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Abstract #1576844

Rapid and Automated Genomic Profiling of Lung Cancer Solid Tumor and Liquid Biopsy

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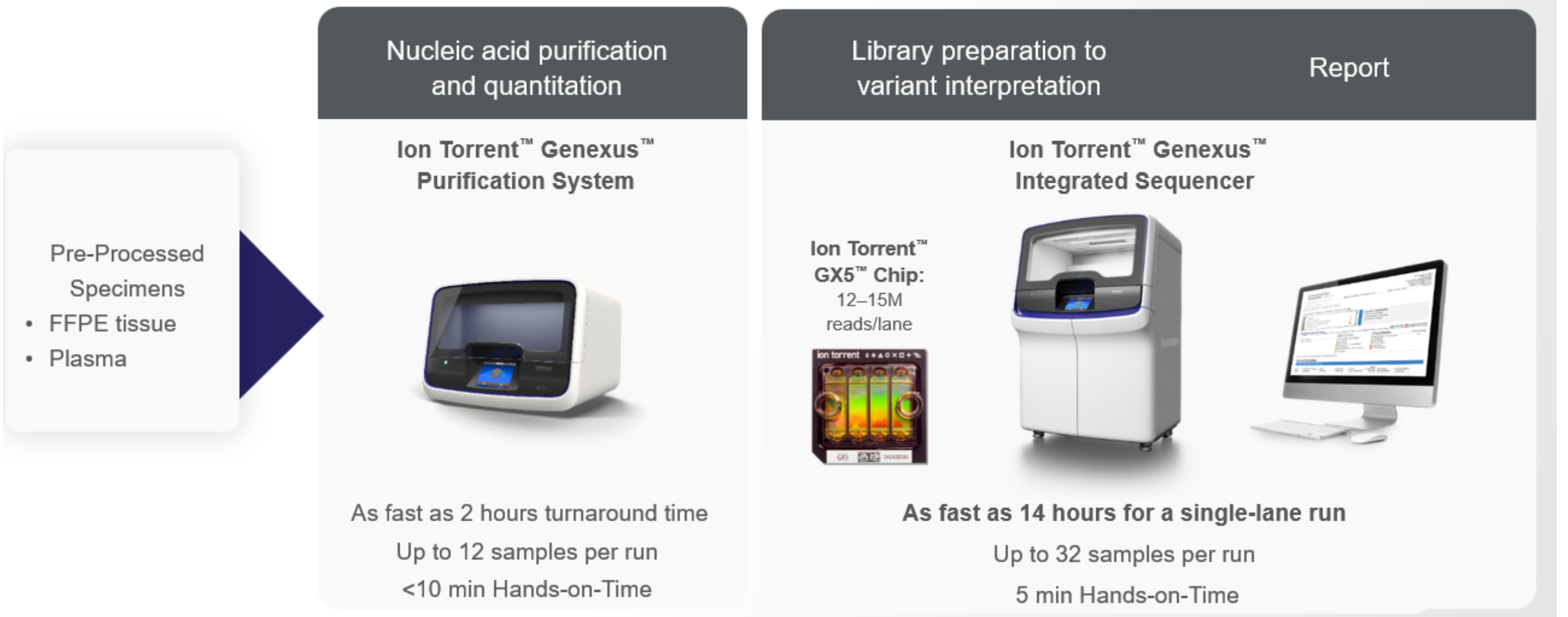
INTRODUCTION

Lung cancer is the leading cause of cancer-related mortality and the second leading cancer type for incidence[1]. Progress has been recently made in the treatment and management of non-small cell lung cancer (NSCLC). Molecular diagnostics plays a critical role in precision medicine of NSCLC, however, the penetrance of comprehensive next-generation sequencing (NGS) is uneven and shows widespread disparities. To address the gaps associated with complexity of NGS workflows and the long turn-around time, we developed a rapid and automated NGS system for FFPE and plasma samples to support oncology research in NSCLC.

