#I014: Analytical Validation of a Homologous Recombination Deficiency Signature (HRDsig) in Pan-Tumor Tissue Samples

Jeffrey A. Leibowitz[*1], *Wenshu Li*[*1], *Shuoguo Wang*[1], *Bahar Yilmazel*[1], *Louisa Walker*[1], *Chang Xu*[1], *Ethan S. Sokol*[1], *Alexa B. Schrock*[1], *Jason Hughes*[1], *Nimesh R. Patel*[1], *Julia Elvin*[1], *Lauren Ritterhouse*[1], *Brennan Decker*[1], *Lucas Dennis*[1]

**These authors contributed equally*

1: Foundation Medicine, Inc., Boston, MA.

Introduction

Homologous recombination repair (HRR) is a cellular pathway for high-fidelity double strand DNA break repair that uses the sister chromatid as a guide to ensure chromosomal integrity and cell viability. Deficiency in the HRR pathway (HRD) can sensitize tumors to poly (ADP-ribose) polymerase inhibitors and platinum-based chemotherapy, offering an avenue to select patients who may benefit from relevant therapies

HRD signature (HRDsig) is a pan-solid tumor biomarker on the FoundationOne®CDx assay. HRDsig does not rely on HRR gene alterations and instead employs a DNA scar-based approach to calculate a score based on copy number features, thus enabling detection of both genomic and non-genomic mechanisms of HRD.

We examine the analytical performance of FoundationOne®CDx assay for detecting HRDsig. The results demonstrate high analytical concordance to an independent HRD biomarker (reversion of biallelic loss of function in an HRR gene), a low false positive rate, high reproducibility, and robustness to interfering substances of the FoundationOne®CDx HRDsig calling methodology.

Methods

Table 1. Study Designs

Study Type	Sample Number	Study Description
Limit of Blank (LoB)	5	• 12 replicates per sample
Limit of Detection (LoD)	3	• Biomarker-positive samples were diluted with matched normal DNA through a series of titration levels • 96 total replicates per sample • Represented Disease Ontologies: Breast

Precision	22	• 11 biomarker-positive samples; 11 biomarker-negative samples
		• 36 replicates per sample
		• Represented Disease Ontologies:
		  • Biomarker-positive: Ovary, Breast, Prostate
		  • Biomarker-negative: Ovary, Breast, Prostate, Lung, Skin, Colon

Interfering Substances	17	• Interfering Substances Assessed: unconjugated and conjugated bilirubin, hemoglobin, triglycerides, xylene, ethanol, proteinase K, MIB, melanin, necrosis. • 5 biomarker-positive samples, 6 biomarker-negative samples, 6 samples with undetermined biomarker status • Represented Disease Ontologies: Ovary, Lung, Breast, Colon, Liver, Prostate, Skin
Concordance	231	• True positives were defined as those with a reversion of loss of function in an HRR gene (101) • True negatives were defined as those that lacked an alteration in any of 14 HRR pathway genes (130) • Represented Disease Ontologies: Breast, Ovary, Prostate, Pancreas, Other

High Concordance to Independent HRD biomarker

Table 2. HRDsig Concordance Study Results

	LOF-REV*	HRR Negative**	Total
HRDsig Positive	90	7	97
HRDsig Negative	10	119	129
HRDsig Unknown	1	2	3
Total	101	128	229
	PPA = 90.00% (90/100)#	NPA = 94.44% (119/126)#	

*Reversion of biallelic loss of function in an HRR gene (e.g., a frameshift mutation restoring the open reading frame of a primary frameshift mutation) was used to define positive truth status. Samples with reversion alterations in *BARD1, BRCA1, BRCA2, PALB2, RAD51B, RAD51C,* and *RAD51D* were included in the analysis.

**Lack of detection of any alteration in any HRR pathway gene was used to define negative truth status

#HRDsig Unknown samples were excluded from concordance analysis

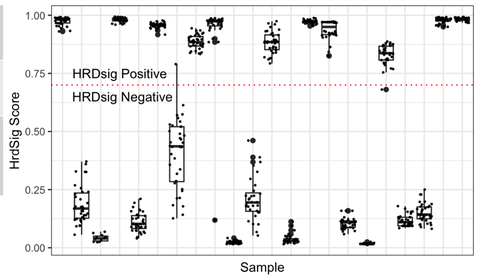
Excellent Precision of HRDsig Calling

Figure 1. HRDsig Precision Study Results

A

Overall Positive Reproducibility	99.49% (389/391) [98.15%, 99.86%]
Overall Negative Reproducibility	99.73% (363/364) [98.46%, 99.95%]
Repeatability Across All Samples	99.19% (368/371) [97.65%, 99.72%]

B



(a) Inter-run reproducibility and intra-run repeatability were evaluated for each sample and all samples combined. (b) The boxplot of HRDsig score of each study sample. The HRDsig scores were largely consistent across 36 replicates for each sample.

