# CareDx Perspective on
# Questions for CAC on the use of Molecular Diagnostic Testing to identify acute rejection with AlloSure in Kidney Transplant recipients

1. Is there sufficient evidence to identify the patient population where molecular diagnostic test should be used? Based on the evidence, the appropriate patient population includes

All kidney transplant recipients, except patients with multi-organ transplant recipients, pregnant patients, patients < 14 days post-transplant.

Since the initial clinical validation in DART study (Bloom 2017), Allosure has been extensively studied in all risk categories, ethnicities including re-transplant patients and pediatric patients. More recently, in a large real-world study of over 1000 patients from 7 kidney transplant centers using Allosure prospectively as standard of care (Bu et al 2021), the study population closely resembled the overall U.S. adult kidney transplant population. This represented the largest published cohort of kidney transplant recipients undergoing surveillance with dd-cfDNA. For context, there are only about 20,000 kidney transplants per year in United States.

There have also been analysis and publications targeted towards specific patient populations such as pediatrics (Puliyanda 2020 and Dandamudi 2022) and retransplant (Sureshkumar 2020).

2. Is there sufficient evidence on the clinical context (i.e. for-cause vs surveillance) in which the molecular diagnostic test should be used

Evidence clearly indicates for-cause and surveillance use.

Allosure has been shown to have clinical utility in differentiating rejection from no-rejection in large diverse cohorts of both for-cause biopsies (clinical rejection) and surveillance biopsies (subclinical rejection). For example, 109 of 219 biopsies in Bu 2021 and 74 of 208 biopsies in Gupta 2022 were performed for surveillance.

In addition to ruling out/diagnosing clinical rejection, AlloSure can be used for-cause to assess response to treatment of rejection, which would otherwise need follow up biopsy for accurate assessment (Gupta 2022).

In addition to ruling out/diagnosing subclinical rejection, surveillance use of AlloSure helps risk stratify patient as risk of poor outcomes such as development of donor specific antibody (Bu 2021). Serial change in AlloSure during surveillance use is also a useful indicator of allograft injury (Bu 2021). It can also be used in conjunction with donor specific antibody monitoring to identify patients who do not need biopsy for subclinical antibody mediated rejection (Jordan 2018).

3. In the existing evidence, what is the level of confidence (or certainty) regarding test performance data reported without any confidence intervals

There is high confidence in the level of evidence.

Based on extensive published data in high impact journals (Bloom 2017, Bu 2021) repeatedly validated with consistent performance across multiple independent analysis in real world studies (Gupta 2022, Obrisca 2022), there is high level of confidence in the data on Allosure performance. Additionally, a large number of transplant physicians across the country utilize AlloSure as standard of care for 5 years now. This provides further confidence in the clinical applicability of the data.

4. Is there sufficient evidence to support the utility of surveillance (i.e. not for-cause) testing in kidney transplant recipients

Yes, there is sufficient evidence to support the utility of AlloSure surveillance testing.

Large multicenter real-world studies have clearly shown that AlloSure can identify subclinical rejection (Bu 2021, Gupta 2022). In another real-world study, AlloSure outperformed all traditional non-invasive tests in diagnosis of rejection, including creatinine, proteinuria, and donor specific antibodies (Obrisca 2022). In addition to identification of and ruling out subclinical rejection, AlloSure has clinical utility in risk stratification of patients when used as surveillance test (Bu 2021), to allow personalized care to optimize allograft and patient outcomes.

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

Month 1,2,3,4,6,9,12 and then quarterly post-transplant, subject to individual clinician judgment based on patient characteristics.

This schedule parallels the usual post-transplant clinical monitoring and follow up schedule developed over the years to correspond with clinically significant time points. Testing with AlloSure is more frequent in the early post-transplant period when there is higher risk of acute rejection and immunosuppression related infectious complications. This period requires frequent immunosuppression titration to balance the benefits, risks, and side-effects of medications. After the first 6 months, quarterly testing would be appropriate.

Bu 2021 validates the utility of AlloSure use for surveillance at this schedule in large diverse patient population. Melancon 2020 also validated AlloSure testing with this surveillance schedule.

These schedules have been reviewed by many transplant centers and clinicians and integrated into their existing standard of care workflow. There is significant variability in surveillance among transplant centers and testing is set by a patient's physician based on medical necessity.

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

Yes, there is sufficient evidence on the ability of Allosure to discriminate acute T-cell mediated rejection from quiescence.

