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Clinical Phenomapping and Outcomes after Heart Transplantation

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Abstract

Background—Survival after heart transplantation (HTx) is limited by complications related to alloreactivity, immune suppression, and side effects of pharmacological therapies. We hypothesize that time-dependent phenomapping of clinical and molecular datasets is a valuable approach to clinical assessments and guiding medical management to improve outcomes.

Methods—We analyzed clinical, therapeutic, biomarker, and outcome data from 94 adult HTx patients and 1557 clinical encounters performed between January 2010 and April 2013. Multivariate analyses were employed to evaluate the association between immunosuppression therapy, biomarkers, and the combined clinical endpoint of death, allograft loss, retransplantation, and rejection. Data were analyzed by K-means clustering (k=2) to identify patterns of similar combined immunosuppression management, and percentile slopes were computed to examine the changes in dosages over time. Findings were correlated with clinical parameters, HLA antibody titers, peripheral blood mononuclear cell gene expression of the AlloMap test genes, and an intragraft, heart tissue gene co-expression network analysis was performed.

Results—Unsupervised cluster analysis of immunosuppressive therapies identified two groups, one characterized by a steeper immunosuppression minimization, associated with a higher

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likelihood for the combined endpoint, and the other by a less pronounced change. A time-dependent phenomap suggested that patients in the higher event rate group had increased HLA class I and II antibody titers, higher expression of the FLT3 AlloMap gene, and lower expression of the March8 and WDNR40A AlloMap genes. Intramyocardial biomarker-related co-expression network analysis of the FLT3 showed an immune system-related network underlying this biomarker.

Conclusion—Time-dependent precision phenotyping is a mechanistically insightful, data-driven approach to characterize patterns of clinical care and identify ways to improve clinical management and outcomes.

Introduction

Despite medical advances in heart transplantation (HTx), the median survival is only 13 years.[1] Survival is limited by effects of the immune system on the cardiac allograft and by clinical consequences related to immunosuppression.[2] Infection, rejection, malignancy, and cardiac allograft vasculopathy (CAV) are common complications after HTx.[3,1] The mechanisms driving these diseases are not well understood, but they generally involve immune-mediated responses against the cardiac allograft. Antibodies to cardiac donor allograft antigens are risk factors for ACR, CAV, and cardiac allograft failure, compromising survival.[4,5,6]

The effect of immunosuppression on determinants of graft survival is not well understood, and current therapies have not been effective in preventing the development and progression of ACR, CAV, and cardiac allograft failure.[7,8,9] Typically, management of immunosuppression involves down-titration of one or more drugs over time, with various combinations of drugs and dosages making it difficult to assess the effects of these combinations.[10,11] Studies that involved combination therapy have shown differential incidences of CAV,[12,13] and studies using monotherapy have been linked to similar outcomes and lack of CAV progression.[14] Changes in the management of various drugs are also associated with differential effects on the immune system of the host.[15] Although physicians strive to make clinical decisions based on the knowledge embodied in medical literature, in the absence of sufficient research guiding clinical practice, decision-making is challenging. Each encounter requires an individualized decision-making process to avoid complications caused by activation, suppression, and positive or negative modulation of the immune system.

The use of noninvasive biomarkers to provide information on the individualized immune response after HTx has guided decision-making and improved clinical care. Yet, no single biomarker is sufficient. Enabled by sophisticated computational tools and algorithms, precision medicine aims to improve prevention and treatment strategies.[16,17] Cluster analysis with dense phenotypic data, “phenomapping,” has been introduced to improve classification of complex heterogeneous phenotypes.[18,19,20] Reports on utilizing large datasets to identify clinical or immune-related biomarkers to guide management optimization after HTx are lacking.

We aimed to improve characterization of longitudinal phenotypes for guiding clinical management, hypothesizing that time-dependent variation in the management of immunosuppression is associated with differential clinical outcomes. We assessed the individual and time-dependent variation in the management of immunosuppression, classifying each patient using an unsupervised, machine-learning algorithm and comparing the relationship of the classification to a predefined composite clinical outcome. Subsequently, we analyzed the immune-specific phenotype by evaluating the differential clinical and biomarker profiles associated with the variation in time-dependent management of immunosuppression.

Methods

Study Population

Data from all adult patients who underwent HTx at the University of California Los Angeles between January 2010 and April 2013 were prospectively collected and retrospectively analyzed. Patients who were not followed regularly after transplant at our program were not included in the analysis. Medical records were accessed to collect variables obtained from each of outpatient HTx clinic visits. Data was obtained manually by a single investigator and through queries of structured data obtained from the electronic health records. Raw data for the AlloMap gene expression test was obtained from the manufacturer of the test. There were a total of 152 adult heart transplants between 2010–2013. Included in the analysis were 94 adult HTx recipients who had their post-transplant follow up at our center. Overall, these patients contributed 1,557 clinical encounters. A significant number of patients during the study period transferred their care to another institution either following their healthcare plan's standard practice of transitioning after 45 days post-HTx or to continue their care under a different care team. For these patients (n= 58), data was not available and therefore could not be included in the study. The number of encounters used in each step of the analysis varies based on data availability as described below. The study was approved by our Institutional Review Board, and a waiver of informed consent was requested given the retrospective nature.

Clinical, Laboratory and Biomarker Data

Clinical and immune-related variables were collected at baseline and longitudinally during post-HTx follow-ups as part of our standardized allograft and immune monitoring protocol, which includes endomyocardial biopsies, brain natriuretic peptide (BNP) measurements, calcineurin inhibitor (CNI) levels, mycophenolate (MMF) and prednisone doses, anti-HLA class I and class II antibody screening by flow panel reactive antibodies (PRA), DSA single-antigen determinations by MFI, CD4 T-Cell ATP measurements (Viracor Eurofins, Lee's Summit, MO), and a molecular classifier (Allomap Molecular Test, CareDx Inc. Brisbane, CA).

Immunosuppression management was accomplished following a standard regimen with a CNI (typically tacrolimus), an antimetabolite (mycophenolate mofetil or MMF), and steroids. Immunosuppression minimization followed a standard protocol in addition to the clinical judgment of the treating clinician.[21] Medication doses for patients treated with either

cyclosporine or controlled-release mycophenolic acid were converted to equivalent levels or doses of either tacrolimus or MMF, respectively.

Endomyocardial biopsies are typically performed during the first 3–6 month post-transplant and are graded by a panel of cardiac pathologists following the consensus guidelines.[22] For clinically stable patients, non-invasive rejection surveillance is implemented using AlloMap gene expression test.

Post-transplant Luminex PRA testing (Gen-Probe, San Diego, California) is usually performed at weeks 1, 2, 3, 4, and 6; months 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 21, and 24; and quarterly thereafter.[11] For patients with positive antibody screening, Luminex bead-based mean fluorescence intensity (MFI) assays (One Lambda, Inc., Canoga Park, CA) are used to detect antibodies against single anti-HLA class I and class II antigens. The presence of CAV is routinely assessed by coronary angiograms and intravascular ultrasounds (IVUS), typically at 6 weeks, 1 year, and yearly thereafter during the first 3 to 5 years after HTx, with variations according to clinical criteria. CAV was graded by interventional cardiologists and independently verified by one of two transplant cardiologists and a member of the research team following published guidelines.[23]

Information on expression profiling of AlloMap (CareDx Inc, CA) molecular test genes were available after day 56 post-HTx and was obtained monthly during the first year post HTx and generally every 3 months during the 2nd and 3rd years. AlloMap testing entails 20 genes, 9 control and 11 informative genes. A molecular score is obtained through the combined analysis of these genes, which run in triplicate including IL1R2, FLT3, ITGAM, MARCH8, WDR40A, PF4, C6orf25, ITGA4, PDCD1, RHOU, and SEMA7A.[24,25,26] We focused this analysis on expression of the individual genes.

