

GENOMIC INSTABILITY

FUSIONS

MSI

TMB

CNVs

A comprehensive genomic profiling solution that integrates *BRCA1/2* mutational status and genomic instability to characterize homologous recombination deficiency in a next-generation sequencing tumor only workflow

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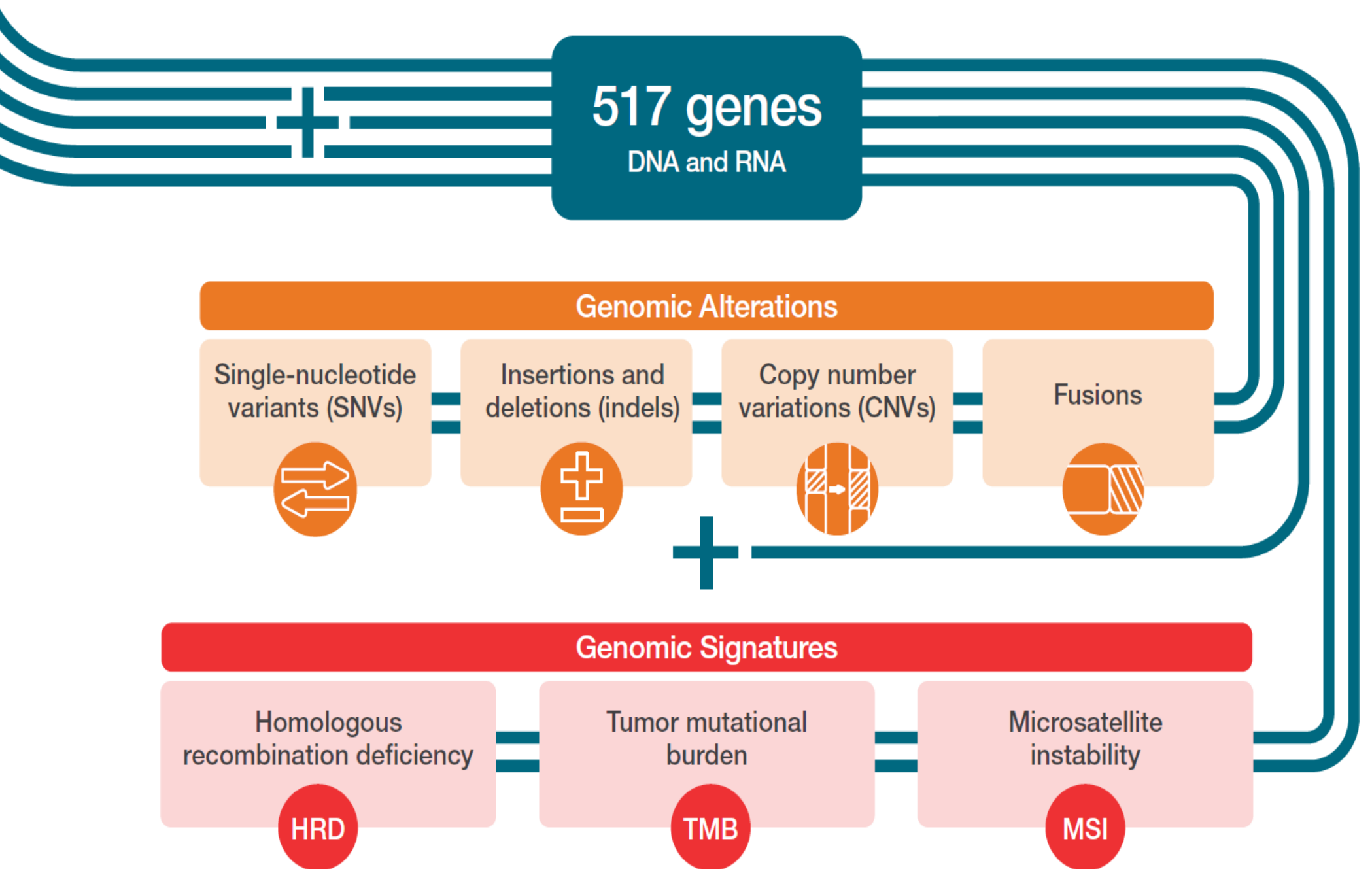
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Introduction

Homologous Recombination Deficiency (HRD) is the inability of cells to repair DNA double strand breaks and is caused primarily by mutations in *BRCA1/2*. The consequence of this impairment in the repair mechanism is genomic instability which is accumulation of errors during DNA replication. These causes and consequences must be collectively interrogated through comprehensive genomic profiling (CGP) for reliable characterization of HRD. We developed a complex biomarker named genomic instability metric (GIM) that summarizes genomic instability and combined with deleterious mutations identified in *BRCA1/2* to enable HRD determination.

