Comprehensive profiling of copy number changes to assess genomic instability

Mohit Gupta, Ying Jin, Vinay Mittal, Dinesh Cyanam, Jennifer Kilzer, Geoffrey Lowman, Elaine Wong-ho, Santhoshi Bandla, Portia Bernardo, Sameh El-Difrawy, Jianjun Guo, Amir Marcovitz, Scott Myrand, Emily Norris, Coleen Nemes, Alex Phan, Loni Pickle, Tanaya Puranik, Michelle Toro, Yu-Ting Tseng, Paul Williams, Derek Wong, Yan Zhu, Eugene Ingerman, Seth Sadis Thermo Fisher Scientific, Ann Arbor, MI, USA; South San Francisco, CA; Carlsbad, CA

INTRODUCTION

- Defects in Homologous Recombination Repair (HRR) pathway interfere with the ability to repair DNA double strand breaks (DSBs), leading to Homologous Recombination Deficiency (HRD).
- One of the consequences of HRD is accumulation of copy number variations (CNVs) leading to genomic instability.
- To better understand the incidence and prevalence of HRD in cancer, and its association with HRR gene defects, we developed a novel metric to measure genome instability in FFPE tissue samples.

METHODS

- Oncomine Comprehensive Assay Plus (OCA Plus) is an on-market assay that interrogates 500+ genes relevant to precision oncology research including BRCA1/2 and 44 other genes in the HRR pathway.
- To evaluate genomic instability with OCAPlus, we measured CNV log-ratio profiles and determined log odds for thousands of single nucleotide polymorphisms (SNPs) with high minor allele frequencies.
- CNV profiles were aggregated into a summary metric to characterize genomic instability called Genomic Instability Metric (GIM).
- We also used an array-based assay Oncoscan to characterize genomic instability using genomic LOH (%LOH).
- OCAPlus testing was typically done on just 20 ng input DNA extracted from ovarian cancer FFPE sections using the Ion Torrent Gene Studio next generation sequencing instrument and Ion Reporter software.

Figure 1. OCA Plus endpoints

HRR and Genomic Instability

- BRCA1 and BRCA2 full gene sequencing
- Genomic Instability (GIM)
- 44 other HRR genes
- Gene level LOH
  - ATM, BARD1, BLM, BRCA1, BRCA2, BRIP1, CDK12, CHEK1, CHEK2, FANCL, NBN, PALB2, POLD1, POLE, PP2R2A, PTEN, RAD51B, RAD51C, RAD51D, RAD54L

Other OCA Plus endpoints

- Small Variants
- Copy Number Variation
- Gene Fusions
- Tumor Mutation Burden
- Microsatellite Instability

Unbalanced copy number change is a strong predictor of genomic instability

Genomic Instability Metric (GIM)

GIM is a quantitative measure based on genome segmentation[1] using CNV log2 ratios and log odds for SNP allele frequencies.

Figure 2. Example of unbalanced CN events characterized with OCA Plus

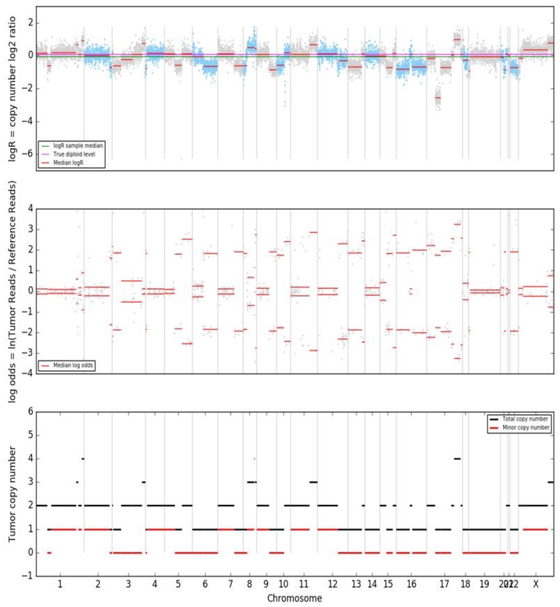
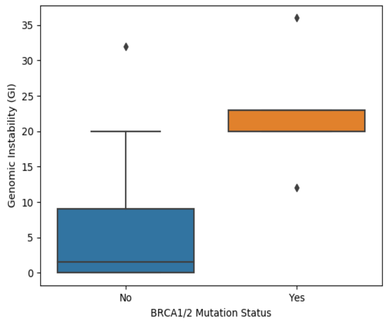


Figure 3. GIM in ovarian tumor FFPE cohort



N=46, p-val = 0.000082

Figure 4. Comparison of %LOH between OCA Plus and Oncoscan on a FFPE tumor cohort (N = 58)

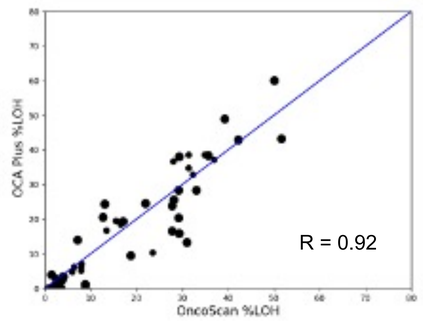


Table 1. GIM performance in breast cancer cell-lines

Cell-line	*BRCA1/2* status	HRD[5]	GIM
HCC38 Breast carcinoma	*BRCA1* promoter methylated[2], Loss of *BRCA1/2* expression[3]	Positive	Positive
HCC1806 Breast carcinoma	Loss of *BRCA2* expression[3]	Positive	Positive
HCC1428 Breast carcinoma	*BRCA2* allelic loss[4] Loss of *BRCA2* expression[3]	Positive	Positive
HCC1954 Breast carcinoma	Loss of *BRCA2* expression3	N/A	Positive
HCC1937 Breast carcinoma	BRCA1 5382insC[3]	N/A	Positive
NA12878	*BRCA 1/2* WT	N/A	Negative

CONCLUSIONS

- Genomic Instability Metric (GIM) was developed to enable the measurement of genomic instability by next generation sequencing
- GIM scores were significantly higher in BRCA1/2 mutant ovarian cancer samples and breast cancer cell lines relative to BRCA WT
- %LOH from OCA Plus was highly correlated with array-based assay
- GIM complements BRCA1/2 sequencing to provide an overall assessment of HRD on OCA Plus

REFERENCES

1. Shen Ronglai et al., "FACETS: allele-specific copy number and clonal heterogeneity analysis tool for high-throughput DNA sequencing", 2016, *Nucleic Acids Research*, Vol.44, No.16
2. Jinhua Xu et al., "CpG island methylation affects accessibility of the proximal BRCA1 promoter to transcription factors", 2010, Breast Cancer Res Treat, 10.1007/s10549-009-0422-1
3. Shari E Smith et al., "Molecular characterization of breast cancer cell lines through multiple omic approaches", 2017, Breast Cancer Res Treat, 10.1186/s13058-017-0855-0
4. Banu Arun et al., "The PARP inhibitor AZD2281 (Olaparib) induces autophagy/mitophagy in BRCA1 and BRCA2 mutant breast cancer cells", 2015, Int J Oncol., 10.3892/ijo.2015.3003
5. Dongju Chen et al., "GSA: an independent development algorithm for calling copy number and detecting homologous recombination deficiency (HRD) from target capture sequencing ", 2021, BMC Bioinformatics, 10.1186/s12859-021-04487-9

ThermoFisher SCIENTIFIC