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

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Introduction

Solid tumor minimal residual disease (MRD) testing using circulating tumor DNA (ctDNA) has gained increasing interest in recent years. In addition to several observational studies, the first clinical utility data for MRD were recently published in stage II CRC demonstrating that chemotherapy use could be reduced by ~50% vs standard of care without impact on recurrence-free survival (1-4).

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, error-free 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.

Objective and Study Design

In this study, we characterized a novel cfDNA-like reference material (n-cfDNA) provided by SensID using the Haystack MRD Test, a personalized next-generation sequencing test powered by Duo™ chemistry, an advanced error correction methodology enabling ultra-high specificity and sensitivity (Figure 1).

Briefly, n-cfDNA and other enzymatically or mechanically sheared cfDNA-like reference materials were evaluated with regards to their conversion efficiency and error rates in the Haystack MRD workflow, as well as to their fragment size distribution (Figures 2 and 3). For functional testing, n-cfDNA derived from a tumor cell line was serially diluted down to 0.5 parts per million in wild-type n-cfDNA and analyzed using several tumor n-cfDNA-specific Haystack MRD panels (Figures 4 and 5).

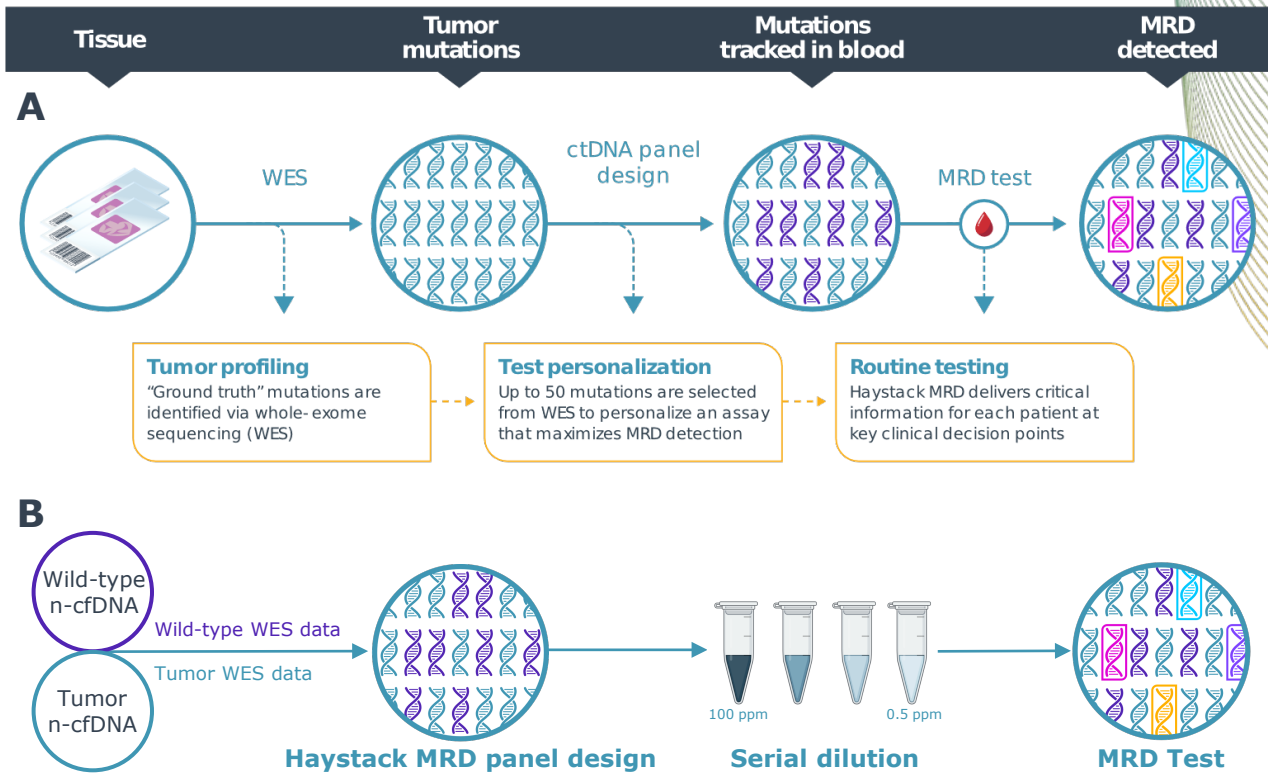

Figure 1: Personalized, tumor-informed Haystack MRD test workflow (A) and study design using serial dilutions of n-cfDNA prepared from wild-type and tumor cell lines (B).

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Error Rates and Conversion Efficiency

Approach: 10,000 genomic equivalents (33 ng) of BDXXP4 n-cfDNA (SensID) and other commercially available reference materials (RMs), sheared either enzymatically or by sonication, were sequenced on a NovaSeq 6000 (Illumina) using Haystack's Duo™ library preparation chemistry which exhibits very low technical noise of ~2 errors per 10^7 bp (data not shown). Measured error rates (mutations per bp sequenced) and library conversion efficiencies (sequenced molecules vs input molecules) were compared to healthy donor (HD) cfDNA as a reference.

