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Development of a novel metric to measure genomic instability using unbalanced copy number changes with fast comprehensive genomic profiling

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Introduction

Comprehensive genomic profiling (CGP) using next generation sequencing (NGS) is becoming increasingly important to allow detection of all relevant cancer biomarkers applicable to varying tumor entities in a single assay. However, CGP is typically associated with a complex manual workflow with many touchpoints and slow turn-around time to results. Additionally, most assays are not true CGP and involve additional steps to evaluate complex biomarkers especially HRD which requires characterization of genomic instability at the sample level. To facilitate adoption of CGP by removing these pain points, we developed the Oncomine™ Comprehensive Assay Plus (OCA Plus) assay on Genexus™ that allows detection of DNA variants like Single Nucleotide Variants (SNVs), Insertions and Deletions (Indels), Copy Number Variations (CNVs), RNA fusions and complex biomarkers like Microsatellite Instability (MSI), Tumor Mutation Burden (TMB) and Homologous Recombination Deficiency (HRD). To evaluate HRD, we developed a novel metric to estimate genomic instability based on unbalanced copy number changes.

Materials and methods

The high-throughput capabilities of the Genexus System enable support for large oncology research panels such as OCA Plus. In addition, Ion AmpliSeq™ technology enables low sample input as low as 30ng of DNA or 20ng of RNA. Hence, the extensive per sample coverage and low sample input allows for comprehensive DNA and RNA genomic profiling of relevant cancer biomarkers in over 500 genes. Performance is characterized using control and FPFE samples.

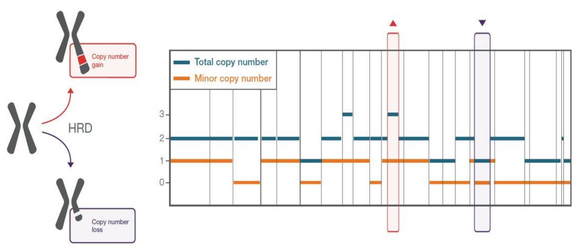
Single Gene and Complex Biomarkers	
500+ genes	Automated tumor fraction calculation
Small Variants (SNVs and Indels)	Genomic Instability Metric (GIM)
Gene Level Copy Number Variants	*BRCA1/BRCA2* large genomic rearrangements
Microsatellite Instability (MSI)	Tumor Mutational Burden (TMB)
Gene Fusions (>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
Arm level Aneuploidy	

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)

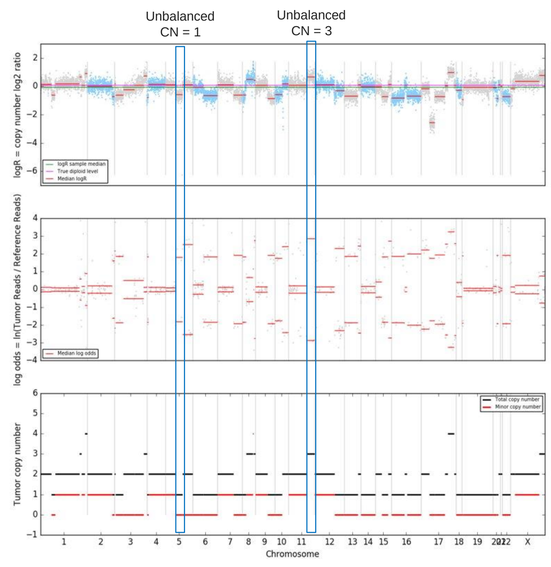
GIM is a novel metric to quantify genomic scars/instability associated with HRD. It is based on genome segmentation[1] using CNV log2 ratios and log odds for SNP allele frequencies which allows to summarize different unbalanced copy number events across the autosomes. It ranges from 0-100, the higher the value, the more genomic instability.

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



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.

