FDA-cleared elio™ tissue complete assay provides rapid, accurate, and actionable insights to guide targeted cancer therapies with a path to reimbursement

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Introduction

- Comprehensive next-generation sequencing (NGS) is changing the diagnostic landscape and empowering precision oncology by identifying actionable mutations and genomic signatures unique to each tumor that help guide targeted treatment decisions.
- Identifying the genetic landscape of each patient's cancer is critical for the development of a personalized treatment plan that takes advantage of the growing number of targeted and immune therapies which have been shown to be safer and more effective than traditional chemotherapies when used in an appropriate patient population.
- Historically, single analyte biomarker testing of limited tumor tissue has been utilized to assess driver mutations thereby risking exhaustion of the samples before completing a comprehensive assessment.
- Comprehensive NGS serves to overcome this barrier by assessing a patient's genomic profile for all actionable mutations and mutational signatures simultaneously from a single tumor sample.
- While clinical NGS has been made available through a few specialized commercial laboratories, there is not yet widespread adoption or accessibility due to a lack of validated and reimbursable assays that can be scaled in local laboratories.
- Here we describe the development and analytical validation of the PGDx elio tissue complete test, an FDA-cleared kitted and reimbursed solution complete with automated bioinformatics analysis that enables comprehensive genomic profiling for use in local laboratories.

Methods

- Non-cancerous and tumor FFPE samples and pre-characterized cells lines were procured from commercial providers as well as from consented patients from Duke University and the National Cancer Institute.
- The PGDx elio tissue complete test, a 2.2 Mb hybrid capture, targeted gene panel assessing somatic mutations resulting in SNVs, indels, gene amplifications and translocations, MSI and TMB from 505 genes, was analyzed in IVD-mode and re-analyzed in RUO-mode.
- Whole-exome sequencing (WES) data were processed, and variants were identified using PGDx's VariantDx custom variant calling software and assigned a confidence score by PGDx Cerebro. In silico data sets containing spiked-in known variants were used to train PGDx's machine learning-based variant calling algorithm, Cerebro, to more accurately distinguish true somatic mutations from artifacts.
- Assessing tumor FFPE samples, SNVs and indels were validated for accuracy through orthogonal testing with FoundationOne (Foundation Medicine, Inc.), MSK-IMPACT (Memorial Sloan Kettering Cancer Center), and PCR-based assays (COBAS) on select SNVs and indels.
- In silico TCGA data analysis and whole-exome sequencing of matched tumor and normal FFPE samples were used for the training, optimizing, and performance comparison of the TMB algorithm for PGDx elio tissue complete. The ThermoFisher Oncomine Tumor Mutation Load assay was also used for performance comparison.
- 68 mononucleotide tracts were identified for PGDx's MSI classification algorithm and an overall weight matrix score was developed to determine the MSS and MSI threshold. Results were compared to an independent multiplex PCR assay.
- Structural variants were detected based on previously described algorithms, Digital Karyotyping for gene amplifications and Personalized Analysis of Rearranged Ends for translocations and results were compared to orthogonal assays including the Foundation Medicine FoundationOne NGS assay, ArcherDx RNA-based NGS assay, and FISH.