Assessment of Clinical Outcomes

We evaluated the combined endpoint of time to either biopsy-proven ≥2R acute cellular rejection (ACR), grades 1 or 2 antibody-mediated rejection (AMR), ≥ISHLT 1 CAV[1], death, allograft failure or re-transplantation.

Statistical Analyses

To characterize the study population and to make comparisons between groups, descriptive statistics (counts, percentages, means, and standard deviations) were generated for baseline demographic and clinical information. Trajectories of the medications and biomarkers were characterized by use of a mixed-effects linear regression model with a linear random and fixed effect for time since transplant (in days) nested within a patient random intercept. Patients were assigned values for their intercepts and slopes based upon the fixed effect intercepts (or slopes) and their individual deviations from the mean value. The intercept values represented the starting levels for the patients, and the slopes the rates of change. These values were estimated by empirical Bayesian posterior means. A Cox proportional hazards model was used to assess the association between clinical endpoints and the estimated slopes and intercepts. These models were fit using data from > 30 days post-HTx until the event time. Since there was no standard measure of the long-term exposure to immunosuppression, the slope and intercept of the time-dependent exposure measure (either

dose for MMF or prednisone or serum level for CNI) were combined by evaluating the percentage of maximum dosage for MMF (max = 3,000 mg), prednisone (max = 20 mg), and CNI serum level (max = 15 ng/ml). After conversion to percentiles, values > 100 percent were rounded to 100. The mixed model was used to create a slope and intercept for each patient for the combined percentile biomarker. Subsequently, K-means clustering (k=2) was used to identify patterns of similar immunosuppression management among patients, based on the MMF, CNI, and prednisone percentile slopes. These analyses and the aforementioned descriptive statistics were conducted using Stata version 14 (StataCorp LP, College Station, TX).

Immunophenomap, the visual arrangement of directly or indirectly involved clinical and immune monitoring variables was performed in GeneSpring 12.6 (Agilent, Santa Clara, CA).[27] The unpaired Mann-Whitney U-test was used to identify statistically significant differences among AlloMap genes over time. A two-sided p-value < 0.05 was considered statistically significant. For residual missing data, imputation was performed on <10 % of the data using the multiple imputation method in the statistical analysis software package, SPSS (IBM Corp, New York, USA). The data were further organized into time-dependent groups, including information for single patients per time points in each group (as available) organized into 8 time points using the first single patient encounter information per time group (timepoint 0 < 30 days; timepoint 1, 30–50 days; timepoint 2, 50–100 days; timepoint 3, 100–150 days; timepoint 4, 150–200 days; timepoint 5, 200–300 days; timepoint 6, 300–365 days; and timepoint 7, ≥365 days). These groups included 76, 82, 89, 70, 66, 66, 54, and 66 patients from timepoints 0 to 7, respectively. There were 94 patients and 561 encounters available to generate clinical and immune-related phenomaps, and 82 patients and 361 encounters for the AlloMap gene expression test genes' phenomap. The number of encounters per patient may vary and not be the same for each patient.

Biomarker-based intramyocardial gene expression network

To correlate clinical findings with intramyocardial gene expression, we used tissue samples collected from 52 HTx patients: 20 patients from the same cohort and 32 independent patients who provided a total 64 heart tissue samples. A description of the methods and cohort is provided in the supplementary material section.

Results

Baseline Characteristics of the HTx Recipients

Data on 94 adult patients who received a HTx were collected. The major demographic and clinical characteristics of the population are summarized in Table 1. Most patients were male (n=71, 73%), with a predominant proportion of Whites (54%). The mean age was 54.9 ± 13.4 years. Of the population, 8.5 % (n=8) had histological evidence of varying degrees of ACR, AMR, or both. There were 6 episodes of AMR, and 3 patients had clinically significant (≥2R) ACR. The mortality rate for patients included in the study was 4% at a median follow-up of 461 days (Table 4).

Relationship between Immunosuppression, Biomarkers, and Clinical Outcomes

The slope for CNI was associated with time to the defined combined endpoint (Table 2); with each 1-unit increase in the slope (i.e., slower rate of minimization) of CNI level, there was a 20% decrease in risk of developing the combined clinical endpoint adjusted by age and gender (hazard ratio 0.80 [0.66, 0.96] p=0.018) (Table 2). Although, in unadjusted models, a slower rate of decline for prednisone was associated with an increased risk of the endpoint (HR=1.05, p=0.036), this was not seen after accounting for the effects of covariates. The prednisone intercept had low variability and could not be modeled. Neither the slopes nor intercepts for the other biomarkers alone were associated with the risk of having the combined endpoint.

Cluster Trends of Immunosuppression

Cluster analyses of the percentile slopes for MMF, CNI, and prednisone were conducted using 2 clusters (k=2), patient groups 1 and 2. A summary of the slope differences between clusters defined for each biomarker is presented in Table 3. The results indicated that group 1 (faster rate of immunosuppression minimization) also had the highest incidence of the combined endpoint (Table 4). The probability of occurrence of the combined endpoint in group 1 was 80% and 67% in group 2 (Table 4). A Kaplan-Meier analysis showing event-free survival is presented in Figure 1. Differences between the groups were compared using a Cox regression model, adjusting for age and sex. These showed that patients with faster rates of immunosuppression minimization (group 1), had a greater risk for the combined endpoint ($p<0.01$) compared to those with a slower rate (group 2**).** There was no significant association with patient groups and occurrence of de novo donor-specific antibodies (dnDSA), ACR (2+), AMR (1+), and mortality. Development of CAV was significantly associated with the patient group, suggesting that the rate of immunosuppression minimization may be involved in the development of allograft-related endpoints. The probability of developing CAV detected through angiography and IVUS was, respectively, 73.6% in group 1 for angiography; 89.2% in group 1 for IVUS; 60% in group 2 for angiography; and 92.9% in group 2 for IVUS (Table 4) with higher time to event rates. All deaths occurred in Group 1, and they were mainly of cardiac allograft origin.

Time-Dependent Phenomapping of Patient Groups

Correlation of the different clinical variables used to guide clinical management and the differences between the two groups are provided in Figure 2. Compared to group 1, group 2, at the time of transplant, had elevated HLA antibodies, which could lead to a slower taper in immunosuppression. For HLA class II antigens, most DRB locus antigen levels had the highest MFI levels; most DP and DQ antigens had intermediate strength; and most HLA class I A, B, C, and Cw had lower levels over time compared to group 1 (Figure 3). In the figures, data is visually displayed as heatmaps, representing only a small subset of patients with no statistical significance. The changes in immunosuppression minimization across patient groups is also provided as a heatmap showing the clinical management of immunosuppression for each group over time. Levels of BNP and CD4-T cell ATP over time are also depicted. There were no statistical differences between the groups, but patterns in the data trends are suggested (Figure 2). Assessment of genes that are part of the AlloMap

Gene Expression Test was accomplished for a subset of 435 samples. Among the 11 AlloMap genes (Table 6), patients in the steep management group were more likely to have higher levels of the FLT3 gene and lower levels of March8 and WDR40A genes (Figure 4).

