# Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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**Gene Expression Profiling for Rejection Surveillance After Cardiac Transplantation**

**SUPPLEMENTARY APPENDIX**

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## SECTION 1: SUPPLEMENTARY METHODS

**1.1 Patients:** Non-consecutive heart transplant recipients from 13 U.S. centers were assessed for eligibility between January 2005 and October 2009. The full list of inclusion and exclusion criteria is presented in Supplementary Table 1. Additionally, some patients who met eligibility criteria were not enrolled due to the preference of the investigator or treating physician for biopsy-based rejection monitoring. A subset of these patients may have been considered to be at higher risk for rejection on the basis of their medical history or clinical assessment. The institutional review board at each participating center approved the study.

**1.2 Biopsy:** Patients in the biopsy group underwent protocol surveillance biopsies at pre-specified and center-specific intervals (refer to Supplementary Table 2). Patients in the gene-expression profiling group underwent biopsies if the gene-expression profiling score was above threshold (see *Thresholds used in the IMAGE study* below). However, patients with consistently elevated gene-expression profiling scores on three consecutive study visits who did not have clinical manifestations of graft dysfunction and who did not have treatable rejection on two consecutive biopsies over a period of 3 to 9 months could be managed without a biopsy on the third or subsequent visit if there was no statistically significant increase in the score during those visits. Two scores were considered statistically different from one another if the previous score did not fall within the 95% confidence interval of the current score.

Patients in both groups underwent endomyocardial biopsy if signs or symptoms of rejection or allograft dysfunction were present at the time of the clinic visit, or if the echocardiogram showed a proportional left ventricular ejection fraction decrease of $\geq$25% compared to the first visit (reference) value. Clinically driven biopsies were permitted in both groups if signs or symptoms of heart failure developed between routine surveillance visits. Following a treated rejection episode, all patients underwent surveillance endomyocardial biopsies per center protocol, regardless of randomization arm, for a period of 2-3 event-free

months. Biopsy specimens were interpreted by the transplant center pathologist in accordance with ISHLT criteria and without knowledge of the patient's gene-expression profiling score.[1]

**1.3 Gene-expression profiling:** Gene-expression profiling is based on the analysis of peripheral blood mononuclear cell messenger ribonucleic acid (mRNA) using real-time polymerase chain reaction (PCR) technology. Previous studies have shown that certain gene-expression profiles correlate with histologic rejection on the endomyocardial biopsy, and their analysis may provide a valuable tool for the non-invasive monitoring of acute rejection.[2, 3, 4]

In the Cardiac Allograft Rejection Gene Expression Observation (CARGO) study, 9 centers enrolled 737 patients and collected clinical data, peripheral blood mononuclear cell samples, and biopsy specimens during 5834 routine and non-routine clinical encounters.[4] The primary objective of the CARGO study was to develop and validate a gene-expression profile test for acute cellular rejection. A linear discriminant equation (classifier) was derived by sequentially fitting the gene-expression data from 145 peripheral blood samples to maximize the agreement with the histology classification on the corresponding biopsy samples. The final classifier, yielding a score between 0 and 40, combined the expression levels of 11 informative genes which best distinguished rejection (ISHLT biopsy grade ≥3A) from non-rejection (ISHLT biopsy grade <3A). An additional 9 genes are included in the test for control and normalization purposes.

The gene-expression profiling test is available commercially as an FDA cleared in vitro diagnostic multivariate index assay (AlloMap®; XDx, Brisbane, CA). The test is intended to aid in the identification of heart transplant recipients with stable allograft function who have a low probability of moderate or severe acute cellular rejection at the time of testing, in conjunction with standard clinical assessment. The AlloMap® test is approved for heart transplant recipients 15 years of age or older who are at least two months (≥55 days) post transplantation.

*Genes and pathways:* The 11 informative genes in the AlloMap® test are involved in pathways hypothesized to play a role in immune activation during acute cellular rejection, including T cell priming, platelet activation, proliferation and mobilization of immature erythrocytes, and steroid responsiveness.[4] Supplementary Table 3 lists the individual genes, their patterns of expression during rejection, predominant source of expression in blood, and their postulated role in immune activation and rejection.

*Performance characteristics:* Supplementary Table 4 presents the performance characteristics for the AlloMap® test across a range of score thresholds in the 2 to 6 month and >6 month post-transplantation periods. The test characteristics reported here differ slightly from previously published reports and reflect updated data utilized for FDA clinical validation studies in 2008. Test characteristics were derived for an independent 300 samples from 154 patients in the CARGO study that were not used in the discovery and development of the classifier.

*Specimen processing and reporting of test results:* The AlloMap® blood samples were processed locally and shipped frozen to the XDx laboratory. An AlloMap® score from 0-40 was reported to the transplant center within 4 days of specimen collection.

