Sensitive detection and monitoring of genetic alterations in circulating cfDNA with an enhanced whole-exome approach

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Introduction

The analysis of tumors using circulating cell-free DNA (cfDNA) is beginning to transform cancer diagnosis, prognosis, response to therapy and to enable disease progression monitoring. Most cfDNA assays are centered around the identification of therapeutically actionable mutations and only cover a limited number of genes, and as such cannot inform on all genetic alterations present in the tumors. 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 20,000 genes. The NeXT LB platform generates comprehensive sequencing data derived from the cfDNA (plasma), the tumor (e.g. FFPE) and the patient's germline (e.g. blood cells), and therefore enable to closely discover and monitor clonal and subclonal evolution of mutations in plasma, and novel genetic mutations following surgery, and/or treatment therapies.

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 247 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 built from a panel of normal individual plasma samples, and custom filters including a dedicated blacklist that is tailored to our NeXT LB technology.

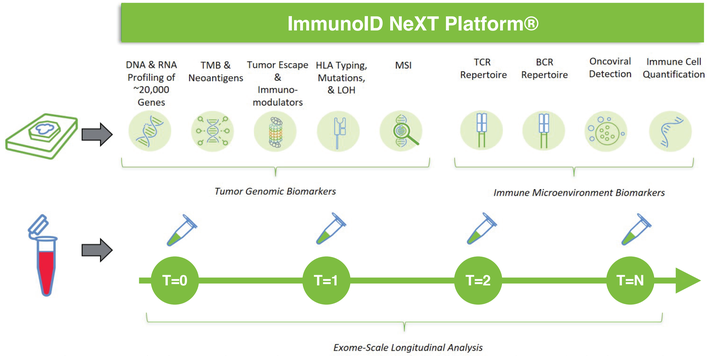

Figure 1: The NeXT LB technology complements the ImmunoID NeXT platform by surveying the mutation landscape and immune status at baseline, as well as the following timepoints.

Results

100% sensitivity achieved for SNVs with AF≥1%

Our NeXT LB platform successfully and consistently detected all 25/25 (100%) known SNVs across all 2% and 1% dilution replicates, and missed only one or two events (94.6% on average) in the 0.5% dilution SeraCare® samples. We also achieved 100% sensitivity in 5%, 2.5%, and 1% Horizon dilution samples. The observed AFs in SeraCare and Horizon sample are well correlated with the expected AFs.

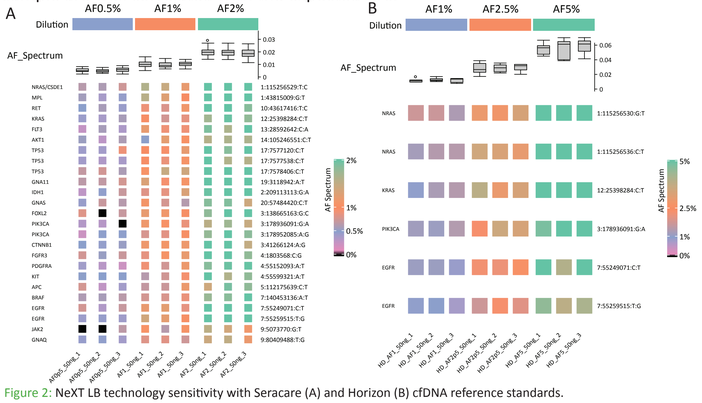

Figure 2: NeXT LB technology sensitivity with Seracare (A) and Horizon (B) cfDNA reference standards.

Whole-exome scale high sensitivity achieved using cell media

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. We developed cell-free media samples from two breast cancer cell lines, HCC1143 and HCC1954, from which curated sets of high-quality known somatic mutations. As demonstrated in Figure 3B and 3C, 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% across co-culture cell media dilutions.

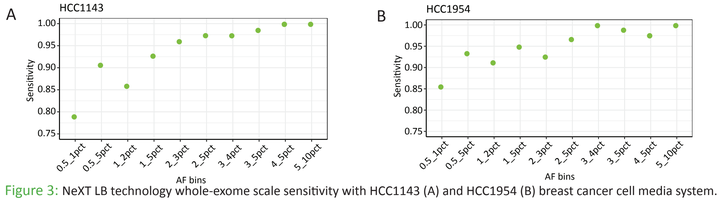

Figure 3: NeXT LB technology whole-exome scale sensitivity with HCC1143 (A) and HCC1954 (B) breast cancer cell media system.

NeXT LB detected more SNVs outside of footprint of targeted panels

Our NeXT LB platform detected 1334 somatic SNVs in a cohort of 9 head and neck cancer plasma samples, 31 of which were in clinically-relevant immuno-oncology genes. The amount of detected plasma SNVs would be significantly less if intersecting with genes included across all commercially available targeted panels. With NeXT LB, we could gain additional insights on IO pathways, such as innate and adaptive immune responses.

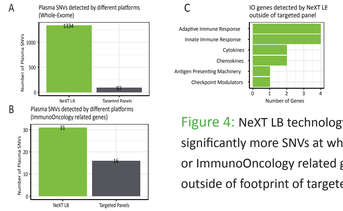

Figure 4: NeXT LB technology detects significantly more SNVs at whole-exome (A) or ImmunoOncology related genes (B, C) outside of footprint of targeted panels.

Non-invasively profiling tumor heterogeneity and clinical relevant somatic mutations

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.

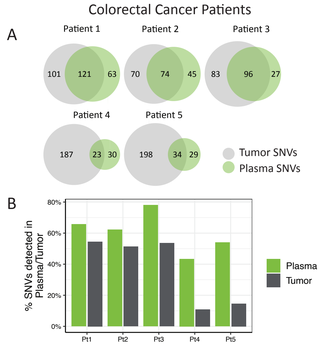

Figure 5: The number (A) and the percentage (B) of tumor-plasma concordant mutations identified from 5 late-stage colorectal cancer patients.

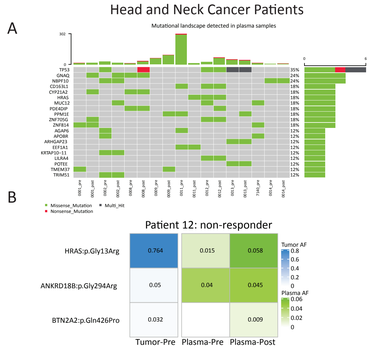

Figure 6:(A)-(B) Frequently mutated genes in HNSCC, such as TP53, GNAQ, HRAS were also detected in plasma samples. Patient 12 who is defined as non-responder by clinical measurement also demonstrated increased AF of HRAS mutation post treatment.

Conclusion

We have developed a whole-exome scale NeXT LB technology that enables sensitive monitoring and novel 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 commercially available targeted-based liquid biopsy platforms. The platform enables broader monitoring of changes in response to cancer therapy, mechanisms of drug resistance, and intra- and inter-tumor heterogeneity.

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