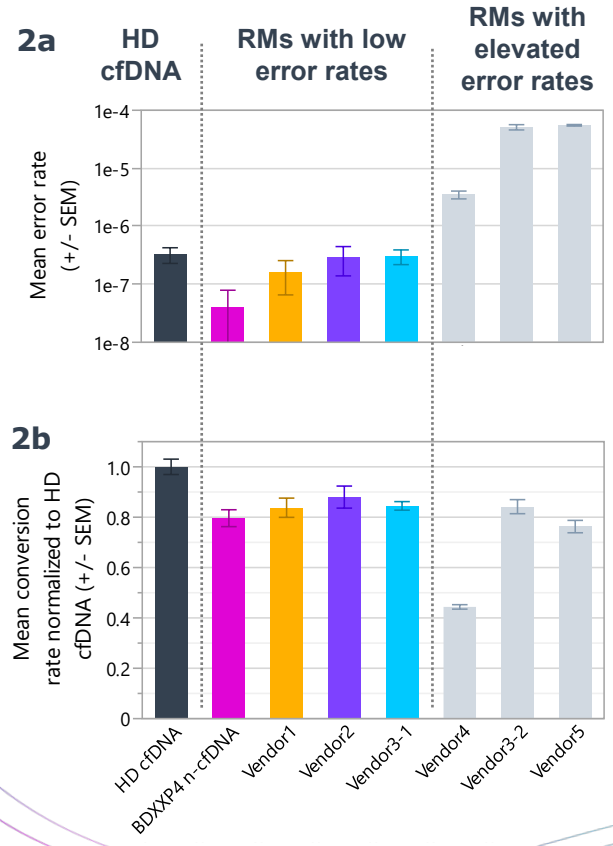

Figure 2a: Mean error rate was determined for different cfDNA-like RMs and healthy donor (HD) cfDNA (black bar) using the Haystack MRD Test. Samples were sequenced at comparable target depths of ~100,000x.

Result: n-cfDNA from SensID (pink bar) and RMs from 3 other vendors show error rates comparable to HD cfDNA or lower. Lowest error rate is observed for n-cfDNA, making it a promising candidate as a reference sample for MRD applications.

Figure 2b: Mean library conversion efficiency was calculated for RMs analyzed with the Haystack MRD Test and was normalized to HD cfDNA conversion results.

Result: cfDNA reveals the highest conversion rate (shown as 100%) and all tested RMs except for the one from Vendor 4 are converted in a range of 75% to 90% compared to HD cfDNA.

Size Distribution

Approach: Fragment size profiles of BDXXP4 n-cfDNA and other RMs with error rates comparable to HD cfDNA were determined on a TapeStation 4200 instrument (Agilent).

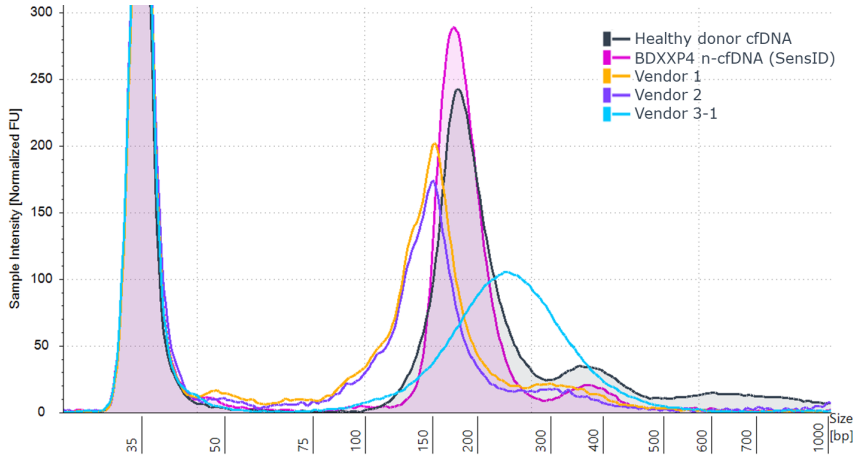

Figure 3: DNA size profiles of HD cfDNA and RMs with lowest error rates (colored bars in Figure 2) were visualized using cfDNA ScreenTapes on a TapeStation 4200 instrument.

Result: BDXXP4 n-cfDNA (shaded pink) exhibits a size distribution most similar to HD cfDNA (shaded black) including a dinucleosomal peak at ~350 bp.

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Robustness of MRD Panel Design

Approach: Five panels tracking 50 variants (50-plex) each were designed based on variants identified in the SID-CL006 tumor cell line (SensID) using the Haystack MRD Test pipeline. An n-cfDNA blend was manufactured by spiking SID-CL006 into wild-type BDXXP4, targeting 5 ctDNA molecules per variant (250 total ctDNA molecules per 50-plex panel). The blend was processed using the Haystack MRD Test applying five 50-plex panels.

