CareDx Perspective on Questions for CAC on the use of Molecular Diagnostic Testing to identify acute rejection with AlloMap, AlloSure, and HeartCare in Heart Transplant recipients

1. ***Is there sufficient evidence to identify the patient population that the molecular diagnostic test could be used? (e.g., risk level, ethnic/cultural demographics, repeat transplant recipients, etc.***

a) *AlloMap:*

   a. *There is sufficient evidence to identify the patient population for which the molecular diagnostic test could be used.*

      i. *The large clinical utility prospective randomized control trial, IMAGE, enrolled a broad range of adult patients (>/= 18-year-old) beginning at 6 months post-transplant and evaluated AlloMap as a surveillance tool.*[1]

         1. *Exclusion criteria included the presence of graft dysfunction, heart failure or a history of antibody mediated rejection. Patients undergoing re-transplantation were not excluded, but only one patient in the AlloMap group was a re-transplant recipient.*
         2. *Overall demographics were representative of the average heart transplant population (mean age 54 years, 82% male) and 18.2% non-White race.*
         3. *Overall, this population resembles the general cardiac transplant patient population eligible for rejection surveillance who are not at elevated risk for antibody mediated rejection.*

      ii. *Following this study, eIMAGE further expanded the clinical utility population for surveillance to include consecutive adult patients (>/= 18 years old) beginning at 2 months post-transplant.*[2]

         1. *Exclusion criteria included graft dysfunction, heart failure, a history of AMR, a history of acute cellular rejection (ACR), donor specific antibodies, or anything thing that would confound the AlloMap score (prednisone greater than 20mg daily, recent whole blood transfusion or hemopoietic growth factors). Pregnant patients were also excluded. Re-transplantations represented 10% of the population. Overall demographics were representative of the average heart transplant population (mean age 53 years, 67% male) and 20% non-White race.*

   b. *Overall, the data supports the guidance that AlloMap is approved to aid in the identification of heart transplant recipients with stable allograft function who have a low probability of moderate/severe acute cellular rejection (ACR) at the time of testing in conjunction with standard clinical assessment.*

b) *AlloSure:*

a. *There is sufficient evidence to identify the patient population for which the molecular diagnostic test could be used.*

i. *The main clinical validation of AlloSure to detect rejection in cardiac transplant recipients occurred in the D-OAR registry.*[3] *The published study included 841 samples from 443 patients that included paired dd-cfDNA and cardiac biopsies. Samples were collected across 26* clinical sites, *from 55 days to >5 years post-transplant in patients >/=15 years old with 81% of samples collected during the first-year post-transplant and 13% collected during the second-year post-transplant. Of the paired samples, 587 were performed as part of surveillance and 254 were for cause based on clinical suspicion for rejection. Multi-organ transplant recipients were excluded. Demographics from the paired dd-cfDNA and biopsy patients were reflective of the general heart transplant population (mean age 59 years, 74% male) and included 23% non-White patients and 1% retransplants.*

ii. *A secondary validation study occurred in the Cedars-Sinai sub-study.*[3] *The mean age of this population was 56 years old and 67% were non-white.*

iii. *Subsequent to the clinical validation studies, there have been several non-randomized clinical utility studies published on AlloSure which also included a broad range of patients.*

1. *Gondi et al.: Included all cardiac transplant patients >/= 15 years old that were not multi-organ recipients or pregnant. The population had a mean age of 55 years and was 75% Male, 13% Black, 2% retransplant, and 76% were more than 6 months post-transplant.*
2. *Feingold et al: AlloSure performed for surveillance in pediatric patients >= 7 months post-transplant without a history of rejection. The population had a mean age of 15 years and was 66% Male and 7% Black.*
3. *Henricksen et al.: AlloSure performed for surveillance in consecutive cardiac transplant patients >/=18 years old beginning at 28 days post-transplant. The population had a mean age of 57 years and was 86% Male, 6.3% Black, 14% Hispanic, and 23% Asian.*
4. *Amadio et al.: AlloSure performed for surveillance in cardiac transplant recipients >/= 17 years old and >/= 6 months post-transplant. The population had a mean age of 46 years and was 68% Male, 16% Black, and 23% Asian.*

b. *Overall, the published peer-reviewed data supports the guidance that AlloSure has been studied in a broad range of patients with the following exceptions where data is absent.*
    i. *Multi-organ transplant recipients*
    ii. *Pregnant patients*

