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ORIGINAL RESEARCH ARTICLE

Evaluation of the Oncomine Comprehensive Assay Plus NGS Panel and the OncoScan CNV Assay for Homologous Recombination Deficiency Detection

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Abstract

Introduction Testing for homologous recombination deficiency (HRD) as a biomarker in relation to poly (ADP-ribose) polymerase inhibitor (PARPi) treatment in ovarian cancer is done by sequencing of the *BRCA1/2* genes and/or by assessing a genomic instability signature. Here we present data obtained with two different methods for genomic instability testing: the Oncomine™ Comprehensive Assay Plus (OCA Plus) NGS panel and the OncoScan CNV assay.

Methods The retrospective analytical study included 80 ovarian cancer samples of patients previously referred to clinical Myriad testing (reference cohort), and 50 ovarian cancer samples from patients collected as part of the Pelvic Mass study. OCA Plus NGS libraries were sequenced with the Ion S5™XL Sequencer and analyzed with the Ion Reporter™ Software v5.20 for calculation of the genomic instability metric (GIM). In addition, all samples were tested with the OncoScan CNV FFPE Assay and analyzed with a previously published *R*-algorithm for generation of an in-house genomic instability score (in-house GIS).

Results The OCA Plus assay had a concordance to the reference of 89% on samples with a tumor fraction ≥ 30% (auto-calculated or via molecular estimation). A total of 15 samples in the reference cohort had a calculated tumor fraction < 30% in the OCA Plus assay. In these, the concordance to reference was only 60%.

For the OncoScan CNV in-house GIS a local cutoff point of ≥ 50 was calculated. This gave a concordance to the reference of 85%, with 91% of the samples in the reference cohort passing quality control (QC) on tumor fraction.

Both assays had a high sensitivity for the detection of genomic instability in samples with pathogenic or likely pathogenic *BRCA1/2* mutations, with 12/13 being GIM positive (OCA Plus assay) and 13/13 being in-house GIS positive (OncoScan CNV assay).

Conclusions The OCA Plus assay and the OncoScan CNV assay show a high but not complete concordance to reference standard homologous recombination deficiency (HRD) detection. The main reason for QC failure or non-concordance in our study was a low tumor fraction estimated in the assay, despite the selection of material by a pathologist with an inclusion criterion of > 30% tumor. QC steps should include careful tumor content evaluation, and results on samples with < 30% tumor should not be reported.

1 Introduction

Ovarian cancer composes a diverse group of neoplasms characterized by distinct clinicopathological and different molecular characteristics. Overall, they are divided due to anatomical origin in epithelial tumors, representing > 90%, or non-epithelial tumors. Epithelial ovarian cancers can be subclassified into high-grade serous carcinomas (70%, HGSC), endometrioid carcinomas (10%), clear-cell carcinomas (10%), mucinous carcinomas (3%), and low-grade serous carcinomas (< 5%) [1, 2]. HGSOC typically presents in advanced stages (FIGO III–IV). Primary treatment is surgery aiming for complete resection of all visible tumor tissue; alternatively, if that is not possible, then neoadjuvant chemotherapy followed by surgery may be the choice. In Denmark all HGSOC due to nationwide guidelines are offered *BRCA1/2* mutational test for genetic counseling and for possible biological treatment. At the molecular level, TP53 mutations

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Key Points

Homologous recombination deficiency (HRD) is a predictive biomarker for response to treatment with poly (ADP-ribose) polymerase inhibitor (PARPi) in platinum sensitive ovarian cancer. Testing for HRD can be done with different techniques that need to be aligned with Myriad myChoice HRD assessment to ensure correct patient stratification.

We evaluated the Oncomine™ Comprehensive Assay Plus (OCA Plus) NGS panel and the OncoScan CNV Assay and found high concordance to the Myriad myChoice assay with both techniques on samples where the analysis pipelines found more than 30% tumor.

It is an important QC step to evaluate the tumor content estimate by the analysis pipelines. For the OCA Plus assay, HRD assessment is not recommended on samples with a tumor fraction below 30%.

are prevalent (96%) in HGSOCs [3]. Many patients with ovarian cancer exhibit homologous recombination repair (HRR) pathway deficiency (HRD) [4], which together with *BRCA1*/2 mutations, serve as a biomarker for poly (ADP-ribose) polymerase inhibitor (PARPi) treatment in in platinum-sensitive ovarian cancer [5–11]. The principle behind PARPi therapy in HRD-positive tumors is synthetic lethality, where simultaneous disruption of two different DNA repair pathways, the homologous recombination repair pathway (double-strand breaks) and the base excision repair (BER) pathway (single-strand breaks), will be lethal for the tumor cells. The mechanism is likely dual: inhibition of PARP catalytical activity will leave DNA single-strand breaks unrepaired, and PARPi/PARP complexes can be trapped at damaged DNA. Both events will lead to double-strand breaks in the DNA, which cannot be sufficiently repaired in the tumor cells due to the underlying homologous recombination deficiency [12, 13].

HRD biomarker-guided PARPi treatment was approved on the basis of centralized testing with the Myriad MyChoice test (Myriad, USA) which uses *BRCA1/2* mutation positivity and/or a genomic instability score (GIS) ≥ 42 to discriminate positive and negative HRD results.

Testing for HRD has in research settings been done with several different technologies including single nucleotide polymorphism (SNP) arrays [14], next-generation sequencing (NGS) panels [15], and exome and whole-genome sequencing [16, 17]. Along with the different technologies, multiple bioinformatic pipelines have been developed for the analysis and subsequent evaluation of genomic instability signatures that are characteristic for HRD-positive tumors [14, 18–20]. Several groups have used combination scores that comprise the levels of loss of heterozygosity (LOH), telomeric allelic imbalances (TAI), and large-scale transitions (LST) in cancer cells [14, 15, 19, 20]. LOH can be quantified in different ways. It can be measured as the number of LOH areas above the size of 15 mega bases (Mb) [14] or as the fraction (%) of the tested genome that exhibits LOH [21]. TAI has been defined as the number of sub-telomeric regions with allelic imbalance that start beyond the centromere and extend to the telomere [14] and are only included if they encompass a certain minimum number of SNPs [22] or are longer than 11 MB in size [23]. LST was initially defined as the number of chromosomal breaks between adjacent regions of at least 10 Mb after filtering out areas < 3 Mb and later modified by adjusting it by ploidy [14, 19, 23, 24].