Evaluation of the FLT3 Molecular Network in Heart Tissue Biopsies

We explored the WGCNA 64 heart tissue biopsy network (described in supplementary material), focusing on the FLT3 gene network, which was upregulated in patients with rejection. In this network, highly correlated genes were aggregated into modules following the WGCNA algorithm and consisted of 19 modules, with each module having several hundred genes. The FLT3-related network was largely part of an immune system module linked to more than 600 genes with over 8800 interactions enriched by genes described in the pathophysiology of the immune response associated with transplantation (Figure 5). Nodes related to FLT3 included the genes CD69, FCGR1C, PTPRCAP, CIITA, and MIR21.[28,29,30] Analysis of the expression of the intramyocardial MIR21 target gene of 117 experimentally proven targets (either by reporter assay, Western blots, or qPCR) showed increased mRNA abundance for 54 targets (46.1%, Figure 6).

Discussion

This study, which involved a complex dataset of various clinical parameters, was designed to understand the multivariable and time-dependent assessment of the relationship between biomarkers and clinical outcomes. An unsupervised cluster analysis was used to group patients into different time-dependent immunosuppressive strategies and to evaluate time-dependent changes in immune markers. Within this cohort, we differentiated two groups of patients with different event-free survival rates and longitudinal therapeutic and immunophenotypic profiles. Patients with higher event rates had more pronounced immunosuppression minimization and were possibly more likely to have HLA class I or class II antibodies, upregulation of the FLT3 gene, and down regulation of March8 and WDR40A genes. As determined in a heart biopsy dataset, intragraft gene expression of the FLT3 biomarker revealed a well differentiated inflammatory network with genes well known to be associated with cardiac allograft rejection.

A limitation in clinical studies is the ability to reconstruct or predict the effect of an intervention, reflecting the common pattern of practice in which a combination of drugs is used and minimized over time. In clinical medicine, predictive models tend to be assessments at a single point in time, rather than a longitudinal time-based information. To overcome this limitation, we conducted an unsupervised cluster analysis to capture information related to the therapeutic management of these patients on combined immunosuppressive therapies. Time-dependent clustering showed that a steeper (faster) decrease in immunosuppression was associated with an increased incidence of the combined endpoint of clinically significant cardiac allograft rejection, CAV, death, or re-transplantation. While these findings do not claim causality, they capture the practice of immunosuppression management over time and provide insights that can be helpful to design data-driven personalized care to guide long-term follow-up after transplantation.

Although immunosuppression minimization after HTx is a common goal of clinical practice, it may be associated with unwanted allograft effects. In the TICTAC study, treatment with the single agent tacrolimus was non-inferior to a combination of tacrolimus and mycophenolate.[31] In a subset of these patients, there was no evidence of greater development of vasculopathy, suggesting that single agent strategy is as safe and effective as standard care. In our study, there were molecular changes in the blood of patients with different management of immunosuppression. These changes included changes in anti-HLA antibody detection over time, mainly due to anti-HLA class II antibodies. Various types of HLA class I and II antibodies have been implicated in rejection and other immune-related diseases.[32,33,34] There was also variability in the expression of the IL2Ra, FLT3, MARCH8, and WDR40A genes. The IL2Ra gene is involved in immune regulation by controlling T cells.[35] Murine heart donors lacking the FLT3 ligand exhibit prolonged survival.[36,37] In patient group 2, the IL1R2 and FLT3 genes were down-regulated during the early period post-transplant. Conversely, in group 1, the MARCH8 gene, which is involved in control of HIV-1 infections,[38] and the WDR40A (DCAF12) gene, which is involved in protein ubiquitination,[39] were down-regulated in a more pronounced way. These findings were evident in the early post-HTx period, at least after the second month. Intramyocardial FLT3 gene expression network revealed the relationship between biomarker and allograft biology.[28,29,30,40]

Our study does not claim causality or provide a standardized framework to guide immunosuppression management. Instead, we reveal patterns hidden in the data and provide the initial step towards personalized, data-driven clinical care. To guide clinical management, time-dependent models and decision support algorithms should be further developed to allow better informed clinical decision making. These models, which would facilitate the transition to a more preventive rather than reactive clinical care, are incrementally perfectible, as more observations and contextual information is obtained while building on a natural experimental framework.[41]

For natural experimentation, clinical scenarios and outcomes are used to automate the creation of these experiments. Data driven approaches of cumulative information extracted from clinical records will contribute to improvement in machine learning and personalized medicine. Eventually, this needs to be validated, and the strategy should be randomly tested in a control clinical trial or replicated independently at the least. Our study provides the first introspection into the complexity of multiscale analysis of clinical data and to the value of data science which can help inform clinical decision making.

Limitations

Our study has to be interpreted in the context of several limitations which include a small sample size, observational study design, variability in the evaluation of rejection and CAV, the variable number of samples in the evaluation, and the inability to support causation. The reason behind each clinical decision is a very important but challenging task to precisely capture in a retrospective, observational study and requires further development. Yet, our findings should be carefully thought as they reveal a group with higher allograft related

event rate characterized by an overall reduced exposure to immunosuppression when observed overtime.

Conclusion

Our data demonstrate that time-dependent experiments allow assessment of the differential clinical outcomes and distinctive characteristics of the underlying immune biology of patients undergoing HTx. Noninvasive phenomapping, a meta-level approach, can produce information to support clinical decision making. Time-series phenomapping is helpful for categorization and identification of trajectories of natural and clinical variables. Such immunophenomapping, combined with other clinically related variables and real-time translational experimentation, is promising for early identification and prediction of clinical events.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Yusen RD, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-second Official Adult Lung and Heart-Lung Transplantation Report—2015; Focus Theme: Early Graft Failure. J Hear Lung Transplant. 2015; 34(10):1264–1277. DOI: 10.1016/j.healun.2015.08.014
2. Kobashigawa J, Crespo-Leiro MG, Ensminger SM, et al. Report from a consensus conference on antibody-mediated rejection in heart transplantation. J Heart Lung Transplant. 2011; 30(3):252–269. DOI: 10.1016/j.healun.2010.11.003 [PubMed: 21300295]
3. Kobashigawa J, Starling R, Mehra M, et al. Multicenter Retrospective Analysis of Cardiovascular Risk Factors Affecting Long-term Outcome of De Novo Cardiac Transplant Recipients. J Hear Lung Transplant. 2006; 25(9):1063–1069. DOI: 10.1016/j.healun.2006.05.001
4. Frank R, Molina MR, Wald JW, Goldberg LR, Kamoun M, Lal P. Correlation of circulating donor-specific anti-HLA antibodies and presence of C4d in endomyocardial biopsy with heart allograft outcomes: A single-center, retrospective study. J Hear Lung Transplant. 2013; 32(4):410–417. DOI: 10.1016/j.healun.2012.12.005
5. Loupy A, Lefaucheur C, Vernerey D, et al. Complement-Binding Anti-HLA Antibodies and Kidney-Allograft Survival From the Paris Translational Research Center for Organ Transplantation (A the Departments of Nephrology and Kidney Transplantation. N Engl J Med. 2013; 13369(26):1215–1226. DOI: 10.1056/NEJMoa1302506
6. Dasari TW, Saucedo JF, Krim S, et al. Clinical characteristics and in hospital outcomes of heart transplant recipients with allograft vasculopathy undergoing percutaneous coronary intervention: Insights from the National Cardiovascular Data Registry. Am Heart J. 2015; 170(6):1086–1091. DOI: 10.1016/j.ahj.2015.09.021 [PubMed: 26678629]
7. Kobashigawa JA. Strategies in immunosuppression after heart transplantation: is less better? Circ Heart Fail. 2011; 4(2):111–113. DOI: 10.1161/CIRCHEARTFAILURE.110.960690 [PubMed: 21406677]
8. Baran DA, Zucker MJ, Arroyo LH, et al. A prospective, randomized trial of single-drug versus dual-drug immunosuppression in heart transplantation: the tacrolimus in combination, tacrolimus alone

compared (TICTAC) trial. Circ Heart Fail. 2011; 4(2):129–137. DOI: 10.1161/CIRCHEARTFAILURE.110.958520 [PubMed: 21216835]

