Enhanced Whole Exome Profiling of Tumor Circulating Cell-Free DNA Enables Sensitive Assessment of Tumor Mutations

#1989

Simo V. Zhang*, Mengyao Tan*, Josette M. Northcott, Shuyuan Ma, Christopher S. Nelson, L. Gordon Bentley, Manju Chinnappa, Devayani P. Bhave, Dan Norton, Jason Harris, Sean M. Boyle, John West, Richard Chen

Personalis, Inc. | 1330 O'Brien Dr., Menlo Park, CA 94025
* Co-first author

Contact:
simo.zhang@personalis.com
mengyao.tan@personalis.com

Introduction

An increasing number of studies have demonstrated the potential use of circulating cell-free DNA (cfDNA) for diagnosis, prognosis, and disease progression monitoring. However, many of these studies utilize assays covering a limited set of well known genes, and therefore can miss biologically and clinically important genetic alterations in DNA repair pathways, immuno-modulatory pathways, mechanisms of resistance and changes in neoantigen status. To address this, we have developed a whole-exome scale cfDNA platform, NeXT Liquid Biopsy, that enables sensitive detection and tracking of mutations in approximately 20000 genes. The NeXT LB platform sequences a triplet of tumor, normal, and plasma samples from a patient, allowing 1) discovery of novel genetic mutations, 2) non-invasively close monitoring of clonal and subclonal evolution in response to treatment of curative intent, and 3) broad interrogation of inter- and intra-tumor heterogeneity.

Methods

Whole-Exome NeXT LB assay and Silencer

To enable sensitive detection across the exome, we developed an enhanced exome assay and chemistry that augments hard to sequence genomic regions such as regions of high GC content, to enable more uniform coverage across the exome. Additionally, we achieve a high average depth of approximately 2000X for the entire exome, with additional boosted depth (5000X) for 248 clinically relevant oncogenic and tumor suppressor genes to further enhance sensitivity. For analysis, we developed a computational algorithm, Silencer, that enables accurate somatic mutation detection without compromising sensitivity in the plasma. Silencer is empowered by an error suppression model estimated from a panel of normal individual plasma samples, and ad hoc filters including a dedicated blacklist that is tailored to our NeXT LB technology.

Sensitivity Evaluation

Sensitivity of the NeXT LB platform was evaluated using three approaches. First, Horizon reference materials at 5%, 2.5%, and 1% allele frequency (AF) dilutions were evaluated. Second, Seracare reference materials at 2%, 1% and 0.5% AF dilutions were analyzed. Both evaluations used 50ng input and have 3 replicates at each AF dilutions. To further demonstrate our sensitive detection at the whole exome scale, we developed a cell culture media system that models the shed and degraded tumor DNA fragments seen in human plasma samples. Two breast cancer cell lines, HCC1143 and HCC1954, were selected as a set of high-quality goldset SNVs were already established as part of the tissue somatic verification. The two cell lines and their corresponding B-lymphoblast cells were cultured in serum-free media. Cell media was collected after 48-72hr culture, and cfDNA was extracted for the downstream NeXT LB assay. Here, sensitivity is defined as the percentage of detected goldset SNVs.

Clinical Application

Lastly, we demonstrated the power of our NeXT LB technology in providing a much broader view in mutation landscape profiling, tumor-plasma concordance evaluation, and disease monitoring using matched tumor, plasma and matched normal samples from nine head and neck cancer patients (with both pre- and post-treatment samples), as well as five late stage colorectal cancer patients.

Results

100% sensitivity achieved for SNVs with AF≥1%

Our NeXT LB platform successfully and consistently detected all 25/25 (100%) known SNVs across each of the 2% and 1% dilution replicates, and detected 24/25 (96%) events in the majority of the 0.5% dilution Seracare sample replicates. Further, we achieved 100% sensitivity in each of the 5%, 2.5%, and 1% Horizon dilution samples. Additionally, the observed AFs were well correlated with the expected AFs in both of the Seracare and Horizon samples.

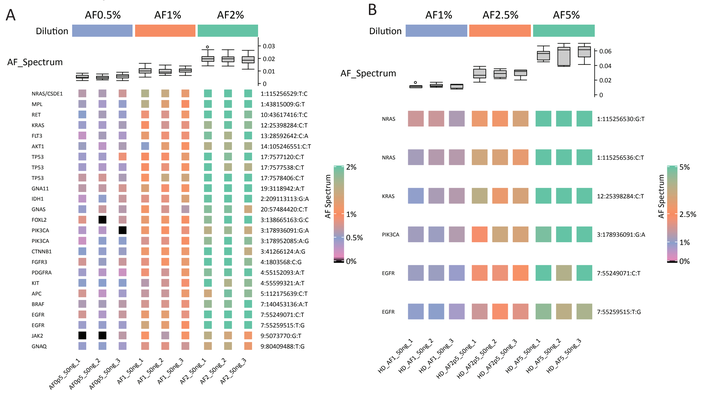

Figure 1: NeXT LB technology sensitivity with Seracare (A) and Horizon (B) ctDNA reference standards.

