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Dear Valued Clients,

On November 1st – 3rd, during the Association of Molecular Pathologist (AMP) 2018 Annual Meeting in San Antonio, Texas, GenPath/ BioReference Laboratories, Inc., were privileged to present a research paper on **Clinical Targeted Next Generation Sequencing Panel Testing in Non-Small Cell Lung Cancer.**

The purpose of our research was to investigate the clinical utility of the **OnkoSight Targeted Next-Generation Sequencing (NGS) panel for Non-Small Cell Lung Cancer.** Specifically we compared the results obtained in lung adenocarcinoma versus lung squamous cell carcinoma. We also defined **our laboratories low QNS rate even in samples with a very low DNA concentration input.**

Please see the following pages for the details of the clinical paper.

Thank you!

GenPath Oncology
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CLINICAL TARGETED NEXT GENERATION SEQUENCING PANEL TESTING IN NON-SMALL CELL LUNG CANCER: SINGLE INSTITUTION EXPERIENCE AT A HIGH SCALE NATIONAL REFERENCE LABORATORY

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Introduction

Clinical targeted next-generation sequencing (NGS) panels are emerging as a mainstream diagnostic test in the routine clinical laboratory setting for comprehensive genomic profiling in non-small cell lung cancer (NSCLC). The National Comprehensive Cancer Network (NCCN) guidelines recommend that biomarker testing in NSCLC be performed as part of a broad molecular panel containing, at a minimum, the following genes: *EGFR, BRAF, KRAS, MET,* and *HER2 (ERBB2)* (NCCN NSCLC, 4.2018). The World Health Organization (WHO) highlights the *RET* gene in addition to these same biomarkers to be included in an NGS sequencing panel for NSCLC (Linderman et al, 2018). In a recent NGS study of NSCLC, 82.4% of samples harbored at least one gene alteration (31.4% *KRAS,* 22.4% *EGFR*) (Fumagalli et al, 2018). Herein, we report one high scale national reference laboratory's experience with clinical targeted NGS panel testing in NSCLC.