9. Kip KE, Hollabaugh K, Marroquin OC, Williams DO. The problem with composite end points in cardiovascular studies: the story of major adverse cardiac events and percutaneous coronary intervention. J Am Coll Cardiol. 2008; 51(7):701–707. DOI: 10.1016/j.jacc.2007.10.034 [PubMed: 18279733]
10. O'Leary JG, Samaniego M, Barrio MC, et al. The Influence of Immunosuppressive Agents on the Risk of De Novo Donor-Specific HLA Antibody Production in Solid Organ Transplant Recipients. Transplantation. 2016; 100(1):39–53. DOI: 10.1097/TP.0000000000000869 [PubMed: 26680372]
11. Guethoff S, Meiser BM, Groetzner J, et al. Ten-year results of a randomized trial comparing tacrolimus versus cyclosporine a in combination with mycophenolate mofetil after heart transplantation. Transplantation. 2013; 95(4):629–634. DOI: 10.1097/TP.0b013e318277e378 [PubMed: 23423270]
12. Hirt SW, Bara C, Barten MJ, et al. Everolimus in heart transplantation: an update. J Transplant. 2013; 2013:683964.doi: 10.1155/2013/683964 [PubMed: 24382994]
13. Zuckermann A, Schulz U, Deuse T, et al. Thymoglobulin induction in heart transplantation: patient selection and implications for maintenance immunosuppression. Transpl Int. 2015; 28(3):259–269. DOI: 10.1111/tri.12480 [PubMed: 25363471]
14. Baran DA, Pieretti J, Gidea CG, et al. Incidence of Allograft Vasculopathy in the TICTAC Trial as Compared to a Real World Cohort. J Hear Lung Transplant. 2013; 32(4):S26.doi: 10.1016/j.healun.2013.01.048
15. O'Leary JG, Samaniego M, Barrio MC, et al. The Influence of Immunosuppressive Agents on the Risk of De Novo Donor-Specific HLA Antibody Production in Solid Organ Transplant Recipients. Transplantation. 2016; 100(1):39–53. DOI: 10.1097/TP.0000000000000869 [PubMed: 26680372]
16. Lau E, Watson KE, Ping P. Connecting the Dots. Circulation. 2016; 134(5)
17. Collins FS, Varmus H. A New Initiative on Precision Medicine. N Engl J Med. 2015; 372(9):793–795. DOI: 10.1056/NEJMp1500523 [PubMed: 25635347]
18. Shah SJ, Katz DH, Selvaraj S, et al. Phenomapping for novel classification of heart failure with preserved ejection fraction. Circulation. 2015; 131(3):269–279. DOI: 10.1161/CIRCULATIONAHA.114.010637 [PubMed: 25398313]
19. Starling RC, Stehlik J, Baran DA, et al. Multicenter Analysis of Immune Biomarkers and Heart Transplant Outcomes: Results of the Clinical Trials in Organ Transplantation-05 Study. Am J Transplant. 2016; 16(1):121–136. DOI: 10.1111/ajt.13422 [PubMed: 26260101]
20. Crespo-Leiro MG, Stypmann J, Schulz U, et al. Performance of gene-expression profiling test score variability to predict future clinical events in heart transplant recipients. BMC Cardiovasc Disord. 2015; 15:120.doi: 10.1186/s12872-015-0106-1 [PubMed: 26452346]
21. Anderson A, Chan M, Desai S, et al. The International Society of Heart and Lung Transplantation Guidelines for the care of heart transplant recipients. HEALUN. 29:914–956. DOI: 10.1016/j.healun.2010.05.034
22. Gruner L. The Journal of Heart and Lung Transplantation. 2013:12–15.
23. Mehra MR, Crespo-Leiro MG, Dipchand A, et al. International Society for Heart and Lung Transplantation working formulation of a standardized nomenclature for cardiac allograft vasculopathy-2010. J Hear Lung Transplant. 2010; 29(7):717–727. DOI: 10.1016/j.healun.2010.05.017
24. Crespo-Leiro MG, Stypmann J, Schulz U, et al. Clinical usefulness of gene-expression profile to rule out acute rejection after heart transplantation: CARGO II. Eur Heart J. 2016; 6(1):150–160. DOI: 10.1093/eurheartj/ehv682
25. Deng MC, Elashoff B, Pham MX, et al. Utility of gene expression profiling score variability to predict clinical events in heart transplant recipients. Transplantation. 2014; 97(6):708–714. DOI: 10.1097/01.TP.0000443897.29951.cf [PubMed: 24637869]
26. Dedrick RL. Understanding Gene Expression Patterns in Immune-Mediated Disorders. J Immunotoxicol. 2007; 4(3):201–207. DOI: 10.1080/15476910701385562 [PubMed: 18958729]
27. AgilentAgilent GeneSpring User Manual

