Abstract # 4005

Complementary use of DNA- and RNA-based NGS assays optimizes detection of clinically relevant translocations for comprehensive genomic profiling

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Background and Objectives

- Oncogenic gene translocations are common in solid cancers and detection of these structural events are a key component of clinical diagnostics to enable precision medicine in oncology.
- Cancer patients harboring certain translocation events can be treated with fusion-specific approved therapies that have proven to be remarkably effective in improving clinical outcomes.
- Several methods such as fluorescence in situ hybridization or RT-PCR have historically been employed, however, next-generation sequencing (NGS)-based comprehensive genomic profiling (CGP) including DNA- and RNA-based sequencing approaches have been validated for this purpose.
- DNA- and RNA-based sequencing approaches have distinct advantages and can be employed in a complementary or reflex manner to comprehensively detect translocation events in cancer patients to optimize targeted treatment strategies.
- Here we explore the complementary nature of these NGS-based methods to enable detection of clinically relevant translocations to guide patient care.

Approach and Cohort

- The following study is a retrospective analysis of 153 advanced or metastatic solid tumor patient cases that were accessioned by PathGroup from 2020 to 2022 for personalized molecular profiling. Indications widely varied and included lung, brain, sarcomas, gynecologic, breast, and several other cancer types (summarized in Figure 1).
- DNA-based genomic profiling was conducted utilizing PathGroup's molecular pathology-directed tumor profiling solution, Endeavor, which is powered by the Personal Genome Diagnostics (PGDx) elio™ tissue complete assay. This test comprehensively queries 505 genes for single nucleotide variants (SNVs) and insertion/deletions (indels), 23 genes for translocations, 28 genes for amplifications, as well as microsatellite instability (MSI) and tumor mutation burden (TMB). Translocations are detected through personalized analysis of rearranged ends (PARE), a proprietary method combining deep sequencing and bioinformatic approaches developed by PGDx, to identify paired end sequencing indicating gene fusion events.[1] By comprehensively tiling across exons and intronic regions, the assay is able to capture well characterized as well as novel fusion events making this a highly sensitive, fusion partner agnostic detection approach.
- RNA-based molecular profiling was conducted using PathGroup's Solid Tumor Fusion Assay, which is powered by Invitae/ArcherDx NGS FusionPlex Solid Tumor v1 assay. The test queries 53 genes specifically for translocations alone.
- Only the 19 shared genes across both panels were used for this performance comparison

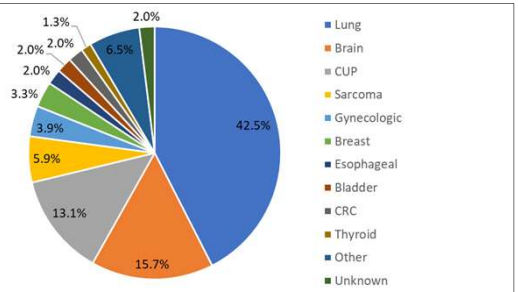

Figure 1. Indications represented in the 153-patient cohort. "Other" cancer category includes gastric, pancreatic, prostate, kidney and neuroendocrine cancers. There were also 3 cases of "unknown" indication. CUP - cancer of unknown primary, CRC - colorectal cancer.

Results

- Translocation events were detected in 23/153 (15%) cases by Endeavor and 17/105 (11.1%) cases by FusionPlex (Table 1).
- For the 135 cases where data was available from both assays, 12 (8.9%) concordant translocation positive cases were detected involving *ALK*, *RET*, *NTRK1*, *NTRK3*, *MET* exon 14 skipping, *EGFR*vIII, and *EWSR1*. Both assays called 109 (80.7%) cases as translocation negative. An overall concordance rate of 89.6% (121/135) was observed.
- The RNA-based FusionPlex assay had a 5x increased failure rate versus the DNA-based Endeavor assay. Two samples that failed with FusionPlex, both cancers of unknown primary (CUP), were found to have 3 distinct fusion events (*EWSR1*, *NTRK3* and *TMPRSS2*) as detected by Endeavor, one of which is actionable and the others which may be useful in tumor characterization and refinement of diagnosis.
- There were no cases where the Endeavor assay failed and the FusionPlex assay detected a fusion event.

Table 1. Translocation profiling results and comparison from the DNA-based Endeavor and RNA-based FusionPlex assays.

	FusionPlex Failure	FusionPlex +	FusionPlex -	Total (%)
Endeavor Failure	0	0	3	3 (2.0%)
Endeavor +	2	12	9	23 (15.0%)
Endeavor -	13	5	109	127 (83.0%)
Total (%)	15 (9.8%)	17 (11.1%)	121 (79.1%)	153 (100%)

- The Endeavor assay detected translocation events in 9 cases (*FGFR1*, *FGFR2*, *ETV4*, *ETV6*, *MYC*, and *NTRK3)* that were not identified by FusionPlex (Table 2 and 4).
- Conversely, FusionPlex identified 5 cases with translocations in *ROS1*, *NTRK2*, and *EGFR*vIII that were not detected by Endeavor (Table 3 and 4).
- Visual inspection was conducted when upstream intermediate files were available. Discrepancies in translocation detection were attributed to variability in panel design and exon coverage, differences in variant calling algorithms and thresholds and underlying biological differences in detectability associated with DNA- and RNA-based methods.