*Thresholds used in the IMAGE study:* The initial protocol specified a gene-expression profiling score of 30 or higher to prompt a required endomyocardial biopsy. This threshold was selected based upon the initial findings from the CARGO study, showing that a score below 30 was associated with a negative predictive value of 99.6% for concurrent ISHLT Grade 3A (2R) or higher rejection. On November 7, 2005, a protocol amendment increased the threshold to 34 to minimize the number of biopsies needed in the gene-expression profiling group without compromising the assay performance. The decision to increase the threshold was based upon additional analyses from the CARGO study showing that the negative predictive value of the gene-expression profiling test remained robust (99.2%) at a higher threshold of 34 while reducing the number of positive tests from 50.8% to 22.3%.[5] The IMAGE investigators

recognized that the higher threshold represented a more pragmatic yet still prudent threshold in order to maintain a low risk of missing asymptomatic rejection episodes while reducing the number of unnecessary biopsies.

**1.4 Rejection therapy:** Treated rejection was defined as the administration of anti-rejection therapy such as pulse steroids, antibody therapy, or plasmapheresis, with or without histological findings of rejection on the endomyocardial biopsy. Rejection therapy was given based upon endomyocardial biopsy results and other conventional diagnostic testing according to center specific practices, which are summarized in Supplementary Table 9.

**1.5 Primary end point:**

*Definition:* The primary outcome was a composite of the following subcomponents: rejection with hemodynamic compromise, graft dysfunction due to other causes, death, or retransplantation. The definitions are presented below:

1. **Rejection with hemodynamic compromise:** Criteria (a) **and** either criteria (b) or (c) must be met.
    a. Presence of hemodynamic compromise: The presence of one or more of the following criteria is required:
        i. Absolute drop in LVEF ≤30% at the time of the rejection episode, as confirmed by the Core Echocardiography Laboratory.
        ii. Proportional decrease in LVEF ≥25% compared to the reference (first study visit) value at the time of the rejection episode, as confirmed by the Core Echocardiography Laboratory.
        iii. Cardiac index < 2 L/min/$m^2$ at the time of the rejection episode.
        iv. Use of inotropic drugs to support circulation at any time during the rejection episode. Use of dopamine at ≤3 mcg/kg/min, when used to enhance renal perfusion, did not count toward this criteria.

b. Supporting histologic or immunologic evidence of rejection, as determined by the local pathologist's review. At least one of the criteria below is required:

   i. Cellular rejection of ISHLT Grade 3A (1990 classification) or Grade 2R (2004 classification).

   ii. Antibody-mediated rejection of ISHLT Grade AMR1 (2004 classification).

   iii. Antibody-mediated rejection as defined by histologic evidence of capillary injury and/or positive immunopathologic evidence of antibody mediated injury (immunofluorescence or immunohistochemistry).

   iv. Mixed cellular and antibody-mediated rejection.

c. Probable rejection: This category was used by the Endpoints Committee to classify events considered to be secondary to rejection in the absence of histologic confirmation on biopsy. An example would include a patient who presents with hemodynamic compromise, no evidence of cardiac allograft vasculopathy or histologic evidence of rejection, and whose condition improves with initiation of anti-rejection therapy.

2. **Graft dysfunction due to other causes.** Criteria (a) **and** either criteria (b), (c), or (d) must be met.

   a. Presence of hemodynamic compromise: The definition is the same as listed above.

   b. Cardiac allograft vasculopathy: The diagnosis requires any one of the following criteria, either prior to or at the time of the event:

      i. Stenosis of ≥50% within any major epicardial coronary vessel or branches on angiography.

      ii. Severe diffuse or distal vessel tapering on angiography.

      iii. Maximal intimal thickness ≥ 0.5 mm in any major epicardial coronary vessel at the time of intravascular ultrasound.

iv. Evidence of significant intimal hyperplasia at the level of the arterioles or intra-myocardial small vessels at autopsy.

v. Evidence of recent ischemic injury on biopsy.

c. Probable cardiac allograft vasculopathy: This category was used by the Endpoints Committee to classify events that are related to cardiac allograft vasculopathy but that do not meet the diagnostic criteria above. An example would include a patient with graft dysfunction and epicardial stenosis of <50% who does not respond to empiric anti-rejection therapy and for which no autopsy information is available.

d. Nonspecific or other: This category was coded if the etiology of graft dysfunction could not be readily classified into one of the two previous categories.

3. **Death or retransplantation.** Death or retransplantation from any cause was considered.

*Rationale for selection of primary end point:* We selected a composite primary end point to include events whose detection, if present, would not be influenced by the specific rejection monitoring method used. For example, rejection with hemodynamic compromise would manifest clinically with symptoms or echocardiographic evidence of graft dysfunction in both the gene-expression profiling and biopsy groups. Since histologic sampling for rejection was performed, by study design, less frequently in the gene-expression profiling group, our end point would also need to identify the sequelae of undetected rejection episodes. Since rejection in the late (>1 year) post-transplantation can theoretically cause graft dysfunction through progressive myocardial fibrosis of intimal hyperplasia of the coronary vasculature, we included graft dysfunction not associated with rejection. Finally, since graft dysfunction can rapidly progress to death or the need for retransplantation, these events were also included in the primary end point.

