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Development of a pan-cancer NGS assay for detection of tumor mutational burden and targeted biomarkers from FFPE samples

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INTRODUCTION

Next-generation sequencing (NGS) is used to support routine clinical research in oncology with a primary focus on evaluating known oncogenic variants. Effective solutions such as targeted NGS assays allow assessment of hundreds of cancer-related genes simultaneously. Although, the primary focus of targeted assays has been to evaluate known oncogenic variants, the advent of cancer immunotherapies requires that clinical research solutions must also address biomarkers such as Tumor Mutational Burden (TMB) and Microsatellite Instability (MSI) for immune checkpoint inhibitors.

In recent years, TMB has emerged as important biomarker for immunotherapy[1]. Several published studies have demonstrated that high TMB is associated with positive response to various immune checkpoint inhibitors.

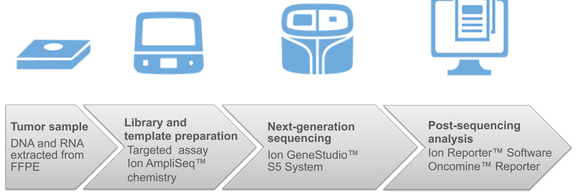
Although TMB can be accurately computed using whole exome sequencing (WES), associated challenges such as high cost, requirement for high starting FFPE material, requirement of tumor matched normal or control samples and limited sample to report software solutions has hindered the adoption of TMB biomarker testing in routine clinical research.

In order to overcome these challenges, we have developed a unified, yet simple multiplex PCR-based target enrichment NGS assay. The assay covers comprehensive targets that are relevant in cancer, has sensitive and specific chemistry to maximize low quantity FFPE tissues, and an automated sample-to-report workflow, that holistically provides an assessment of important cancer biomarkers, including TMB, in a time sensitive manner.

METHODS

Gene content was prioritized based on the relevance and variant prevalence of biomarkers in solid tumors. Additional genomic regions were added to supplement the coding sequence footprint to support TMB. The assay used Ion AmpliSeq™ technology with automated templating on the Ion Chef™ system and sequencing on the Ion GeneStudio™ S5 sequencing platform. An automated tumor-only workflow for variant calling, TMB and MSI estimation and sample quality reporting was provided within Ion Reporter Software. Streamlined access to decision support software is enabled by Oncomine™ Reporter[2].

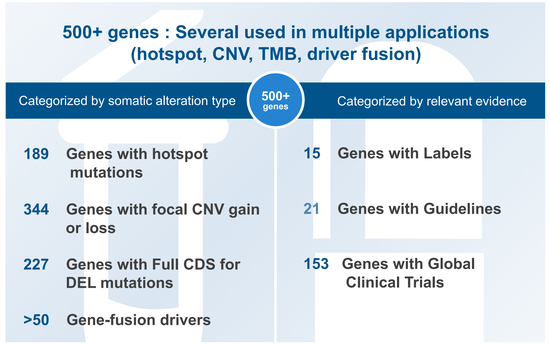
Figure 1. Schematic flow-diagram of the complete workflow



RESULTS

Over 500 genes with DNA based alterations and over 50 RNA fusion drivers are included. More than 13,000 DNA amplicons cover a comprehensive genomic targeted region with a large (>1 MB) coding sequence (CDS) footprint to support high-confidence TMB. FFPE tumor samples from a variety of tissue types were sequenced using the assay. The assay displays high (>95%) uniformity and consistent read depth (>2200x) to support robust variant calling at low allele frequency. In-silico assessment of TMB using publicly available whole-exome cancer sequencing data resulted in high correlation (R^2 > 0.90, 0-40 mut/mb) in pan-cancer and specific cancer types including lung, colorectal and melanoma.