METHODS

Simultaneous testing of multiple key biomarkers with limited tumor tissue material, complex workflows, long turnaround times, and numerous user touch points of most sequencing platforms remain a challenge for NGS based targeted assays. We developed the Oncomine^{TM} Precision Assay (OPA)[2] on the automated Genexus^{TM} sequencing platform[3]. OPA delivers genomic profiling across 50 key genes relevant to NSCLC using 10 ng of DNA and RNA from FFPE and 10 – 30 ng nucleic acid from plasma. Nucleic acid extraction, purification, and quantitation were done in a single workflow on the Genexus Purification System. Library preparation, templating, and sequencing used the automated workflow on the Genexus Integrated Sequencer. Bioinformatics analysis and reporting used the Genexus System software version 6.8. To demonstrate the performance of OPA in NSCLC, we profiled >2100 NSCLC FFPE and >3500 plasma research samples from different cohorts to characterize the landscape of relevant somatic alterations in lung cancer.

Fully automated Genexus^{TM} NGS system



Pre-Processed Specimens
- FFPE tissue
- Plasma

Nucleic acid purification and quantitation

Ion Torrent™ Genexus™ Purification System

As fast as 2 hours turnaround time
Up to 12 samples per run
<10 min Hands-on-Time

Library preparation to variant interpretation

Report

Ion Torrent™ Genexus™ Integrated Sequencer

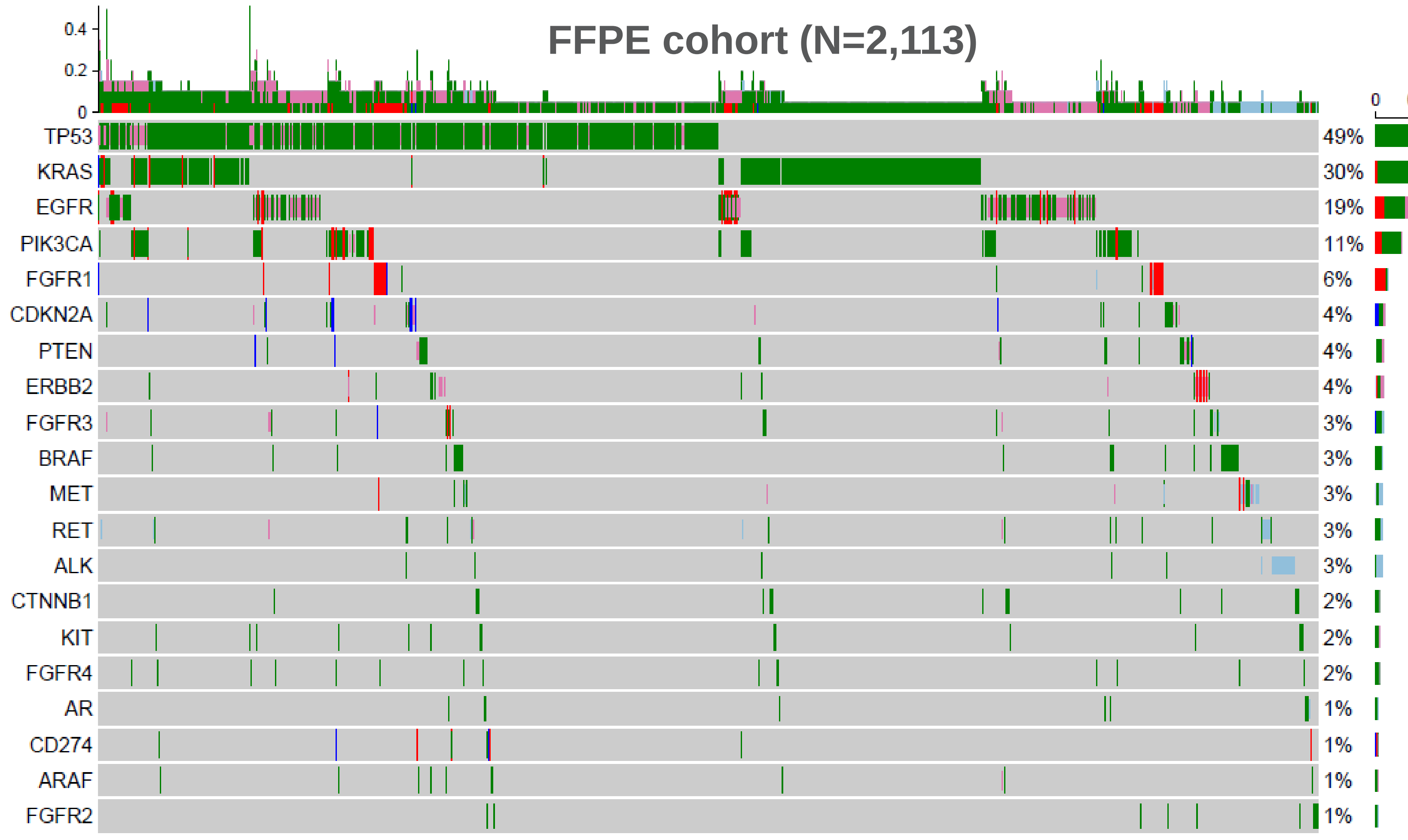
Ion Torrent™ GX5™ Chip: 12–15M reads/lane

As fast as 14 hours for a single-lane run
Up to 32 samples per run
5 min Hands-on-Time

An automated end-to-end NGS workflow from specimen to report supported by Genexus^{TM} system. Turn around time is less than one day. Single sequencing kit can be used for FFPE or plasma samples.

RESULTS

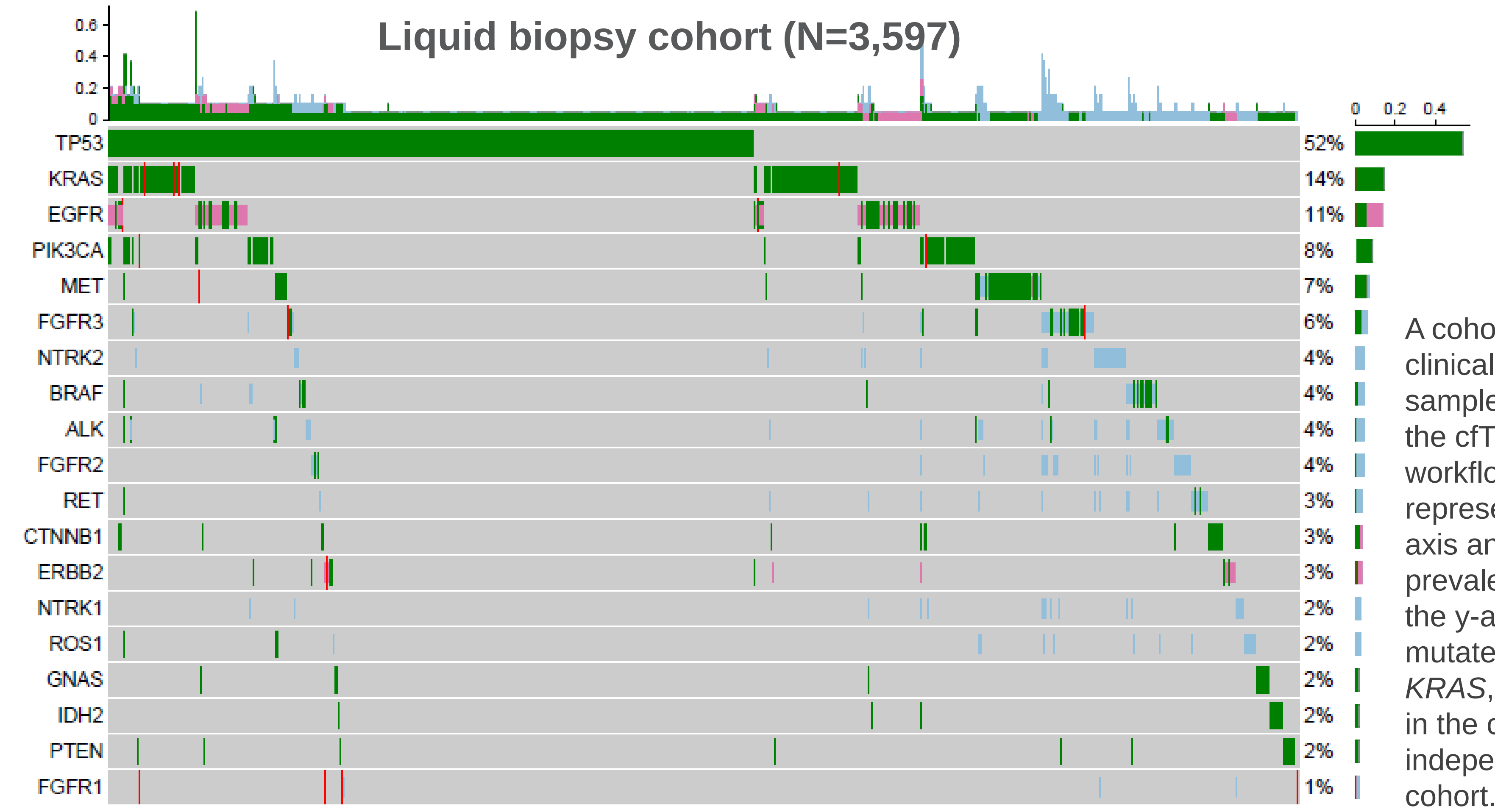
Landscape of somatic mutations in cohorts of FFPE and liquid biopsy NSCLC samples



FFPE cohort (N=2,113)

Minimum VAF 5%; MAPD (median absolute pairwise difference) for CNV<0.5; fusion threshold >20 reads

A cohort of 2,113 FFPE NSCLC clinical research samples were profiled using the solid tumor workflow. Samples are represented on horizontal axis and genes with >1% prevalence are shown on the y-axis using Oncoprint plots[4]. Most frequently mutated genes were *TP53*, *KRAS*, *EGFR* and *PIK3CA* in the cohort.

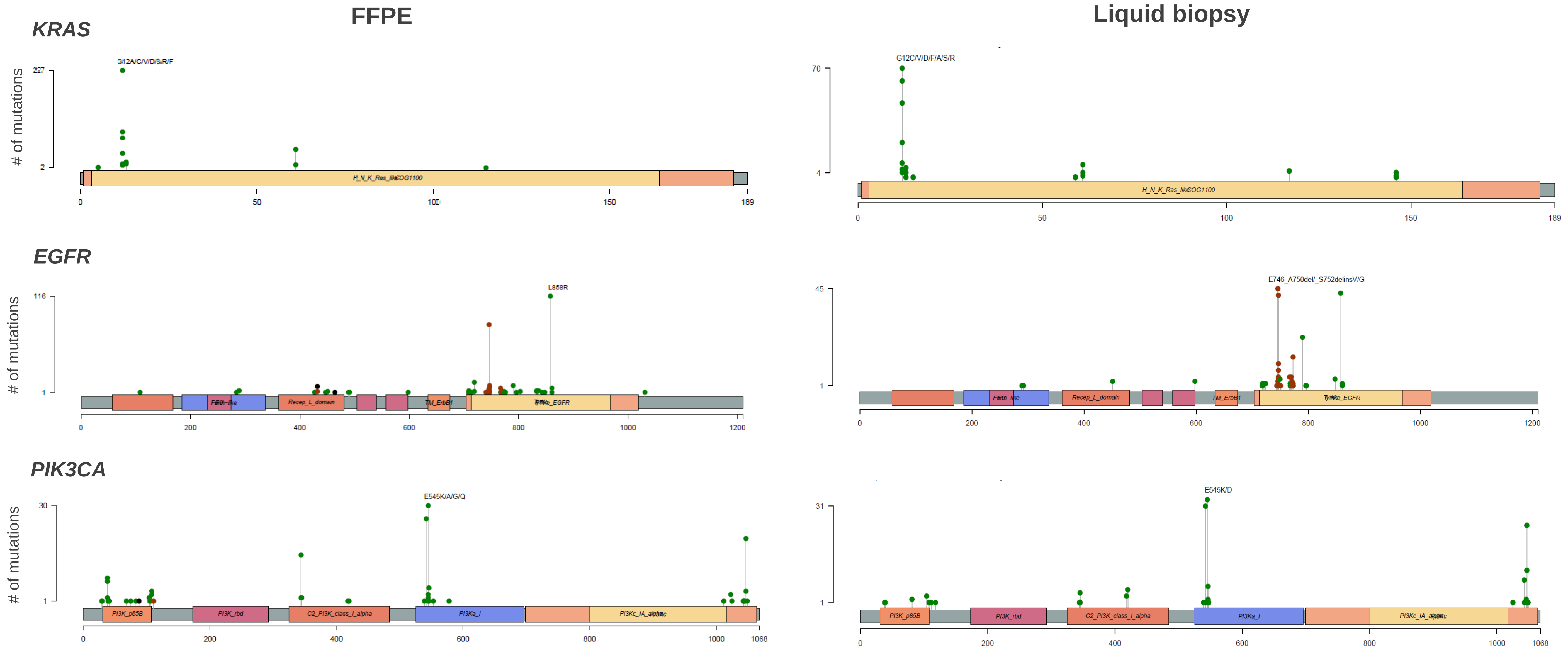


Liquid biopsy cohort (N=3,597)

Minimum VAF 0.3%; MAPD for CNV <0.5; fusion threshold >20 reads and molecular count > 2

A cohort of 3,597 NSCLC clinical research plasma samples was profiled using the cfTNA (liquid biopsy) workflow. Samples are represented on horizontal axis and genes with >1% prevalence are shown on the y-axis. Most frequently mutated genes were *TP53*, *KRAS*, *EGFR* and *PIK3CA* in the cohort. This cohort is independent of the FFPE cohort.