**1.6 Quality of life and patient satisfaction:** We assessed the effects of rejection monitoring strategy on patients' quality of life using the Medical Outcomes Study 12-item Short Form (SF-12) General Health Survey (version 2). The SF-12 survey measures health status in eight core domains, and the results are expressed in terms of two meta-scores. The Physical Component Summary assesses physical functioning, bodily pain, physical role functioning, and general health, while the Mental Component Summary assesses vitality, social functioning, emotional role functioning, and mental health. The SF-12 summary scores for mental health and physical health have a range of 0 to 100, with higher scores indicating better functioning. They were designed to have a mean score of 50 and a standard deviation of 10 in a representative sample of the U.S. population. We also assessed patient satisfaction with the method of rejection monitoring using an ordinal scale ranging from 1 (very unhappy) to 10 (very happy). The SF-12 health survey and patient satisfaction questionnaire were administered at enrollment, 1 year in the study, and at the completion of 2 years in the study.

**Supplementary Table 1:** IMAGE eligibility criteria

| **Inclusion criteria** |
| --- |
| 1. Heart transplant recipients who are between >6 months to 5 years (>6-60 months) post-transplantation.<br>2. Age ≥18 years.<br>3. Stable outpatient being seen for routine monitoring of rejection. Stability is defined as absence of prior or current evidence of either severe cardiac allograft vasculopathy (CAV) or antibody-mediated rejection (AMR) with associated hemodynamic compromise.<br>a. Severe CAV is defined as either A) >50% left main stenosis; B) ≥50% stenosis in ≥2 primary vessels (proximal 1/3 or middle 1/3 of the LAD or LCx, RCA to takeoff of PDA in right-dominant coronary circulations) or C) isolated branch stenoses of >50% in all 3 systems (diagonal branches, obtuse marginal branches, distal 1/3 of LAD or LCx, PDA, PLB, and RCA to takeoff of PDA in non-dominant systems).<br>b. AMR with associated hemodynamic compromise is defined as AMR (defined according to local criteria) with either A) a left ventricular ejection fraction (LVEF) ≤ 30% or at least 25% lower than the baseline value, B) a cardiac index < 2 L/min/$m^2$, or C) the use of inotropic agents to support circulation.<br>4. Left ventricular ejection fraction ≥45% by Echocardiography, Multiple Gated Acquisition (MUGA) scan, or ventriculography at study entry (baseline / enrollment study) |

**Supplementary Table 1 (Continued):** IMAGE eligibility criteria

| **Exclusion criteria** |
|---|
| 1. Patients <7 months after heart transplantation. |
| 2. Any symptoms or clinical signs of impaired allograft function: |
| a. Symptoms of Congestive Heart Failure (CHF) at the enrollment visit. |
| b. Signs of decompensated heart failure, including the development of a new S3 gallop at the enrollment visit. |
| c. Elevated right heart pressures with diminished cardiac index <2.2 L/min/$m^2$ that is new compared to a previous measurement within 6 months. |
| d. Decrease in LVEF as measured by echocardiography: ≥25% compared to prior measurement within 6 months. |
| 3. Rejection therapy for biopsy-proven ISHLT Grade 3A or higher during the preceding 2 months. |
| 4. Major changes in immunosuppression therapy within previous 30 days (e.g., discontinuation of calcineurin inhibitors, switch from mycophenolate mofetil to sirolimus or vice versa). |
| 5. Unable to give written informed consent. |
| 6. Patient receiving hematopoietic growth factors (e.g. Neupogen, Epogen) currently or during the previous 30 days. |
| 7. Patients receiving ≥ 20 mg/day of prednisone equivalent corticosteroids at the time of enrollment |
| 8. Patient enrolled in a trial requiring routine surveillance endomyocardial biopsies. |
| 9. Patient received transfusion within preceding 4 weeks. |
| 10. Patients with end-stage renal disease requiring some form of renal replacement therapy (hemodialysis or peritoneal dialysis). |
| 11. Pregnancy at the time of enrollment. |