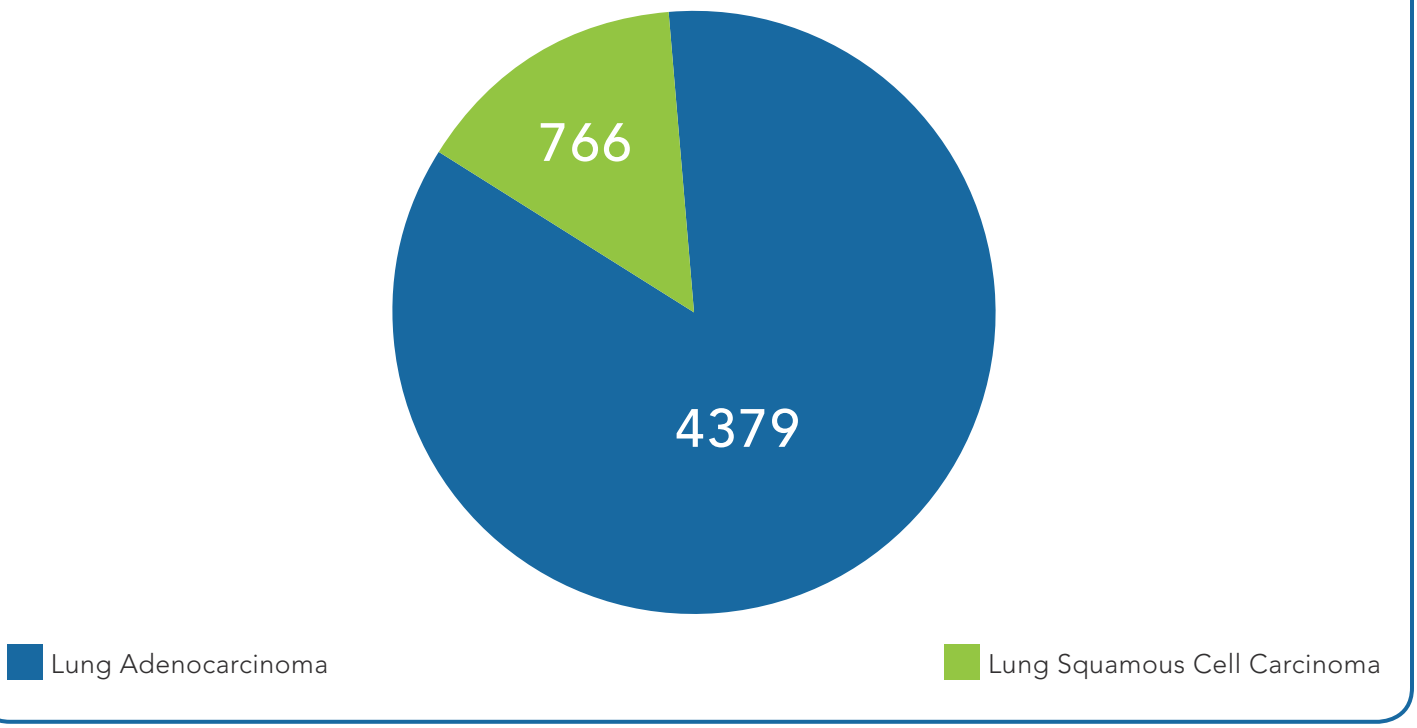
Methods

We performed a retrospective analysis of the 5,145 cases of NSCLC specimens (containing at least 10% tumor burden) profiled using the GenPath OnkoSight™ 18-gene lung tumor NGS panel from January 2015 to April 2018. Somatic mutational landscape was compared for NSCLC subtypes including adenocarcinomas and squamous cell carcinomas.

Results

Of the total 5,145 cases analyzed, 4,379 (85%) were lung adenocarcinoma and 766 (15%) were lung squamous cell carcinoma (Figure 1).

FIGURE 1: TOTAL NUMBER OF LUNG ADENOCARCINOMA AND LUNG SQUAMOUS CELL CARCINOMA CASES ANALYZED



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Results

Overall, 4,117 cases (80.0%) were found to have at least one somatic alteration. A total of 151 cases (2.9%) were deemed quantity/quality not sufficient (QNS) (Figures 2 and 3).

FIGURE 2: RESULT SUMMARY OF LUNG ADENOCARCINOMA CASES

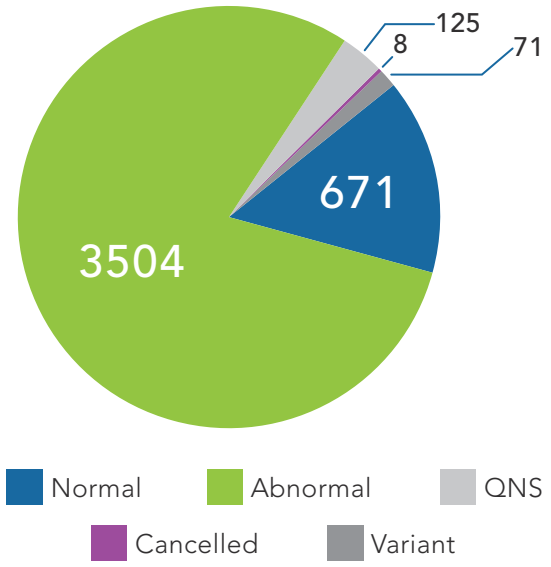
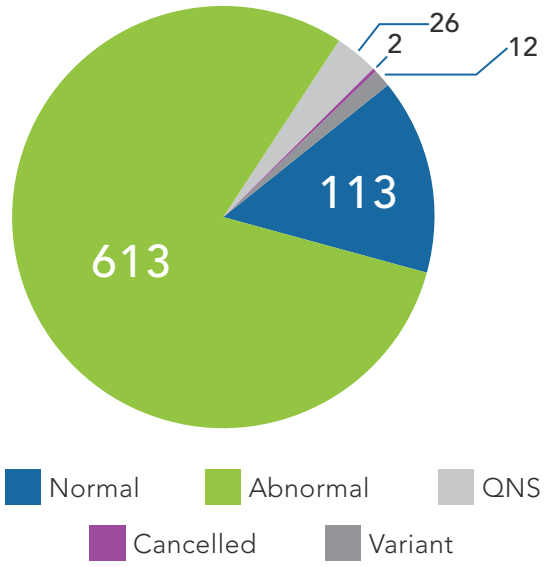


FIGURE 3: RESULT SUMMARY OF LUNG SQUAMOUS CELL CARCINOMA CASES



A result was obtained in 91% of lung adenocarcinoma and lung squamous cell carcinoma cases analyzed with only 1 ng/uL of DNA or less (Tables 1 and 2). Nine lung adenocarcinoma cases had an inadequate input tumor cellularity (<10% tumor cellularity) therefore testing was not performed.