HRD evaluation with the OncoScan CNV array has recently been described by several groups [25–28]. This assay lacks an HRD analysis pipeline provided by the manufacturer, and users therefore rely on previously published or in-house developed analytical pipelines.

Recently, Thermo Fisher Scientific introduced the genomic instability metric (GIM), a measurement that quantifies genomic scarring associated with HRD, available for data from the Oncomine Comprehensive Assay Plus NGS panel (OCA Plus) with a recent update of the Ion Reporter™ Software to version 5.20. It is therefore possible with this assay to assess HRD in parallel mutation analysis with an analysis pipeline available from the kit manufacturer. It is, however, still important to validate the assay locally on clinical samples. The German HRD assay Harmonization Consortium recently published a concordance study on a range of HRD assays, but the study did not include the OCA Plus assay [29].

Here, we evaluate the HRD assessment obtained with the OCA Plus assay on 130 ovarian cancer patient samples with orthogonal HRD results obtained by Myriad myChoice ($n = 80$) and a local OncoScan CNV assay ($n = 130$).

2 Material and Methods

The study included 80 samples from patients with ovarian cancer previously evaluated by Myriad for diagnostic HRD assessment. Before referral, these patients all tested negative for *BRCA1/2* mutation as part of the local routine evaluation. The sample material was either from FFPE samples (archived DNA, $n = 23$; excess slides returned from Myriad, $n = 36$; or new extraction of DNA from the same FFPE blocks, $n = 19$) or fresh frozen tumor ($n = 2$). Material selection was performed by a pathologist, who estimated the tumor content to be $> 30\%$ in the selected material.

DNA was extracted using the Maxwell RSC DNA FFPE kit and automated extraction as instructed by the manufacturer (Promega).

In addition, data from a cohort of 50 ovarian cancer patient samples previously described [30] were included in the study (The Pelvic Mass cohort). The cohort consisted of 39 high-grade serous adenocarcinoma, 4 clear cell adenocarcinoma, 4 mucinous adenocarcinoma, and 3 endometrioid adenocarcinoma.

2.1 Oncomine Comprehensive Assay Plus

Library preparation for the OCA Plus assay was performed manually according to manufacturer's instructions MAN0018490 (Revision D.0) and quantified using Ion Library TaqMan Quantitation kit (Thermo Fisher Scientific, Waltham, MA, USA). Manually prepared libraries that ended up with a Median Absolute Pairwise Difference (MAPD) > 0.5 were repeated with library preparation on the Ion Chef™System. All libraries were adjusted to 50 pM before template preparation and 550™chip loading using the Ion Chef™System according to manufacturer's instructions [Ion 550™–Chef, MAN0017275 (Revision C.0)]. Sequencing was performed using the Ion S5™XL Sequencer (Thermo Fisher Scientific, Waltham, MA, USA). Data were analyzed using the Ion Reporter™ Software version 5.20 (Thermo Fisher Scientific, Waltham, MA, USA) with the Oncomine Comprehensive Plus w3.1 DNA single sample workflow and data filtering with the Oncomine Extended (5.20) filter. The tumor fraction (tumor cellularity percentage) was either auto-calculated by the ion reporter software or estimated on the basis of the variant allele frequency (VAF) of detected variants, if the tumor fraction could not be auto-calculated by the software. For the molecular estimation, VAFs for TP53 mutations or other informative somatic variants should be > 30% (or compound heterozygous > 15%) to support a tumor fraction > 30%.

Samples from the Pelvic Mass cohort were sequenced with OCA Plus as part of a previous study [30]. The BAM files from this study were downloaded and reanalyzed with the Ion Reporter™ Software (v.5.20) for GIM determination and updated variant detection. The cutoff for HRD positivity was a GIM of ≥ 16.

2.2 OncoScan CNV Assay

The OncoScan CNV assay was set up according to the manufacture's user guide (User Guide OncoScan® CNV FFPE Assay Kit, P/N 703302 Rev. 1). Initial data analysis was done with chromosome analysis suite software version 4.3 (Applied biosystems, Thermo Fisher Scientific, Waltham, MA, USA) on the basis of hg19 and with probe signals normalized using the OncoScan.CNV.FFPE.NA33.r2.REF model for FFPE samples and OncoScan.CNV.REF103.NA33.r2.REF model for fresh frozen tissue samples. Quality control (QC) files and all probe results files were downloaded for further analysis in R.

Data analysis in R was performed using the algorithm described by Marquart et al. [14], kindly provided by Nicolai Birkbak. In addition, local adjustments were made to comply with local data folder structures. In short, the analysis used the ASCAT R package (version 3.0.0) to infer tumor purity, ploidy, and allele-specific copy number profiles [31]. It calculated LOH and LST according to Abkevich et al. [32] and Popova et al. [24], and TAI with cutoffs as defined by Birkbak et al. [22]. The combined genomic instability score was calculated as the unweighted sum of LOH + LST + TAI as defined by Telli et al. [15]. QC of the OncoScan CNV data included a MAPD ≤ 0.350, snpQC ≥ 10, and an ASCAT aberrant cell fraction < 1 for samples with pathologist estimated tumor fraction ≤ 50% (pathologist estimate only available for the Myriad cohort)

2.3 Statistical Analyses

Concordance between methods were calculated as percentage of concordant results as well as Kappa statistics. For the OncoScan CNV assay, the receiver operating characteristic (ROC) was calculated to determine a suitable cutoff point. To describe the precision of the analyses, sample mean, and standard deviation (SD) were calculated. Estimates are presented with 95% confidence intervals (CI).