28. Schowengerdt KO, Fricker FJ, Bahjat KS, Kuntz ST. Increased expression of the lymphocyte early activation marker CD69 in peripheral blood correlates with histologic evidence of cardiac allograft rejection. Transplantation. 2000; 69(10):2102–2107. [Accessed January 14, 2017] http://www.ncbi.nlm.nih.gov/pubmed/10852605. [PubMed: 10852605]
29. Posselt AM, Vincenti F, Bedolli M, Lantz M, Roberts JP, Hirose R. CD69 Expression On Peripheral CD8 T Cells Correlates with Acute Rejection In Renal Transplant Recipients. Transplantation. 2003; 76(1):190–195. DOI: 10.1097/01.TP.0000073614.29680.A8 [PubMed: 12865808]
30. Spivey TL, Uccellini L, Ascierto M, et al. Gene expression profiling in acute allograft rejection: challenging the immunologic constant of rejection hypothesis. J Transl Med. 2011; 9(1):174.doi: 10.1186/1479-5876-9-174 [PubMed: 21992116]
31. Baran DA. New directions in immunosuppression after heart transplantation. Nat Rev Cardiol. 2013; 10(7):422–427. DOI: 10.1038/nrcardio.2013.63 [PubMed: 23629437]
32. Miyadera H, Ohashi J, Lernmark Å, et al. Cell-surface MHC density profiling reveals instability of autoimmunity-associated HLA. J Clin Invest. 2015; 125(1):275–291. DOI: 10.1172/JCI74961 [PubMed: 25485681]
33. Tsuchiya N. Genetics of ANCA-associated vasculitis in Japan: a role for HLA-DRB1*09:01 haplotype. Clin Exp Nephrol. 2013; 17(5):628–630. DOI: 10.1007/s10157-012-0691-6 [PubMed: 23180035]
34. Ayala García MA, González Yebra B, López Flores AL, et al. The Major Histocompatibility Complex in Transplantation. J Transplant. 2012; 2012:1–7. DOI: 10.1155/2012/842141
35. Goudy K, Aydin D, Barzaghi F, et al. Human IL2RA null mutation mediates immunodeficiency with lymphoproliferation and autoimmunity. Clin Immunol. 2013; 146(3):248–261. DOI: 10.1016/j.clim.2013.01.004 [PubMed: 23416241]
36. Wang Z, Castellaneta A, De Creus A, Shufesky WJ, Morelli AE, Thomson AW. Heart, but Not Skin, Allografts from Donors Lacking Flt3 Ligand Exhibit Markedly Prolonged Survival Time. J Immunol. 2004; 172(10)
37. Deol A, Sengsayadeth S, Ahn KW, et al. Does FLT3 mutation impact survival after hematopoietic stem cell transplantation for acute myeloid leukemia? A Center for International Blood and Marrow Transplant Research (CIBMTR) analysis. Cancer. 2016; 122(19):3005–3014. DOI: 10.1002/cncr.30140 [PubMed: 27315441]
38. Tada T, Zhang Y, Koyama T, et al. MARCH8 inhibits HIV-1 infection by reducing virion incorporation of envelope glycoproteins. Nat Med. 2015; 21(12):1502–1507. DOI: 10.1038/nm.3956 [PubMed: 26523972]
39. Angers S, Li T, Yi X, Maccoss MJ, Moon RT, Zheng N. Molecular architecture and assembly of the DDB1–CUL4A ubiquitin ligase machinery Protein ubiquitination is a common form of post-translational modification that regulates a broad spectrum of protein substrates in diverse cellular pathways.
40. Konig H, Levis M. Is Targeted Therapy Feasible in Acute Myelogenous Leukemia? Curr Hematol Malig Rep. 2014; 9(2):118–127. DOI: 10.1007/s11899-014-0198-1 [PubMed: 24599573]
41. Craig P P Manager, Cooper C Director, et al. Using natural experiments to evaluate population health interventions: new MRC guidance Europe PMC Funders Group. Public Heal Popul Heal UK J Epidemiol Community Heal. 2012; 66(1210):1182–1186. DOI: 10.1136/jech-2011-200375

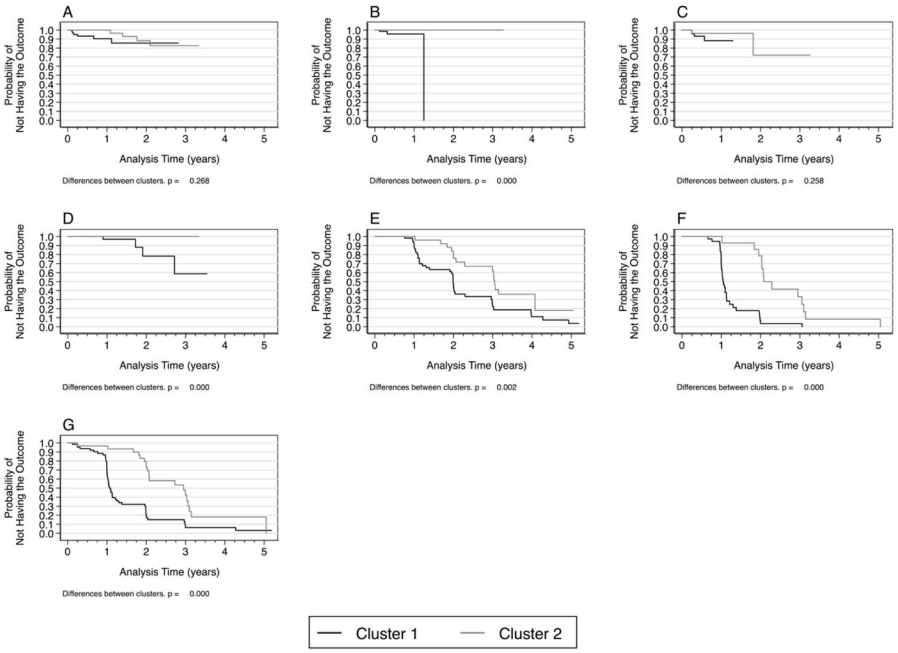

Figure 1. Associations of clusters (patient groups) with time to individual events and the combined endpoint

Kaplan-Meier curves. Differences between patient groups adjusted for age and sex were assessed by Cox regressions. Panel A, dnDSA Outcome; B, ACR 2+; C, AMR 1+; D, Death; E, Angio 1+; F, IVUS 1+; G, Combined Endpoint.

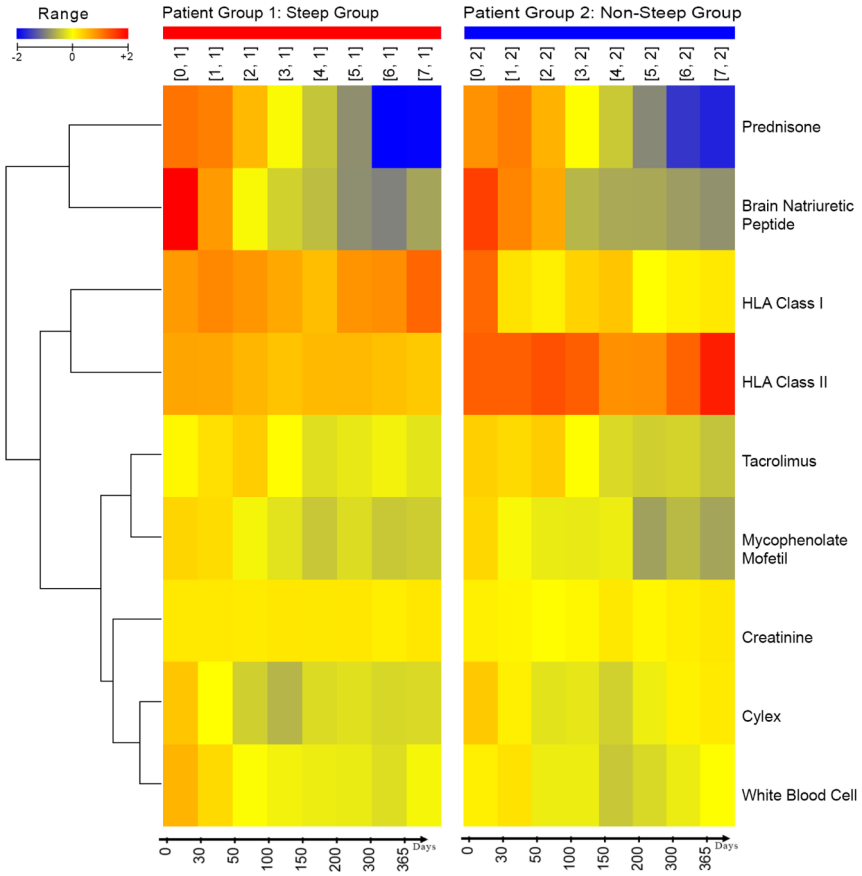

Figure 2. Phenomap of clusters (patient groups) and immune variables over time

Phenotype Heatmap (PhenoMap) of immune variables over time. Columns represent individual patients in clusters and time. Rows represent individual phenotypes. 0, < 30 days; 1, 30–50 days; 2, 50–100 days; 3, 100–150 days; 4, 150–200 days; 5, 200–300 days; 6, 300–365 days; and 7, ≥365 days. Patient Group 1: Patients assigned to this cluster based on unsupervised statistical analysis; Patient Group 2: Patients assigned to this cluster based on unsupervised statistical analysis. Red=increased value of phenotype; Blue: decreased value of phenotype; HLA human leukocyte antigen; Cylex denotes Immuknow.