TABLE 1: FFPE DNA CLINICAL SAMPLE CONCENTRATIONS IN LUNG ADENOCARCINOMA AND SQUAMOUS CELL CARCINOMA

Concentration (ng/uL)	Total Cases	% of Cases
<1	1394	27%
1-5	1832	36%
5-10	428	8%
10-20	349	7%
>20	1134	22%
	5137	100%

TABLE 2: QNS RATE IN LUNG ADENOCARCINOMA AND LUNG SQUAMOUS CELL CARCINOMA FOR EACH DNA CONCENTRATION

Concentration (ng/uL)	QNS case	% of Cases in [] range
<1	125	9%
1-5	16	1%
5-10	1	0.2%
10-20	0	0%
>20	0	0%

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Results

Disease-associated alterations were detected in 17/18 genes included in the panel (no abnormalities were identified in *EGFR1*) (Figure 4 and 5). Clinically actionable hotspot alterations in *KRAS, BRAF,* and *EGFR,* as well as alterations in *MET* and *HER2* (exon 20 insertions), together accounted for 43.7% of the total disease-associated alterations (Figure 6 and 7). When assessed by subtype, the most frequently altered genes among adenocarcinomas include, *TP53* (42.9%), *KRAS* (32.0%), *EGFR* (13.9%) and *BRAF* (4.5%) (Figure 4). The most frequently altered genes in squamous cell carcinomas include, *TP53* (79.2%), *PIK3CA* (8.9%) and *KRAS* (7.0%) (Figure 5). The other genes on the panel collectively accounted for the remaining 3.5% of the total disease associated alterations detected among NSCLC cases studied.