3 Results

3.1 Concordance to Reference Standard

In 80 patients, the previously obtained Myriad testing results were used as the reference standard to evaluate the HRD testing results of the OCA Plus assay and the OncoScan CNV assay, respectively. The cohort included 23 HRD-positive patients and 57 HRD-negative patients with a wide range of Myriad GIS, including 24 samples within ± 10 score points of the Myriad GIS cutoff point at 42. With the OCA Plus assay, a GIM score was only calculated by the software on samples with a MAPD < 0.5. Manually prepared libraries that ended up with a MAPD > 0.5 were therefore repeated with library preparation on the Ion Chef™ System, which gave successful MAPD improvements in 96% (27/28 of samples). Therefore,

in total, GIM scores were available for 99% of samples (79/80). The tumor fraction in the analyzed material was > 30% in 64 samples (auto-calculated or molecular estimation based on mutation VAF). Among these, a concordance of 89% [95% CI 81–97%, Kappa: 72% (95% CI 53–92%)] was found between HRD assignment on the basis of OCA Plus GIM and HRD assignment on the basis of Myriad GIS; Table 1 and Fig. 1a. In 15 samples, the tumor fraction was < 30%. In these, the concordance in HRD assignment was only 60% (95% CI 35–85%, Kappa not done, test for equal marginal frequencies, $p = 0.01$, McNemar's test) and none of the Myriad HRD-positive samples were detected as positive with the OCA plus assay; Table 1 and Fig. 1b.

In the OncoScan CNV analysis, seven samples failed in QC because the ASCAT algorithm predicted a homogeneous sample (aberrant cell fraction = 1) while the tumor fraction estimated by the pathologist was ≤ 50%. These samples were excluded. The correlation between reference Myriad GIS and in-house GIS on the remaining 73 samples (91%) are presented in Fig. 1c. The concordance was 85% [95% CI 75–92%, Kappa: 67% (95% CI 49–84%)]. The QC on the ASCAT tumor fraction estimate was implemented because the tumor fraction may be lower than estimated by the pathologist and therefore not recognized by ASCAT. This is an important QC step as the GIS may otherwise be underestimated, as demonstrated in Fig. 1d.

To determine a cutoff point for HRD positivity with the OncoScan CNV analysis, receiver operating characteristic (ROC) was calculated, as shown in Fig. 2. If the calculated cutoff point of the in-house GIS at 49.75 (rounded to ≥ 50) was used, we found a diagnostic accuracy of 85%, with 12.3% false positive samples and 1.7% false negative samples. Among the samples with discordant results, 77% (7/9) of the samples were analyzed on excess slides returned from Myriad.

The OncoScan results of the 15 samples that had a tumor fraction estimate < 30% in the OCA Plus analysis is presented in Supplementary Table 1; 5 of the samples also failed in the Oncoscan QC with an ASCAT aberrant cell fraction of 1, and 7 samples passed with an ASCAT aberrant cell fraction > 30%. In three samples the ASCAT aberrant cell fraction estimate was 20–28%; the results of these samples were in concordance with the Myriad results (HRD high).

3.2 Concordance Between OCA Plus and OncoScan CNV

Concordance between OCA Plus and OncoScan CNV assay was evaluated in a total of 107 samples (Fig. 3). Samples were only included in the comparison if the tumor fraction calculation in the OCA Plus assay was ≥ 30% and the OncoScan CNV assay passed the QC ($n = 45$ from the Pelvic Mass cohort and $n = 62$ from the Myriad cohort; a graphical summary of samples that passed our QC settings with each assay is shown in Fig. 4).

HRD assignment based on OCA Plus GIM and HRD assignment based on OncoScan in-house GIS showed a concordance of 83% [95% CI 76–90%, Kappa: 64% (95% CI 49–79%)]. In Fig. 3, samples with pathogenic or likely pathogenic *BRCA1/2* variants were depicted in red and variants of unknown significance (VUS) were shown in blue. HRD-positive scores were detected with both assays in 92% (12/13) of samples with pathogenic or likely pathogenic *BRCA1/2* mutations. In 77% (10/13) of the samples, homozygosity for *BRCA1/2* mutation was evident based on tumor fraction and mutation VAF. In one sample the pathogenic *BRCA2* mutation was found with a VAF indicating heterozygosity. In the remaining two samples the tumor fractions and *BRCA1/2* VAFs were close to 50 and supplementary sequencing of *BRCA1/2* on corresponding blood samples revealed that for one of these samples the BRCA1 mutation was germline with either an unidentified second hit or lack of a second hit. This sample was negative with a GIM of 0 in the OCA Plus assay and positive with the OncoScan SNV assay with an in-house GIS of 59. Interestingly, the LOH detected in this sample with the OncoScan CNV assay was 0 and the high GIS score was primarily driven by a high LST value of 42. An OncoScan CNV LOH of 0 was not observed in any other HRD-positive samples in our cohort.

3.3 Repeatability

The repeatability of the OCA Plus assay was evaluated by inclusion of the same sample in 5 different runs with libraries prepared on the Ion Chef system (Fig. 5a). The inter-run mean GIM was 15.8 (SD 3.8).

