Use of a highly sensitive and specific minimal residual disease test to characterize novel cell-free DNA reference material

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Background & objectives

Solid tumor minimal residual disease (MRD) testing using circulating tumor DNA (ctDNA) has gained increasing interest in recent years. The first clinical utility data for MRD were recently published in stage II CRC and are supportive of routine use in this patient population (1). With the advent of molecular tests capable of detecting a single ctDNA molecule in the presence of a million or more wild-type cell-free DNA (cfDNA) molecules, the need for standardizable reference materials for development and quality control (QC) purposes that mimic the properties of cfDNA yet are available in larger quantities than patient-derived material has become increasingly important.

In this study, we characterized a novel enzymatically fragmented cfDNA-like reference material using Haystack MRD, a personalized next-generation sequencing test with advanced error correction to detect ctDNA with high sensitivity and specificity.

Methods

DNA size distribution, conversion efficiency, and DNA damage (visualized as error rate) were evaluated and compared to native circulating DNA as well as commercially available reference materials that were fragmented by sonication. To demonstrate the potential use as an MRD reference material, cfDNA-like specimens prepared from a tumor cell line were serially diluted in material prepared from a wild-type cell line down to a tumor fraction of 0.0001%. Whole-exome sequencing results of both cell lines informed the design of several personalized sequencing panels, each of which interrogated 50 tumor-cell-line-specific mutations in the dilutions. All DNA samples were subjected to Haystack MRD library preparation and target enrichment using personalized panels and were sequenced on a NovaSeq 6000.

Results

Our results demonstrate that the tumor DNA can be reproducibly detected in the low parts-per-million range, and that the novel cfDNA reference material's size distribution, conversion

efficiency, and error rates are highly comparable to native cfDNA and thus superior to sonicated materials.

Conclusion

In conclusion, these properties suggest that the novel cfDNA reference material is an abundant source of patient-like material and demonstrates superior functional characteristics versus fragmentation using sonication. Thus, the material may be useful to support the development, validation, and production testing QC of even the most sensitive MRD tests.

(1) Tie, J. et al. Circulating Tumor DNA Analysis Guiding Adjuvant Therapy in Stage II Colon Cancer. New Engl. J. Med. 386, 2261–2272 (2022)