The Validation of a Homologous Recombination Deficiency Assay into Clinical Practice within the NHS

FPN: 3P

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1. Introduction

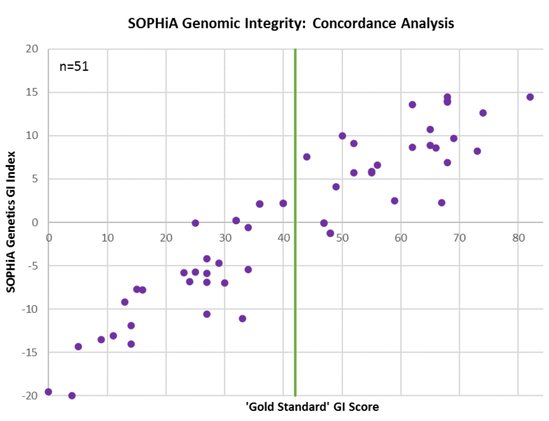
- Homologous Recombination Deficiency (HRD) testing is available for all NHS patients with newly diagnosed, advanced high-grade epithelial ovarian cancer to determine eligibility for PARP inhibitors as an option for maintenance treatment.
- **HRD status is determined by combining BRCA1/2 mutation status and a genomic instability score (GIS).**
- Patients with HRD-positive tumours show an increased sensitivity to PARP inhibitors leading to significant improvements in progression-free survival.
- HRD referrals were previously sent to Myriad (US) for testing, but as of April 2024 testing was taken over by each NHS Genomic Laboratory Hub (GLH).

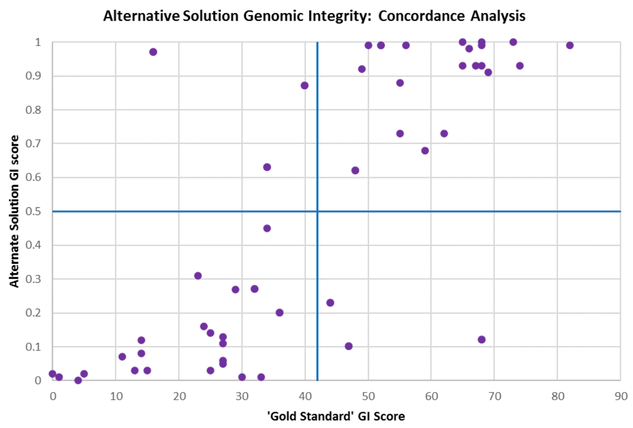
2. Methods

- The Royal Marsden, as part of the NT-GLH, carried out a product evaluation to investigate the most suitable replacement for this service.
- **Four assays** were chosen for the initial evaluation comparing 23 FFPE samples: **2 new wet-lab solutions and 2 bioinformatic solutions** utilising the Marsden's routine NGS service for the wet-lab work.
- The two bioinformatic solutions were chosen for further investigation; this being carried out on a larger dataset of 59 samples. The bioinformatics solutions showed comparable concordance to the wet-lab solutions and could run alongside the Royal Marsden's current in-house DNA NGS panel (RMH200, Roche) negating the need to set-up an additional wet-lab NGS service.

3. SOPHiA GENETICS' GIInger™ Validation Results

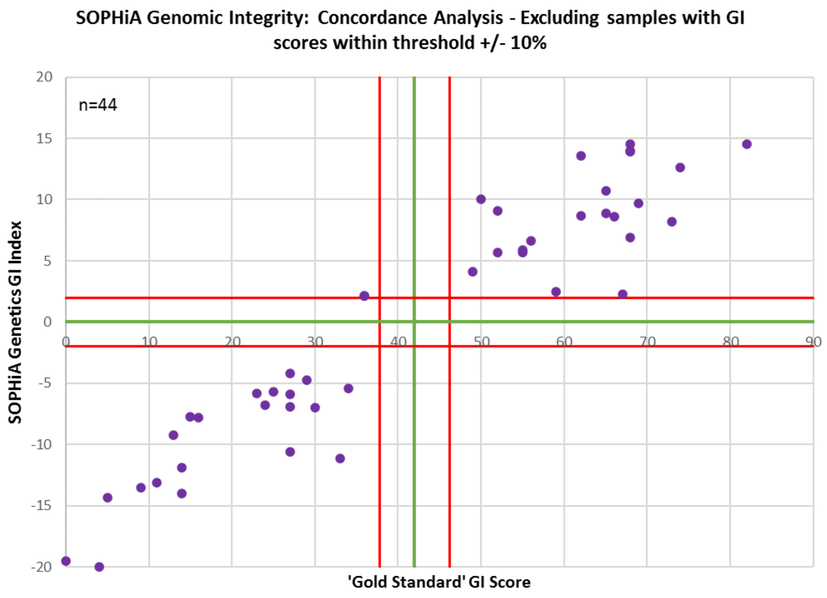
- For routine diagnostic use, the NT-GLH selected the **SOPHiA DDM™ GIInger Genomic Integrity Solution** (or **GIInger™**) pipeline, which utilises low amplification WGS in conjunction with a deep learning algorithm to produce a Genomic Integrity Index (GII).
- **The GII score generated is combined with tBRCA status determined using Royal Marsden's in-house somatic DNA NGS panel to produce a complete HRD status.**
- This pipeline was chosen as when both bioinformatic solutions were compared against the gold standard, the genome instability scores from GIInger™ showed greater concordance.





GI Scoring systems:
Myriad ≥42 is GI positive, SOPHiA DDM™ ≥0 is GI positive, Alternative Bioinformatics Solution ≥0.5 is GI positive

- **The full validation of GIInger™ showed 88% overall percentage agreement (OPA) to previously reported samples (Myriad, AZ), increasing to 97.7% when samples +/- 10% of positivity threshold were excluded.**



- The pipeline reproducibility and repeatability exhibited 100% concordance.

Reproducibility	Run 1 GI Index	Run 1 GI Status	Run 2 GI Index	Run 2 GI Status	Concordant Yes/No
23SP-087M0054	-11.1	Negative	-10.7	Negative	Yes
23SP-095M0048	6.9	Positive	7.7	Positive	Yes
23SP-087M0056	8.9	Positive	9	Positive	Yes
23SP-097M0001	4.1	Positive	4.5	Positive	Yes
23SP-108M0054	2.1	Positive	2.8	Positive	Yes
23SP-124M0046	10.7	Positive	11.1	Positive	Yes

Repeatability	Repeat 1 GI Index	Repeat 1 GI Status	Repeat 2 GI Index	Repeat 2 GI Status	Concordant Yes/No
23SP-087M0054	-10.7	Negative	-10.6	Negative	Yes
23SP-097M0001	4.5	Positive	4.2	Positive	Yes

4. Comparing GIInger™ with SOPHiA DDM™ Dx HRD

- **SOPHiA DDM™ Dx HRD Solution** is a CE-marked HRD solution which utilises SOPHiA GENETICS' preferred library preparation and capture protocol for the wet-lab work.
- The Royal Marsden wanted to ensure that the **GIInger™** bioinformatics pipeline ran using libraries prepared with RMH protocols gave comparable results to SOPHiA DDM™ Dx HRD Solution.
- 23 samples were run through both solutions to ensure concordance: 100% comparability was seen.

	GIInger™		SOPHiA DDM™ Dx	
22/00170	6.6	Positive	6.8	Positive
22/00352	-9.2	Negative	-9.8	Negative
22/00673	13.1	Positive	11.8	Positive
22/00678	8.7	Positive	8.7	Positive
22/00770	-7.0	Negative	-7.6	Negative
22/00773	10.0	Positive	10.4	Positive
22/00866	-14.8	Negative	-15.2	Negative
22/01018	8.2	Positive	7.1	Positive
22/01019	14.5	Positive	15.2	Positive
22/01020	-14.3	Negative	-14.7	Negative
22/01022	2.3	Positive	2.3	Positive
22/01024	-13.1	Negative	-13.6	Negative
22/01028	0.2	Positive	1.0	Positive
22/01185	-11.9	Negative	-11.6	Negative
22/01187	-7.7	Negative	-8.0	Negative
22/01188	-4.2	Negative	-3.5	Negative
22/01355	5.7	Positive	6.2	Positive
22/01607	2.5	Positive	2.7	Positive
22/01791	13.6	Positive	13.9	Positive
22/01796	5.9	Positive	6.4	Positive
22/02013	14.0	Positive	15.5	Positive

* 22/01028 was classed as negative by Myriad, but is called positive by both SOPHiA DDM™ Dx HRD Solution and GIInger™