The inter-run precision of the OncoScan CNV in-house GIS was calculated on a positive control (DNA from a local HRD positive FFPE sample) included once in each run for 6

Table 1. Concordance between OCA Plus and reference assay

Tumor fraction	Reference positive OCA Plus positive	Reference negative OCA Plus negative	Reference positive OCA Plus negative	Reference negative OCA Plus positive	Concordance
≥ 30%	14	43	3	4	89% (95% CI 81–97%)
< 30%	0	9	6	0	60% (95% CI 35–85%)

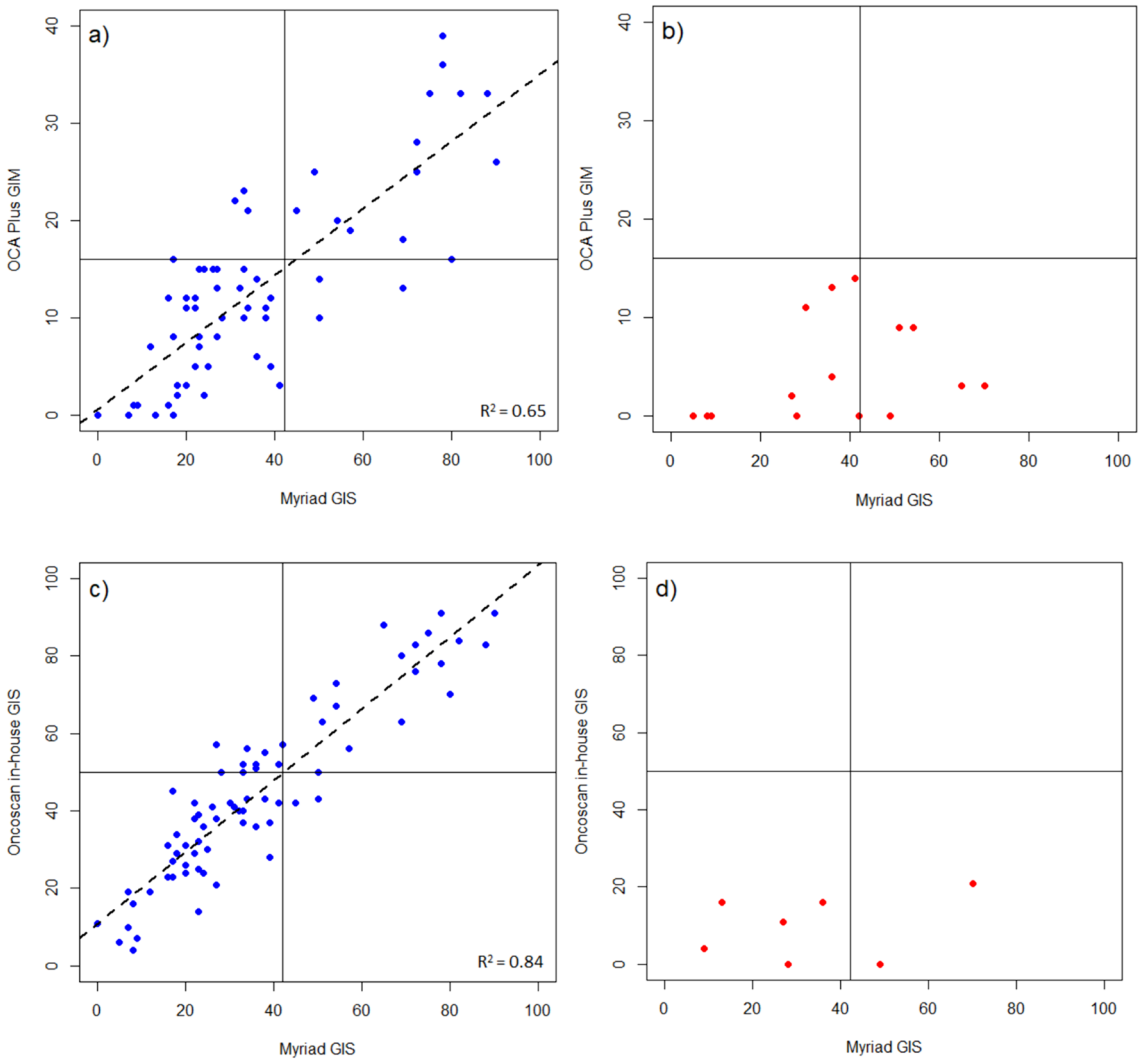

Fig. 1. Myriad GIS versus OCA plus GIM and OncoScan in-house GIS. Correlation between Myriad GIS and **a** OCA Plus assay GIM for samples with a tumor fraction ≥30% (auto-calculated or molecular estimate), **b** OCA Plus assay GIM for samples with a tumor fraction <30%, **c** OncoScan in-house GIS for samples with an acceptable QC on the ASCAT tumor fraction estimate, and **d** OncoScan in-house GIS for samples that failed in our ASCAT QC (where the pathologist estimated 30–50% tumor but ASCAT predicted a homogenous sample). The black lines show the cut points for positivity (Myriad GIS ≥42, OCA Plus GIM ≥16, in-house GIS ≥50)

months ($n=24$, Fig. 5b). The inter-run mean GIS was 81.4 (SD 3.3.)

3.4 DNA input requirement

The OCA Plus assay only requires 20 ng of DNA as input material, which is achievable for most clinical samples. In contrast, the recommended input for the OncoScan CNV assay is 80 ng (6.6 µL of DNA with a concentration of 12 ng/µL). This might be challenging to obtain from small FFPE biopsies, which additionally may be the only tissue available for analyses from patients administered neoadjuvant therapy. Therefore, four samples (samples 1, 4, 5, and 6) were tested in the OncoScan assay with input concentrations of 12 ng/µL, 6 ng/µL, 3 ng/µL, and 1 ng/µL, respectively, and two samples (samples 2 and 3) were tested with input concentrations of 12 ng/µL, 3 ng/µL, and 1 ng/µL, respectively. The results are shown in Fig. 6. Sample 1 mean GIS was 12.0, SD 0.0; sample 2 mean GIS was 29.3, SD 6.1; sample 3 mean GIS was 27.0, SD 3.6; sample 4 mean GIS was 57.8, SD 1.3; sample 5 mean GIS was 48.5, SD 1.8; and sample 6 mean GIS was 85.8, SD 1.1. The SD on GIS obtained with different DNA input concentrations were within or close to the inter-run precision SD except for one sample (sample 2) that showed more deviation for the low level 1 ng/µL dilution.