**Supplementary Table 2:** Rejection surveillance schedule at IMAGE centers

| Study Center | >6-12 months | Year 2 | Year 3 | Year 4 | Year 5 |
|---|---|---|---|---|---|
| Intermountain Medical Center, UT | 7, 8.5, 10, 12 | 3, 6, 9, 12 | 4, 8, 12 | 6, 12 | 12 |
| University of Chicago Medical Center, IL | 7, 8, 9, 10, 11, 12 | 3, 6, 12 | 12 | 12 | 12 |
| Hospital of the University of Pennsylvania, PA | 7, 8, 9, 10, 11, 12 | 3, 6, 9, 12 | 6, 12 | 6, 12 | 6, 12 |
| St Luke's Hospital, Kansas, MO* | 7, 8, 9, 10, 11, 12 | 3, 6, 9, 12 | 6, 12 | — | — |
| Barnes Jewish Hospital, MO | 7, 8, 9, 10, 11, 12 | 3, 6, 9, 12 | 6, 12 | 12 | 12 |
| Columbia University Medical Center, NY | 8, 10, 12 | 3, 6, 9, 12 or 4, 8, 12 | 4, 8, 12 or 12 | 6, 12 or 12 | 6, 12 or 12 |
| Cleveland Clinic, OH | 8, 10, 12 | 3, 6, 9, 12 | 3, 6, 9, 12 | 6, 12 | 6, 12 |
| University of Pittsburgh Medical Center, PA* | 8, 10, 12 | 3, 6, 9, 12 | 3, 6, 9, 12 | 12 | 12 |
| VA Palo Alto Medical Center, CA† | 8, 10, 12 | 4, 8, 12 | 4, 8, 12 | 6, 12 | 6, 12 |
| Northwestern University Medical Center, IL | 8, 10, 12 | 3, 6, 9, 12 or 4, 8, 12 | 6, 12 | 6, 12 | 6, 12 |
| Stanford University Medical Center, CA† | 9, 12 | 4, 8, 12 | 4, 8, 12 | 6, 12 | 6, 12 |

| | | | | | |
|---|---|---|---|---|---|
| Texas Heart Institute, TX‡ | 9, 12 | 6, 12 | 12 | 12 | 12 |
| Newark Beth Israel Medical Center, NJ | § | 6, 12 | 6, 12 | 12 | 12 |

**Footnotes for Supplementary Table 2:**

* Patients >3 years post-transplant were followed-up for surveillance monitoring using echocardiogram and physical exams, instead of by biopsy. Therefore no gene-expression profiling testing was done for patients >3 years post-transplant.

† Patients >5 years post-transplant were followed-up for surveillance monitoring using echocardiogram and physical exams instead of by biopsy. Therefore no gene-expression profiling testing was done for patients >5 years post-transplant.

‡ The site did not biopsy patients who were 1 year post-transplant, therefore no gene-expression profiling testing was done after 1 year post-transplant.

§ Patients in this time frame excluded from IMAGE due to competing clinical study at this center.

**Supplementary Table 3:** Genes and pathways contained in AlloMap® test

| AlloMap Genes | Expression Change with ACR | Predominant Source of Expression in Blood | Role in Immune Activation and Rejection |
|---|---|---|---|
| IL1R2, FLT3, ITGAM | Decreased | Monocytes | Steroid response |
| MARCH8, WDR40A | Increased | Reticulocytes | Proliferation and mobilization of erythrocytes |
| PF4, C6orf25 | Decreased | Platelets | Platelet activation |
| RHOU | Increased | T cells and Monocytes | Unknown |
| PDCD1 | Increased | T cells | T cell priming |
| ITGA4 | Increased | T cells | T cell priming |
| SEMA7A | Increased | T cells, B cells and Immature Neutrophils | Unknown |

**Supplementary Table 4: AlloMap® performance metrics** estimated from the CARGO patient population. Performance is given by time post-transplantation.

| AlloMap Score | >2 – 6 Months (N=166 samples) | | | | | >6 Months (N=134 samples) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | % Pts Below | PPV ≥3A(2R) | PPV Std. Err. | NPV <3A(2R) | NPV Std. Err. | % Pts Below | PPV ≥3A(2R) | PPV Std. Err. | NPV <3A(2R) | NPV Std. Err. |
| 19 | <22.4 | ≤2.7% | ≤0.1% | 100.0% | 0.0% | ≤5.4 | ≤1.8% | 0.0% | 100.0% | 0.0% |
| 20 | 24.3% | 2.8% | 0.2% | 100.0% | 0.0% | 8.1% | 1.8% | 0.1% | 100.0% | 0.0% |
| 21 | 33.6% | 2.5% | 0.4% | 98.8% | 0.8% | 9.8% | 1.9% | 0.1% | 100.0% | 0.0% |
| 22 | 38.8% | 2.7% | 0.5% | 98.9% | 0.7% | 11.0% | 1.9% | 0.1% | 100.0% | 0.0% |
| 23 | 41.8% | 2.9% | 0.5% | 99.0% | 0.6% | 14.1% | 2.0% | 0.1% | 100.0% | 0.0% |
| 24 | 47.5% | 3.2% | 0.6% | 99.1% | 0.6% | 18.4% | 2.1% | 0.1% | 100.0% | 0.0% |
| 25 | 56.0% | 3.8% | 0.7% | 99.3% | 0.5% | 22.1% | 2.2% | 0.1% | 100.0% | 0.0% |
| 26 | 61.4% | 3.8% | 0.9% | 99.0% | 0.5% | 26.8% | 2.3% | 0.1% | 100.0% | 0.0% |
| 27 | 63.6% | 3.4% | 1.0% | 98.7% | 0.5% | 31.6% | 1.9% | 0.4% | 98.7% | 0.9% |
| 28 | 68.3% | 3.3% | 1.1% | 98.5% | 0.5% | 39.1% | 2.1% | 0.5% | 98.9% | 0.7% |
| 29 | 73.7% | 4.0% | 1.3% | 98.6% | 0.4% | 40.8% | 2.1% | 0.5% | 99.0% | 0.7% |
| 30 | 77.2% | 4.6% | 1.6% | 98.6% | 0.4% | 50.6% | 2.1% | 0.6% | 98.7% | 0.6% |
| 31 | 81.0% | 3.3% | 1.6% | 98.2% | 0.4% | 54.1% | 2.3% | 0.7% | 98.8% | 0.6% |
| 32 | 85.6% | 2.9% | 2.0% | 98.0% | 0.3% | 63.1% | 2.9% | 0.9% | 99.0% | 0.5% |
| 33 | 89.4% | 4.0% | 2.7% | 98.1% | 0.3% | 72.4% | 3.8% | 1.3% | 99.1% | 0.4% |
| 34 | 91.7% | 5.0% | 3.5% | 98.2% | 0.3% | 79.1% | 4.1% | 1.7% | 98.9% | 0.4% |
| 35 | 94.5% | 5.7% | 4.8% | 98.1% | 0.2% | 84.1% | 4.0% | 2.2% | 98.7% | 0.4% |
| 36 | 97.3% | 7.6% | 13.8% | 98.1% | 0.2% | 90.2% | 5.4% | 3.2% | 98.7% | 0.3% |
| 37 | 97.8% | 9.5% | 21.1% | 98.1% | 0.2% | 91.7% | — | – | 98.4% | 0.2% |
| 38 | 100.0% | — | – | 97.9% | 0.0% | 96.5% | — | – | 98.2% | 0.0% |
| 39 | 100.0% | — | – | 97.9% | 0.0% | 97.7% | — | – | 98.3% | 0.0% |

**Supplementary Table 5:** Rejection therapy protocols at IMAGE centers

| ISHLT biopsy grade | Hemodynamic compromise absent | Hemodynamic compromise present |
|---|---|---|
| 0, 1A (1R), 1B (1R), 2 (1R) | No treatment except at Columbia University, where Grade 1B (1R) rejection during the first year-transplantation was treated with oral corticosteroids. | High dose (500-1000mg) IV corticosteroids<br>**with or without**<br>Cytolytic antibody therapy (OKT3, Thymoglobulin, ATGAM)<br>**Consider empiric treatment for antibody mediated rejection** |
| 3A (2R) | 1 mg/kg oral corticosteroids with rapid taper **or**<br>High dose (500-1000mg) IV corticosteroids x 3 days | High dose (500-1000mg) IV corticosteroids x 3 days<br>**with or without**<br>Cytolytic antibody therapy |
| 3B, 4 (3R) | High dose (500-1000mg) IV corticosteroids<br>**with or without**<br>Cytolytic antibody therapy (Thymoglobulin 1.5 mg/kg up to 120 mg daily x 3 days) | High dose (500-1000mg) IV corticosteroids<br>**with or without**<br>Cytolytic antibody therapy |
| AMR positive | No treatment | Apheresis<br>**with or without**<br>IV immune globulin<br>High dose IV (500-1000mg) corticosteroids<br>Rituximab infusion |

## SECTION 2: SUPPLEMENTARY RESULTS

**2.1 Patient population:** The baseline characteristics of the IMAGE cohort is compared against a cohort of pediatric and adult heart transplant recipients in 2007 as reported to the Organ Procurement and Transplantation Network (OPTN) and United Network of Organ Sharing (UNOS) (See Supplementary Table 6). Compared to the OPTN/UNOS cohort, the IMAGE cohort contained a greater proportion of men and a lower proportion of African-American patients. The higher proportion of patients with coronary artery disease and lower proportion of patients with congenital heart disease in the IMAGE population likely reflects the exclusion of pediatric patients. The use of induction therapy was also higher in the IMAGE cohort, particularly with respect to the use of antithymocyte globulin and interleukin-2 receptor antagonists.

**2.2 Immunosuppression:** Overall immunosuppression intensity was similar in the gene-expression profiling and biopsy arms throughout the study. The mean cyclosporine 12-hour trough level was higher in the gene-expression profiling group compared to the biopsy group at baseline (177 ng/mL vs. 141 ng/mL, P=0.02); however, the difference between the groups narrowed during the study such that the mean drug levels were similar when averaged throughout the study (142 ng/mL in the gene-expression profiling group vs. 131 ng/mL in the biopsy group, P=0.28). The mean tacrolimus drug levels in the gene-expression profiling group, compared to the biopsy group, was numerically higher at study entry (8.8 ng/mL vs. 8.1 ng/mL, P=0.08) and throughout the study (8.1 ng/mL vs. 7.6 ng/mL, P=0.06), but the differences were marginally statistically significant and not clinically meaningful.

