Abstract #G009

Use of Synthetic Internal Standards to Measure Very Low Frequency TP53, PIK3CA, and BRAF Somatic Mutations in Normal Airway Epithelial Field of Injury Associated with Lung Cancer Risk

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Background

Inter-individual variation in lung cancer risk is based on variation in both exposure to environmental factors and genetic predisposition. As such, it is reasonable to hypothesize that prevalence of mutations in airway epithelium will vary based on combined effects of these lung cancer risk factors. Genes most commonly mutated in lung cancer were recently reported from The Cancer Genome Atlas (TCGA) project. The purpose of this study was to use targeted deep sequencing to assess prevalence of low variant allele frequency (VAF) mutations in normal airway epithelial cells (AEC) of individuals at high demographic risk for lung cancer.

Experimental Plan

- Develop and implement a method for measurement of low frequency (LOD: 0.05% VAF) somatic variants in AEC DNA specimens.
  - Mix each DNA specimen with synthetic internal standards to control for technical artifacts associated with library preparation, NGS platform-specific error, and failure of variant callers to filter.
- Characterize low frequency (VAF as low as 0.05%) somatic variants in targeted regions of AEC DNA from cancer smoker (CA/SM), non-cancer smoker (NC/SM), or non-cancer/non-smoker (NC/NS) subjects with respect to prevalence, type, and variant allele frequency.
- Test for association of measured variant characteristics with CA diagnosis and/or SM status.

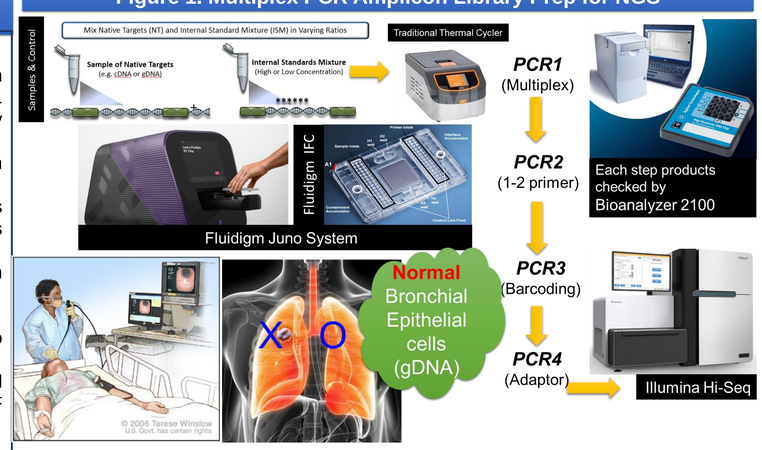
Methods

Subjects/Specimens: We enrolled 19 subjects who were undergoing standard-of-care bronchoscopy into an IRB-approved research study, including 11 CA/SM, 5 NC/SM, and 3 NC/NS. For each subject, after obtaining informed consent we collected 4-5 bronchial brush biopsies (about 1 million AEC/subject) and extracted genomic DNA within 1 hour of collection. DNA representing at least 50,000 genome copies was loaded into each NGS library preparation, enabling reliable measurement of variant allele fraction (VAF) as low as 0.05%.
Synthetic Internal standard (IS) spike-ins: IS and PCR primers for 12 actionable mutations in seven genes (BRAF, EGFR, ERBB2, NOTCH1, KRAS, PIK3CA, and TP53) commonly observed in lung cancer based on TCGA results were synthesized at Accugenomics, Inc. (ACG), including. IS were synthesized with dinucleotide variants every 50 bp to distinguish from native sequence, cloned into pUC vectors, confirmed to be wild-type sequence except at dinucleotides, linearized, quantified, and combined into an IS mixture with each IS at known genome copy concentration.

Variant Call Relative to IS-Spike In

- IS mixture added to each AEC DNA specimen at 1:1 genome copy prior to library prep.
- Multiplex PCR Amplicon library prep (Fig. 1)
- Target reads and IS reads separated into different bins using custom splitter.
- Pipeline analysis on Qiagen CLC Genomics Workbench.
- Variant calls
  - IS variants assumed to be technical error.
  - Contingency tables used to identify significant VAF in sample compared to IS

Figure 1. Multiplex PCR Amplicon Library Prep for NGS



Subject Demographics

Sample ID	Diagnosis	Subtype	Smoking Status	Pack Years (PY)	Age	Sex
1	CA	NSCLC	Former	45	55	F
2	CA	NSCLC	Smoker	50	60	F
3	CA	SCLC	Former	45	61	M
4	CA	NSCLC	Former	46.5	64	F
5	CA	NSCLC	Smoker	28	70	F
6	CA	SCLC	Smoker	90	73	M
7	CA	NSCLC	Former	60	74	M
8	CA	NSCLC	Smoker	Unknown	75	M
9	CA	SCLC	Former	75	76	M
10	CA	NSCLC	Former	15	79	M
13	CA	NSCLC	Smoker	40	50	F
11	NC	Non-Cancer	Smoker	34	40	M
12	NC	Non-Cancer	Never	0	46	F
14	NC	Non-Cancer	Former	30	52	M
15	NC	Non-Cancer	Smoker	100	60	M
16	NC	Non-Cancer	Never	0	65	F
17	NC	Non-Cancer	Smoker	20	69	M
18	NC	Non-Cancer	Former	54	77	M
19	NC	Non-Cancer	Never	0	81	M