2. ***Is there sufficient evidence on the clinical context (i.e., for-cause vs. surveillance) in which the molecular diagnostic test could be used?***
    a. *AlloMap: There is sufficient evidence to use AlloMap for surveillance and for cause. Both clinical utility studies (IMAGE and eIMAGE) were performed in the rejection surveillance context. CARGO and CARGO-II had matched AlloMap and biopsies that were performed for cause. We note the ISHLT guidelines indicate that AlloMap should be used for surveillance.*
    b. *AlloSure: There is sufficient evidence to use AlloSure for surveillance and for cause. The clinical validation study, D-OAR, demonstrated a statistically significant difference in dd-cfDNA levels in matched samples that were performed for cause. All of the aforementioned clinical utility studies for AlloSure included patients undergoing rejection surveillance.*

3. ***In the existing evidence, what is the level of confidence (or certainty) regarding test performance data reported without any confidence intervals?***
    a. *AlloMap: Testing performance data was generally reported with confidence intervals and/or standard deviation in the CARGO registry.* For example, in the primary validation cohort, at a threshold of an AlloMap score of 20, sensitivity was 84% (95% CI of 66-94%) and specificity was *38% (95% CI 22–56%). ROC curve analysis in the secondary validation cohort demonstrated an area under the curve of 0.8 +/- 0.114 at >/=6 months or 0.86 +/- 0.09 at >/=12 months.*
    b. *AlloSure: Confidence intervals were also reported in the D-OAR registry. For example, in the for-cause biopsy group, operating characteristics were reported as follows: Sensitivity was 53.8% (33.3-75.1%), specificity was 76.1% (71.6%-80.2%), PPV was 11.6% (7.6%-16.6%), NPV was 96.6% (95.2%-98.2%), and AUC was 68.5% (48.0%-86.4%) for identifying rejection.*

4. ***Is there sufficient evidence to support the utility of surveillance (i.e., not for-cause) testing in heart transplant recipients?***
    A) There is sufficient evidence to support surveillance testing for AlloMap, AlloSure and HeartCare.
    a. *AlloMap surveillance was shown to be non-inferior to standard of care surveillance biopsies in the landmark IMAGE trial and in the eIMAGE trial.*
    b. *In a recent study published by Alam et al., Allosure alone was able to detect 22 out of 23 episodes of histological rejection. Moreover, AlloSure without AlloMap was used in the clinical utility study for surveillance published by Feingold et al.*

The strategy of replacing surveillance biopsies with dd-cfDNA did not lead to any short-term adverse events.

c. *HeartCare as surveillance was shown to be a safe alternative to endomyocardial biopsy surveillance by Gondi et al., Henricksen et al and Amadio et al.*

4b. If yes, what is the appropriate testing schedule based on the published evidence?

The most contemporary published evidence for a surveillance schedule comes from Henricksen et al which performed HeartCare surveillance at 4 and 6 weeks followed by monthly for months 2 -6, then every other month from 8-12 months, then every 3 months from months 12-24. We note that there is significant variability in surveillance frequency amongst cardiac transplant centers and that testing schedules/intervals should be set by a patient's physician based on medical necessity and their independent assessment of the patient.

5. ***Is there sufficient evidence on the ability of the molecular diagnostic test (or combination of tests) to discriminate rejection (acute T-cell-mediated or antibody-mediated) from quiescence?***

   There is sufficient evidence of AlloMap and AlloSure to discriminate rejection from quiescence.

   a. *AlloMap: In the secondary validation cohort in the CARGO study, the sensitivity and specificity to detect rejection at 6 months with a cutoff of a score of 28 was 71% and 87% respectively. The overall area under the ROC curve was 0.8 at 6 months and 0.87 at 1 year. Similar operating characteristics of the test to discriminate rejection from quiescence was confirmed outside of the United States in the CARGO II registry.*

   b. *AlloSure: In the D-OAR registry, the median AlloSure in quiescent samples was 0.07% and in rejection samples was 0.17% ($p<0.001$). At a cutoff of 0.2, the sensitivity and specificity to detect histological evidence of rejection was 44% and 80% respectively. In the Cedars-Sinai study, AlloSure in quiescent samples was 0.17% and 0.5 in samples with AMR ($p=0.004$). The sensitivity and specificity to detect AMR at a cutoff of 0.2% was 88% and 62% respectively. Finally, in an independent study published by Alam et al., the sensitivity and specificity to detect histological evidence of rejection for AlloSure at a cutoff of 0.2% was 96% and 66% respectively.*

   c. *While there is no published data on combination AlloMap and AlloSure (HeartCare)'s ability to discriminate rejection from quiescence, given that the tests are evaluating rejection via 2 different mechanisms, the combination of the two tests would likely lead to an improved ability to discern rejection from quiescence.*