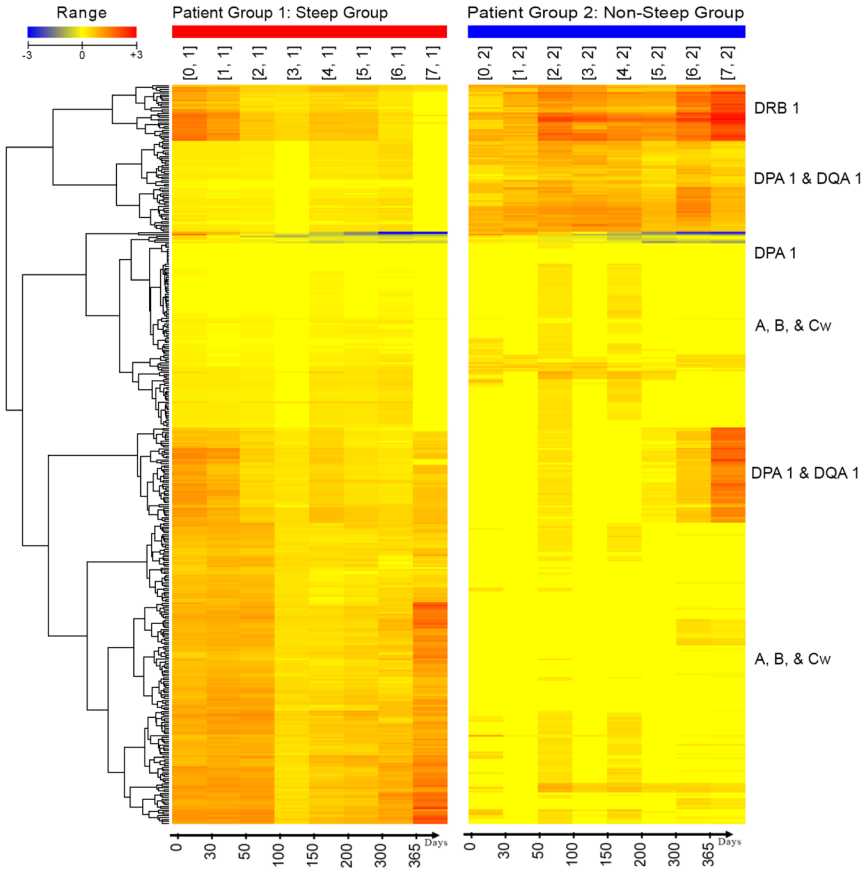

Figure 3. Phenomap of clusters (patient groups) and HLA genes over time

Phenotype Heatmap (PhenoMap) of anti human leukocyte antigen (HLA) antibody levels over time. Columns represent individual patients in clusters and time. Rows represent single antigens. “0, < 30 days; 1, 30–50 days; 2, 50–100 days; 3, 100–150 days; 4, 150–200 days; 5, 200–300 days; 6, 300–365 days; and 7, ≥365 days.” Patient Group 1: Patients assigned to this cluster based on unsupervised statistical analysis; Patient Group 2: Patients assigned to this cluster based on unsupervised statistical analysis. Red=increased value of phenotype; Blue=decreased value of phenotype Single Antigen Bead MFI for each HLA locus and alleles assessed. Only higher order denominations provided. Number of samples for each locus and alleles are variable. DQA, human leukocyte antigen class II; Allomap, molecular expression testing; and A, B, C, Cw, human leukocyte antigen class I Clinical variables (*) are provided for referenced and provided in more detail in figure 2.

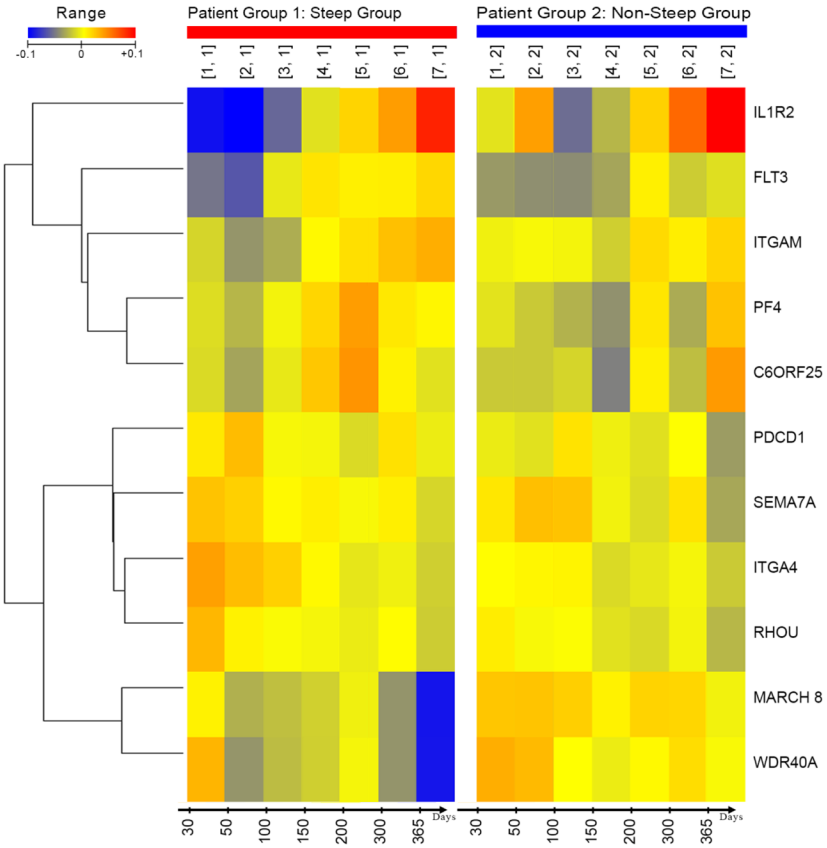

Figure 4. Phenomap of clusters (patient groups) and Allomap genes over time

Phenotype Heatmap (PhenoMap) of Allomap genes over time. Columns represent individual patients in clusters and time. Rows represent individual genes. “1, 50–70 days; 2, 70–100 days; 3, 100–150 days; 4, 150–200 days; 5, 200–300 days; 6, 300–365 days; and 7, ≥365 days.” Patient Group 1: Patients assigned to this cluster based on unsupervised statistical analysis; Patient Group 2: Patients assigned to this cluster based on unsupervised statistical analysis. Red=increased value of phenotype; Blue: decreased value of phenotype subset of patients and encounters for whom a sample of the AlloMap molecular test was available. HLA class I and class II average % for each group at different timepoints are provided.