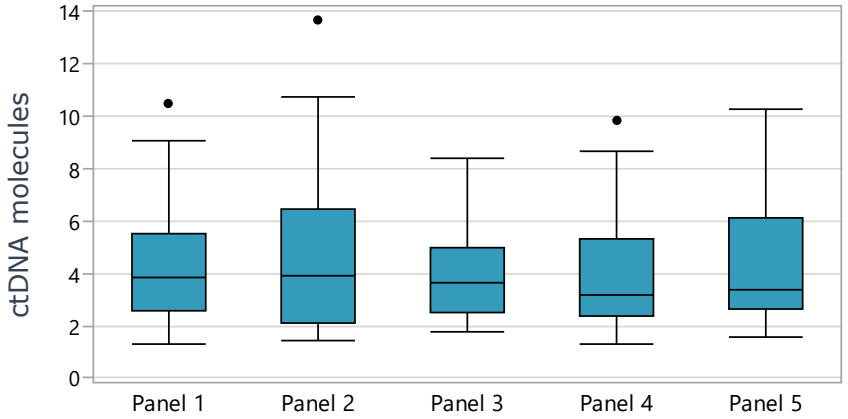

Figure 4: Box-plots showing ctDNA molecules detected across tracked variants for each of the five 50-plex panels. Whiskers: 1.5x IQR.

Result: The distributions of ctDNA molecules detected across tracked mutations is highly consistent across all five 50-plex panels, and approximately matches the expected 5 ctDNA molecules.

n-cfDNA Tumor in Wild-type Serial Dilution

Approach: The initial blend containing 250 total ctDNA molecules per sample was serially diluted in wild-type BDXXP4 n-cfDNA down to 0.5 parts per million (ppm), creating 3 additional ctDNA concentration tiers (Table 1). Per tier and 50-plex panel, 50,000 genomic equivalents (165 ng) were sequenced in a total of 5 to 10 replicates depending on the dilution level. Haystack MRD Test results observed per dilution tier were compared against the expected values.

Tier	Total ctDNA molecules	Tumor fraction	ppm
1	250	1.00E-04	100
2	25	1.00E-05	10
3	5	2.00E-06	2
4	1.25	5.00E-07	0.5

Table 1: Expected ctDNA molecule counts and concentrations of the four dilution tiers are listed. Numbers refer to one 50-plex panel tracking 50 variants at 50,000 genomic equivalents DNA input.

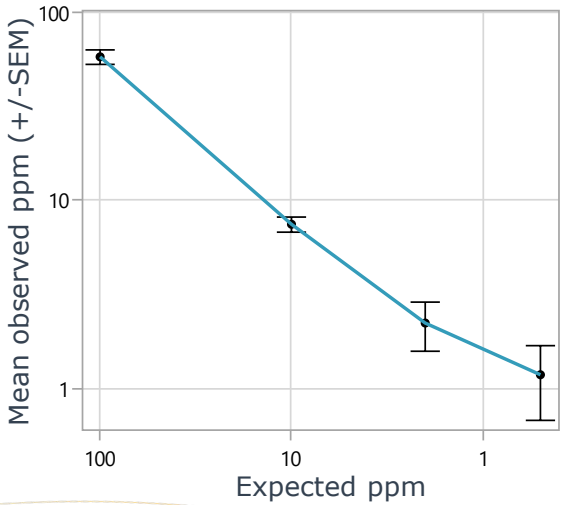

Figure 5: The mean observed tumor fraction in parts per million (ppm) across all 5 panels is plotted against the expected value per sample tier.

Result: The tumor-informed Haystack MRD Test can detect single ctDNA molecules at tumor fractions down to 0.5 ppm in samples with 165 ng DNA input. The blend made of n-cfDNA specimens from two cell-lines provided by SensID shows excellent functional properties as demonstrated by the high correlation between observed and expected tumor fraction.

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Conclusions

- Depending on how cfDNA-like reference materials are manufactured (e. g. type of fragmentation), they can exhibit significant differences in error rates and performance in various assay systems.
- Due to the extremely low inherent error rate of the underlying Duo™ chemistry, the Haystack MRD Test is ideally suited to characterize MRD reference material.
- It is demonstrated that n-cfDNA, a novel cfDNA reference material, exhibits the lowest error rate of all RMs tested using Haystack MRD, similar conversion efficiency, and a fragment size distribution that very closely mimics cfDNA.
- These properties make n-cfDNA a potentially suitable reference material to establish technical noise (limit of blank) and the limit of detection of highly sensitive MRD tests.
- Feasibility for this approach was shown by serially diluting n-cfDNA prepared from a tumor cell line in n-cfDNA prepared from a wild-type cell line down to 0.5 ppm. Haystack MRD was able to detect a positive signal in even the lowest tier, demonstrating sensitivity in the single ppm range.

In summary, the novel n-cfDNA reference material from SensID is an abundant source of patient-like material. Thus, n-cfDNA may be useful to support the development, validation, and production testing QC of even the most sensitive MRD tests, like Haystack MRD.

References

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Acknowledgements

This study was internally funded by Haystack Oncology and SensID. We would like to thank the lab teams at Haystack Oncology and SensID for their outstanding work. Special thanks go to Caroline Brohmeyer, Dagmar Küntzlin-Schierloh and Nastasia Rülcker for sample preparation and sequencing.

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