79, 1.24)	0.917	0.93 (0.78, 1.12)	0.466
CM CD8 +¶	0.43 (0.07, 0.79)	**0.020**	0.32 (0.03, 0.61)	**0.033**	1.03 (0.69, 1.55)	0.878	0.71 (0.45, 1.12)	0.138	1.15 (0.89, 1.49)	0.292
EM CD8 +¶	0.09 (0.01, 0.17)	**0.023**	0.03 (−0.03, 0.09)	0.368	1.00 (0.96, 1.03)	0.872	1.03 (0.94, 1.12)	0.565	1.01 (0.98, 1.05)	0.485
TMRA CD8 +¶	0.07 (0.00, 0.14)	**0.038**	0.06 (0.01, 0.11)	**0.030**	1.03 (1.00, 1.05)	**0.060**	0.94 (0.88, 1.00)	**0.070**	1.01 (0.98, 1.04)	0.564
CD4+ PD1 + CD57 +	−0.04 (−0.15, 0.07)	0.506	−0.05 (−0.14, 0.04)	0.252	1.06 (1.01, 1.11)	**0.026**	0.97 (0.86, 1.08)	0.565	1.00 (0.95, 1.06)	0.969
CD8+ PD1 + CD57 +	0.05 (−0.09, 0.19)	0.486	−0.02 (−0.13, 0.08)	0.656	1.01 (0.97, 1.05)	0.593	0.94 (0.86, 1.04)	0.222	1.02 (0.98, 1.07)	0.331
CD8+ CD28−	0.04 (0.00, 0.08)	**0.077**	0.04 (0.00, 0.07)	**0.045**	1.00 (0.98, 1.02)	0.825	1.00 (0.95, 1.05)	0.937	1.01 (0.98, 1.03)	0.630
CD8+ KLRG1 +	0.02 (−0.03, 0.08)	0.404	0.00 (−0.04, 0.04)	0.958	1.00 (0.98, 1.02)	0.797	0.99 (0.95, 1.03)	0.598	1.00 (0.98, 1.03)	0.667
CD8+ KRLG1+CD28−	0.06 (−0.03, 0.15)	0.211	0.02 (−0.04, 0.09)	0.474	0.99 (0.97, 1.02)	0.565	1.01 (0.96, 1.06)	0.738	1.00 (0.98, 1.03)	0.998
CD4+ CD38 +	0.06 (−0.02, 0.15)	0.151	0.05 (−0.01, 0.12)	0.129	0.98 (0.94, 1.02)	0.309	1.03 (0.96, 1.11)	0.377	0.95 (0.91, 0.99)	**0.026**
CD8+ CD38 +	−0.02 (−0.08, 0.05)	0.614	−0.01 (−0.06, 0.03)	0.589	1.00 (0.98, 1.02)	0.869	1.00 (0.96, 1.05)	0.988	0.99 (0.96, 1.01)	0.373
NKT+ KLRG1 +	0.04 (−0.02, 0.09)	0.235	0.04 (0.01, 0.08)	**0.022**	0.99 (0.97, 1.01)	0.285	0.96 (0.91, 1.01)	0.128	1.03 (1.01, 1.06)	**0.012**

Diff.: Difference; CM, Central Memory; EM, Effector Memory, TMRA, Terminally differentiated RA+.

*BTT: Bridge to Transplant, excluding 4 patients with MCS implant as destination therapy.

§Compared to CD4+ CCR7+RA+ reference group.

¶Compared to CD8+ CCR7+RA+ reference group.

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Table 5b

Multivariate analysis of association of immune phenotype and clinical outcomes, adjusted by age; p values < 0.100 in bold.