Genome Segmentation



HRD biomarker using OCA Plus

OCA Plus assay covers causes and consequences for HRD research by interrogating 46 homologous recombination repair pathway genes and by estimating genomic instability through GIM. *BRCA1* and *BRCA2* genes are enabled for SNV/Indel calling as well as CNVs, gene LOH and large genomic rearrangements such as exon-level deletions and duplications. Pathogenic mutations in *BRCA1*/*BRCA2* and/or GIM above the threshold of 16 allow determining HRD status in ovarian cancer samples.

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.

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.

MSI performance in Reference Controls and FFPE samples

Sample Type	Samples	Sensitivity	Specificity
Ref Controls	76	100%	100%
FFPE	352	100%	99.3%

Reference Controls used are HD-830/HD-831/CRL-2577. FFPE samples comprise of colorectal, endometrial and stomach cancer.

TMB score correlation with TMB Mix controls

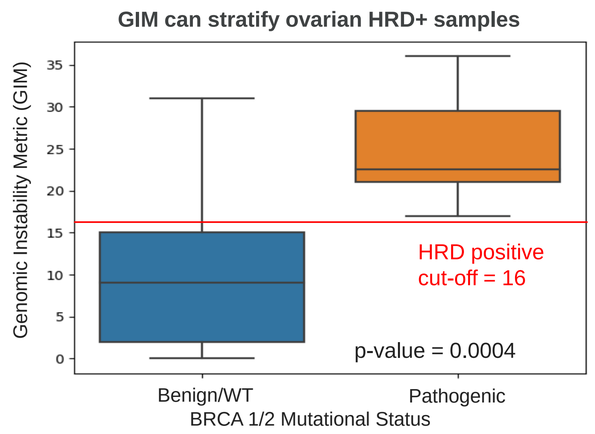
TMB Control	Expected (mut/Mb)	Measured (mut/Mb)
TMB-7	7.2 ± 0.2	8.54
TMB-9	9.5 ± 0.4	9.50
TMB-20	20.1 ± 0.2	22.30

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

GIM analytical performance

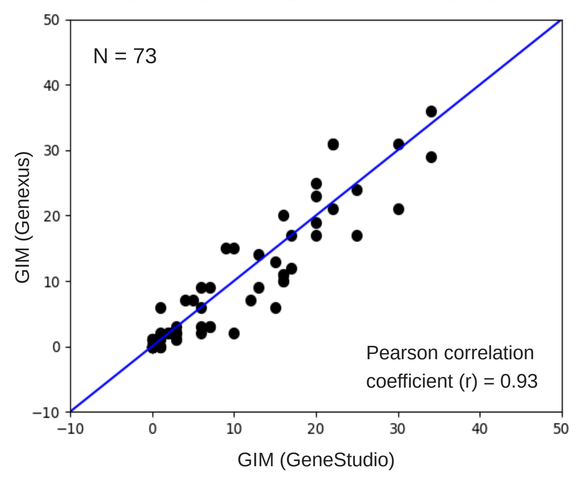
Ovarian cancer samples with *BRCA1/2* mutations are HRD positive. GIM can stratify *BRCA1/2* mutated samples from *BRCA1/2* WT samples.

GIM can stratify ovarian HRD+ samples



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.

Correlation of GeneStudio and Genexus Platforms



GIM compared to Genomic Instability (GI) scores from a reference assay shows concordance of 88.2% across 85 ovarian FFPE samples sequenced on GeneStudio S5.[2]

Ovarian Cancer Samples (N=85)	GIM Positive (≥ 16)	GIM Negative (< 16)
Reference GI Positive	47	2
Reference GI Negative	8	28
Sensitivity	95.9%	
Specificity	77.8%	
Agreement	88.2%	

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

Taking into account also *BRCA1/2* status, overall HRD status classification from OCA Plus compared to the reference assay shows an **agreement of 90.7%.**[2]

Ovarian Cancer Samples (N=86)	OCA Plus HRD+	OCA Plus HRD-
Reference HRD+	51	1
Reference HRD-	7	27
Sensitivity	98.1%	
Specificity	79.4%	
Agreement	90.7%	

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

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.