ABSTRACT

Background: The diagnosis of infections in stem-cell transplant (SCT) patients is challenging. There is a need for improved diagnostics that can quickly and accurately detect a broad range of viruses, bacteria, and fungi that can infect these patients to guide targeted therapy.

Methods: We enrolled 20 patients in a prospective study evaluating the Karius plasma next-generation sequencing (plasma NGS) test to detect infections in SCT patients. Patients had baseline plasma samples drawn prior to transplant followed by weekly collections during engraftment and at onset of febrile episodes. Samples were transferred to the Karius CLIA/CAP laboratory (Redwood City, CA) where cell-free DNA was extracted from plasma and NGS performed. Human sequences were removed and remaining reads were aligned against a curated pathogen database. Organisms present at a significance level above a predefined threshold were reported.

Results: Cytomegalovirus (CMV) was identified in 12/20 patients. Using a nearest-neighbor method, 97 pairs of observations were identified for a comparison of Karius plasma NGS and CMV qPCR collected within 1 day of each other. Positive agreement (PPA) between Karius plasma NGS and CMV qPCR was 81.8% (45/55). PPA was 100% (26/26) when values below the lower limit of quantitation (<137 IU/ml) for CMV qPCR were excluded. In addition to CMV, plasma NGS detected EBV, HHV8, BK virus and Torque teno viruses. The Karius plasma NGS test also detected pathogens prior to conventional testing being ordered in two patients with subsequently confirmed acute infections. Staphylococcus aureus was detected one day and one week prior to blood cultures in one patient and Chlamydia trachomatis 30 days prior to targeted testing in a second patient. These two cases highlight the potential of the Karius plasma NGS test to detect pathogens in SCT patients early when used as a monitoring tool.

Conclusions: Karius has developed a novel NGS plasma test that can simultaneously identify pathogens in SCT patients. The test had 100% concordance with CMV qPCR above the lower level of quantitation. Further work is ongoing to determine the lower limits of detection for the Karius plasma NGS test. Using plasma NGS to monitor SCT patients for infection could permit earlier detection of pathogens, enabling earlier targeted therapy for this vulnerable population.

BACKGROUND AND METHODS

Study Design

Plasma Sampling at Febrile Episodes

Weekly Plasma Sampling until Post-SCT Discharge

Monthly Plasma Sampling

0 7 14 Month 1 Month 2 Month 12

Sample Processing and Workflow

Plasma

Library Preparation

Sequencing

Analysis

Report

RESULTS

Detection of CMV by Karius Plasma NGS and CMV qPCR

| Plasma NGS vs CMV qPCR (all positive values) |
| Karius CMV Comparator |
| Positive | Negative |
| Positive | 45 | 7 |
| Negative | 10 | 35 |

Positive Agreement: 81.8%
Negative Agreement: 83.3%

Karius Plasma NGS can Identify Concurrent Pathogens

Subject 1 – HHV6 and CMV

Subject 2

Chlamydia trachomatis Detected by Karius

Chlamydia trachomatis Detected by urine NAAT

Lower Limit of CMV qPCR Quantitation (LLOQ)

Serial Cell-free Karius Plasma NGS can Detect Pathogens Earlier than Conventional diagnostics

Subject #2 Case Details:

21yo M with AML s/p allogeneic stem cell transplant. Patient was admitted on HD#60 with urinary pain and acute kidney injury. Underwent ureteroscopy on HD#63 which showed diffuse bladder inflammation. R ureter dilated and stent placed. HD#70 urine Chlamydia testing positive

Plasma NGS positive for Chlamydia trachomatis on HD#39 and HD#50 (31 days before clinical testing)

Subject #3 Case Details:

69yo F with myelodysplastic syndrome s/p allogeneic stem cell transplant. Transplant complicated by VOD, renal failure, respiratory failure. MRSA bacteremia on HD#26 which was treated and cleared. MRSA bacteremia again on HD#53.

Plasma NGS positive for Staphylococcus aureus on HD#25 and HD#46 (1 day and 7 days before clinical testing)

CONCLUSIONS

- In stem-cell transplant patients, the Karius cell-free plasma NGS test shows good concordance with CMV qPCR
- Serial Karius plasma NGS sampling in stem-cell transplant patients has the potential to detect a broad range of pathogens earlier than conventional diagnostics