Methods

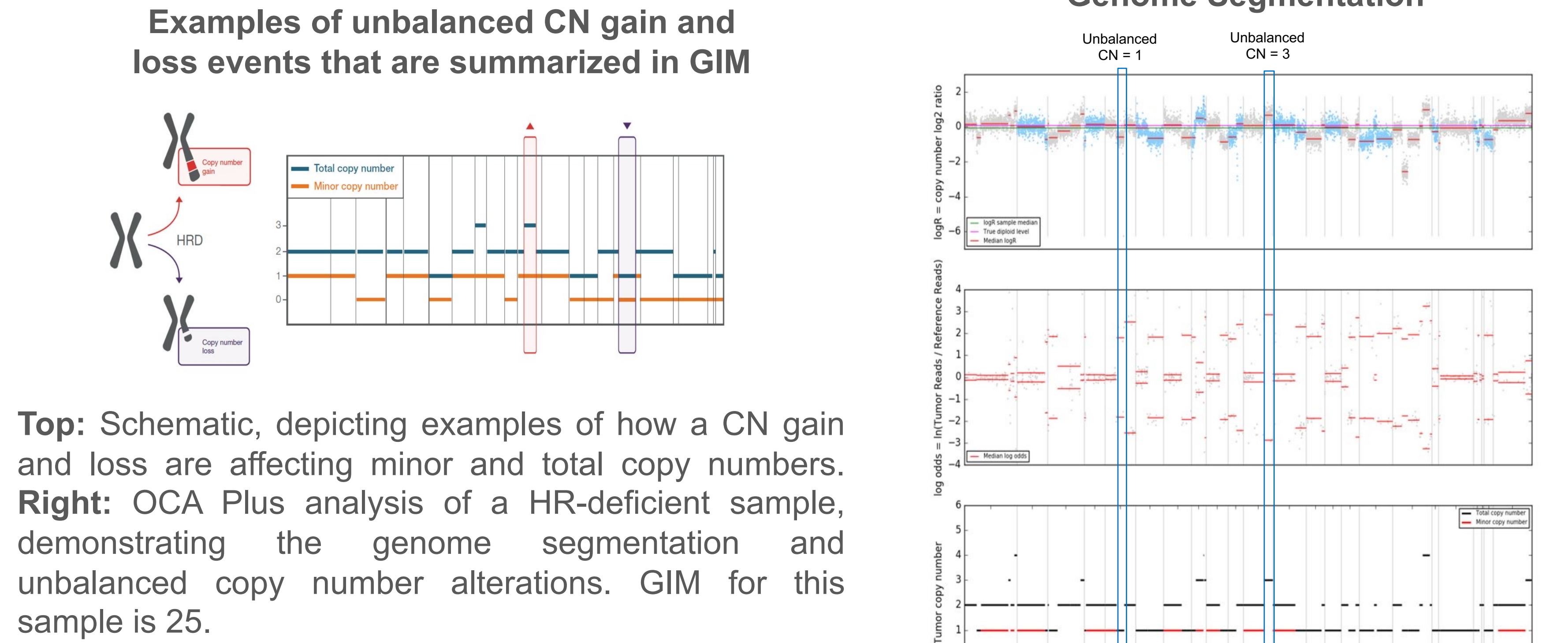
We developed a next generation sequencing based research assay, Oncomine™ Comprehensive Assay (OCA) Plus to allow comprehensive genomic profiling of solid tumors using Ion Ampliseq™ technology, automated templating on Ion Chef™ system and sequencing on the Ion GeneStudio™ S5 platform. The assay is on-market and interrogates 500+ genes relevant to precision oncology research including *BRCA1/2* and 45 other genes in Homologous Recombination Repair (HRR) pathway. In addition, many high minor allele frequency single nucleotide polymorphisms (SNPs) have been included that are evenly spread across the genome and prevalent in various ethnicities to allow genome segmentation¹ and characterize genomic instability which in turn enables HRD detection when combined with *BRCA1/2* mutations. The assay detects a broad range of genomic alterations including single nucleotide variants (SNVs), insertions and deletions (Indels), copy number variations (CNVs), and fusions as well as complex biomarkers or genomic signatures such as tumor mutational burden (TMB) and microsatellite instability (MSI).