FIGURE 4: GENE MUTATIONS DETECTED IN LUNG ADENOCARCINOMA

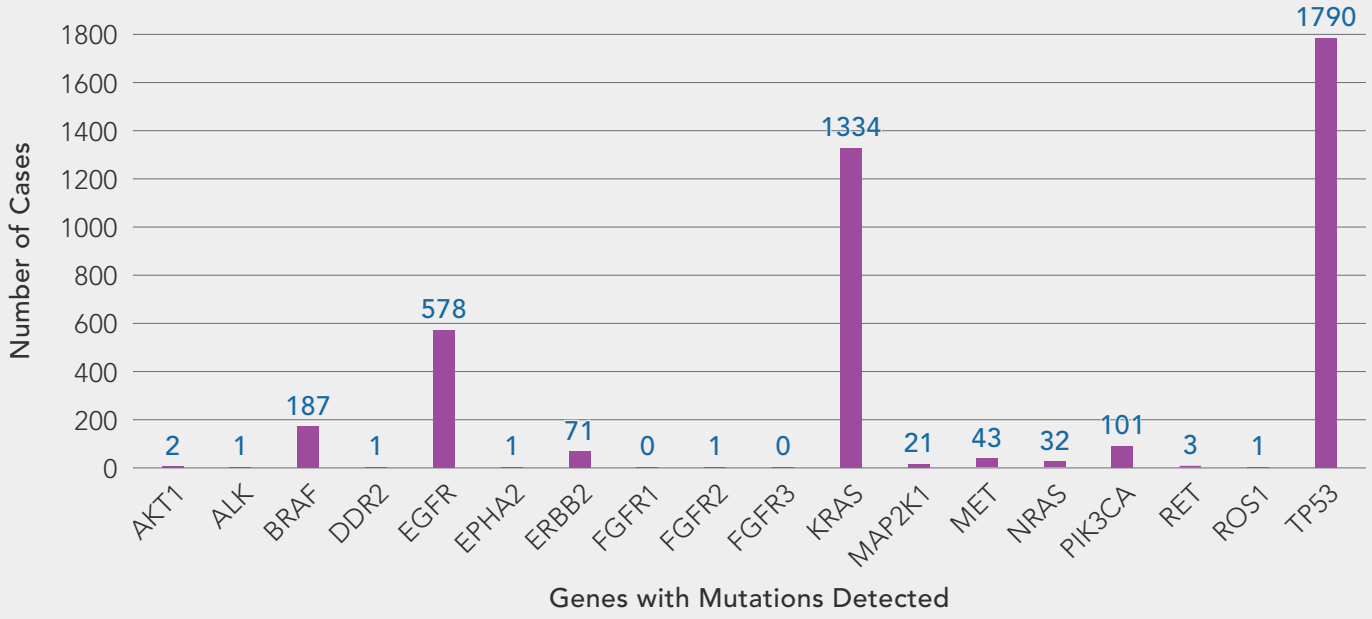
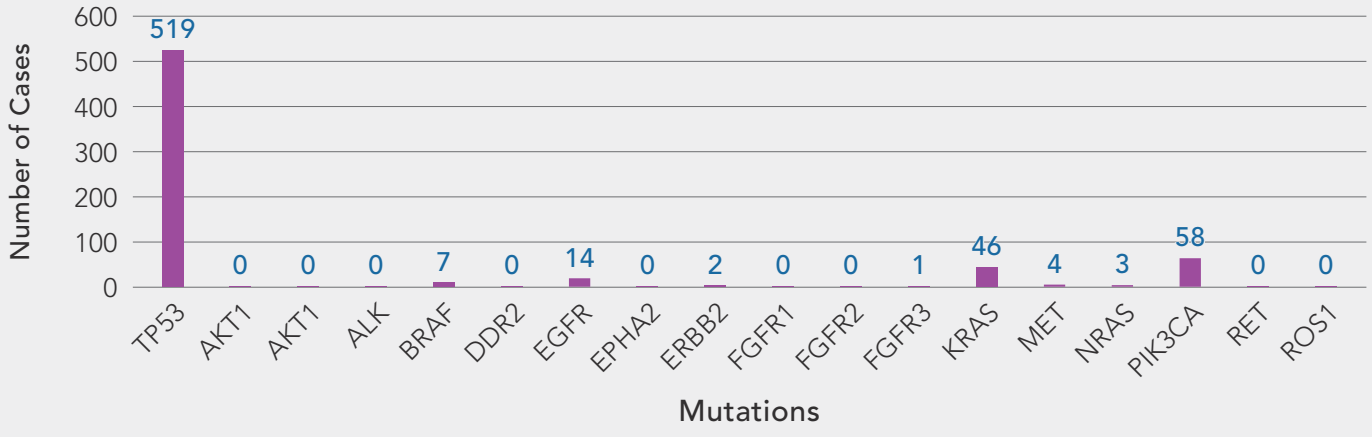


