OncoExTra™ Test: Validation Summary

Test Overview

The OncoExTra test is an ultra-comprehensive genomic profiling assay that incorporates whole-exome sequencing utilizing germline (normal DNA) subtraction and tumor whole-transcriptome* (RNA) sequencing to identify alterations/biomarkers in individuals diagnosed with advanced cancers.

Key Technical Highlights[1,2]

- Test analyzes 19,396 genes + 169 introns.
- Average depth of sequencing coverage for this assay is 180X for matched normal specimens and 800x for the 440 cancer related genes in the tumor.
- Tumor transcriptome analysis includes 100 million (50m) paired reads.
- Reports clinically actionable mutations, copy number alterations, indels, fusions/transcript variants through DNA and RNA analyses. Findings are mapped to a knowledgebase of FDA approved targeted treatment options as well as relevant clinical trial options.
- Microsatellite instability (MSI), tumor mutation burden (TMB) and telomerase reverse transcriptase (TERT) promoter region are also reported, providing a total picture of actionable alterations.

Analytic Validation

- The analytic performance characteristics are summarized in **Table 1 below** and were determined using a variety of tumor-derived cell lines, and standards from commercially available sources commonly used to validate across multiple NGS platforms as well as clinical patient samples using orthogonal testing methods.
- Minimum tumor content for tumor samples was 20%.[1]
- Tumor tissue was evaluated for neoplastic content and macrodissected when necessary.

Table 1: Overall performance of OncoExTra Test

	Overall	SNVs	Indels	Copy number	Fusions
Analytic Sensitivity	98.8%	99.6%	96.8%	97.7%	93.1%
Analytic Specificity	>99.9%	>99.9%	>99.9%	>99.9%	100%
PPV	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%
Precision (variants of clinical significance): 100%					
Limit of Detection: 100% recovery of hotspot variants down to 1% allele frequency					
Analytic Specificity: 96.3% agreement with non-interferant replicates					
MSI Concordance: >99.9%; TMB Concordance: 91%					

SNV: single-nucleotide variant; PPV: positive predictive value; MSI: microsatellite instability; TMB: tumor mutational burden

Transcriptome (RNA) Sequencing[1]

- In a multicenter retrospective analysis of 1,261 solid tumor and heme patients tested with the OncoExTra test, 75 actionable fusions were detected (5.9%).
- 41% of fusions were supported by RNA sequencing alone and were not detected at the DNA level.
- 100% of RNA-only detected fusions were clinically actionable.

*Whole-transcriptome sequencing with select variants reported in New York State

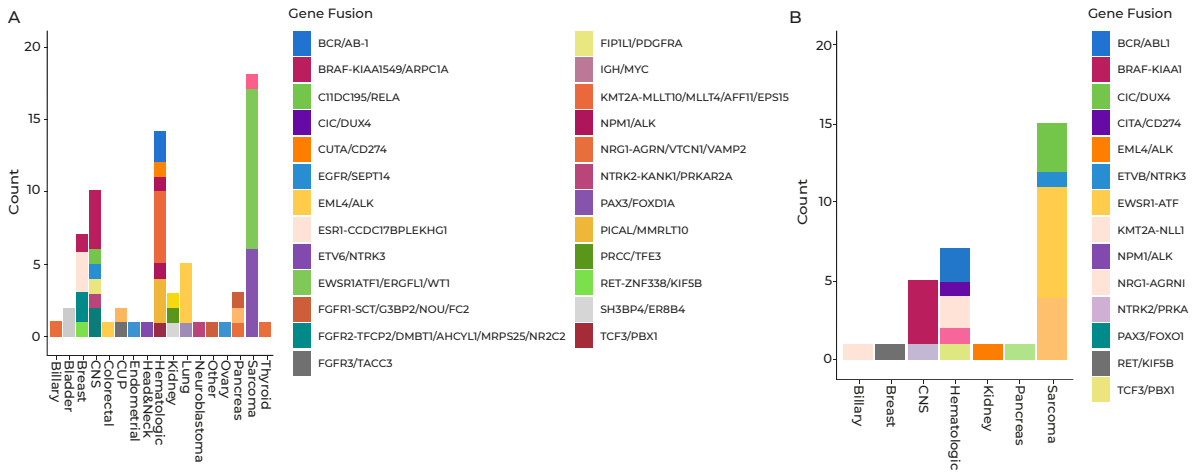

Figure 1: Fusions detection in the OncoExTra test. (A) Fusions detected by tumor type. (B) Fusions detected in tumor types with RNA only findings[1].