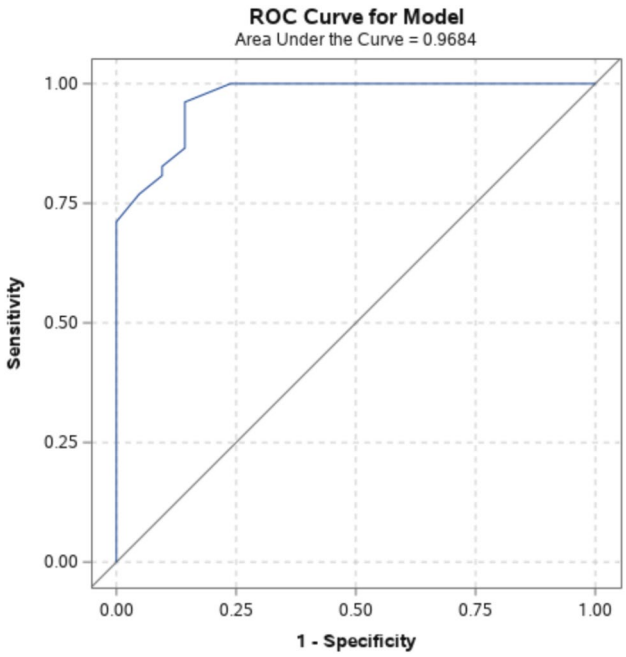

Fig. 2 ROC curves for OncoScan in-house GIS testing against myriad GIS. ROC curve used for cutoff point calculation. An OncoScan in-house GIS cutoff point of 49,75 gives a diagnostic accuracy of 85% with 12.3% false positive and 1.7% false negative; AUC = 0.968

4 Discussion

Here we present the HRD assessment with the OCA Plus NGS panel and the OncoScan CNV assay, respectively. Both assays showed a high concordance to the reference assay (Myriad) in samples with a tumor content ≥ 30%. With the OCA Plus assay, we found a concordance to the reference of 89% on the accepted samples (80% samples passed QC on MAPD and tumor fraction), while the concordance of the OncoScan CNV assay was 85% (91% samples passed QC). Recently, Kang et al. compared HRD results obtained with the OCA Plus assay and the Sophia DDM HRD solution and found a concordance of 0.911 on HRD status, which supports the reliability of the assay, however, the study did not include samples analyzed with an approved reference test [33]. Dumur et al. has presented a validation study of the OCA Plus assay including HRD testing using SeraCare HRD controls and 11 clinical samples previously tested at reference laboratories (3 HRD positive—all *BRCA1/2* mutation positive, and 8 HRD negative samples) and found 100% concordance to reference laboratory results [34]. With a larger number of samples and inclusion of *BRCA1/2* mutation negative, HRD positive samples in our study, it is not unexpected that we find a lower concordance, and the concordance reported here is in the same rate from 80% to 90%

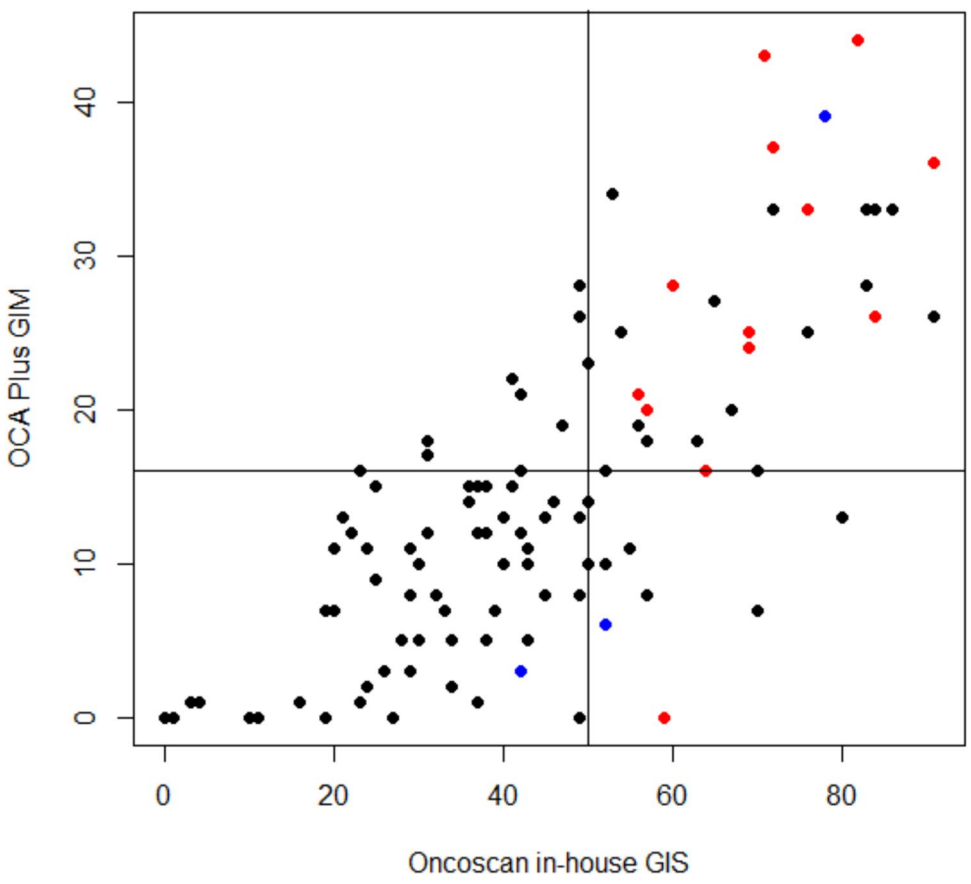

Fig. 3. OCA plus GIM versus OncoScan in-house GIS. Plot of OCA Plus GIM and OncoScan in-house GIS for the entire cohort ($n = 107$). The black lines show the cutoff points for positivity (in-house GIS ≥ 50, OCA Plus GIM ≥ 16). Samples with pathogenic or likely pathogenic *BRCA1/2* mutations are shown in red, while samples with variants of unknown significance in *BRCA1/2* are shown in blue

