Karius Test for the Detection and Monitoring of CMV Viremia

#558: Use of the Quantitative Karius® Plasma Next Generation Sequencing Cell-Free Pathogen DNA Test to Detect and Monitor Cytomegalovirus Infection in Allogeneic Stem-Cell Transplant Recipients

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The Karius Test may be used to monitor CMV viral load in patients while also being able to detect and quantitate 1,000+ pathogens from a single blood draw.

PATIENT POPULATION / STUDY DESIGN

Analytical comparison and validation of the Karius Test included 125 residual plasma samples that were negative or positive for CMV using two separate FDA approved assays. Quantitative CMV results from the Karius Test were compared and correlated with results from the two quantitative PCR tests for CMV monitoring.

To determine clinical utility of the Karius Test for monitoring CMV infection levels, plasma samples from 70 allo-SCT recipients with CMV viremia were tested.

RESULTS

The Karius Test showed high correlation with two FDA-approved CMV viral load tests. It had 94% agreement with the cobas CMV test using its cut-off for negative samples (<37.5 IU/ml) and 95% using a clinical utility cut-off (<2000 IU/ml). There is 95% agreement between the CAP/CTM and cobas CMV tests for both cut-offs.

Among 70 allo-SCT patients with known CMV viremia, the Karius Test showed potential clinical utility as a CMV monitoring assay with significant mean differences in CMV quantities (MPM) when compared between Day 0 and ≥ Day-7 as well as between Day 0 and ≥D14 of antiviral treatment.
ABSTRACT

Background: Allogeneic stem-cell transplant (allo-SCT) recipients are at risk of developing severe CMV infection. The pre-emptive approach to CMV prevention, weekly monitoring with CMV qPCR with treatment for positive viral load (VL), is standard care. The quantitative Karius plasma Next Generation Sequencing test (KT) can identify DNA from >1,000 human pathogens. We report on the clinical validation and utility of KT for detecting and monitoring CMV viremia in allo-SCT patients.

Methods: Plasma samples negative or positive by CMV PCR (COBAS® AmpliPrep/COBAS® TaqMan® CMV Test [CAP/CTM] or cobas 8800/8800 [cobas CMV]) were tested with KT for analytical comparison. For KT, after cell-free DNA (cfDNA) was sequenced, human-derived reads were excluded bioinformatically, and remaining DNA sequences were aligned to a curated microbial pathogen database. KT results were reported as molecules per microliter (MPM). Linear regression was used to correlate log10 transformed CMV VL results within the overlapping quantifiable range of comparison assays. Using the regression equation to convert KT results from MPM to IU/mL, percent agreement was calculated and assay result agreement was analyzed with Bland-Altman plots. KT virome viremia results were selected from the prospective observational DISCOVER trial (NCT02804464), and their plasma specimens were tested with KT. Mean differences in MPM between 17 days before start of antiviral treatment (D-7) and start of treatment (D0), and between D0 and ≥D14 days after start of treatment (≥D14) were examined with Wilcoxon rank-sum tests. Among 125 residual samples tested by all 3 assays, 1 sample failed KT sequencing and was excluded from the analysis. Percent agreement between KT and cobas CMV (n = 124) was 94% using the cobas CMV cut-off for negative samples (VL < 3.5 IU/mL) and 95% using a clinical utility cut-off (VL < 2,000 IU/mL). Comparatively, the percent agreement between CAP/CTM and cobas CMV was 95% for both cut-offs. Correlation coefficients were 0.921 for qKT vs CAP/CTM (n = 74) and 0.951 for KT vs cobas CMV (n = 87), with result differences ranging 0.90 to 0.90 log10 and 0.81 to 0.81 log10, respectively. Among 70 DISCOVER trial patients, those with detectable KT CMV showed significant mean differences in MPM between D0-7 and D0 of antiviral treatment (n = 11; p = 0.042) and between D0 and ≥D14 of antiviral treatment (≥D14) were examined with Wilcoxon rank-sum tests.

Results: Among 125 residual samples tested by all 3 assays, 1 sample failed KT sequencing and was excluded from the analysis. Percent agreement between KT and cobas CMV (n = 124) was 94% using the cobas CMV cut-off for negative samples (VL < 3.5 IU/mL) and 95% using a clinical utility cut-off (VL < 2,000 IU/mL). Comparatively, the percent agreement between CAP/CTM and cobas CMV was 95% for both cut-offs. Correlation coefficients were 0.921 for qKT vs CAP/CTM (n = 74) and 0.951 for KT vs cobas CMV (n = 87), with result differences ranging 0.90 to 0.90 log10 and 0.81 to 0.81 log10, respectively. Among 70 DISCOVER trial patients, those with detectable KT CMV showed significant mean differences in MPM between D0-7 and D0 of antiviral treatment (n = 11; p = 0.042) and between D0 and ≥D14 of antiviral treatment (n = 10; p = 0.009).

Conclusions: High correlation was observed between KT and two FDA-approved CMV VL assays. KT identified significant increases in CMV clinical utility of VL at start of treatment and significant decreases at ≥D14 post-treatment in allo-SCT recipients, showing potential KT as a monitoring assay, with the added value of detecting >1,000 pathogens in the same blood draw.

BACKGROUND AND METHODS

Sample Processing and Workflow

Conflict of Interest Disclosures:

This study was funded by Karius, Inc. MF and PCH received a research grant from Karius to conduct the DISCOVER Study. AL and JY have no conflict of interest. JY has no conflict of interest. HS, AP, DH, DKH, LB, SZ are employees of Karius, Inc.