KARIUS® | STUDY SUMMARY

Karius Test for Rapid Noninvasive Pathogen Detection in Immunocompromised Patients

534 Next Generation Sequencing of Microbial Cell-Free DNA for Rapid Noninvasive Diagnosis of Infectious Diseases in Immunocompromised Hosts

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POSTER SESSION II - Infectious Diseases and Cytotoxic T Lymphocytes
Saturday, February 23rd: 6:45 - 7:45 PM

The Karius® Test provides a rapid NGS-based assay to help diagnose infections in immunocompromised patients with a noninvasive blood draw.

PATIENT POPULATION / STUDY DESIGN

This case series includes ten adult immunocompromised patients with febrile illness or documented infection being followed at a cancer center and receiving antimicrobials at the time of sample collection. The enrollment spanned a period of approximately two months. Patients were 60% male and ranged in age from 20-65 years. Nine patients had underlying hematological disease and/or had received a hematopoietic cell transplant while the tenth patient was a kidney transplant recipient.

As part of the diagnostic workup, plasma samples isolated from 5 mL of peripheral blood were sent for the Karius Test, which uses next generation sequencing of microbial cell-free DNA to identify over 1,000 pathogens including bacteria, DNA viruses, fungi, and parasites.

RESULTS

Karius Test results showed 100% correlation where a microbiological diagnosis was established with other methods. This included detection of *Aspergillus* species, *Pneumocystis jirovecii*, *Stenotrophomonas maltophila*, CMV and Adenovirus. Among four hematological patients with culture-negative sepsis, The Karius Test identified causes of bacterial sepsis in two cases.

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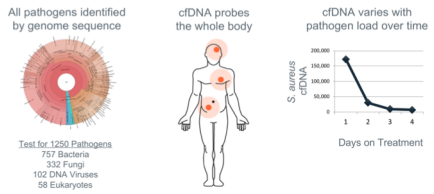
Background

- Infection is a leading cause of mortality among chemotherapy and hematopoietic cell transplantation (HCT) recipients
- Establishing a microbiological diagnosis is often challenging due to a number of reasons including: i) prior exposure to antibiotics and antifungals ii) low sensitivity of cultures; and iii) tissue biopsy often precluded due to thrombocytopenia or clinical instability
- Next-generation whole-genome sequencing of circulating microbial DNA is a non-invasive testing strategy that enables powerful clinical insights in diagnostically-challenging cases

Three advantages of sequencing microbial cell-free DNA



Study design

- Case series of adult patients receiving chemotherapy or transplant followed at the Sylvester Comprehensive Cancer Center who were enrolled (July 31 - October 2, 2018) at the time of febrile illness or documented infection (e.g., positive blood cultures, clinical/radiographic evidence of pneumonia). Most of the patients had an established diagnosis of infection prior to NGS testing.
- Blood samples (5 mL) were collected in BD vacutainer plasma preparation (PPT) tubes. Samples were collected at the time of suspected or confirmed infection diagnosis. Samples were shipped overnight to the Karius, Inc. CLIA/CAP laboratory(Redwood City, CA).
- All febrile patients had blood cultures collected within 24 hours of plasma sample collection for NGS. All of them were on antimicrobials prior to blood sample collection.

The Karius® Test

- The Karius next-generation quantitative sequencing (NGS) assay identifies microbial cell-free DNA (cfDNA) in plasma from bacteria, DNA viruses, yeasts, mold, and protozoa
- Next day results are reported from a single blood draw processed at CAP-accredited and CLIA-licensed laboratory

Results

- Among patients with hematological malignancy in whom a microbiological diagnosis was established (n=5), cfDNA NGS testing correlated with other methods in all cases (100% sensitivity). This included patients with proven/probable invasive aspergillosis, PJP, *Stenotrophomonas maltophilia* bacteremia, CMV and adenovirus viremia.
- Among four patients with hematological malignancy with negative standard laboratory testing, the NGS test identified causes of bacterial sepsis in two patients.

Table 1. Clinical characteristics of study subjects and results of next-generation sequencing of microbial cell-free DNA