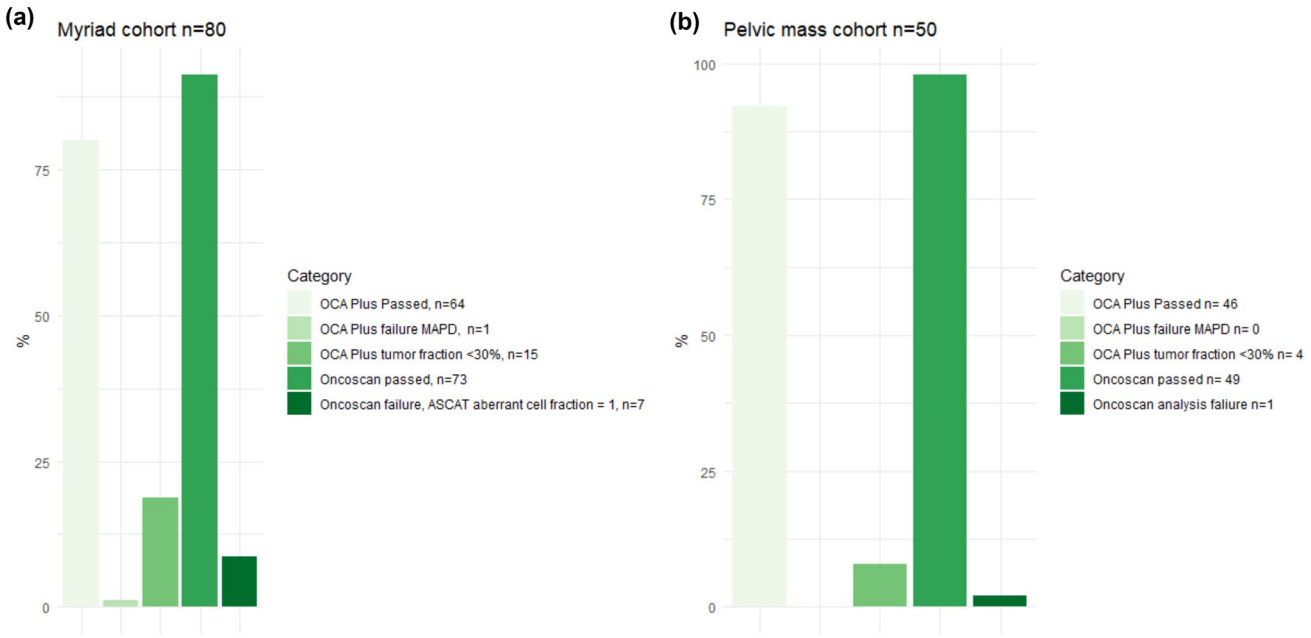

Fig. 4. Graphical summary of analyzed samples and failure rates. Analyzed samples that passed our QC criteria and failure rates are shown for the Myriad cohort (**a**) and the Pelvic Mass cohort (**b**). In the pelvic mass cohort, one sample failed in the Oncoscan analysis due to a run failure, with no sample material left to repeat the analysis

as reported for other assays, including the AmoyDx® HRD Focus Panel and the SOPHiA DDM HRD Solution [25, 35].

The main reason for QC failure or non-concordance samples in our study was a low tumor fraction estimated in the assay, despite the selection of material by a pathologist with an inclusion criterion of > 30% tumor. Assessment of tumor fraction by the pathologist can be challenging, and large interobserver variability is well known [36, 37]. Some of the samples that failed in QC on tumor fraction had been successfully analyzed at the reference laboratory. However, the tumor fraction may vary between different FFPE sections, and inclusion based on a hematoxylin and eosin (HE)-stained section with a proficient tumor fraction level does not guarantee a sufficient tumor fraction in subsequent sections. Our data emphasize the need for careful selection of tumor material for HRD detection, preferably with a tumor fraction well above the limit of 30%, as this could increase the number of samples that pass QC. For the OCA Plus assay, the QC criterion of a tumor fraction estimate > 30% was set by us because of the low concordance ratio on samples < 30%. This gave a high failure rate of the assay that could potentially be optimized by macrodissection of sample material to enrich for tumor cells. For the OncoScan CNV assay, the three samples with an ASCAT tumor fraction estimate at 20–28% had complete concordance to the previous Myriad result. However, the samples where ASCAT could not identify an aberrant cell fraction had low concordance. Even though some samples may pass with tumor fractions below 30%, it is essential for this assay to have a high tumor fraction to ensure a low failure rate. The tumor fraction estimates with the two assays differed for some samples (Supplementary Table 1). As it is an essential parameter for SNP copy number determination, this can impact the final HRD score.

The HRD score is a combinational genomic score relying on the results from large numbers of SNPs (that differs between different assays) and calculated estimates of tumor fraction to determine SNP zygosity and copy numbers (in our OncoScan pipeline done by ASCAT). Variations in this large dataset for each sample means that the score may not be the exactly the same with different analysis techniques or each time a sample is analyzed with the same method. The calculation of our local cutoff point for the OncoScan HRD analysis showed that a direct application of the Myriad cutoff point of 42 for positivity would result in a higher false positive rate. It is therefore important to calibrate local analyses to the reference method. It is, however, also important to note that even though the Myriad My Choice test HRD results are currently reported on the basis of the cutoff of 42, a decreased cutoff point of 33 has been reported to increase the sensitivity of detecting a response to PARP inhibitors [8].