5. Integration of SOPHiA DDM™ GI status with tBRCA Generated from In-house Analysis Pipeline

Example SOPHiA DDM™ GI plot and run report

// Genomic Integrity

Genomic Integrity status (for ovarian cancer)
Positive

Genomic Integrity Index
8.2

Genomic Integrity QA Status
High

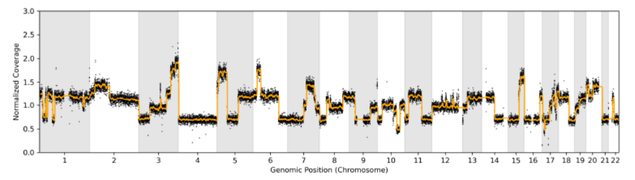


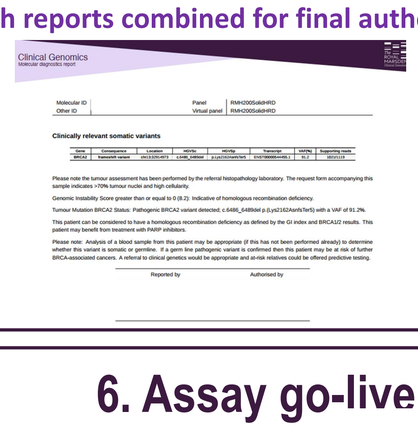
Figure 1: Normalized WGS coverage profile (black) and smoothed normalized WGS coverage profile (orange). White and gray shading denotes chromosome boundaries.

Example RMH200 Variant Report

PCGR annotation report

VARIANT_REPORT	COMMENT	GENE_NAME	POSITION	REFERENCEALLELE	ALTALLELE	PROTEIN	HGVS	MUTATIONEFFECT	TRANSCRIPTS	TUMOR.DEPTH	TUMOR.ALTDEPTH	TUMOR.ALTFREQ
PASS	Pathogenic Variant,Pathogenic Variant,PVS1 VSTR,PM2 Mod	BRCA2	chr13:32914973	GACAA	G	p.(Lys2162AsnfsTer5)	c.6486_6489del	frameshift_variant	ENST00000544455.1	1119	1021	0.91242

Both reports combined for final authorisation


Clinical Genomics
Molecular diagnostics report

Molecular ID
Other ID
Panel RMH200SolidHRD
Virtual panel RMH200SolidHRD

Clinically relevant somatic variants

Gene	Consequence	Location	HGVSc	HGVSp	Transcript	VAF[%]	Supporting reads
BRCA2	frameshift variant	chr13:32914973	c.6486_6489del	p.Lys2162AsnfsTer5	ENST00000544455.1	91.2	1021/1119

Please note the tumour assessment has been performed by the referral histopathology laboratory. The request form accompanying this sample indicates >70% tumour nuclei and high cellularity.

Genomic Instability Score greater than or equal to 0 (8.2): Indicative of homologous recombination deficiency.

Tumour Mutation BRCA2 Status: Pathogenic BRCA2 variant detected; c.6486_6489del p.(Lys2162AsnfsTer5) with a VAF of 91.2%.

This patient can be considered to have a homologous recombination deficiency as defined by the GI index and BRCA1/2 results. This patient may benefit from treatment with PARP inhibitors.

Please note: Analysis of a blood sample from this patient may be appropriate (if this has not been performed already) to determine whether this variant is somatic or germline. If a germ line pathogenic variant is confirmed then this patient may be at risk of further BRCA-associated cancers. A referral to clinical genetics would be appropriate and at-risk relatives could be offered predictive testing.

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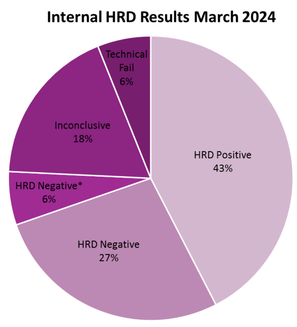
6. Assay go-live

- **In-house HRD testing was implemented at the Royal Marsden in December 2023, with 106 samples tested internally by 1st April 2024.**
- In March 2024, 33 samples were tested with an average turnaround time of 16.76 days.



7. Conclusion

The SOPHiA GENETICS' GIInger™ bioinformatics Pipeline for GI status, alongside our in-house RMH200 panel for tBRCA status provides a suitable HRD solution for testing patients with newly diagnosed, advanced high-grade epithelial ovarian cancer to determine PARP inhibitor eligibility.

Disclosure Statement: In relation to this poster presentation and the associated work, the author declares that there are no conflicts of interest

8. References

1) Miller RE et al., 2020. ESMO recommendations on predictive biomarker testing for homologous recombination deficiency and PARP inhibitor benefit in ovarian cancer. Ann Oncol. 2020 Dec;31(12):1606-1622
2) Pozzorini et al.,2023. GIInger predicts homologous recombination deficiency and patient response to PARPi treatment from shallow genomic profiles,Cell Reports Medicine,Volume 4, Issue 12,2023,101344,ISSN 2666-3791

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