**2.3 Gene-expression profiling scores and biopsy results:** The mean test score for patients in the gene-expression profiling arm was 29.9 ± 4.9. Among the 1190 gene-expression profiling scores reported, 302 (25%) of scores were ≥34 (see Supplementary Figure 1). Biopsies were

performed in conjunction with 274 (91%) elevated scores, either in response to the elevated score in asymptomatic patients (265 biopsies) or due to the presence of both an elevated score and clinical evidence of graft dysfunction (9 biopsies). A biopsy was not required by the protocol in 28 instances (9%) of persistently elevated gene-expression profiling scores occuring in patients with no history of rejection on prior biopsies.

Among the 265 biopsies performed in response to elevated gene-expression profiling scores (≥34), 143 (54%) biopsies revealed no evidence of rejection (ISHLT Grade 0), 111 (42%) biopsies revealed ISHLT Grade 1R rejection, 8 (3%) biopsies revealed ISHLT Grade 2R rejection, and 3 (1%) biopsy revealed ISHLT Grade 3R rejection.

**2.4 Rejection rates:** There were 81 discrete treated rejection episodes (34 in the gene-expression profiling group and 47 in the endomyocardial biopsy group) observed in 61 patients. Among patients in the gene-expression profiling group, 20 (59%) treated rejection episodes were prompted by overt heart failure and/or by echocardiographic evidence of graft dysfunction, 7 (21%) episodes were associated with both clinical manifestations and elevated gene-expression profiling scores, and 6 asymptomatic episodes (18%) were detected solely on the basis of elevated gene-expression profiling scores. In contrast, 22 (47%) of the treated rejection episodes in the endomyocardial biopsy group were asymptomatic and detected by routine endomyocardial biopsy (see Supplementary Table 9).

The incidence of acute cellular rejection (ISHLT grades 2R or 3R) or mixed rejection (acute cellular rejection with antibody-mediated rejection) in our study was 6.1% of biopsies (5.6% of patients) in the gene-expression profiling group and 3.0% of biopsies (9.3% of patients) in the endomyocardial biopsy group. Antibody-mediated rejection was observed in 6.1% of biopsies (5.2% of patients) in the gene-expression profiling group and in 1.7% of biopsies (5.1% of patients) in the endomyocardial biopsy group.

**Supplementary Table 6: Baseline characteristics** of the IMAGE study population compared to OPTN/UNOS pediatric and adult heart transplant recipients from January 1, 2007 – December 31, 2007*

| | IMAGE Study Cohort | UNOS/OPTN Cohort |
|---|---|---|
| | **(N=602)** | **(N=2207)** |
| **Age at transplant – %** | | |
| 12 – 17 years | 0.8 | 4.8 |
| 18 – 34 years | 11.1 | 10.6 |
| 35 – 49 years | 20.8 | 19.9 |
| 50 – 64 years | 52.5 | 43.5 |
| 65+ years | 14.8 | 11.1 |
| **Male gender – (%)** | 81.9 | 73.7 |
| **Race – (%)** | | |
| White | 77.7 | 67.5 |
| Hispanic | 6.5 | 9.4 |
| African American | 11.8 | 19.3 |
| Asian or Pacific Islander | 2.2 | 3.2 |
| Other | 1.8 | 0.7 |
| **Indication for heart transplantation – no. (%)** | | |
| Coronary artery disease | 42.7 | 35.6 |
| Non-ischemic cardiomyopathy | 51.0 | 53.4 |
| Valvular heart disease | 1.8 | 1.5 |
| Congenital heart disease | 3.0 | 7.9 |
| Graft vasculopathy / Retransplant | 0.7 | Not available |

| | | |
|---|---|---|
| Other | 0.8 | 1.4 |
| **Induction therapy use – (%)** | | |
| OKT3 | 1.5 | 3.2 |
| Antithymocyte globulin | 17.4 | 4.2 |
| Basiliximab | 12.1 | 14.2 |
| Daclizumab | 21.4 | 12.7 |
| Alemtuzumab | 4.2 | 2.6 |
| Other | 1.3 | -- |
| **Immunosuppression – (%)†** | | |
| Cyclosporine | 26.9 | 33.0 |
| Tacrolimus | 72.4 | 64.9 |
| Mycophenolate mofetil / mycophenolic acid | 80.9 | 85.6 |
| Azathioprine | 6.8 | 2.8 |
| Sirolimus | 19.6 | 1.2 |
| Prednisone | 42.2 | 88.7 |

**Footnotes for Supplementary Table 6:**

* Data obtained from www.ustransplant.org

† Denotes all medications while in IMAGE study. Note: Medications in the UNOS/OPTN cohort reflect maintenance immunosuppression use prior to discharge from the transplant hospitalization.