Figure 2. Summary of the assay content

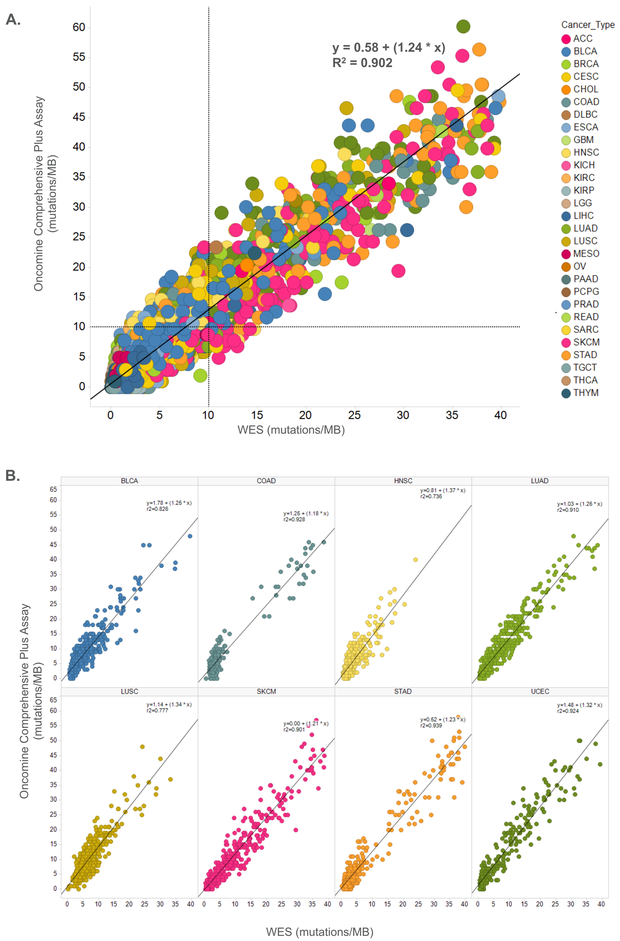


Out of the DNA genes, 189 genes cover important cancer hotspots, 344 cover copy number variants (CNV), while 227 genes have full coding sequence (CDS) coverage for detection of deleterious variants. In addition to fusion drivers, this assay also includes seven genes with intragenic variants. The assay was also designed to maximize the genomic footprint to support high-confidence TMB estimation. The total genomic coverage of the assay is 1.50MB with 1.03MB of coding sequence.

Table 1. Oncomine Comprehensive Plus Assay Cancer Gene Targets

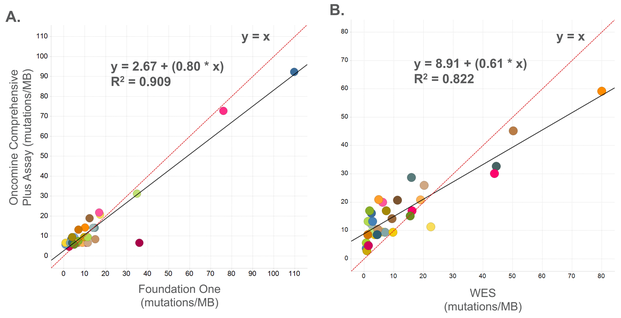
CNV Gain Genes					Full CDS Coverage					
	Hotspot Genes									
CNV Gain Only	CNV Gain and Hotspot			Hotspot Only	CNV Loss					CDS Only
ABCB1	ABL1	FOXA1	PPP6C	ACVR1	NTRK2	ABRAXAS1	CDKN1A	FANCA	MAP3K1	POLE	SOX9	CALR
CTNND2	AKT1	GATA2	PTPN11	ATP1A1	NUP93	ACVR1B	CDKN1B	FANCC	MAP3K4	POT1	SPEN	CIITA
DDR1	AKT2	GNAS	PXDNL	BCR	PAX5	ACVR2A	CDKN2A	FANCD2	MAPK8	PPM1D	STAG2	CYP2D6
EMSY	AKT3	H3F3A	RAC1	BMP5	PIK3CD	ADAMTS12	CDKN2B	FANCE	MEN1	PPP2R2A	STK11	ERCC5
FGF3	ALK	H3F3B	RAF1	BTK	PIK3CG	ADAMTS2	CDKN2C	FANCF	MGA	PRDM1	SUFU	FAS
FGF4	AR	IKBKB	RARA	CACNA1D	PTPRD	AMER1	CHEK1	FANCG	MLH1	PRDM9	TAP1	ID3
FGF9	ARAF	IL7R	RET	CD79B	RGS7	APC	CHEK2	FANCI	MLH3	PRKAR1A	TAP2	KLHL13
FGF19	AURKA	KIT	RHEB	CSF1R	RHOA	ARHGAP35	CIC	FANCL	MRE11	PTCH1	TBX3	MTUS2
FGF23	AURKC	KLF5	RICTOR	CTNNB1	RPL10	ARID1A	CREBBP	FANCM	MSH2	PTEN	TCF7L2	PSMB8
FYN	AXL	KRAS	RIT1	CUL1	SIX1	ARID1B	CSMD3	FAT1	MSH3	PTPRT	TET2	PSMB9
GLI3	BCL2	MAGOH	SF3B1	CYSLTR2	SIX2	ARID2	CTCF	FBXW7	MSH6	RAD50	TNFRSF8	PSMB10
IGF1R	BCL2L12	MAPK1	SETBP1	DDR2	SNCAIP	ARID5B	CTLA4	FUBP1	MTAP	RAD51	TNFAIP3	RNASEH2C
MCL1	BCL6	MAX	SF3B1	DNMT3A	SOS1	ASXL1	CUL3	GATA3	MUTYH	RAD51B	TNFRSF14	RPL5
MDM2	BRAF	MDM4	SLCO1B3	E2F1	SOX2	ASXL2	CUL4A	GNA13	NBN	RAD51C	TP53	RPL22
MYCL	CARD11	MECOM	SMC1A	EPAS1	SRSF2	ATM	CUL4B	GPS2	NCOR1	RAD51D	TP63	RUNX1T1
RPS6KB1	CCND1	MEF2B	SPOP	FGF7	STAT3B	ATR	CYLD	HDAC2	NF1	RAD52	TPP2	SDHC
RPTOR	CCND2	MET	SRC	FOXL2	TAF1	ATRX	CYP2C9	HDAC9	NF2	RAD54L	TSC1	SOCS1
YAP1	CCND3	MPL	STAT3	FOXO1	TGFBR1	AXIN1	DAXX	HLA-A	NOTCH1	RASA1	TSC2	STAT1
YES1	CCNE1	MTOR	STAT6	GLI1	TRRAP	AXIN2	DDX3X	HLA-B	NOTCH2	RASA2	USP9X	TMEM132D
	CDK4	MYC	TERT	GNA11	TSHR	B2M	DICER1	HNF1A	NOTCH3	RB1	VHL	UGT1A1
	CDK6	MYCN	TOP1	GNAQ	WAS	BAP1	DNMT3A	INPP4B	NOTCH4	RBM10	WT1	ZBTB20
	DDR2	MYD88	TPMT	HIF1A		BARD1	DOCK3	JAK1	PALB2	RECQL4	XRCC2	
	EGFR	NFE2L2	U2AF1	HIST1H2BD		BCOR	DPYD	JAK2	PARP1	RNASEH2A	XRCC3	
	EIF1AX	NRAS	USP8	HIST1H3B		BLM	DSC1	JAK3	PARP2	RNASEH2B	ZFHX3	
	ERBB2	NTRK1	XPO1	HRAS		BMPR2	DSC3	KDM5C	PARP3	RNF43	ZMYM3	
	ERBB3	NTRK3	ZNF217	IDH1		BRCA1	ELF3	KDM6A	PARP4	RPA1	ZRSR2	
	ERBB4	PCBP1	ZNF429	IL6ST		BRCA2	ENO1	KEAP1	PBRM1	RUNX1		
	ESR1	PDGFRA		IRF4		BRIP1	EP300	KMT2A	PDCD1	SDHA		
	EZH2	PDGFRB		IRS4		CASP8	EPCAM	KMT2B	PDCD1LG2	SDHB		
	FAM135B	PIK3C2B		KLF4		CBFB	EPHA2	KMT2C	PDIA3	SDHD		
	FGFR1	PIK3CA		KNSTRN		CD274	ERAP1	KMT2D	PGD	SETD2		
	FGFR2	PIK3CB		MAP2K2		CD276	ERAP2	LARP4B	PHF6	SLX4		
	FGFR3	PIK3R2		MED12		CDC73	ERCC2	LATS1	PIK3R1	SMAD2		
	FGFR4	PIM1		MYOD1		CDH1	ERCC4	LATS2	PMS1	SMAD4		
	FLT3	PLCG1		NSD2		CDH10	ERRFI1	MAP2K4	PMS2	SMARCA4		
	FLT4	PPP2R1A		NT5C2		CDK12	ETV6	MAP2K7	POLD1	SMARCB1		