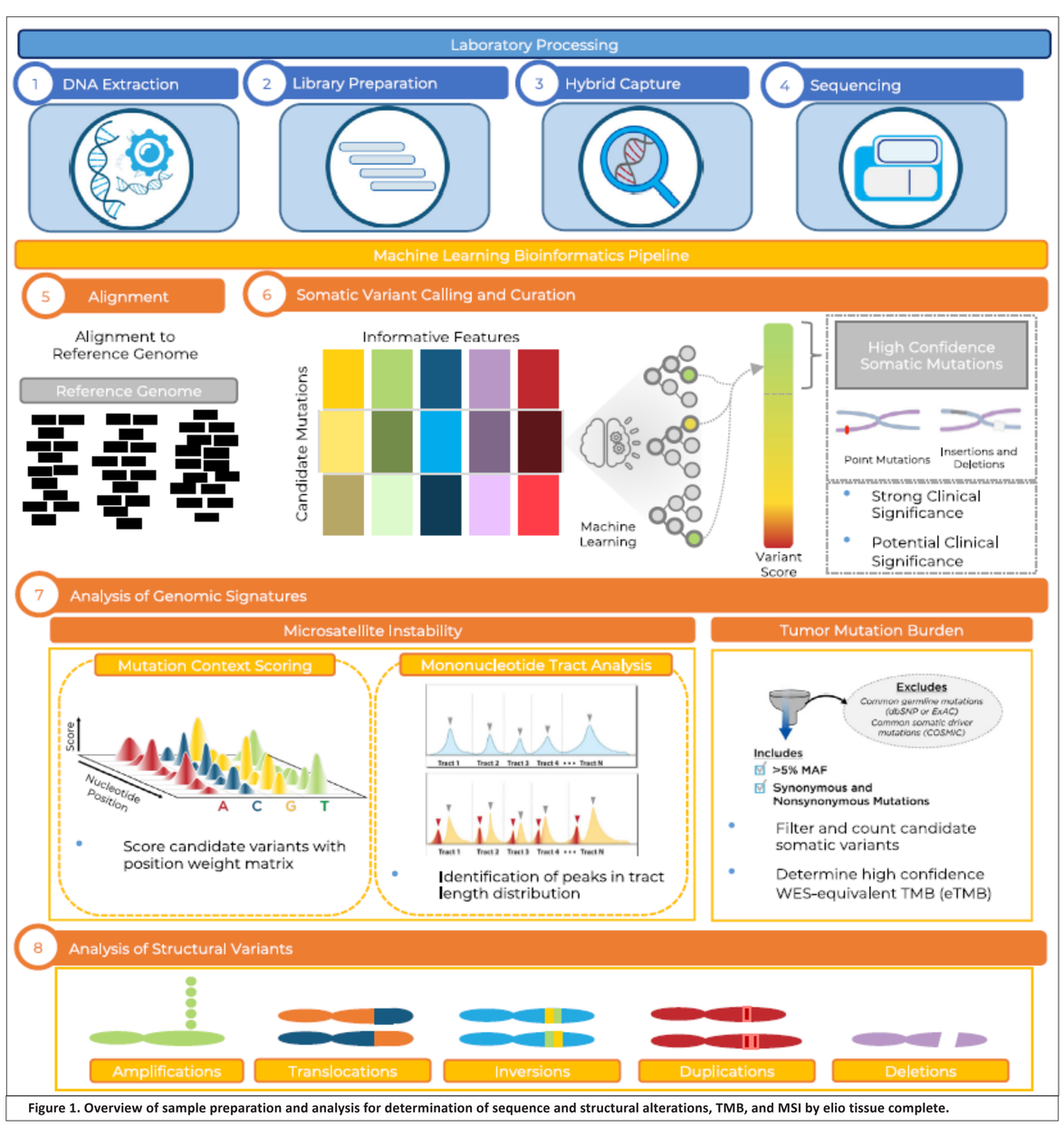

Figure 1. Overview of sample preparation and analysis for determination of sequence and structural alterations, TMB, and MSI by elio tissue complete.

Conclusions

- Independent analyses of clinically and biologically relevant sequence changes across 170 clinical tumor samples using MSK-IMPACT, FoundationOne, and PCR-based methods reveals a positive percent agreement of >97%.
- We observe high concordance with whole-exome sequencing for evaluation of tumor mutational burden for 307 solid tumors (Pearson r = 0.95) and comparison of the elio tissue complete microsatellite instability detection approach with an independent PCR assay for 223 samples displays a positive percent agreement of 99%.
- Evaluation of amplifications and translocations against DNA- and RNA-based approaches exhibits >98% negative percent agreement and positive percent agreement of 86% and 82%, respectively.
- PGDx elio tissue complete enables pan-solid tumor comprehensive genomic profiling of sequence alterations, structural variants, and genomic signatures with high analytical performance.

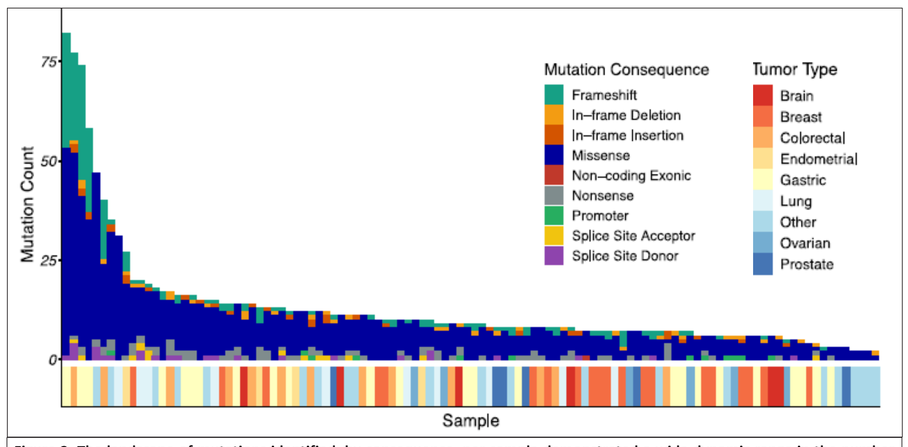

Figure 2. The landscape of mutations identified, by consequence, per sample demonstrated a wide dynamic range in the number and type of variants identified across the cohort, with the tumor types indicated below each case.