**Supplementary Table 7:** Selected adverse events during the study

| Event | Gene profiling | Biopsy | P value |
|---|---|---|---|
| | (N=297) | (N=305) | |
| **Cardiac*** | 7 (2.4) | 8 (2.6) | 0.12 |
| Angina pectoris | 0 | 1 (0.3) | |
| Arrhythmia, palpitations, or tachycardia | 3 (1.0) | 3 (1.0) | |
| Congestive heart failure | 1 (0.3) | 1 (0.3) | |
| Cardiomegaly | 0 | 1 (0.3) | |
| Coronary artery disease | 2 (0.7) | 1 (0.3) | |
| Pericardial effusion | 1 (0.3) | 1 (0.3) | |
| **Infections** | 53 (17.8) | 43 (14.1) | 0.22 |
| Bacterial | 6 (2.0) | 3 (1.0) | |
| Fungal | 0 | 1 (0.3) | |
| Viral (cytomegalovirus, herpes simplex, herpes zoster) | 9 (3.0) | 3 (1.0) | |
| Viral (other) | 5 (1.7) | 6 (2.0) | |
| Other / unspecified | 33 (11.1) | 30 (9.8) | |
| **Neoplasms** | 11 (3.7) | 10 (3.3) | 0.83 |
| Skin cancer | 4 (1.3) | 4 (1.3) | |
| Squamous cell carcinoma | 1 (0.3) | 2 (0.7) | |
| Basal cell carcinoma | 1 (0.3) | 0 | |
| Malignant melanoma | 1 (0.3) | 2 (0.7) | |
| Unspecified | 1 (0.3) | 0 | |
| Breast cancer | 1 (0.3) | 0 | |

| | | |
|---|---|---|
| Colon cancer | 1 (0.3) | 1 (0.3) |
| Lung cancer | 1 (0.3) | 1 (0.3) |
| Prostate cancer | 4 (1.3) | 3 (1.0) |
| Pituitary adenoma | 0 | 1 (0.3) |

**Footnote for Supplementary Table 7:**

* Not meeting endpoint definition and not biopsy-related.

**Supplementary Table 8:** Biopsy use during study*

| | Gene-expression Profiling | Biopsy |
|---|---|---|
| | (N=287) | (N=292) |
| **Total biopsies -- no. biopsies** | 409 | 1249 |
| Routine per-protocol surveillance | N/A | 1125 |
| Performed due to elevated GEP[†] | 274 | N/A |
| Clinically driven[‡] | 70 | 31 |
| Performed within 90 days of rejection treatment | 52 | 58 |
| Off-protocol[§] | 13 | 35 |
| **Frequency of biopsies per patient year of follow-up – no. patients (%)** | | |
| 0 biopsies/patient year[‖] | 142 (50) | 4 (1.4) |
| 1 – 2 biopsies/patient year | 108 (38) | 94 (32) |
| 3 – 4 biopsies/patient year | 21 (7.3) | 135 (46) |
| ≥ 5 biopsies/patient year | 16 (5.6) | 59 (20) |
| **Number of biopsies per patient year of follow-up stratified by time post-transplantation – median (min – max)** | | |
| Overall | 0.5 (0.0 – 15.9) | 3.0 (0.0 – 22.1) |
| 6 – 12 months post-transplantation | 0.7 (0.0 –15.9) | 5.1 (2.8 – 22.1) |
| 12 – 36 months post-transplantation | 0.5 (0.0 – 12.0) | 3.0 (0.0 – 7.5) |
| 36 – 60 months post-transplantation | 0.2 (0.0 – 7.1) | 1.9 (0.0 – 4.5) |

**Footnotes for Supplementary Table 8:**

* The analyses include patients who completed at least 1 study visit and were followed for a minimum of 30 days in the study. Both scheduled study visits and non-scheduled outpatients visits were included.

† As mandated by the study protocol. 9 biopsies were performed for scores ≥30 prior to the protocol amendment on November 7, 2005. 265 biopsies were performed for scores ≥34.

‡ Clinically driven biopsies were performed, per-protocol, for clinical signs or symptoms of congestive heart failure or for graft dysfunction, defined by a decrease in the LVEF of ≥25% compared to the first visit (reference) value.

§ Off protocol biopsies included additional biopsies performed that were not mandated by the protocol and biopsies for which no reason was given.

‖ The number of patients who had exactly 0 biopsies on-study was 133 in the gene-expression profiling group and 2 in the biopsy group. An additional 9 patients in the gene-expression profiling and 2 patients in the biopsy group were also included in this category because they had 0.49 biopsies per patient year of follow-up, which was rounded down to 0 per year.

**Supplementary Table 9:** Treated rejection episodes

| | Gene-expression Profiling | Biopsy |
|---|---|---|
| | (N=34 Episodes) | (N=47 Episodes) |
| **Biopsy Histology** | | |
| Grade 0 | 2 | 5 |
| Grade 1R rejection | 8 | 8 |
| Grade 2R rejection | 11 | 22 |
| Grade 3R rejection | 2 | 3 |
| Antibody-mediated rejection | 8 | 6 |
| Mixed rejection | 3 | 3 |
| **Presentation** | | |
| Clinical signs or symptoms | 6 | 8 |
| Graft dysfunction on echocardiogram | 2 | 7 |
| Clinical signs/symptoms and graft dysfunction on echocardiogram | 12 | 10 |
| Clinical signs/symptoms and elevated GEP score | 6 | N/A |
| Clinical signs/symptoms, graft dysfunction on echocardiogram, and elevated GEP score | 1 | N/A |
| Elevated GEP score | 6 | N/A |
| Asymptomatic | 0 | 22 |
| Other* | 1 | 0 |

**Footnote for Supplementary Table 9:**

* One patient underwent endomyocardial biopsy due to a rising gene-expression profiling score that did not meet the threshold for biopsy (protocol violation).