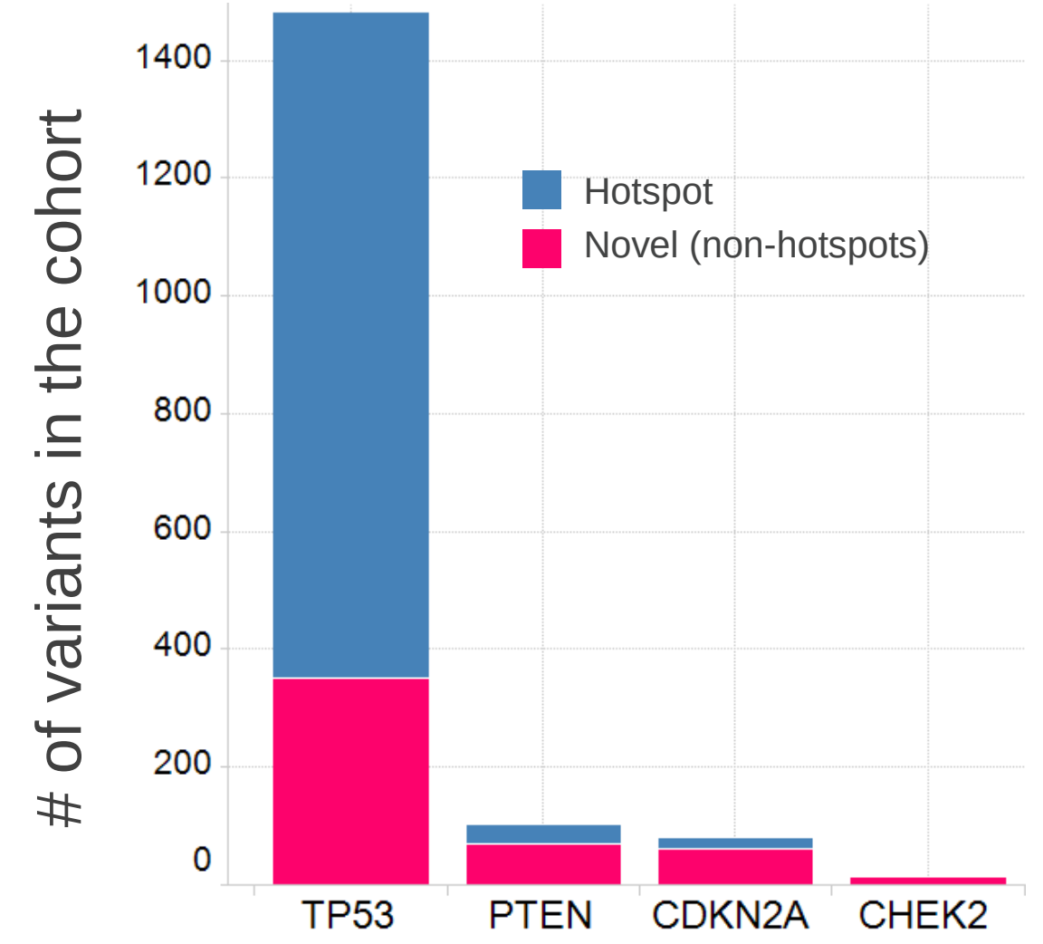
Distribution of somatic mutations in common NSCLC driver genes



Small variant mutations in NSCLC driver genes *KRAS*, *EGFR* and *PIK3CA* in FFPE and liquid biopsy NSCLC cohorts. Variant distribution is displayed using MAF tools lollipop plots[5]. Mutational distribution frequency was similar to prior molecular profiling studies (TCGA, cBio). Lollipop plot legend: missense, truncated, splice, in frame; vertical axis: number of mutations, horizontal axis: length of the gene transcript.

Novel (non-hotspot) variant detection in FFPE workflow

Truncating variants in tumor suppressor genes



Novel (a.k.a non-hotspot) variant detection is enabled for all 50 genes in the OPA FFPE workflow which allows assessment of entire target regions for variant calling.
Relative distribution of truncating hotspot (blue) and novel (red) variants in tumor suppressor genes in the FFPE NSCLC cohort. Higher proportion of novel variants was detected for PTEN, CDKN2A and CHEK2.

Variant calling performance measured using control samples

Hotspot

Type	Sensitivity	PPV
SNVs	98.6%	100%
Indels	100%	100%

Non-hotspot

Type	Sensitivity	PPV
SNVs	95.23%	100%
Indels	100%	100%

Two replicates of Acrometrix^{TM} Oncology Hototspot Control[6] (with 75 hotspots and 22 non-hotspot variants) were used to measure the analytical performance. PPV is positive predictive value to assess false positives variants.

References:
1. Barsouk *et al*. *Contemporary Oncology*. 2021. PMID: 339119812
2. www.thermofisher.com/us/en/home/clinical/preclinical-companion-diagnostic-development/oncomine-oncology/oncomine-precision-assay.html
3. www.thermofisher.com/us/en/home/life-science/sequencing/next-generation-sequencing/ion-torrent-next-generation-sequencing-workflow/ion-torrent-next-generation-sequencing-run-sequence/ion-torrent-genexus-system.html
4. Schlesner *et al. Bioinformatics.* 2016. PMID: 27207943
5. Koeffler *et al. Genome Research.* 2018. PMID: 30341162
6. www.thermofisher.com/order/catalog/product/969056

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