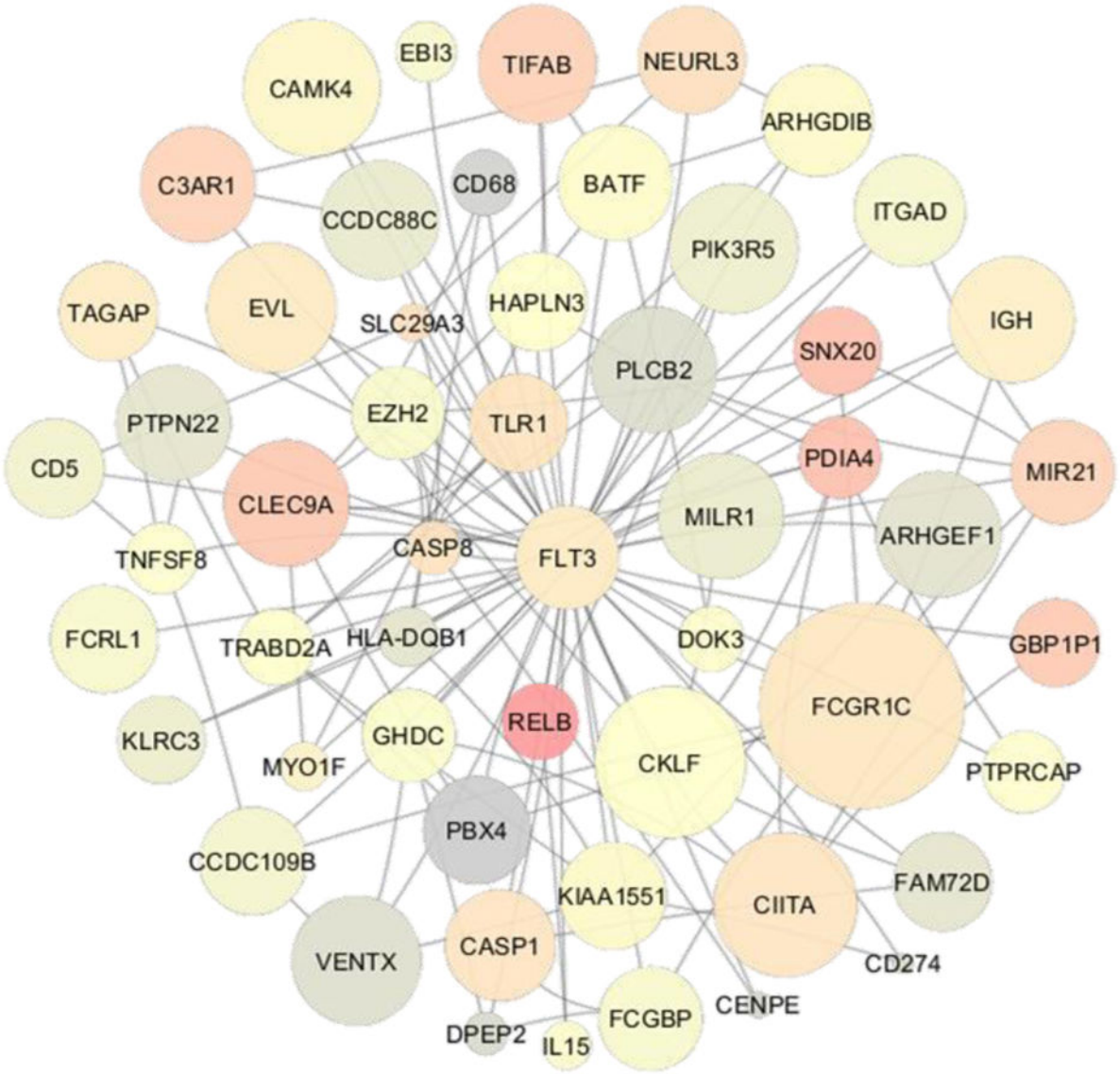

Figure 5. Intramyocardial FLT3 coexpression network

Intramyocardial FLT3 first-neighbor co-expression network, where nodes are genes and edges represent interactions among them. Size of the node reflects the degree or number of connections and the color denotes the gene's network connectivity as inferred by gene co-expression network analysis. Visualization was accomplished in Cytoscape. Second neighbors were removed for clarity.

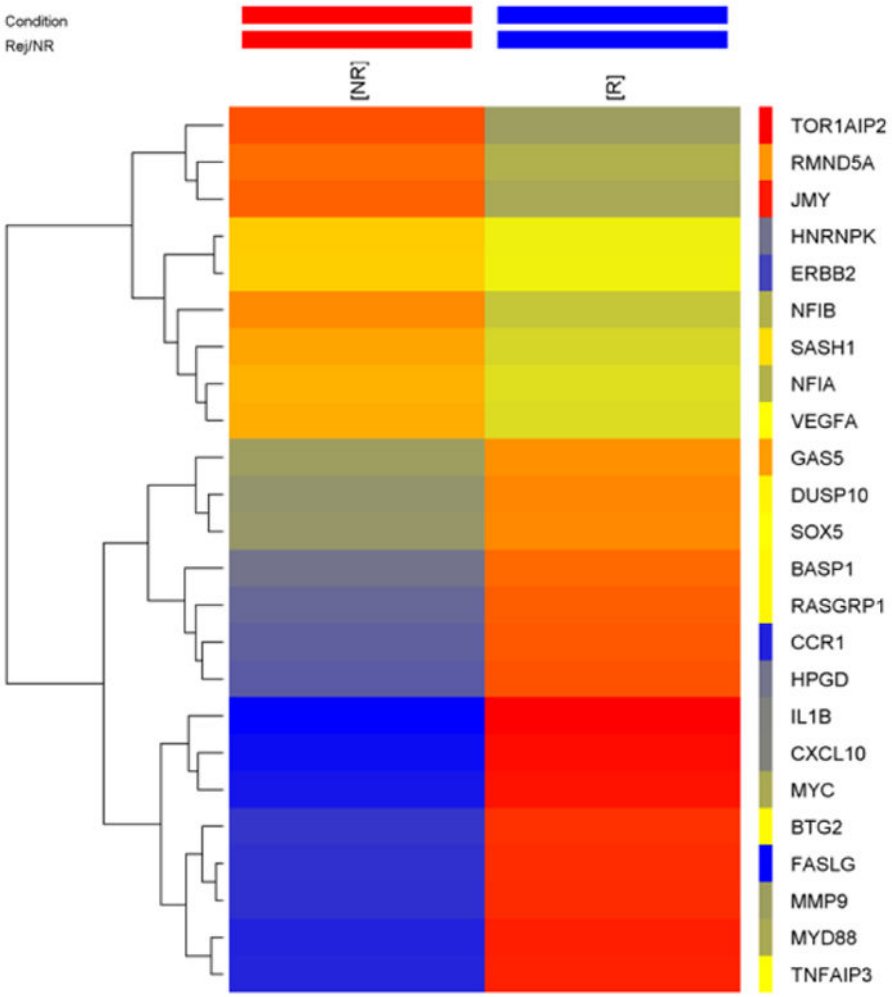

Figure 6. Intramyocardial gene expression of MIR21 targets for patients with and without rejection

Gene expression of MIR21 targets are differentially expressed in patients with cardiac allograft rejection and those without.