OCA Plus on Ion Torrent GeneStudio™ allows HRD detection using CGP together with DNA variants and RNA fusions and complex biomarkers like TMB and MSI with low input in a tumor only workflow.

Genomic Instability Metric (GIM)

GIM is a novel metric to quantify genomic scars/instability associated with HRD. It is based on genome segmentation¹ 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.



OCAPlus Endpoint Results

Variant Type	Sensitivity	PPV
SNVs	99.6%	97.6%
Indels	99.5%	98.1%
CNV Gain	97.6%	99.9%
CNV Loss	86.7%	99.9%
Fusions	100%	97.5%

Table 1. SNVs, Indels, CNVs and Fusions analytical performance in controls and FFPE samples

The AcroMetrix™ Oncology Hotspot Control (AOHC) was used to evaluate SNVs/Indels analytical performance. Evaluation of CNV gain (CN >= 8) and CNV loss (homozygous loss) were performed by sequencing FFPE samples (N=82) of varying tumor types with Oncoscan™ Affymetrix array as the reference assay and tumor cellularity ranging from 40% to 100%. For RNA fusions, we sequenced FFPE samples (N=40) harboring ALK, ROS, NTRK1, RET, FGFR2 and FGFR3 fusions as well as MET exon-14 skip. High sensitivity and PPV were achieved for all these endpoints as shown in Table 1.