6. ***Is there sufficient evidence to indicate that in patients without signs and symptoms of rejection, use of the molecular diagnostic test (or combination of tests) would preclude the need for endomyocardial biopsy?***

There is sufficient evidence that in patients without signs or symptoms of rejection, use of HeartCare, AlloMap and/or AlloSure can obviate the need for a biopsy.

a. *HeartCare: In 3 recent real-world studies evaluating HeartCare, investigators were able to document a significant reduction in biopsies without an increase in adverse events when AlloSure was combined with AlloMap. The reduction in biopsies ranged from 12.7% to 55% (Gondi, Henricksen and Amadio).*
b. *AlloMap: The IMAGE study and eIMAGE study were clinical utility studies that demonstrated that surveying patients with AlloMap was non-inferior to surveying patients with biopsies. In IMAGE, the AlloMap group received 67% fewer biopsies and in eIMAGE the AlloMap group received 83% fewer biopsies.*
c. *AlloSure: The clinical utility study by Feingold et al. demonstrated an 81%* reduction in biopsies with no increase in short-term adverse events.

7. ***Is there sufficient evidence to indicate that in patients with signs and symptoms of rejection, use of the molecular diagnostic test (or combination of tests) would preclude the need for endomyocardial biopsy?***
Due to the inherent risk of missing rejection in patients with signs and symptoms of rejection, studies have not been designed where biopsies are not performed in this clinical context.

8. ***Is there sufficient evidence on the ability of the molecular diagnostic test (or combination of tests) to guide clinical management without endomyocardial biopsy?***
Yes, there is sufficient evidence of the ability of HeartCare, AlloMap and AlloSure to guide clinical management without an endomyocardial biopsy.
8b. If "Yes" for any of the above, what aspect of your clinical management would be influenced by the test result?
a. *HeartCare: In the 3 recent real-world clinical utility studies, investigators were able to manage patients primarily by reducing immunosuppressive therapies without biopsies. In Amadio et al, the authors documented that in 42% of patients being surveilled with HeartCare, immunosuppressive therapy could be safely reduced without biopsies.*
b. *AlloMap: In eIMAGE, the authors specifically highlighted that steroid weaning successfully occurred in 91% of patients undergoing AlloMap surveillance.*
c. *AlloSure: In the clinical utility study by Feingold et al., AlloSure alone facilitated the reduction in immunosuppression including the transition to calcineurin free regimens.*

9. ***Would you perform an endomyocardial biopsy if the molecular diagnostic test indicates rejection, but the patient exhibits no signs and symptoms of rejection?***
As histological rejection is usually asymptomatic, even if asymptomatic, a biopsy should be considered in the context of an abnormal AlloMap, AlloSure or HeartCare. This is

based on positive predictive values noted below from registries and real-world published studies. We do note that the positive predictive values depend on rejection prevalence.

a. *AlloMap: The positive predictive value for acute cellular rejection at a score of 30 was 6.8% in the CARGO registry. We note that AlloMap may also be elevated in the context of infection.*
b. *AlloSure: In the surveillance analysis of D-OAR, AlloSure's positive predictive value for acute rejection was 8.7% at a cutoff of 0.2%.*
c. *HeartCare: In the real-world analysis by Henricksen et al, when both AlloMap and AlloSure were positive, rejection was discovered in 2 of 9 biopsies (PPV of 22%). In Gondi et al., when both AlloMap and AlloSure were positive, rejection was discovered in 2 of 27 biopsies (PPV of 7.4%).*

10. How confident are you in the evidence that, for AlloMap, an elevation in the AlloMap score indicates rejection?

There is high confidence that AlloMap will be elevated in clinically significant rejection. AlloMap is a sensitive marker of immune activity and can also be elevated in some other inflammatory conditions such as CMV infection. In cases of elevated AlloMap, there is high confidence that it indicates rejection or other immune activation. The confidence level for rejection will be further increased if the patient has a high pre-test probability of rejection. Please see PPVs values for question 9a.

11. How confident are you in the evidence that, for AlloSure, an elevation in donor-derived cell-free DNA indicates rejection?

There is high confidence that AlloSure will be elevated in clinically significant rejection. AlloSure is a sensitive marker of allograft rejection and is also an injury marker that may increase in some other inflammatory conditions. In cases of elevated AlloSure, there is high confidence that it indicates rejection or other graft injury. The confidence level for rejection will be further increased if the patient has a high pre-test probability of rejection. Please see PPV values for question 9b.