Tumor Shedding into Cell-free DNA (cfDNA) is Associated with High-mortality Cancers

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INTRODUCTION

- Estimated cell-free DNA (cfDNA) tumor fraction (ECTF), the proportion of tumor molecules in a cfDNA sample, is a direct measurement of signal for cfDNA applications.
- To determine the limit of detection (LOD) requirements across cancer types, there is a need for increased understanding of the nature of ECTF and factors influencing its abundance in cfDNA.
- Previous studies indicated that ECTF varies among cancer types and individuals; however, these studies were limited by small sample sizes and surveyed few cancer types.
- This analysis leverages data from the Circulating Cell-free Genome Atlas (CCGA) study (NCT02889978), a prospective, multicenter, observational, case-control study, to examine ECTF across stages in >20 tumor types.

METHODS

Cancer Detection Classification on cfDNA

- Independent of tumor sequencing, cfDNA classifiers of cancer vs non-cancer for whole-genome bisulfite (WGBS) and whole genome (WGS) were assessed.
- Independent of tumor sequencing, cfDNA classifiers of cancer vs non-cancer for whole-genome bisulfite (WGBS) and whole genome (WGS) (both sequenced at 30x coverage) were assessed.
- Examine ECTF across stages in >20 tumor types.

RESULTS

Tissue Sample Processing

- Formalin-fixed, paraffin-embedded (FFPE) tumor biopsy samples were provided from CCGA clinical trial sites.
- Tissue was microdissected from unstained slides to yield a tumor nuclear volume between 0.5 to 1.5 mm³ from a tissue region that was at least 80% tumor as assessed by review of adjacent H&E slides.
- Matched WBC and tumor samples were processed for DNA extraction and WGS (target sequencing depth: 30x WBC and 60x biopsy).

Tumor Biology Variant Calling and cfDNA Mutant Allele Counting

- Tumor variants were called using WBC to control for germline variant contamination through the GATK HaplotypeCaller tool.
- Variant calls were further refined using in-house filters to jointly optimize the recovery of putative true-positive somatic variants while maintaining retention of known false-positives identified from recurrent events in the cfDNA and tissue samples.
- Targeted sequencing was applied to matched cfDNA covering 267 genes and interrogated regions. Reads were collapsed and matched to high accuracy fragment sequences using unique molecular identifiers and fragment positions (mean collapsed coverage: 3,000x).
- Given the limited variant calls for a tumor sample, the corresponding collapsed cfDNA fragments were assessed for mutant allele support and the unique molecule depth recorded.

Statistical Modeling of Estimated cfDNA Tumor Fraction Based on Tumor Variants

- The ECTF for each sample was calculated using the fraction of mutant reads contributed from the tumor to the cfDNA sample.
- For each participant, we computed the likelihood of observing the cfDNA tumor mutant allele counts corresponding to the alleles found in the tumor for a given value of the ECTF.
- Allele counts were approximated as a Poisson distribution where the rate is controlled by local sequencing depth, ECTF, and allele frequency in the tumor.
- Individual variants were treated as independent for purposes of aggregating the total likelihood.
- We summarize the likelihood by reporting the median ECTF value per sample and include approximate confidence intervals from the same density.
- The observed sample-to-sample variation in ECTF levels was very large. Consequently, small technical corrections were not implemented.

Cancer Type Mortality

- Incidence-weighted 5-year survival was obtained from Surveillance, Epidemiology, and End Results (SEER) (https://seer.cancer.gov), and used to create survey cancer types into high mortality (bottom 20th percentile: esophageal, gastric, hepatobiliary, lung, pancreas) and low mortality (top 20th percentile breast, prostate, thyroid).

CONCLUSIONS

- Characterizing ECTF using the fraction of mutant reads contributed from the tumor to the cfDNA sample in high-depth sequencing indicated that:
  - ECTF was strongly associated with cfDNA classifier performance.
  - Tumor shedding combined with LOD of a given classifier and assay appeared to drive the observed differences in detection among cancer types.
  - Cancers with high mortality appeared to have higher ECTF.
- Together, these data suggest that cfDNA-based assays may detect high mortality cancers at earlier stages due to increased cfDNA shedding among those cancer types.

REFERENCES


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