Figure 3. In-silico comparison of the Oncomine Comprehensive Plus Assay with Whole Exome Sequencing (WES)



Scatter plots (Fig. 3) showing the correlation between the targeted assay (y-axis) and WES (x-axis) mutation counts. WES data was downloaded from TCGA MC3[3]. In-silico analysis was performed to characterize TMB performance of the targeted sequencing assay. Rate of nonsynonymous somatic mutations was computed for WES TMB. Mutations were limited to the targeted assay for predicted TMB. WES TMB strongly correlated (R^2 > 0.9) with the assay TMB in pan-cancer analysis (Fig. 3A). Figure 3B displays similar distribution for selected cancer types. Strong correlation (R^2 > 0.9) were observed for various cancer types with variations in Bladder Urothelial Carcinoma (BLCA) and Lung squamous cell carcinoma (LUSC).

Figure 4. Comparison of the Oncomine Comprehensive Plus Assay with Foundation One and Whole Exome Sequencing



Scatter plots (Fig. 4) showing the correlation between the Oncomine Comprehensive Plus Assay and Foundation One (Fig 4A) and Whole Exome Sequencing (Fig 4B). OCA Plus Assay shows strong correlation (R^2 > 0.9) with Foundation One panel (Fig 4A). For comparison with WES, 35 FFPE tumor samples with matched normal were sequenced on the Illumina platform and the TMB scores were determined. The matched tumor samples were then sequenced on the Oncomine Comprehensive Plus assay using IonTorrent sequencing technology and TMB scored were determined. These scores were then compared with the WES TMB scores (Fig 4B).

CONCLUSIONS

A targeted NGS assay was developed to support comprehensive genomic profiling and routine clinical research in oncology that includes endpoints for TMB and MSI. The design and informatics workflow support characterization of mutational signatures and provides normalized TMB estimates with a strong correlation with orthogonal methods. The assay also supports detecting relevant RNA structural alterations from solid tumor FFPEs. Minimal input material requirement and rapid sample to report time will have a high impact on clinical research.

REFERENCES

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