Effect	MELD-XI		SOFA		Time to infection		BTT*		Time to death	
	Diff. (95% CI)	p	Diff.(95% CI)	p	HR (95% CI)	p	OR (95% CI)	p	HR (95% CI)	p
CM CD4 +§	0.09 (−0.03, 0.21)	0.138	0.02 (−0.08, 0.12)	0.689	1.00 (0.94, 1.07)	0.919	0.94 (0.83, 1.07)	0.373	1.06 (0.99, 1.13)	**0.091**
EM CD4 +§	0.00 (−0.08, 0.07)	0.938	0.01 (−0.04, 0.07)	0.644	1.02 (0.99, 1.05)	0.228	1.00 (0.92, 1.08)	0.908	1.02 (0.98, 1.05)	0.348
TMRA CD4 +§	0.25 (−0.04, 0.54)	**0.094**	0.04 (−0.19, 0.27)	0.748	1.09 (0.98, 1.22)	0.105	0.99 (0.76, 1.30)	0.952	0.93 (0.78, 1.12)	0.463
CM CD8 +¶	0.35 (−0.04, 0.73)	**0.082**	0.25 (−0.07, 0.57)	0.125	1.14 (0.74, 1.76)	0.547	0.82 (0.50, 1.33)	0.417	1.21 (0.88, 1.65)	0.239
EM CD8 +¶	0.08 (0.00, 0.16)	**0.043**	0.02 (−0.04, 0.08)	0.501	1.01 (0.97, 1.05)	0.774	1.05 (0.95, 1.16)	0.355	1.01 (0.98, 1.05)	0.468
TMRA CD8 +¶	0.04 (−0.03, 0.12)	0.262	0.04 (−0.03, 0.10)	0.248	1.07 (1.02, 1.13)	**0.006**	0.98 (0.90, 1.07)	0.686	1.02 (0.97, 1.07)	0.428
CD4+ PD1 + CD57 +	−0.06 (−0.17, 0.05)	0.316	−0.06 (−0.15, 0.02)	0.141	1.06 (1.01, 1.12)	**0.026**	0.96 (0.83, 1.12)	0.629	1.00 (0.94, 1.06)	0.963
CD8+ PD1 + CD57 +	0.01 (−0.13, 0.15)	0.893	−0.06 (−0.16, 0.05)	0.277	1.01 (0.96, 1.06)	0.687	0.99 (0.88, 1.10)	0.795	1.02 (0.97, 1.08)	0.365
CD8+ CD28−	0.03 (−0.01, 0.08)	0.119	0.03 (0.00, 0.07)	**0.066**	1.00 (0.98, 1.02)	0.790	1.01 (0.95, 1.07)	0.865	1.01 (0.98, 1.03)	0.627
CD8+ KLRG1 +	0.03 (−0.03, 0.08)	0.322	0.00 (−0.04, 0.04)	0.862	1.00 (0.98, 1.02)	0.686	0.99 (0.95, 1.03)	0.715	1.01 (0.98, 1.03)	0.594
CD8+ KRLG1+CD28−	0.05 (−0.03, 0.14)	0.232	0.02 (−0.04, 0.08)	0.543	0.99 (0.96, 1.02)	0.499	1.02 (0.95, 1.10)	0.598	1.00 (0.98, 1.03)	0.966
CD4+ CD38 +	0.07 (−0.02, 0.15)	0.111	0.06 (−0.01, 0.12)	**0.076**	0.98 (0.94, 1.03)	0.403	1.04 (0.95, 1.12)	0.395	0.95 (0.91, 1.00)	**0.031**
CD8+ CD38 +	−0.01 (−0.07, 0.05)	0.680	−0.01 (−0.05, 0.04)	0.772	1.00 (0.98, 1.02)	1.000	1.01 (0.96, 1.06)	0.763	0.99 (0.96, 1.01)	0.349
NKT+ KLRG1 +	0.03 (−0.03, 0.09)	0.288	0.03 (0.00, 0.07)	**0.029**	0.99 (0.96, 1.01)	0.198	0.97 (0.92, 1.02)	0.253	1.03 (1.01, 1.06)	**0.012**

Diff.: Difference; CM, Central Memory; EM, Effector Memory, TMRA, Terminally differentiated RA+.

*BTT: Bridge to Transplant, excluding 4 patients with MCS implant as destination therapy.

§Compared to CD4+ CCR7+RA+ reference group.

¶Compared to CD8+ CCR7+RA+ reference group.

Table 6

Factors including immunologic markers impacting patient outcome after MCSD implantation. Patients with older age and/or immunologic dysfunction more likely to experience adverse clinical outcomes including markers of multiorgan system dysfunction (MELD-XI, SOFA), failure to bridge to transplantation, infection, and death. Younger patients and those with potential for immunologic adaptation, as manifested by increased frequency of naïve cells, are more likely to experience good clinical outcomes after MCSD implantation.

Associated with improved outcomes	Associated with worse outcomes
Younger patient age	Older patient age
Increased frequency naive CD4+ and CD8+ T cells	Increased frequency terminally differentiated and effector memory CD8+ T cells
INTERMACS 3/4 at implantation	Increased frequency exhausted and senescent CD8+ T cells
Lower MELD-XI and SOFA scores	Increased frequency NKT and NKT KLRG1+ cells