Table 1

Characteristics of the population

Characteristic	All (N=94) Patients	Patient Group 1 (N=64)	Patient Group 2 (N=30)
Age, *mean ± SD*	54.9 ± 13.4	54.6 ± 12.6	53.1 ± 13.8
Sex			
Male, *no. (%)*	67 (71.3)	47 (73.4)	20 (66.7)
Race/Ethnicity			
White, *no. (%)*	51 (54.3)	35 (54.7)	16 (53.3)
Hispanic, *no. (%)*	16 (17.0)	10 (15.6)	6 (20.0)
African American, *no. (%)*	12 (12.8)	8 (12.5)	4 (13.3)
Asian/Southeast Asian, *no. (%)*	12 (12.8)	9 (14.1)	3 (10.0)
Others, *no. (%)*	3 (3.2)	2 (3.1)	1 (3.3)
Induction therapy, no (%)	26 (27.6)	17 (25.7)	9 (32.1)
Desensitization no (%)	3 (3.2)	2 (3.0)	1 (3.6)
Steroid duration	355 ± 224	269 ± 128	538 ± 272
Tacrolimus	9.85 ± 4.00	9.81 ± 3.70	9.93 ± 4.65
Mycophenolate Survival Outcome [1]	1,811 ± 716	1,818 ± 631	1,795 ± 882
Survived, *no. (%)*	90 (95.7)	60 (93.8)	30 (100.0)
Infections. [† 1]			
Yes, *no. (%)*	28 (33.3)	14 (25.0)	14 (50.0)
CMV/IgG [1]			
Yes, *no. (%)*	33 (42.9)	18 (35.3)	15 (57.7)
Rejection. [† 2]			
Yes, *no. (%)*	8 (8.5)	6 (9.4)	2 (6.7)
Donor specific antibodies anti-HLA Class [1]			
None, *no. (%)*	77 (84.6)	54 (88.5)	23 (76.7)
HLA class I only, *no. (%)*	1 (1.1)	1 (1.6)	
HLA class II only, *no. (%)*	7 (7.7)	3 (4.9)	4 (13.3)
Both HLA class I & II, *no. (%)*	6 (6.6)	3 (4.9)	3 (10.0)

Notes:

[1] Total sample size less than 94;

[2] Rejection, either AMR only, ACR only, or both.

CMV, Cytomegalovirus (the data denotes CMV serology and not CMV viremia); HLA, human leukocyte antigen, values above 15% and those increased above 15% after 120 days included; infection includes any type of viral, bacterial, or fungal infection.

[†] The data is not baseline and reflects the differences in the number of patients included in each step of the analysis related to the availability of information.

Table 2

Biomarker Slope and Intercept Associations with Time to Combined Endpoint

	Unadjusted		Adjusted for Age and Sex	
	HR (95% CI)	**P**	**HR (95% CI)**	**P**
MMF				
Intercept	1.06 (0.82, 1.36)	0.658	1.01 (0.78, 1.32)	0.918
Slope	0.97 (0.78, 1.19)	0.752	0.96 (0.77, 1.19)	0.697
Prednisone				
Intercept‡				
Slope	1.05 (1.00, 1.10)	0.036	1.03 (0.95, 1.11)	0.538
CNI				
Intercept	1.00 (0.82, 1.20)	0.967	0.95 (0.78, 1.16)	0.615
Slope	0.77 (0.65, 0.92)	0.004	0.80 (0.66, 0.96)	0.018
BNP				
Intercept	0.95 (0.75, 1.21)	0.690	0.95 (0.73, 1.24)	0.703
Slope	0.85 (0.68, 1.07)	0.173	0.84 (0.66, 1.08)	0.173
Creatinine				
Intercept	0.84 (0.62, 1.13)	0.252	0.79 (0.58, 1.08)	0.137
Slope	1.16 (0.81, 1.67)	0.413	1.20 (0.79, 1.81)	0.386
WBC				
Intercept	0.98 (0.79, 1.20)	0.816	0.96 (0.77, 1.20)	0.743
Slope	0.89 (0.75, 1.05)	0.151	0.90 (0.77, 1.06)	0.204
Immuknow				
Intercept	0.90 (0.72, 1.13)	0.377	0.92 (0.72, 1.18)	0.519
Slope‡				

Notes: Effects are reported per 1 standard deviation increase in intercept or slope.

HR, hazard ratio; CI, confidence interval

‡Insufficient variability in the intercept/slope for modeling

Table 3

Slope differences between clusters (patient groups) defined for the combined endpoint. Note that, although untransformed slopes are presented, patient groups were defined based upon slopes of the change in biomarker percentile.

Biomarker	Patient Group 1 (N=64)	Patient Group 2 (N=30)
≠ MMF slope, *mg/90 Days*	−133 ± 60	−84 ± 68
≠ Prednisone slope, *mg/90 Days*	−2.0 ± 0.3	−1.3 ± 3.0
≠ CNI slope, *ng per ml/90 Days*	−0.61 ± 0.06	−0.58 ± 0.14
BNP slope, *pg per ml/90 Days*	−26.2 ± 12.1	−20.3 ± 29.0
Creatinine slope, *mg per dL/90 Days*	0.013 ± 0.195	0.005 ± 0.049
WBC slope, *# x 10^9 per L/90 Days*	−0.15 ± 0.04	−0.11 ± 0.09

≠ indicates biomarkers used to define the patient groups

Table 4

Observed Probability of Events among patient groups

Events	Patient Group 1 (N=64)	Patient Group 2 (N=30)
dnDSA, *no. (%)*[1]	(6) 10.0%	(4) 13.8%
ACR 2+, *no. (%)*	(3) 4.7%	
AMR 1+, *no. (%)*	(4) 6.3%	(2) 6.7%
Death, *no. (%)*[1]	(4) 6.3%	
CAV by Angio 1+, *no. (%)*[1]	(39) 73.6%	(15) 60.0%
CAV by IVUS 1+, *no. (%)*[1]	(33) 89.2%	(13) 92.9%
Combined Endpoint, *no. (%)*	(51) 79.7%	(20) 66.7%

Notes:

[1]Total sample size less than 94;

dnDSA indicates Donor Specific Antibodies, CAV Cardiac Allograft Vasculopathy, Angio, angiogram; ivus, intravascular ultrasound; combined end point is defined as time to either death, re-transplantation, ACR, 2+, AMR, 1+, ISHLT CAV 1+ by angiogram, or ISHLT CAV 1+ by intravascular ultrasound.

Table 5

CAV by Angiogram and IVUS

Events	Patient Group 1	Patient Group 2
CAV by Angio:		
Normal, *no. (%)*	14 (26.4)	10 (40.0)
Mild, *no. (%)*	36 (67.9)	14 (56.0)
Moderate, *no. (%)*	3 (5.7)	
Severe, *no. (%)*		1 (4.0)
CAV by IVUS:		
Normal, *no. (%)*	4 (10.8)	1 (7.1)
Mild, *no. (%)*	27 (73.0)	12 (85.7)
Moderate, *no. (%)*	5 (13.5)	1 (7.1)
Severe, *no. (%)*	1 (2.7)	

Notes: CAV indicates Cardiac Allograft Vasculopathy, Angio angiogram; ivus, intravascular ultrasound;

Table 6

P-values of Allomap molecular test genes across time points

Allomap Genes	Timepoint 1	Timepoint 2	Timepoint 3	Timepoint 4	Timepoint 5	Timepoint 6	Timepoint 7
IL1R2	<.001	<.001	0.919	0.489	0.958	0.295	0.457
FLT3	0.666	0.282	0.182	0.015	0.999	0.074	0.055
ITGAM	0.059	<.001	0.023	0.020	0.784	0.063	0.076
PF4	0.838	0.658	0.222	0.006	0.175	0.055	0.535
C6ORF25	0.724	0.418	0.719	0.002	0.107	0.209	0.078
PDCD1	0.119	0.008	0.373	0.833	0.883	0.321	0.030
SEMA7A	0.002	0.502	0.013	0.263	0.215	0.591	0.040
ITGA4	<.001	0.015	0.200	0.017	0.942	0.787	0.837
RHOU	0.002	0.313	0.933	0.333	0.356	0.567	0.626
MARCH 8	0.064	0.029	<.001	0.092	0.021	<.001	<.001
WDR40A	0.816	0.013	0.020	0.479	0.551	<.001	0.006

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