Limitations on Mutation Detection for Early Detection of Cancer

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INTRODUCTION
The prevalence of inherited genetic alterations in circulating cfDNA [ctDNA] circulating in the bloodstream of patients with cancer has led to development of blood-based assays for cancer prognosis and early detection.

- Circulating tumor [ctDNA] is present in many different malignancies, making it potentially useful for the early detection of cancer (Figure 1A).
- While current methods rely on low deep sequencing, few genes can be detected in ctDNA (Figure 1B).

- For detection of tumor-derived mutations, it is critical to estimate the tumor fraction [ratio of ctDNA to cfDNA], and the tumor variant allele frequency (VAF) of the DNA extracted [a mutation known as ‘present in tissue’]. Both tumor burden and VAF vary relying on tumor type and stage.

- Numerous and ongoing studies [including Aravanis and colleagues] have resulted in the development of several ctDNA-based liquid biopsy assays.

OBJECTIVES

1. To assess the sensitivity of the ctDNA-based liquid biopsy assay on our physiologic and economic requirements
2. To determine the limits of sequence typing and diagnostic utility of the assay
3. To assess the diagnostic utility and potential for patient care using these results

METHODS

A binomial model was used to assess and infer depths and input requirements, with parameters derived from published data on ctDNA sequencing2,3.

- Model parameters:
  - No more than 5% of samples may fail because of insufficient cfDNA quantity
  - Depth (the number of molecules assayed) is a significant factor when detecting “$1000 genome” sequencing costs: US $1000/(30 x 3 Gbp) of sequencing bandwidth

B. Biologic limitations driven by recently discovered somatic heterogeneity in healthy tissue

$83,000
30,000x
100% on-target rate in target enrichment (WES, whole exome sequencing).
$140
0.01%

C. Limitations on Mutation Detection for Early Detection of Cancer

- The model suggests that early detection may be infeasible:
  - Small patients (e.g., the 98% (T17) low add and wide sequencing costs, but low input type of ctDNA of which only one is present)
  - Larger patients (e.g., the 99% [T4] panel reported by ten of the US) show significantly higher sequencing requirements

D. Alternatives to ctDNA Mutation Detection

- There are many alternative methodologies that may be useful for early cancer screening (Table 1)

- Repurposing current sequencing to achieve unique opportunities to improve screening with longitudinal monitoring

FIGURE 2C. Statistical Model for ctDNA Sequencing. cfDNA Is Less Frequent in Healthy Individually and Stage I/II Cancer Patients

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TABLE 2. Biologic Components Other Than ctDNA With Potential for Cancer Screening

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ACKNOWLEDGMENTS

We thank Christina Curtis, Adam Drake, Kate Niehaus, Girish Putcha, and David Weinberg for their suggestions and feedback on the data and methodology used in this study. This work was presented at the American Association for Cancer Research (AACR) Annual Meeting, April 14–18, 2018; Chicago, Illinois, USA.