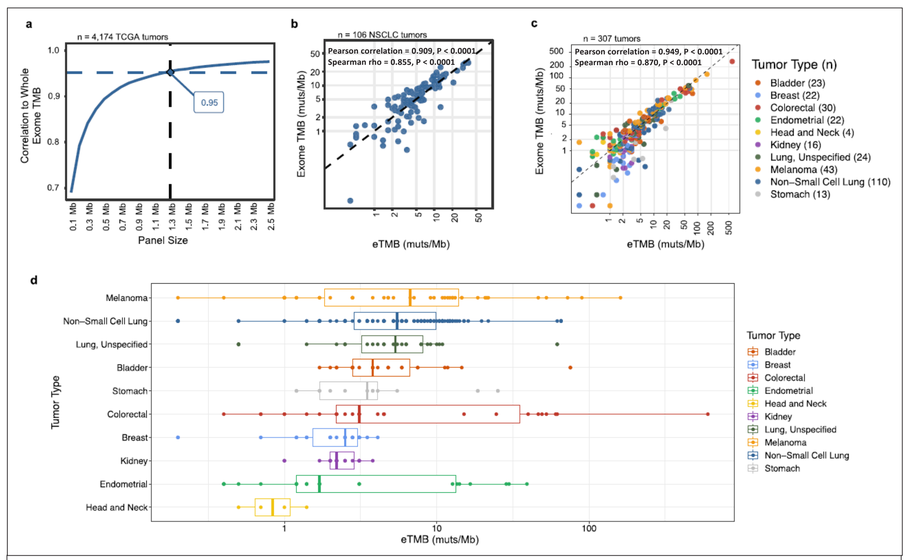

Figure 3. In silico and experimental comparison of the targeted panel and whole-exome TMB performance. a The correlation of in silico predicted TMB for panels of various size to observed WES TMB for samples in TCGA suggested that panels of >1 Mb provide accurate TMB measurements. **b** Comparison of the elio-predicted exome TMB (eTMB) using elio tissue complete and WES of tumor and matched-normal samples in a cohort of 106 non-small cell lung cancer (NSCLC). **c** Evaluation of eTMB in an independent cohort of 307 FFPE-derived pan tumor samples demonstrated high correlation to WES. **d** Distribution of eTMB scores in the independent cohort of 307 FFPE-derived tumors by tumor type, with the number of each tumor type captured in **(c).**

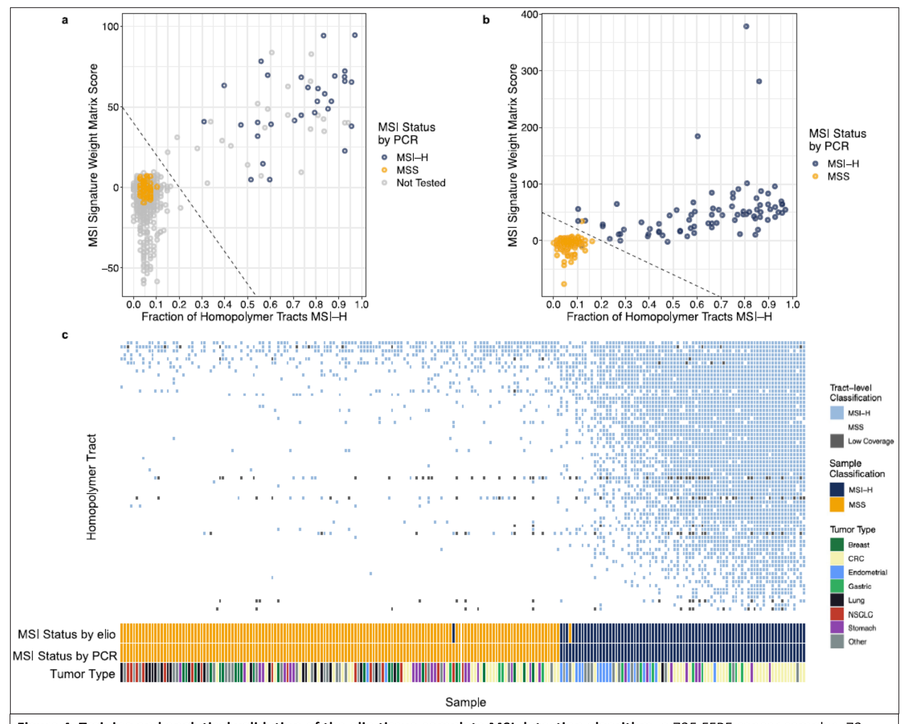

Figure 4. Training and analytical validation of the elio tissue complete MSI detection algorithm. a 725 FFPE cancer samples, 73 with confirmed MSI status via PCR, were evaluated using the combination tract-based and substitution score MSI algorithm. Plotting tracts versus signature weight matrix score, a decision boundary was determined to separate the cluster of known MSS cases from known MSI-H cases. **b** An independent cohort of 223 FFPE cancer samples with confirmed MSI status was analyzed with the elio tissue complete MSI algorithm. **c** Detailed analyses of each of the 68 mononucleotide tracts employed demonstrated >40% of tracts have perfect specificity in MSS cases and that a combination of high-sensitivity and high-specificity tracts was employed in the peak finding algorithm.