Age, Sex	Underlying Disease	Clinical Scenario	Days of antibiotics/ antifungals prior to Karius sample	Conventional Diagnostic Method Results[b]	Microbial cfDNA pathogen results	Molecules per Microliter (MPM)	Reference Values[c] (MPM)	Correlation[d]
65F	Kidney transplant	Abdominal abscess	18/182	*Aspergillus fumigatus* detected by PCR and culture in abdominal fluid	Negative (*Aspergillus fumigatus*[e])	15[e]	<10	No[e]
21M	NHL, HCT day +342	Mediastinal LAD	0/8	Negative fungal serologies and antigens BAL and lymph node tissue cultures negative	Negative			Yes
20M	AML, HCT day +9	Neutropenic fever, diarrhea	8/2	CMV detected <137 IU/mL (subsequently peaked at 2,621 IU/mL) Blood cultures and C. difficile PCR negative	Cytomegalovirus	108	<10	Yes
64F	B-ALL MMUD day +291	Fever, cough, lung mass	6/5	*Pneumocystis jirovecii* BAL PCR+	*Pneumocystis jirovecii*	263	<10	Yes
37M	Relapsed DLBCL after CAR-T	Neutropenic fever, diarrhea, cough	21/5	Adenovirus 480 copies/mL (subsequently peaked at 2,600 copies/mL)	Adenovirus	845	<10	Yes
56M	AML, MMUD day +290	Pulmonary nodules, SOB	6/21	CMV detected <137 IU/mL (subsequently peaked at 440 IU/mL) Repeat BAL negative	Cytomegalovirus	93	<10	Yes
44M	DLBCL	Fevers, pulmonary nodules	3/3	Blood cultures negative	*Rothia mucilaginosa*	20	<10	No
60F	MDS, HCT day+160, GI-GVHD	Septic shock, multi-organ failure	15/10	Blood cultures negative	*Escherichia coli* *Lactobacillus rhamnosus* Torque teno virus	2,492 308 91	<17 <10 <10	No
55F	Multiple myeloma	Pneumonia	2/0	Negative BAL studies	Negative			Yes
58M	AML	Neutropenic fever, pulmonary nodules, sepsis	120/129	*S. maltophilia* in blood cultures Pan-*Aspergillus* PCR+ in BAL Serum galactomannan+	*Stenotrophomonas maltophilia* *Aspergillus oryzae* *Staphylococcus epidermidis*	236,594 11,533 9,673	<83 <10 <17	Yes

[a] *Refers to empiric or targeted therapy only. It does not include days of antimicrobial prophylaxis.* [b] *Blood cultures were obtained within 24h of plasma sample for NGS in all patients and resulted as negative unless specified otherwise in the table.* [c] Reference value is the 97.5th percentile in self-reported healthy adults for whom the Karius® Test was performed [d] Correlation between Karius® Test and standard laboratory methods [e] *Aspergillus fumigatus* reads were present in the raw data but below the threshold for a positive test result.
ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BAL, bronchoalveolar lavage; CAR-T, chimeric antigen receptor-modified T-cell immunotherapy; cfDNA, cell-free DNA; CMV, cytomegalovirus; CVC, central venous catheter; DLBCL, diffuse large B cell lymphoma; GI-GVHD, gastrointestinal graft-versus-host disease; HCT, hematopoietic cell transplantation; F, female; M, male; MPM, molecules per microliter; NGS, next-generation sequencing; NLH, Non-Hodgkin lymphoma; SOB, shortness of breath.

Conclusions

- The Karius® Test demonstrated clinical utility in identifying causative pathogens in hematological patients with proven/probable invasive aspergillosis, PJP, *Stenotrophomonas maltophilia* bacteremia, CMV and adenovirus viremia.
- NSG seems like a valuable tool for the rapid noninvasive diagnosis of infectious complications following chemotherapy or transplantation, especially in cases where conventional diagnostics have limited sensitivity or invasive procedures for sampling are precluded.
- Larger studies are needed to validate the use of this technology in the HCT population

REFERENCES

Blauwkamp T, et al. Analytical and Clinical Validation of a Microbial Cell-Free DNA Sequencing Test for Infectious Disease. Nature Microbiology in press
Ballen K, et al. Infection Rates among Acute Leukemia Patients Receiving Alternative Donor Hematopoietic Cell Transplantation. Biol Blood Marrow Transplant 2016;22(9):1636-45
Gudiol C, et al. Etiology, clinical features and outcomes of pre-engraftment and post-engraftment bloodstream infection in hematopoietic SCT recipients. Bone Marrow Transplant 2014;49(6):824-30.
Fukuda T, et al. Risks and outcomes of invasive fungal infections in recipients of allogeneic hematopoietic stem cell transplants after nonmyeloablative conditioning. Blood 2003;102(3):827-33
Clancy CJ, Nguyen MH. Finding the "missing 50%" of invasive candidiasis: how nonculture diagnostics will improve understanding of disease spectrum and transform patient care. Clin Infect Dis 2013;56(9):1284-92
Hong DK, et al. Liquid biopsy for infectious diseases: sequencing of cell-free plasma to detect pathogen DNA in patients with invasive fungal disease. Diagn Microbiol Infect Dis 2018;92(3):210-3.
De Vlaminck I, et al. Noninvasive monitoring of infection and rejection after lung transplantation. Proc Natl Acad Sci U S A 2015;112(43):13336-41.