Donor-Derived Cell-Free DNA Normal Range and Biological Variation Defined in a Reference Population

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BACKGROUND

• The standard test to differentiate rejection and injury in kidney transplants is the allograft biopsy
• Donor-derived cell-free DNA (dd-cfDNA) is a noninvasive test of allograft cell injury that may enable more frequent, quantitative and safer assessment of allograft status1-3

OBJECTIVES

• The Circulating Donor-Derived Cell-Free DNA in blood for diagnosing Acute Rejection in Kidney Transplant Recipients (DART) study was designed to validate that plasma levels of dd-cfDNA can discriminate active rejection status and to characterize reference ranges for dd-cfDNA stable transplant recipients.
• By establishing clinical reference ranges of dd-cfDNA in stable renal transplant recipients and identifying normal biological variation in serial testing, clinical interpretation of dd-cfDNA results can be enhanced.

METHODS

• Blood specimens were prospectively collected from kidney recipients at scheduled post-transplant intervals (1,2,3,4 and 6 months).
• Patients with stable allograft function across at least 3 serial visits >14 days post transplant were selected as the reference population.
• Reference Population Selection Criteria

<table>
<thead>
<tr>
<th>Inclusion</th>
<th>Exclusion</th>
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<tbody>
<tr>
<td>Well Functioning grafts</td>
<td>Increase in Serum Creatinine ≥ 0.5 mg/dL Since Prior Testing</td>
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<tr>
<td>No Clinical Suspicion of Rejection</td>
<td>Delayed Graft Function Defined by Ongoing Need for Dialysis in the Post-Transplant Period</td>
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<td>Stable and Acceptable Serum Creatinine Values</td>
<td>Clinically indicated Video Renal Transplant Biopsy for Allograft Dysfunction</td>
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<tr>
<td>No Significant Proteinuria</td>
<td>Allograft Acute Rejection Event</td>
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<tr>
<td>No Infections</td>
<td>Active Urinary tract Cytomegalovirus (CMV) or Polyomavirus Type BK (BKV) infections.</td>
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<tr>
<td>No Acute Cardiovascular Changes or Other Acute Clinical Event</td>
<td>Prior Organ Transplant Remained in situ</td>
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• dd-cfDNA was measured using a validated clinical-grade targeted NGS method with an established analytical coefficient of variation (CV) of 6.8%.4
• Reference change values (RCV) and Coefficient of Variations (CV) computed using standard formulas.5

RESULTS

Of 384 patients with 1272 samples, 93 patients met the inclusion/exclusion criteria for reference population with ≥3 sample for a total of 380 samples that met dd-cfDNA test and sample QC

Biologic variability computed in 18 of 93 patients where dd-cfDNA ≥0.2% (limit of detection)

dd-cfDNA is below 1% in 96% of the samples in stable reference population

CONCLUSIONS

• Stable, healthy renal transplant recipients have a median dd-cfDNA level of 0.21%.
• 96% of dd-cfDNA results for stable, healthy renal transplant patients are below 1%.
• The reference change value (RCV), for dd-cfDNA is 61%. This reference change value defines the relative change, between two sequential results from an individual that is greater than would be expected of biological variation in a stable patient.

REFERENCES