PY: pack-years of smoking (packs/day x number of years smoked), one pack equals 20 cigarettes. **CA**: cancer; **NC**: Non-cancer; **NSCLC**: non-small cell lung cancer; **SCLC**: small cell lung cancer

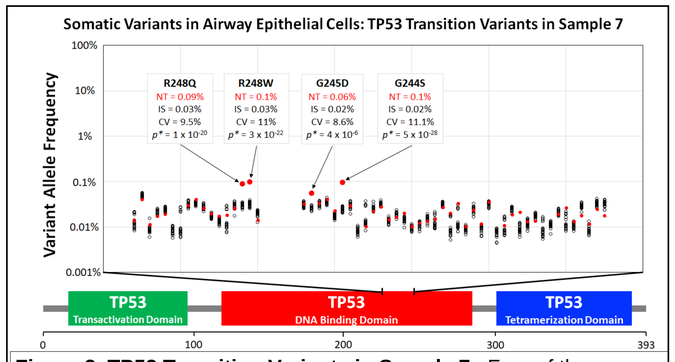


Figure 2. TP53 Transition Variants in Sample 7. Four of the variants indicated in Figure 2 are highlighted here; specifically, four TP53 somatic mutations significantly above background in Sample 7 AEC. Variants were identified at each other site (red dots), but they were not above technical background limit of the blank (LOB) measured by the 10 synthetic internal standard replicates (black dots).

Synthetic Internal Standard Spike-Ins Control for Site-Specific Technical Error And Enable Identification of Low Frequency Variants

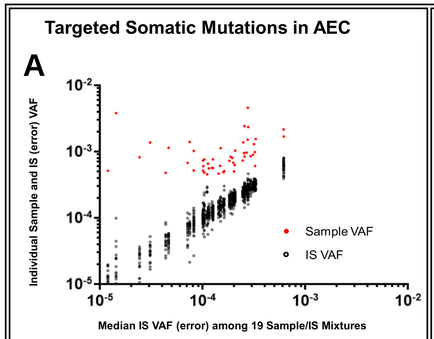


Figure 1A. Use of IS to determine variant-specific signal-to-noise ratio

- 19 replicate IS site specific VAF measurements enabled signal to noise determination for each variant.
- For targeted variants, error VAF was <0.0005 (0.05%).

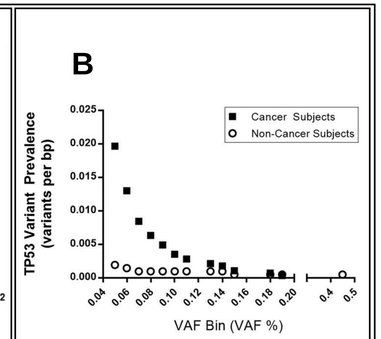


Figure 1B. Prevalence of somatic mutations increases with decreasing VAF threshold.

- Increasing differential between CA and NC subjects between 0.5 and 0.05% VAF
- Very few variants above 0.5% VAF.

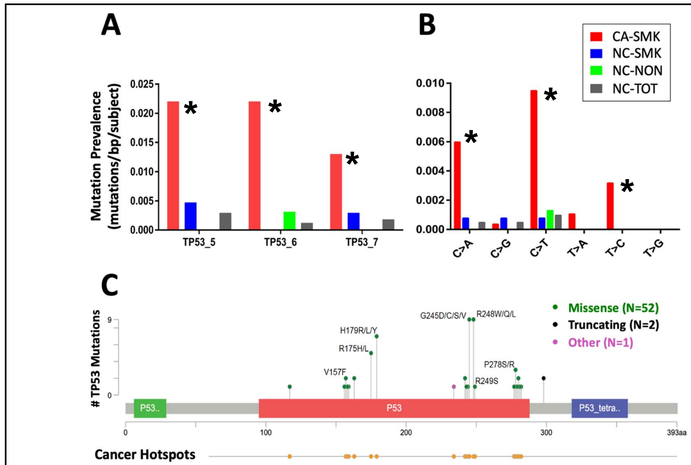


Figure 3A. Among the three measured exons of TP53, the prevalence (mutations/bp/subject) of substitution mutations was 10.4-fold higher ($p<0.05$. *) in AEC from CA-SMK subjects relative to NC-SMK subjects matched for smoking and age. **and C.** These mutations had clear cigarette smoke signature of increased C>A and C>T **(3B)** and were enriched in TP53 hotspots **(3C).**

Summary

-TP53 mutation prevalence was significantly higher in AEC of CA Smokers compared with non-cancer smokers.
-These mutations were enriched in TP53 hotspots, and had clear cigarette smoke signature of increased C>A and C>T.

Disclosures/Acknowledgments

Acknowledgements: Studies supported by HL108016 and CA148572, and The George Isaac Cancer Research Fund. Tests and technology licensed to Accugenomics, Inc., Wilmington. **Competing Interests:** J.Willey serves as consultant to and has significant interest in Accugenomics, Inc. The views presented in this article do not necessarily reflect current or future opinion or policy of the US Food and Drug Administration. Any mention of commercial products is for clarification and not intended as endorsement.