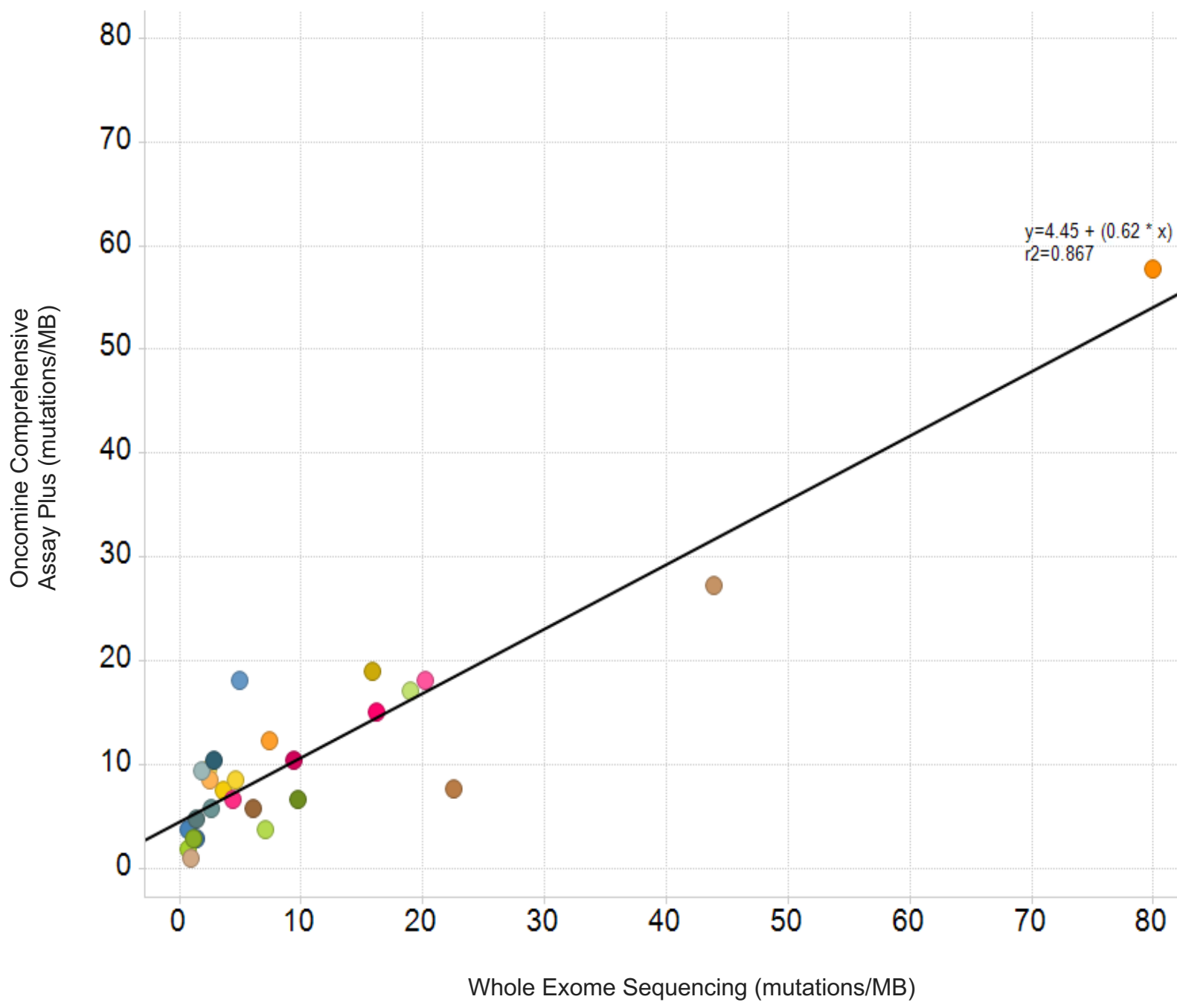
Complex Biomarkers: MSI and TMB

MSI performance was evaluated in a cohort of 119 FFPE colorectal samples. High sensitivity and PPV was observed for this tumor type.

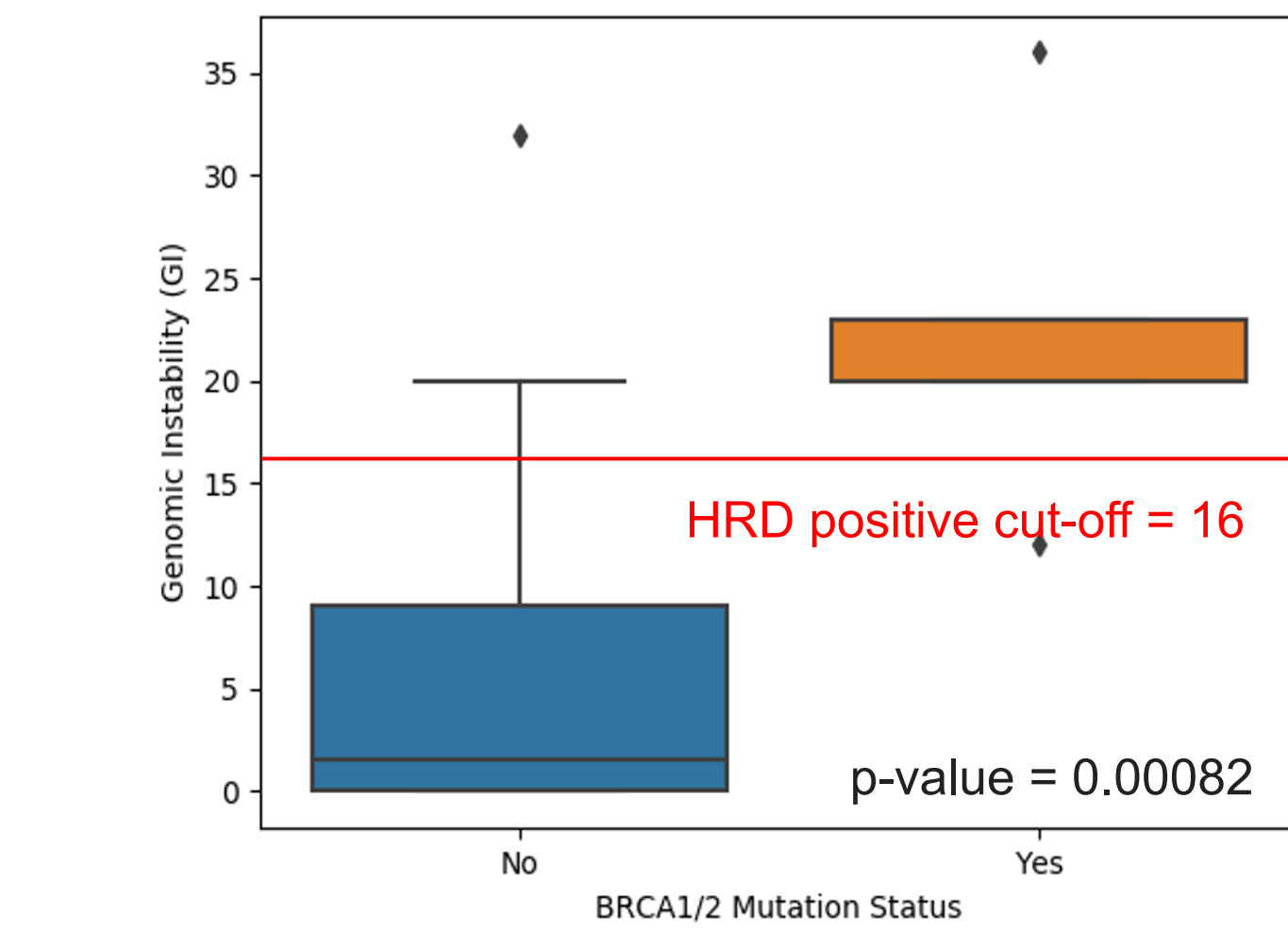
Sample Type	Sensitivity	PPV
FFPE	98%	100%

