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Abstract #1577128

Rapid and Automated Comprehensive Genomic Profiling to Assess Single-gene and Complex Biomarkers Including Genomic Instability

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Introduction

Comprehensive genomic profiling (CGP) assays are important to advance research into precision medicine, which aims to deliver the right drug to the right patient at the right time. However, CGP is typically associated with a complex manual workflow with many touchpoints and slow turn-around time (TAT) to results. To facilitate adoption of CGP, an automated workflow with rapid TAT is required. Hence, we developed a 500 gene targeted amplicon enrichment-based oncology research panel that delivers DNA small variants, copy number alterations (CNA), RNA-based gene fusions and complex biomarkers including microsatellite instability (MSI), tumor mutation burden (TMB), and homologous recombination deficiency (HRD) on the Ion Torrent Genexus™ automated sequencing system.

Methods

The Oncomine™ Comprehensive Assay (OCA) Plus is being developed on the Genexus™ automated sequencing platform using 30ng of DNA and RNA as input. The Genexus sequencer provides automated library preparation, templating, sequencing and variant reporting in a typical TAT of 24 hr. Cell lines, reference controls and orthogonally tested FFPE research samples are used to evaluate various endpoints for sensitivity and PPV. For HRD, we developed a novel algorithm (GIM, genomic instability metric) to summarize unbalanced CN segments to measure genomic instability and compared the scores to same samples sequenced using on-market OCA Plus assay on GeneStudio™ platform.

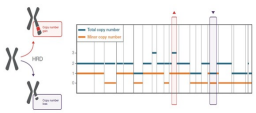
Single Gene and Complex Biomarkers	
500+ genes	Automated tumor fraction calculation
Small Variants (SNVs and Indels)	Genomic Instability Metric (GIM)
Gene Level Copy Number Variants	Microsatellite Instability (MSI)
Arm-Level Aneuploidy	Tumor Mutational Burden (TMB)
Gene Fusions (>1300 isoforms)	Gene LOH for BRCA1/2 and other HRR genes
MET exon skipping detection at DNA and RNA level	Full coverage of DNA repair pathway genes including HRR and MMR

OCA Plus on Genexus™ allows CGP with next day results for DNA variants and RNA fusions including TMB, MSI and HRD with low input in a single assay

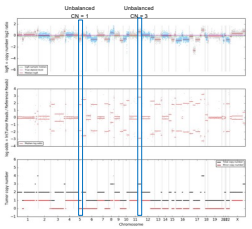
Genomic Instability Metric (GIM)

GIM is a novel metric to quantify genomic scars/instability associated with HRD. It is based on genome segmentation[1] using CNV log2 ratios and log odds for SNP allele frequencies which allows to summarize different unbalanced copy number events across the autosomes. It ranges from 0-100, the higher the value, the more genomic instability. For ovarian cancer, we derived a threshold of 16, equal to or above which the sample is classified as genomic instability high and vice versa.

Examples of unbalanced CN gain and loss events that are summarized in GIM



Genome Segmentation



Top: Schematic, depicting examples of how a CN gain and loss are affecting minor and total copy numbers. **Right:** OCA Plus analysis of a HR-deficient sample, demonstrating the genome segmentation and unbalanced copy number alterations. GIM for this sample is 25.

Results

SNV/Indel performance in AOHC samples

Variant Type	Sensitivity	PPV
SNVs	99.5%	99.4%
Indels	99.0%	98.5%

The AcroMetrix™ Oncology Hotspot Control (AOHC) was sequenced to evaluate OCA Plus SNV and Indel variant calling performance.

SNV/Indel performance in FFPE samples

Variant Type	Concordant Calls	Genexus Only	GeneStudio Only	PPA
SNVs	38	0	1	97.6%
Indels	3	0	0	100%

TMB score correlation with TMB Mix controls

TMB Control	Expected (mut/Mb)	Measured (mut/Mb)
TMB Mix-7	7.2 ± 0.2	7.62
TMB Mix-9	9.5 ± 0.4	9.53
TMB Mix-13	12.6 ± 0.02	12.29
TMB Mix-20	20.1 ± 0.2	20.90

Evaluation of TMB score performance by sequencing SeraCare® FFPE TMB Reference Mix samples, with known TMB scores.

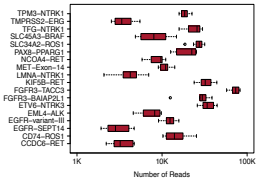
SNV/Indel variant calling performance in FFPE samples was evaluated using a cohort of (N=26) samples containing hotspot variants and comparing them against the variant calls made in the same samples sequenced using on-market OCA Plus assay on GeneStudio™ platform. High percent positive agreement (PPA) was observed between the two platforms.