Table 2. Visual Inspection of Endeavor Positive and FusionPlex Negative Cases

Case #	Fusion Detected by Endeavor	Intermediate File Visual Inspection		Reason for Discordance
		Endeavor	FusionPlex	
19	*FGFR2-ITPR2*	XX supporting reads	Not detected; TBD	TBD, timing uncertain
29	*ETV4-ETV4*	N/A	Not detected; N/A	Unknown
35	*FGFR1-FGFR1*	116 supporting reads	Not detected; N/A	Not detected by FusionPlex, reason unknown
52	*SV2B-NTRK3*	N/A	Not detected; TBD	TBD, timing uncertain
79	*ETV6-ETV6*	N/A	Not detected; TBD	TBD, timing uncertain
88	*ANK1-FGFR1*	57 supporting reads	Not detected, exon not covered	Not detected by FusionPlex due to fusion location
143	*NRG1-MYC* *FGFR1-PXDNL*	99 supporting reads 11 supporting reads	Not detected, exon not covered	Not detected by FusionPlex due to fusion location
27	*PLB1-NTRK3*	8 supporting reads	Not detected; TBD	TBD, timing uncertain
39	*NTRK3-MFGE8*	XX supporting reads	Not detected; TBD	TBD, timing uncertain

N/A: Intermediate file not available

Table 3. Visual Inspection of FusionPlex Positive and Endeavor Negative Cases

Case #	Fusion Detected by FusionPlex	Intermediate File Visual Inspection		Reason for Discordance
		Endeavor	FusionPlex	
40	*SDC4-ROS1*	No evidence of ROS1 fusion	N/A	Not detected by Endeavor, likely RNA-specific fusion event
78	*EGFR vIII*	Detected, did not meet threshold	137 unique start sites	Detected but not reported by Endeavor
112	*EGFR vIII*	Detected, did not meet threshold	37 unique start sites	
134	*EGFR vIII*	Detected, did not meet threshold	233 unique start sites	
147	*KANK1-NTRK2*	Not detected, exon not covered	102 unique start sites	Not detected by Endeavor due to fusion location

N/A: Intermediate file not available

Conclusions and Future Direction

- In this study, comparison of translocation detection using DNA- and RNA-based NGS approaches revealed a high concordance between the two assays and were equally valuable for identifying actionable targets.
- To better understand the clinical implications of the discordant findings from both assays, Table 4 highlights the clinical actionability of each discordant fusion and ties the findings to FDA-approved or guideline-supported therapies as well as opportunities for enrollment into clinical trials.
- Of the 14 discordant cases, the DNA-based Endeavor assay identified 4 cases with indication-specific clinically actionable targets whereas the RNA-based FusionPlex assay identified 2 cases (Table 4, light blue rows).
- These findings provide confirmatory support for the complementary use of and possibly a reflex strategy for DNA- and RNA-based NGS approaches to most accurately identify clinically relevant translocations thereby providing more comprehensive results to help guide cancer treatment strategies.
- Current guidelines for NSCLC as a representative example recommend that when feasible, testing should be performed via a broad panel-based approach, most typically by NGS and for patients who, through broad panel testing, do not have an identifiable driver oncogene, physicians may consider RNA-based NGS to maximize fusion detection.[2]
- Of note, performing concurrent RNA analysis on all samples increases complexity, cost, and failure rates, which can lead to fewer patients receiving tumor profiling results[3] and should therefore be taken into consideration when devising a molecular testing strategy.
- This study will conclude with a comprehensive assessment of the discordant results as 75% of these ambiguous cases have residual DNA and RNA available for orthogonal testing and will be evaluated using a third-party NGS assay that can assess both analytes.

Table 4. Discordant Cases and Clinical Utility of Findings

Case #	Diagnosis	Fusion Findings	Identifying Assay	Indication-specific Treatments*	Other Potential Treatments**
19	Cancer of unknown primary	*FGFR2-ITPR2*	Endeavor	None	Futibatinib, Erdafitinib, Infigratinib, Pemigatinib
29	Lung squamous cell carcinoma	*ETV4-ETV4*	Endeavor	None	None
35	Diffuse astrocytoma	*FGFR1-FGFR1*	Endeavor	None	Pemigatinib, AZD4547, Erdafitinib, Debio1347, Infigratinib
52	Non-small cell lung cancer	*SV2B-NTRK3*	Endeavor	Larotrectinib (1), Entrectinib (1), Repotrectinib (3A)	None
79	Non-small cell lung cancer	*ETV6-ETV6*	Endeavor	None	None
88	Breast carcinoma (hormone receptor -, HER2+)	*ANK1-FGFR1*	Endeavor	None	Pemigatinib, AZD4547, Erdafitinib, Debio1347, Infigratinib
143	Lung Adenocarcinoma	*NRG1-MYC* *FGFR1-PXDNL*	Endeavor	Zenocutuzumab (3A)	Seribantumab, Pemigatinib, AZD4547, Erdafitinb, Debio1347, Infigratinib
27	Spindle cell sarcoma	*PLB1-NTRK3*	Endeavor	Larotrectinib (1), Entrectinib (1), Repotrectinib (3A)	None
39	Prostate carcinoma	*NTRK3-MFGE8*	Endeavor	Larotrectinib (1), Entrectinib (1), Repotrectinib (3A)	None
40	Lung adenocarcinoma	*SDC4-ROS1*	FusionPlex	Crizotinib (1), Entrectinib (1), Ceritinib (2), Lorlatinib (2), Repotrectinib (3A)	None
78	Non-small cell lung cancer	*EGFRvIII*	FusionPlex	None	None
112	Glioblastoma	*EGFRvIII*	FusionPlex	None	None
134	Glioblastoma	*EGFRvIII*	FusionPlex	None	None
147	Glioneuronal tumor	*KANK1-NTRK2*	FusionPlex	Larotrectinib (1), Entrectinib (1), Repotrectinib (3A)	None

* Therapeutic level of evidence: 1, 2, and 3A
**Therapeutic level of evidence: 3B and 4

1 Keefer et al. Nat Commun 2022
2 NCCN Non-Small Cell Lung Cancer Version 7.2021
3 Benayed et al. Clin Cancer Res 2019