FIGURE 5: GENE MUTATIONS DETECTED IN LUNG SQUAMOUS CELL CARCINOMA



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Results

FIGURE 6: HOTSPOT GENE MUTATIONS IN LUNG ADENOCARCINOMA CASES

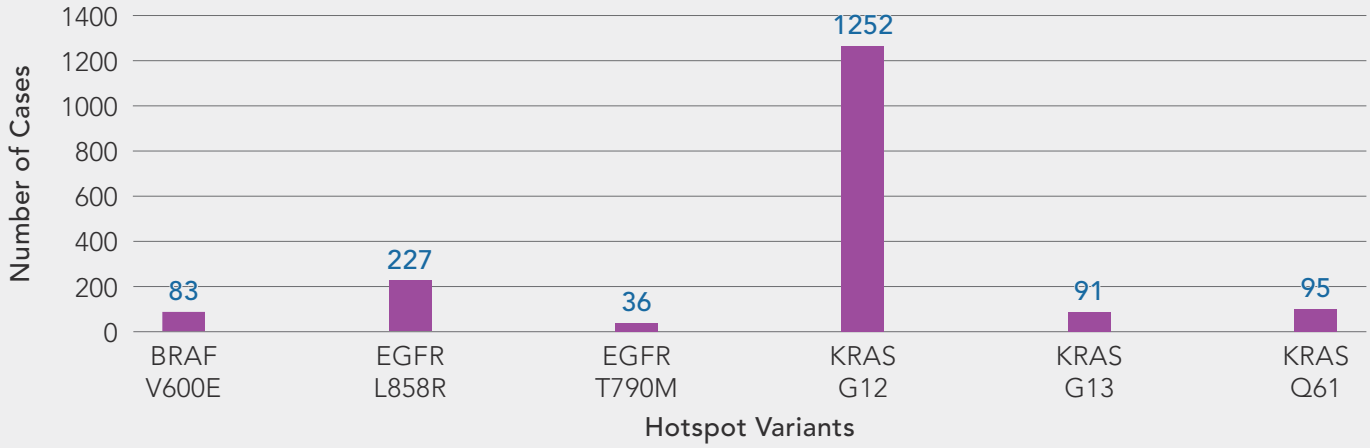
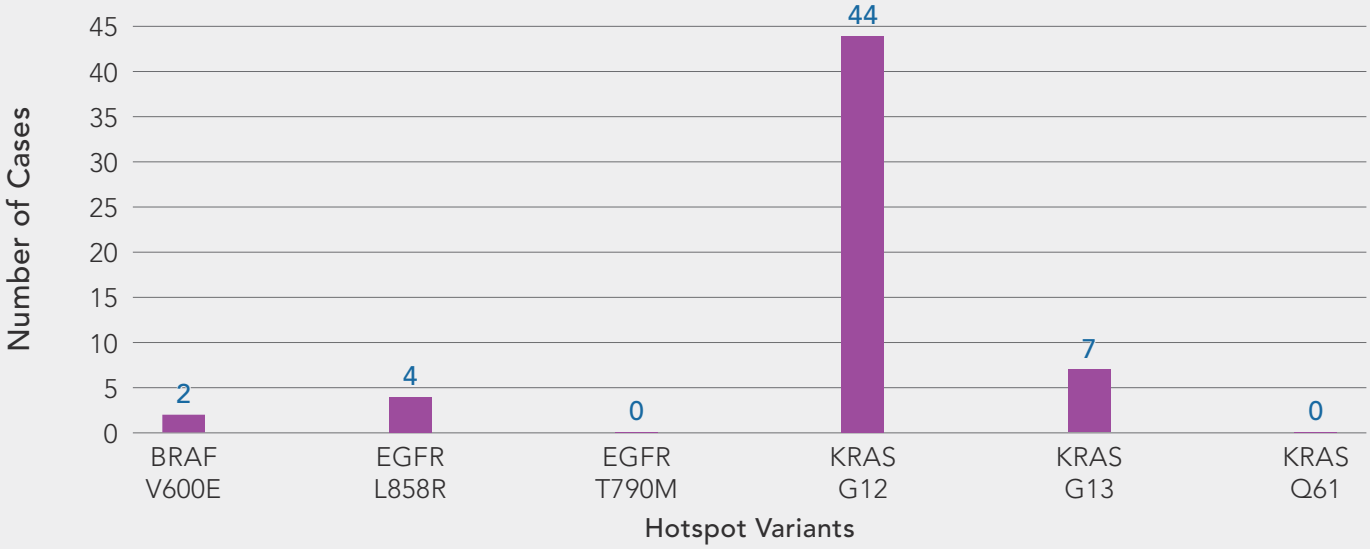


FIGURE 7: HOTSPOT GENE MUTATIONS IN LUNG SQUAMOUS CELL CARCINOMA CASES



Conclusion

This data demonstrates significant clinical utility of NGS panel testing in NSCLC. Potentially actionable findings were noted among multiple genes, with a very low QNS rate. The hotspot alterations in *KRAS, EGFR* and *BRAF,* as well as the alterations in *MET* and *HER2,* account for more than one third of all the alterations detected and clinically actionable results. Hotspot mutations typically associated with adenocarcinoma were detected among a moderate subset of squamous cell carcinoma cases, raising the possibility of a histologically under-represented or under-reported adenocarcinoma component of disease, and further expanding the scope and practical utility of NGS testing in NSCLC to include cases with exclusively squamous histology. Rates of detection using this assay are consistent with similar smaller studies reported in the literature (Fumagalli et al, 2018). Although *TP53* alterations are not referenced in the NCCN guidelines, several clinical trials are now available for NSCLC patients with a *TP53* alteration, suggesting that limiting an NGS panel to the minimum recommended genes by the NCCN, could miss alterations that may have clinical utility.

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