MSI performance in Reference Controls and FFPE samples

Sample Type	# of Samples	Sensitivity	Specificity
Reference Controls	76	100%	100%
FFPE	352	100%	99.3%

The OCA Plus assay was used to evaluate MSI calls in controls as well as >350 colorectal, endometrial, and stomach FFPE samples. The concordance in FFPE samples was 99.4% with sensitivity of 100% and specificity of 99.3%.

Fusion detection in Reference Controls



The SeraSeq® Fusion RNA Mix v4 control contains 18 important gene fusions. The OCA Plus assay successfully and reproducibly detects all 18 fusions (2M reads per sample).

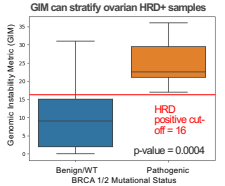
CNV performance in control and FFPE samples

Variant Type	Sensitivity	PPV
CNV Gain	95.0%	98.8%
CNV Loss	92.0%	100%

Evaluation of CNV gain (CN >=6) and CNV loss (homozygous loss) were performed by sequencing FFPE samples of varying tumor types with Oncoscan™ Affymetrix array as the reference assay.

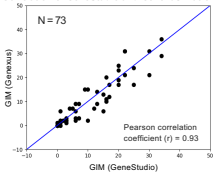
GIM analytical performance

GIM can stratify ovarian HRD+ samples



Ovarian cancer samples with *BRCA1/2* mutations are HRD positive. GIM can stratify *BRCA1/2* mutated samples from *BRCA1/2* WT samples.

Correlation of GeneStudio and Genexus Platforms



FFPE samples and cell-lines from different cancer types (N=73) were sequenced on both GeneStudio and Genexus platforms. We found GIM to be highly correlated on the two platforms.

HRD calling using OCA Plus was evaluated in two different ovarian tumor FFPE cohorts and exhibited very high concordance in *BRCA1/2* variant calling and genomic instability scoring using GIM as well as combining them to derive HRD status when compared to two different reference assays as shown in Table 1[2] and Table 2[3].

	OCA Plus *BRCA1/2* (N=93)			OCA Plus GIM (N = 86)			OCA Plus HRD (N = 86)	
	Mut	WT		GIM +	GIM -		HRD +	HRD -
Reference Assay 1	Mut 29	3	GI +	47	2	HRD +	51	1
	WT 1	60	GI -	8	28	HRD -	7	27
Positive Percent Agreement (PPA)	90.6%			95.9%			98.1%	
Negative Percent Agreement (NPA)	98.3%			77.8%			79.4%	
Overall Percent Agreement (OPA)	**95.7%**			**88.2%**			**90.7%**	

Data courtesy of Dr. Normanno, Istituto Nazionale Tumori-IRCCS-Fondazione G. Pascale, Naples, Italy

Table 1. OCA Plus *BRCA1/2* variant calling, GIM and HRD concordance to Reference Assay 1

	OCA Plus *BRCA1/2* (N = 75)			OCA Plus GIM (N = 77)			OCA Plus HRD (N = 79)	
	Mut	WT		GIM +	GIM -		HRD +	HRD -
Reference Assay 2	Mut 25	3	GI +	48	2	HRD +	54	2
	WT 0	47	GI -	7	20	HRD -	5	18
Positive Percent Agreement (PPA)	89.2%			96%			96.4%	
Negative Percent Agreement (NPA)	100%			74.1%			78.2%	
Overall Percent Agreement (OPA)	**96%**			**88.3%**			**91.1%**	

Data courtesy of Dr. Ahwon Lee, Department of Hospital Pathology, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Republic of Korea

Table 2. OCA Plus *BRCA1/2* variant calling, GIM and HRD concordance to Reference Assay 2

References

1. Shen Ronglai et al., 2016, *Nucleic Acids Research*, Vol.44, No.16
2. C. Roma et al., Poster abstract EACR23-1096, EACR Congress 2023.
3. Jun Kang, Kiyong Na, Haeyoun Kang, Uiju Cho, Sun Young Kwon, Sohyun Hwang, Ahwon Lee. 2023. Prediction of Homologous Recombination Deficiency from Oncomine Comprehensive Assay Plus Correlating with SOPHiA DDM HRD Solution. medRxiv doi: 10.1101/2023.08.09.23293743

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