Bloom 2017, Stites 2020, Melancon 2020, Bu 2021, Gupta 2022 demonstrate the ability of Allosure to discriminate acute TCMR from quiescence. In addition, Stites 2020 showed that within the borderline and low-grade TCMR cohort, AlloSure is elevated in patients at higher risk of poor immunological outcomes. Gupta et al demonstrated that AlloSure correlates even better with molecular transcriptome of TCMR than histology, suggesting that some of the low grade TCMR on histology may not be true rejections and rather represent a nonspecific inflammation and a "response to wounding" (Gupta 2022).

6. Is there sufficient evidence on the ability of the molecular diagnostic test (or combination of tests) to discriminate acute antibody mediated rejection from quiescence

Yes, there is sufficient evidence on the ability of AlloSure to discriminate acute antibody mediated rejection from quiescence.

Bloom 2017, Melancon 2020, Bu 2021, Obrisca 2022, Gupta 2022 demonstrate the ability of AlloSure to discriminate acute antibody mediated rejection from quiescence.

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

Yes, there is sufficient evidence to indicate that in patients without signs and symptoms of rejection, use of AlloSure would preclude the need for kidney biopsy.

The NPV of AlloSure at a score below 0.5% is 90% (Bu 2021). With the median AlloSure score in stable kidney transplant recipients of 0.21% (IQR 0.12%–0.39%) (Bromberg 2018), this remarkably high NPV would preclude the need for surveillance biopsies. This includes surveillance biopsies when performed in all kidney transplant recipients for subclinical rejection and those performed in high-risk populations such as those with donor specific antibodies.

8. Is there sufficient evidence to indicate that in patients with signs and symptoms of rejection, use of molecular diagnostic test (or combination of tests) would preclude the need for kidney biopsy

Yes, there is sufficient evidence to indicate that in patients with signs and symptoms of rejection, use of Allosure would preclude the need for kidney biopsy.

The high negative predictive value of AlloSure to rule out rejection, as noted above in Question 7, would preclude the need for for-cause biopsy being performed for concern of allograft rejection.

9. Is there sufficient evidence on the ability of the molecular diagnostic test(or combination of tests) to guide clinical management without a biopsy

Yes, there is sufficient evidence on the ability of AlloSure to guide clinical management without a biopsy.

9b) If yes for any of the above, what aspect of your clinical management would be influenced by the test result

With a low AlloSure in for-cause testing, clinicians can look for and optimize other causes of elevated creatinine and/or proteinuria. For example: pre-renal causes such as hypovolemia, elevated tacrolimus level, or conditions without acute inflammation such as diabetic nephropathy. In surveillance testing, with a low AlloSure score, clinicians can continue with titration of immunosuppression to balance the risks and benefits as well as personalize care such as frequency of follow up visits and monitoring.

An elevated AlloSure in patients getting treated for rejection will indicate inadequate response to therapy and suggest need for further treatment.

10. Would you perform a kidney biopsy if the molecular diagnostic test indicates rejection, but the patient exhibits no signs and symptoms of rejection

Yes, I would likely perform a kidney biopsy if AlloSure indicates rejection even though the patient exhibits no signs and symptoms of rejection.

Subclinical rejection is common in kidney transplant recipients. Thus, in most cases, clinicians would perform a biopsy with elevated AlloSure even with no signs and symptoms of rejection. The positive predictive value of Allosure depends on degree of AlloSure elevation and patient risk factors (Bu 2021, Jordan 2018). This can influence the decision to biopsy. For example, in very low risk patients with mild Allosure elevations, clinicians may opt for closer monitoring instead of biopsy straightaway.

11. How confident are you in the evidence that, for this test, the elevation in donor-derived-cell free DNA indicates T-cell mediated rejection

Very confident that AlloSure will be elevated in clinically significant TCMR. AlloSure is a sensitive marker of allograft rejection, TCMR and ABMR. AlloSure is also an injury marker that can increase in some other inflammatory conditions such as BK virus nephropathy (Kant 2021, Bu 2021). 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 high pre-test probability of rejection.

12. How confidence are you in the evidence that, for this test, the elevation in donor-derived-cell free DNA indicates antibody mediated rejection

Very confident that AlloSure will be elevated in clinically significant ABMR. AlloSure is a sensitive marker of allograft rejection and is also an injury marker that can increase in some other inflammatory conditions such as BK virus nephropathy (Kant 2021, Bu 2021). 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 high pre-test probability of rejection.