Clinical Utilization[1]

- 1,509 clinical reports generated during 2018-2019 for a total of 1,435 patients.
- 83.9% of reports (1,261) included both DNA and RNA profiling.
- 83.9% of tumor samples harbored at least one clinically actionable alteration (defined as positive); 1,267 positive and 242 negative.
- A median of 2 clinically actionable mutations were identified per tumor.
- Patient samples were ~75% FFPE and ~25% were fresh-frozen, cell pellets or bone marrow aspirates.

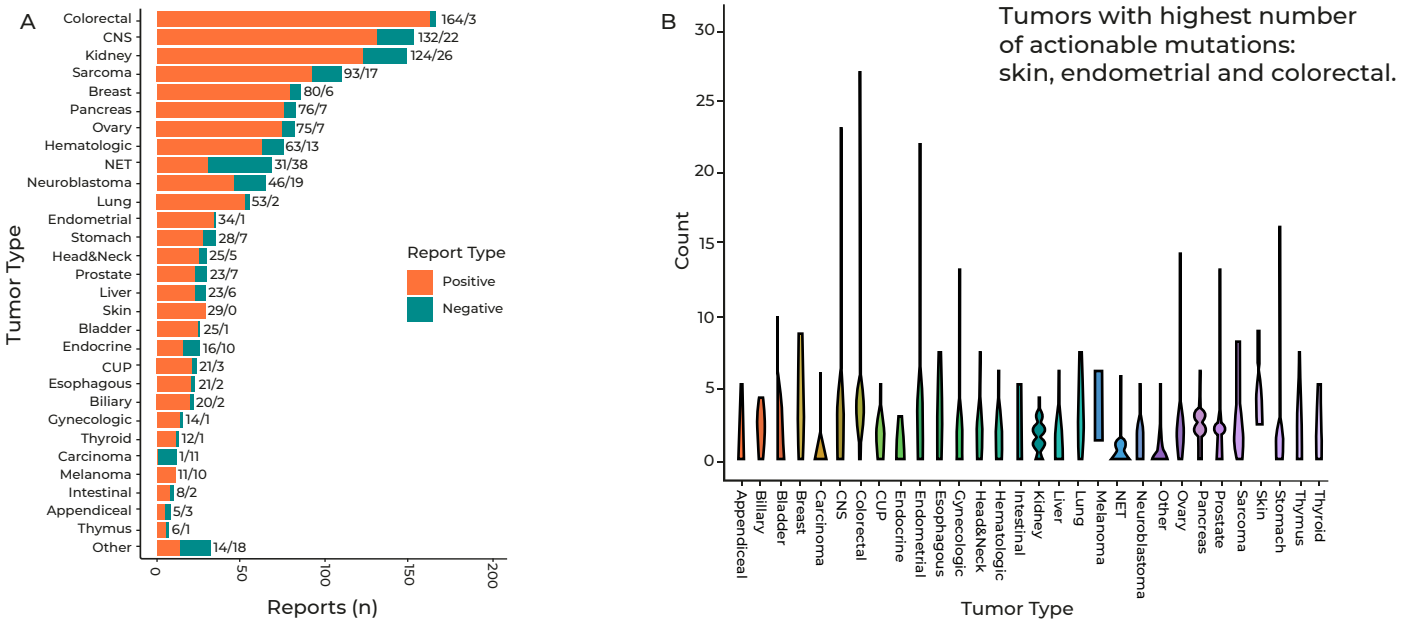

Figure 2: Performance Characteristic of the OncoExTra test (A) Tumor specific positivity (B) Violin plot of the number of clinically actionable events reported per tumor type[1].

References:
1. White T, Szelinger S, LoBello J, et al. Analytic validation and clinical utilization of the comprehensive genomic profiling test, GEM ExTra™. Oncotarget. 2021;12:726-739. 2. Bonneville R, Krook MA, Kautto EA, et al. Landscape of microsatellite instability across 39 cancer types. JCO Precis Oncol. 2017;2017:PO.17.0073.

OncoExTra has been validated according to the guidelines set forth by the New York State Department of Health. Whole exome (DNA) events have been validated to include point mutations, indels, and copy number alterations, as well as MSI analysis and TMB calculation. Whole transcriptome (RNA) has been validated to report on select fusion genes and special transcripts.

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