High sensitivity acheived at the whole-exome scale using cell media system

Our internally developed cell media system is whole-exome scale and built upon well-characterized cancer cell lines, providing a far broader assessment of accuracy than the commercially available reference standards. Using this system to measure accuracy across 137 (HCC1143) and 185 (HCC1954) variants, our NeXT LB platform achieved >95% sensitivity for variants with AF>=2%, and between 85% to 92% sensitivity for mutations with AF between 1% to 2%.

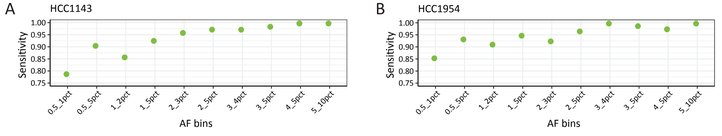

Figure 2: NeXT LB technology whole-exome scale sensitivity with HCC1143 (A) and HCC1954 (B) breast cancer cell media system.

NeXT LB detected a substantial number of distinct variants outside of the footprint of targeted panels in a head and neck cancer patient cohort

NeXT LB technology was applied to profile somatic events in a cohort of 9 head and neck cancer patients. NeXT LB detected 1,334 plasma SNVs across the platform, 31 of which were in immuno-oncology related genes. Application of our exome-scale approach detected 1,241 somatic events which are outside of commercially available targeted cfDNA panel genes, 17 of which are in immuno-oncology related genes. These observations highlight the extent of somatic evidence observable through an exome-scale cell free approach. In addition to IO pathways, NeXT LB provides other important insights across the entire exome, such as innate and adaptive immune responses.

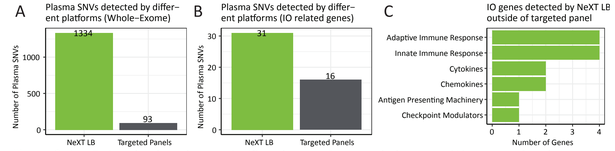

Figure 3: NeXT LB technology detects significantly more SNVs at whole-exome (A), ImmunoOncology related genes (B), and ImmunoOncology related pathways (C) outside of footprint of targeted panels.

Non-invasively profiling tumor heterogeneity and clinical relevant somatic mutations on NeXT LB

We next applied NeXT LB to assess tumor-plasma concordance and cfDNA cancer driver mutations in two independent colorectal cancer and head and neck cancer cohorts. NeXT LB consistently detected both tumor concordant and plasma distinct mutations from cancer patient samples, both of which have implications for non-invasive monitoring of patient response to treatment and profiling tumor heterogeneity. Further, we observed dynamic allele frequency changes in cancer driver genes in checkpoint blockade non-responders, detecting increasing allele frequency over the course of therapy, highlighting NeXT LB's utility in non-invasive response monitoring.

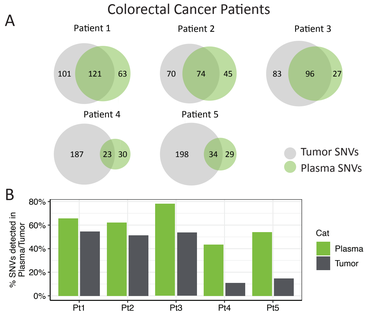

Figure 4: The number (A) and the percentage (B) of tumor-plasma concordant mutations identified from 5 late-stage colorectal cancer patients.

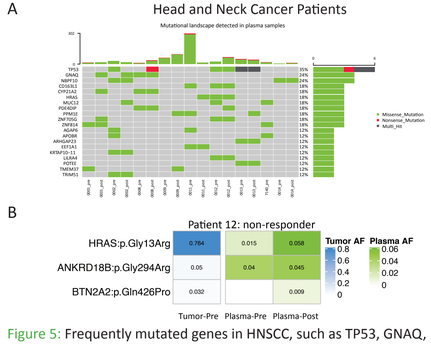

Figure 5: Frequently mutated genes in HNSCC, such as TP53, GNAQ, HRAS were also detected in plasma samples (A). Patient 12 who is defined as non-responder clinically also demonstrated increased AF of HRAS mutation post treatment (B).

Conclusion

We have developed a whole-exome scale NeXT LB technology that enables sensitive monitoring and detection of somatic SNVs from cfDNA. The NeXT LB platform generates a much broader view of the tumor mutational landscape from the plasma than typical targeted liquid biopsy platforms. The platform enables broader monitoring of changes in response to cancer therapy, acquired mechanisms of drug resistance, and intra- and inter-tumor heterogeneity.

Personalis