One of the factors that may affect the calculated instability score is the number of SNPs included in the assay. This was recently demonstrated by Tsantikidi et al., who reported that SNP down-sampling of OncoScan data gave a higher correlation with the Myriad HRD test; the highest

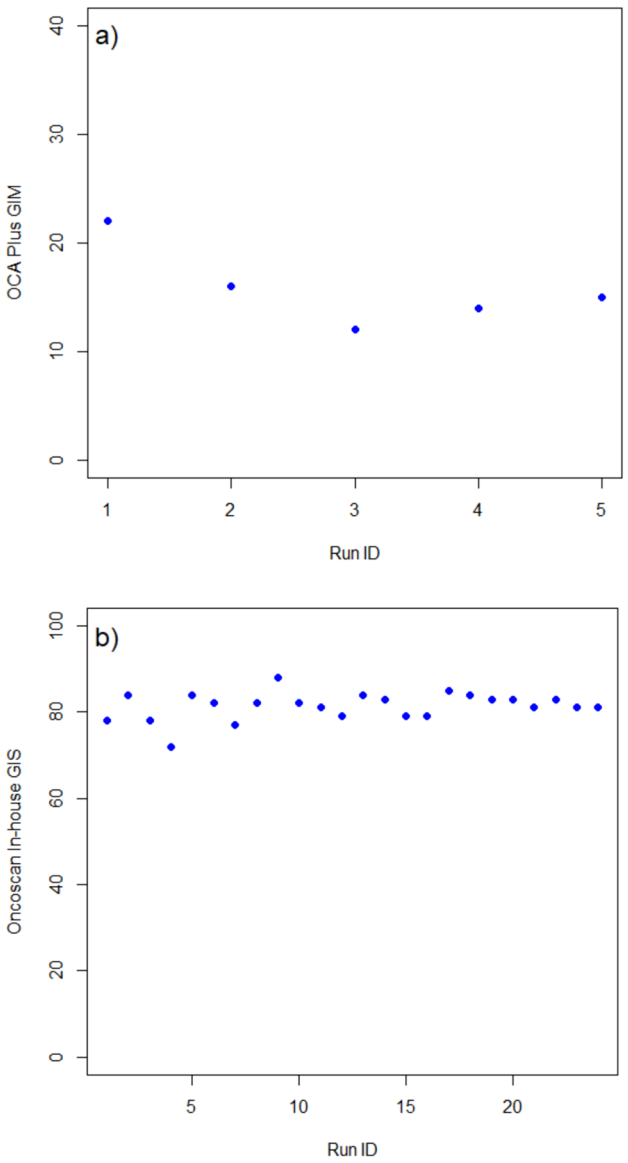

Fig. 5. Inter-run precision. **a** OCA Plus GIM for sample repeated in 5 individual runs and **b** OncoScan in-house GIS of the positive control analyzed in 24 different runs over 6 months

correlation was observed when only 10% of the SNPs were used, by only including one of every 10 SNPs across the chromosomes [19].

The variation in the analysis results using the same method was demonstrated in the repeatability studies. For the OncoScan CNV assay, the inter-run precision evaluation showed a SD of 3.3 in the positive control sample with a mean GIS of 81.4, which we consider acceptable. However, a similar variation around the cutoff point would result in a sample fluctuating between being HRD positive and HRD negative. In the OCA Plus assay the repetition of the same sample five times gave a mean GIM of 15.8 and SD of 3.8, which resulted in HRD assessment on both sides of the GIM cutoff of 16. This is, however, not unique for the OncoScan CNV or OCA Plus test. According to the Myriad myChoice® HRD Technical Specifications (PB 278 REV 0), the overall 95% confidence interval for the true proportion of majority calls across samples corresponds to 81.5–100% for the genomic instability score portion of the assay, with samples with genomic instability status being low positives. Therefore, assay results close to the cutoff point should be interpreted with caution regardless of the assay. It should also be noted that even though the SD in the OncoScan CNV assay and the OCA Plus assay were close, the OCA Plus assay had a narrower range of GIM score values (0–44) than the OncoScan CNV assay GIS scores in our cohort (0–91). Thus, the SD values are not directly comparable. In addition, the precision was only evaluated in a limited number of runs and only at one level for the OCA plus assay.

BRCA1/2 mutated tumor samples were genomic instability positive in 100% (13/13) and 92% (12/13) of the samples for the OncoScan CNV and OCA Plus Assay, respectively, which support the accuracy of the assays. The discrepant sample (from the Pelvic Mass cohort), which was positive in the OncoScan CNV assay and negative in the OCA Plus assay, had a germline *BRCA1* p.E23Vfs*17 mutation with either an unidentified second hit or lack of a second hit. Interestingly, this variant has been shown to result in the production of a BRCA1 protein that lacks the N-terminal really interesting new gene (RING) domain due to translation initiation at an alternative start site, and in mouse models of mammalian cancer were linked to decreased response to platinum and PARPi [38–40]. Recently, an exploratory subgroup analysis of the PAOLA-1 study found that the benefit from maintenance therapy with olaparib and bevacizumab in women with *BRCA1* mutations located in the RING domain was lower than in those with mutations in the DNA-binding domain [41]. However, the effect on HRR and the response to PARPi for *BRCA1* p.E23Vfs*17 positive tumors in the real-life clinical setting needs further investigation.

In the input concentration experiment, we challenged the OncoScan CNV assay with dilutions of FFPE DNA. FFPE tissue samples with an intrinsic low DNA output (in contrast to diluted samples) may, however, be more likely to be of lower DNA quality. Our results indicate that despite reproducible GIS scores in some samples, the OncoScan CNV assay quality may be challenged by a low input concentration of 1 ng/µL. It is therefore recommended only to use samples with DNA concentrations ≥ 3 ng/µL. Samples between 1 and 3 ng/µL in DNA concentration should be analyzed with caution if the result is close to the cutoff. We have recently shown that low-yield DNA can be up-concentrated to sufficient levels for NGS analysis by vacuum centrifugation [42]. A similar strategy may be applicable for HRD analyses for challenging samples.