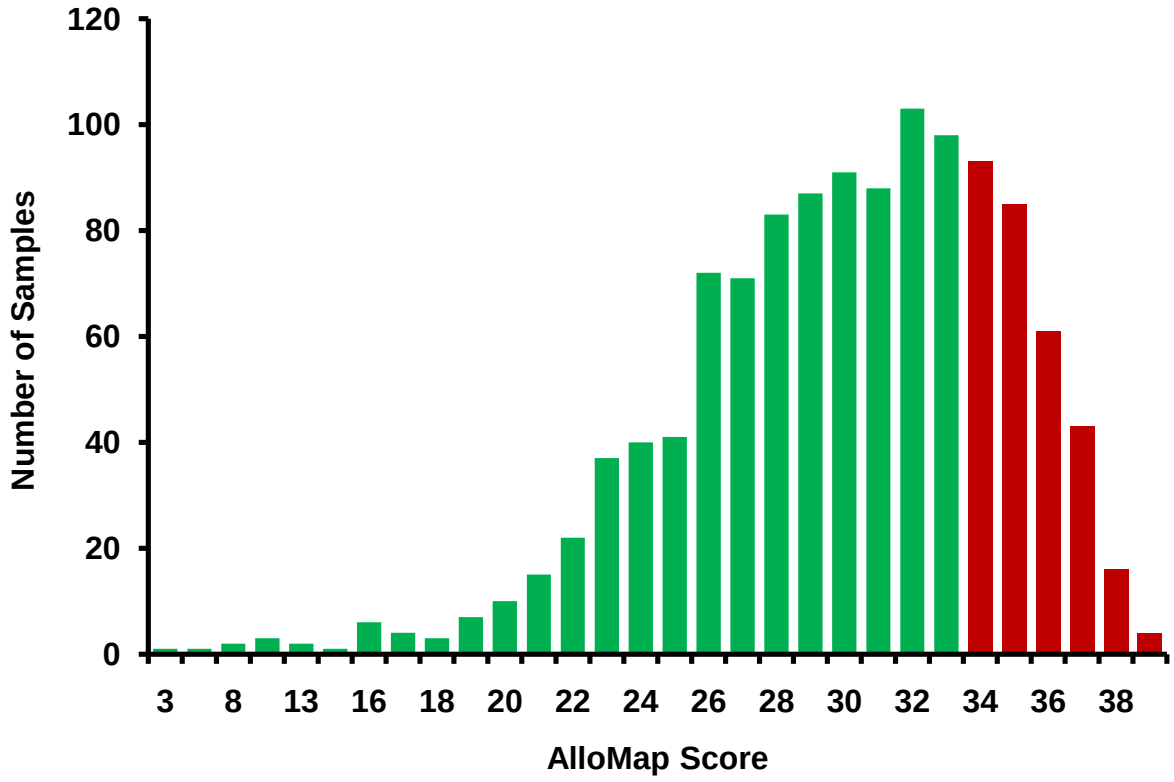
**Supplementary Table 10:** Quality of life and patient satisfaction scores.

| | Gene-expression Profiling | | | Biopsy | | | P-values* | | |
|---|---|---|---|---|---|---|---|---|---|
| | Enrollment | Year 1 | Year 2 | Enrollment | Year 1 | Year 2 | Enrollment | Year 1 | Year 2 |
| Patients on-study | N = 297 | N = 209 | N = 101 | N = 305 | N = 211 | N = 91 | | | |
| Patients completing SF-12 Survey | N = 249 | N = 148 | N = 89 | N = 239 | N = 146 | N = 83 | | | |
| SF-12 Mental health summary score | 51.6±10.1 | 50.3±10.8 | 50.8±10.1 | 52.4±8.9 | 51.7±9.7 | 50.7±9.8 | 0.33 | 0.23 | 0.66 |
| SF-12 Physical health summary score | 45.5±10.6 | 44.7±11.4 | 45.1±11.6 | 46.8±9.0 | 47.3±9.6 | 46.2±10.9 | 0.13 | 0.03 | 0.52 |
| Patients completing satisfaction questionnaire | N = 269 | N = 153 | N = 92 | N = 263 | N = 155 | N = 91 | | | |
| Patient satisfaction score | 6.86±2.75 | 8.15±2.95 | 8.74±1.90 | 6.74±2.71 | 6.64±2.98 | 6.66±2.81 | 0.61 | <0.001 | <0.001 |

**Footnote for Supplementary Table 10:**

* P values were obtained from the two-sample t-test.

**Supplementary Figure 1:** Distribution of AlloMap® scores in the gene-expression profiling group



## SECTION 3: REFERENCES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