Table 1 Summary of amplification and translocation detection analytical performance.

Gene	Variant type	PPA (%) (*n*/*N*)	NPA (%) (*n*/*N*)	Orthogonal assay	Reference	Analysis mode
Aggregate (*n* = 12 genes)	Translocation	82.4% (42/51)	99.9% (1220/1221)	N/A	N/A	N/A
Aggregate (*n* = 16 genes)	Amplification	86.4% (121/140)	98.8% (2106/2132)	N/A	N/A	N/A
ALK	Translocation	92.9% (13/14)	98.2% (56/57)	Vysis ALK Break-Apart FISH Probe	This study	IVD and RUO
BRAF	Translocation	100% (2/2)	100% (145/145)	FoundationOne	Deak et al.[24]	RUO
CCND1	Amplification	72.7% (8/11)	99.3% (146/147)	FoundationOne	Deak et al.[24]	RUO
CCND2	Amplification	75.0% (3/4)	99.3% (146/147)	FoundationOne	Deak et al.[24]	RUO
CCND3	Amplification	50.0% (1/2)	100% (147/147)	FoundationOne	Deak et al.[24]	RUO
CCNE1	Amplification	92.9% (13/14)	98.6% (145/147)	FoundationOne	Deak et al.[24]	RUO
CD274	Amplification	100% (2/2)	98.6% (145/147)	FoundationOne	Deak et al.[24]	RUO
CDK4	Amplification	66.7% (2/3)	100% (147/147)	FoundationOne	Deak et al.[24]	RUO
EGFR	Amplification	100% (7/7)	99.3% (146/147)	FoundationOne	Deak et al.[24]	RUO
EGFR	Translocation	50% (1/2)	100% (147/147)	FoundationOne	Deak et al.[24]	RUO
ERBB2	Amplification	87.0% (40/46)	95.9% (71/74)	LSI HER2/neu FISH Probe	This study	IVD and RUO
EWSR1	Translocation	100% (2/2)	100% (147/147)	FoundationOne	Deak et al.[24]	RUO
FGFR1	Amplification	100% (11/11)	100% (147/147)	FoundationOne	Deak et al.[24]	RUO
FGFR1	Translocation	100% (1/1)	100% (147/147)	FoundationOne	Deak et al.[24]	RUO
FGFR2	Amplification	100% (1/1)	99.3% (146/147)	FoundationOne	Deak et al.[24]	RUO
FGFR3	Translocation	100% (1/1)	100% (147/147)	FoundationOne	Deak et al.[24]	RUO
MDM2	Amplification	88.3% (5/6)	98.0% (144/147)	FoundationOne	Deak et al.[24]	RUO
MET	Amplification	100% (5/5)	97.3% (143/147)	FoundationOne	Deak et al.[24]	RUO
MYC	Amplification	76.2% (16/21)	95.2% (140/147)	FoundationOne	Deak et al.[24]	RUO
MYCN	Amplification	100% (4/4)	99.3% (146/147)	FoundationOne	Deak et al.[24]	RUO
NTRK1	Translocation	75.0% (3/4)	100% (147/147)	FoundationOne	Deak et al.[24]	RUO
NTRK2	Translocation	100% (1/1)	100% (69/69)	FoundationOne	This study	IVD and RUO
NTRK3	Translocation	66.7% (2/3)	100% (12/12)	Archer Solid Tumor FusionPlex	This study	IVD and RUO
PDGFRA	Amplification	100% (1/1)	N/A	FoundationOne	This study	RUO
PIK3CA	Amplification	100% (2/2)	100% (147/147)	FoundationOne	Deak et al.[24]	RUO
RET	Translocation	55.6% (5/9)	100% (18/18)	Vysis 10q11 RET Break-Apart FISH	This study	IVD and RUO
ROS1	Translocation	100% (1/1)	100% (36/36)	Archer Solid Tumor FusionPlex	This study	RUO
TMPRSS2	Translocation	90.9% (10/11)	100% (147/147)	FoundationOne	Deak et al.[24]	RUO

Table 1. Analysis of >340 FFPE-derived tumor specimens comparing elio tissue complete to other DNA- and RNA-based orthogonal assays. elio tissue complete achieved 86.4% (121/140) PPA and 98.8% (2106/2132) NPA for gene amplifications compared to orthogonal NGS and fluorescence in situ hybridization (FISH)-based assays as well as 82.4% (42/51) PPA and 99.9% (1220/1221) NPA for translocations compared to independent DNA- and RNA-based approaches.

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