Table 2. MSI performance in colorectal FFPE samples

OCA Plus assay with a genomic footprint of 1mb correlated well to whole exome sequencing for TMB in a large cohort of FFPE samples of varying tumor types.



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 in a cohort of 46 ovarian FFPE samples

HRD calling using OCA Plus was evaluated in different ovarian tumor FFPE cohorts by our collaborators 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 three different HRD reference assays as shown in Table 3², Table 4³ and Table 5.

	OCA Plus <i>BRCA1/2</i> (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
		WT	1	60	GI -	8	28	HRD -	7
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 3. OCA Plus *BRCA1/2* variant calling, GIM and HRD concordance to Reference Assay 1

	OCA Plus <i>BRCA1/2</i> (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
		WT	0	47	GI -	7	20	HRD -	5
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 4. OCA Plus *BRCA1/2* variant calling, GIM and HRD concordance to Reference Assay 2

	OCA Plus <i>BRCA1/2</i> (N = 20)			OCA Plus GIM (N = 20)			OCA Plus HRD (N = 20)		
		Mut	WT		GIM +	GIM -		HRD +	HRD -
Reference Assay 3		Mut	2	0	GI +	5	3	HRD +	5
		WT	0	18	GI -	0	12	HRD -	0
Positive Percent Agreement (PPA)	100%			62.5%			62.5%		
Negative Percent Agreement (NPA)	100%			100%			100%		
Overall Percent Agreement (OPA)	100%			85%			85%		

Data courtesy of Dr. Erika Tóth, Department of Surgical and Molecular Pathology, National Tumor Biology Laboratory, National Institute of Oncology, Budapest and Dr. Erzsébet Csemák, Department of Surgical and Molecular Pathology, National Institute of Oncology, Budapest

Table 5. OCA Plus *BRCA1/2* variant calling, GIM and HRD concordance to Reference Assay 3

GIM and HRD PPA with Reference Assay 3 was lower compared to other two cohorts since most of the discordant samples were either at the genomic instability cutoff of the Reference assay 3 or the samples had very low tumor cellularity.

References

- Shen Ronglai et al., 2016, *Nucleic Acids Research*, Vol.44, No.16
- C. Roma et al., Poster abstract EACR23-1096, EACR Congress 2023.
- 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