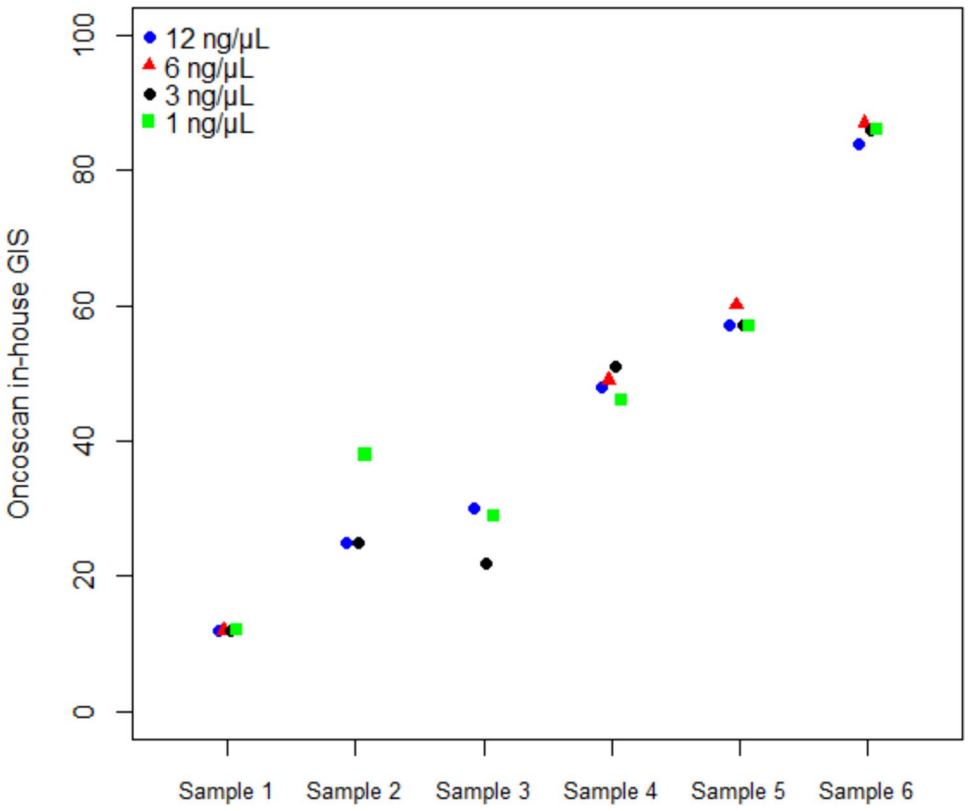

Fig. 6. DNA input concentration test in the OncoScan CNV analysis. OncoScan in-house GIS was obtained with different DNA input concentrations for 6 different samples. Samples 1, 4, 5, and 6 were analyzed in all four concentrations, while samples 2 and 3 were analyzed with input concentrations of 1 ng/µL, 3 ng/µL, and 12 ng/µL due to limited sample material

Our study has some limitations. The fraction of samples that failed QC were between 9 and 20% depending on the assay. This reflects that the study included real-world samples that have the challenges related to material used in routine settings, including scarcity and low tumor fraction for some cases. Improvement of the failure rate related to low tumor fraction may be achievable by selecting sample material further using macrodissection or by implementing computer-assisted tumor fraction assessment as described by Frei et al. [37]. Another limitation of our study is that we do not have access to clinical follow-up data on the Myriad cohort with respect to response to PARPi treatment, and the Pelvic mass cohort was established before the introduction of PARPi in the clinic. We are therefore not able to comment on the predictive value of the HRD assignments.

Furthermore, this is not a clinical validation of any of the methods. When a treatment has been approved on the basis of a biomarker, evaluation of other technologies to determine the biomarker status can be done by comparing the new method to the reference method as presented here. Such method comparison studies are general practice for clinical laboratories according to method validation guidelines [43]. A clinical validation would require access to patient samples from the original clinical study as described for the OncoScan nLST test [27], or initiation of new randomized treatment protocols, which is not always applicable when a successful biomarker has already been approved. This does not mean that the original biomarker with a given cutoff is always correct or the best method to predict the clinical response.

In conclusion, the OCA Plus assay and the OncoScan CNV assay show a high but not complete concordance to reference standard HRD detection. QC steps should include careful tumor content evaluation, with selection of sample material with a high tumor cell fraction being recommended, and results of samples with < 30% tumor should not be reported.

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Declarations

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Conflicts of Interest/Competing Interests (include appropriate disclosures) L.S., T.S.P., L.K.V., I.J.C., E.H.: None.

Ethics Approval This retrospective study was conducted on already available data or biological material and approved by The Danish National Committee on Health Research Ethics (Case ID 2212528) and the Danish Capital Region Knowledge Center for Data Reviews (Case ID P-2023-13).

Consent for Publication/Participation Not applicable.

Availability of Data and Material (Data Transparency) The datasets generated during and/or analyzed during the current study are not publicly available, which is in accordance with the rules concerning the processing of personal data set out in the EU General Data Protection Regulation (GDPR) and the Danish Data Protection Act. Nonetheless, may a researcher have an interest in our data, they are welcome to contact us for collaborations. The data that support the findings of this study can be requested from The National Secretariat for Bio- and Genome Bank Denmark, RBGB.sekretariat.herlev-og-gentofte-hospital@regionh.dk, Herlev Hospital, Borgmester Ib Juuls Vej 73, 2730 Herlev, Denmark.

Author Contributions Estrid Høgdall, Lone Schejbel, and Tim Svenstrup Poulsen contributed to the study conception and design. Next generation sequencing and data collection and analysis were performed by Lone Schejbel and Lau Kræsing Vestergaard. Statistical analyses were performed by Ib Jarle Christensen. The first draft of the manuscript was written by Lone Schejbel and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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