High Analytical Sensitivity

Figure 2. HRDsig Analytical Sensitivity Study

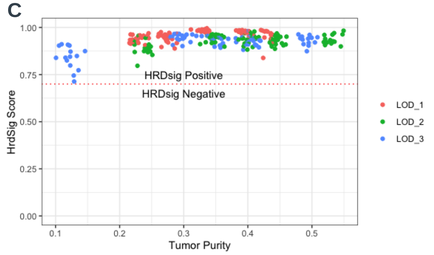
A

Sample	False Positive Rate
LoB_1	0.00% (0/12)
LoB_2	0.00% (0/12)
LoB_3	0.00% (0/12)
LoB_4	0.00% (0/12)
LoB_5	0.00% (0/12)
Overall	0.00% (0/60)

B

Sample	Dilution Level	Hit Rate (%)	Tumor purity
LoD_1	0.2	100.00 (20/20)	23.04%
LoD_1	0.25	100.00 (19/19)	27.41%
LoD_1	0.3	100.00 (20/20)	33.05%
LoD_1	0.35	100.00 (20/20)	39.32%
LoD_1	0.4	100.00 (13/13)	43.09%
LoD_2	0.2	100.00 (18/18)	24.51%
LoD_2	0.3	100.00 (19/19)	34.67%
LoD_2	0.35	100.00 (20/20)	40.14%
LoD_2	0.4	100.00 (20/20)	44.50%
LoD_2	0.5	100.00 (14/14)	53.13%
LoD_3	0.2	100.00 (15/15)	12.21%
LoD_3	0.3	100.00 (18/18)	29.60%
LoD_3	0.35	100.00 (17/17)	35.52%
LoD_3	0.4	100.00 (20/20)	40.61%
LoD_3	0.5	100.00 (14/14)	49.28%

C



(a) LoB was confirmed by a false positive rate < 5%. (b) LoD was determined as the lowest tumor purity at which a ≥ 95% hit rate was achieved. (c) The distribution of HRDsig scores of LoD study samples

Limited Impact of Interfering Substances

Table 3. HRDsig Interfering Substances Study Results

Sample	Baseline HRDsig Status	Interfering Substances	Percent Agreement
IF_1	Negative	Conjugated Bilirubin, DMSO Control, Hemoglobin, Triglycerides, Unconjugated Bilirubin	100.00% (12/12)
IF_2	Negative	Conjugated Bilirubin, DMSO Control, Hemoglobin, Triglycerides, Unconjugated Bilirubin	100.00% (12/12)
IF_3	Negative	Conjugated Bilirubin, DMSO Control, Hemoglobin, Triglycerides, Unconjugated Bilirubin	100.00% (12/12)
IF_4	Positive	Molecular Index Barcodes, Proteinase K	100.00% (10/10)
IF_5	Positive	Melanin, Molecular Index Barcodes, Proteinase K	100.00% (12/12)
IF_6	Positive	Unconjugated Bilirubin	100.00% (5/5)
IF_7	Positive	Molecular Index Barcodes, Proteinase K	100.00% (10/10)
IF_8	Positive	Triglycerides, Xylene	100.00% (7/7)
IF_9	Undetermined	Necrotic 5%	100.00% (2/2)
IF_10	Undetermined	Necrotic 10%	100.00% (2/2)
IF_11	Undetermined	Necrotic 15%	100.00% (2/2)
IF_12	Undetermined	Necrotic 25%	100.00% (2/2)
IF_13	Undetermined	Necrotic 40%	100.00% (2/2)
IF_14	Undetermined	Necrotic 50%	50.00% (1/2)
IF_15	Negative	Melanin, Proteinase K	100.00% (8/8)
IF_16	Negative	Melanin, Molecular Index Barcodes, Proteinase K	100.00% (12/12)
IF_17	Negative	Melanin, Molecular Index Barcodes, Proteinase K	100.00% (12/12)

Single discordance at the highest necrosis level (50%):

- 50% necrotic content potentially had an impact on HRDsig status calling
- Basline HRDsig status in necrotic sample was not determined
- The HRDsig score in the two replicates was close to the HRDsig positivity cut-off of 0.7, with the negative replicate presenting with a score of 0.6774

Conclusion

The analytical validation results demonstrate high analytical concordance compared to an independent HRD biomarker, a low false positive rate, high reproducibility, and robustness to interfering substances of HRDsig calling.

Author contact: