Masterarbeit

Establishment of *BRCA* mutation analysis in formalin-fixed paraffin-embedded ovarian cancers in a Lower Austria hospital network

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Wien, am 1. September 2017

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Zusammenfassung

Das Ovarialkarzinom ist weltweit der siebenthäufigste maligne Tumor der Frau und die achtzehnthäufigste Krebsart überhaupt. Patientinnen, die an einem Ovarialkarzinom erkrankt sind und eine Keimbahn- oder somatische Mutation in den *BRCA1* oder *BRCA2* Genen aufweisen, sprechen besser auf eine platinhaltige Chemotherapie und auf PARP-Inhibitoren an. Deshalb ist die effiziente und kostengünstige Bestimmung des *BRCA*-Mutationsstatus sowohl für Keimbahn- als auch für somatische Mutationen aus formalinfixierten-paraffineingebetteten (FFPE) Gewebeproben wichtig für lokale Krankenanstaltenverbunde. Wir untersuchten die Durchführbarkeit einer solchen Bestimmung aus DNA von FFPE-Proben von drei serösen high-grade Ovarialkarzinomen, einem Tubenkarzinom und an nicht-erkranktem Ovargewebe unter Verwendung eines kommerziell verfügbaren Genpanels auf einer Next Generation Sequencing (NGS)-Plattform. Es wurde eine pathogene Mutation in *BRCA1* sowie eine bisher nicht beschriebene variant of unknown significance (VUS) in *BRCA2* im FFPE-Gewebe von zwei Patientinnen (40%) gefunden, weiters Polymorphismen in *BRCA1* und *BRCA2* bei vier Patientinnen. Zusammenfassend zeigt unsere Studie, dass die Verwendung von NGS mit einem kommerziell verfügbaren Genpanel für die Detektion von Mutationen, VUS und Polymorphismen in den *BRCA* Genen aus DNA von FFPE-Gewebe möglich ist und sich das Verfahren damit als verlässliche Routinemethode für unseren Krankenanstaltenverbund anbietet.

Abstract

Ovarian cancer is the seventh most common cancer in women and the 18 most common cancer overall. Ovarian cancer patients holding a heriditary or somatic mutation in *BRCA1* or *BRCA2* genes show a better response to platinum-based chemotherapy and to PARP inhibitors. Thus efficient and cost-effective testing of *BRCA* mutational status on formalin-fixed paraffin-embedded (FFPE) tissue samples for detection of somatic as well as of heriditary mutations is of importance for local networks of hospitals. We assessed the feasibility of using a commercially available gene panel on a next generation sequencing (NGS) platform on DNA from FFPE samples of three high-grade serous ovarian cancers, one fallopian tube cancer, and of non-cancerous ovarian tissue. A pathogenic mutation in *BRCA1* and a variant of unknown significance (VUS) in *BRCA2* that has not been described up to now were found in two patients (40%), together with polymorphisms in *BRCA1* and *BRCA2* genes in four patients. In summary, our study demonstrates that using a commercially available gene panel on a NGS platform is efficient for detection of mutations, VUS and polymorphisms in *BRCA* genes using DNA from routinely available FFPE tissue. This method offers our local hospital network a reliable procedure for *BRCA* mutation detection.

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Abbreviations

BER, base excision repair

CGH, array comparative genomic hybridization

CNV, copy number variation

DSB, double strand break

EMA, European Medicines Agency

FDA, Food and Drug Administration

FFPE, formalin-fixed paraffin-embedded

FIGO, International Federation of Gynecology and Obstetrics

GQN, genomic quality number

GRCh38, Genome Reference Consortium Human Build 38

HBOC, Hereditary breast and ovarian cancer syndrome

IARC, International Agency for Research on Cancer

NCCN, National Cancer Comprehensive Network

NGS, Next-Generation Sequencing

PARP, poly(ADP-ribose) polymerase

PBSO, prophylactic bilateral salpingo-oophorectomy

PCR, polymerase chain reaction

PFS, progression free survival

qRT-PCR, quantitative reverse transcription polymerase chain reaction

ROI, region of interest

SSB, single strand brake

TNBC, triple-negative breast cancer

VUS, variant of unknown significance

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1 Material and Methods

1.1 BRCA1 and BRCA2 mutations in ovarian cancer

Ovarian cancer is the seventh most common cancer in women and the 18 most common cancer overall with 239,000 new cases and 152,000 deaths worldwide in 2012 (1). In the same year there were 65,500 newly diagnosed patients and 42,700 deaths from ovarian cancer in Europe (2). In Austria, ovarian cancer has a 33% share of all malignant tumors of the female reproductive organs, and in 2014 there were 635 newly diagnosed patients and overall 3,640 patients. For breast cancer there were 5,454 new cases and 72,453 prevalent cases in Austria, making the prevalence for breast cancer the highest among all tumors (3).

BRCA1 and *BRCA2*, two similar tumor suppressor genes, are most commonly mutated in high-grade ovarian cancers (which includes fallopian tube and primary peritoneal cancers), which are responsible for most deaths from ovarian cancer (4), and also in triple-negative breast cancer (TNBC), which has an unfavorable outcome despite polychemotherapy based systemic treatment (5). About 20% of women under the age of 60 diagnosed with TNBC will have *BRCA1* or *BRCA2* mutations. *BRCA1* and *BRCA2* genes play an important role in the homologous recombination DNA repair system, together with *ATM, BARD1, BRIP1, CHEK1, CHEK2, FAM175A, MRE11A, NBN, PALB2, RAD50, RAD51C*, and *RAD51D* (6).

Hereditary breast and ovarian cancer syndrome (HBOC) is most commonly caused by germline mutations in *BRCA1* and *BRCA2*. These mutations increase the risk for breast, ovarian, pancreatic, prostate, melanoma and possibly other cancers (7). About 10% of breast cancer cases exhibit a higher familial incidence, and functional mutations in *BRCA1* or *BRCA2* are responsible for the development of breast cancer in approximately half of these cases. Women with a germline mutation in either of the two genes have a lifetime risk of up to 85% to develop breast cancer, and of up to 60% risk to develop ovarian cancer. Germline mutations and somatic mutations in homologous recombination genes occur in 31% of ovarian cancer patients. Approximately 75% of these mutations are found in *BRCA1* and *BRCA2* genes (6, 8, 9). Estimated carrier frequencies of *BRCA1* and *BRCA2* mutations, respectively, are 0.32% and 0.69% (10). Therefore,

international guidelines recommend that women with a high familial incidence of breast and/or ovarian cancer should undergo genetic counseling and genetic testing. A recently published guideline defines the individual and familial tumor constellations that represent an indication for *BRCA1* and *BRCA2* germline testing. It also describes the therapeutic options of being included in an early detection programme versus the option of having prophylactic surgery that arise from the result of the *BRCA* mutational analysis (11).

The current standard of treatment for patients with advanced ovarian cancer has been established in light of the results from various clinical trials. After debulking surgery, a combination of carboplatin and paclitaxel is considered to be the best systemic treatment option in terms of survival and quality of life (12). However, the majority of patients with ovarian cancer will relapse despite state-of-the-art first-line surgery and chemotherapy. There are two subgroups of patients with recurrent ovarian cancer: those with platinum-resistant disease and those with platinum-sensitive disease. Re-treatment with single-agent platinum has long been considered standard therapy for patients with platinum-sensitive disease, and, based on its favorable therapeutic profile, carboplatin has become the treatment agent of choice. High response rates are seen with platinum agents used in combination with paclitaxel or gemcitabine. Therefore combination chemotherapy is considered the standard treatment of recurrent platinum-sensitive ovarian cancer. The choice of treatment needs to take into account the increase in side effects when using combination chemotherapy compared with carboplatin monotherapy, and the different toxicities of the two combination regimens (13).

Ovarian cancer patients holding a hereditary or somatic mutation in *BRCA1* or *BRCA2* genes have a better response to platinum-based chemotherapy and a better overall survival (9, 14, 15). Patients with TNBC holding a hereditary mutation in *BRCA1* or *BRCA2* genes respond better to neoadjuvant platinum-based chemotherapy (16). Additionally, olaparib, a poly(ADP-ribose) polymerase (PARP) inhibitor, shrunks or stops the growth of tumors from ovarian cancers caused by mutations in the *BRCA1* and *BRCA2* genes (17). The abovementioned clinical guideline further provides recommendations regarding therapeutic aspects that arise from the selective use of PARP inhibitors in patients with known *BRCA1* or *BRCA2* mutations. PARP1 and PARP2 are important proteins in DNA repair

pathways especially the base excision repair (BER). BER is involved in DNA repair of single strand breaks (SSBs). If BER is impaired by inhibiting PARP, SSBs accumulate. When SSBs are encountered by replication forks, they generate double strand breaks (DSBs) that need to be repaired by other pathways, mainly the HR and the nonhomologous end joining (18). Hence, cells with defective homologous recombination caused by *BRCA* deficiency or malfunction are even more susceptible to impairment of the BER pathway. Therefore, inhibitors of PARP like olaparib lead to selective killing of cancer cells with *BRCA1/2* mutations, a principle referred to as synthetic lethality (19). Also, PARP inhibitors increase cytotoxicity by inhibiting repair in the presence of chemotherapies that induces SSBs (20).

A study published in July 2014 by Ledermann et al. shows that maintenance monotherapy with the PARP inhibitor olaparib significantly prolonged progression-free survival (PFS) versus placebo in patients with platinum-sensitive recurrent serous ovarian cancer. Olaparib is most likely to benefit patients with a *BRCA* mutation. Data from the second interim analysis of overall survival and a retrospective, preplanned analysis of data by *BRCA* mutation status from the authors' randomised, double-blind, phase 2 study that assessed maintenance treatment with olaparib 400 mg capsules twice daily versus placebo in patients with platinum-sensitive recurrent serous ovarian cancer who had received two or more platinum-based regimens and who had a partial or complete response to their most recent platinum-based regimen were shown. The primary endpoint of analysis was PFS, analyzed for the overall population and by *BRCA* status. Between August, 2008, and February, 2010, 136 patients were assigned to olaparib and 129 to placebo. BRCA status was known for 131 (96%) patients in the olaparib group versus 123 (95%) in the placebo group, of whom 74 (56%) versus 62 (50%) had a deleterious or suspected deleterious germline or tumor *BRCA* mutation. Of patients with a *BRCA* mutation, median PFS was significantly longer in the olaparib group than in the placebo group (11.2 months [95% CI 8.3-not calculable] vs 4.3 months [3.0-5.4]; HR 0.18 [0.10-0.31]; $p<0.0001$); similar findings were noted for patients with wild-type BRCA, although the difference between groups was lower (7.4 months [5.5-10.3] vs 5.5 months [3.7-5.6]; HR 0.54 [0.34-0.85]; $p=0.0075$). At the second interim analysis of overall survival (58%

maturity), overall survival did not significantly differ between the groups (HR 0.88 [95% CI 0.64-1.21]; p=0.44); similar findings were noted for patients with mutated *BRCA* (HR 0.73 [0.45-1.17]; p=0.19) and wild-type *BRCA* (HR 0.99 [0.63-1.55]; p=0.96). These results supported the hypothesis that patients with platinum-sensitive recurrent serous ovarian cancer with a *BRCA* mutation have the greatest likelihood of benefiting from olaparib treatment (21).

Following the publication of these results the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) approved the PARP inhibitor olaparib for treatment of *BRCA* mutated ovarian cancer on December, 2014 (22, 23). Testing ovarian cancer patients for the presence of mutations in *BRCA* genes has therefore an important role for finding heriditary cancer syndromes and to get information before administration of a therapy containing PARP inhibitors.

1.2 Next generation sequencing

Various mutations and polymorphisms arise in the *BRCA1* and *BRCA2* genes and their characterization by Sanger sequencing is a time consuming, cost intensive and complicated process (24). Next generation sequencing (NGS) allows fast simultaneous analysis of DNA with increasing applications in clinical medicine and research (25, 26). These applications include also the detection of *BRCA1/2* mutations. Furthermore, protocols and software solutions for the analysis and interpretation of NGS data, as well as fast growing knowledge bases, allow pathologists and clinicians to use genomic informations more easily for their patients (27).

1.3 Formalin-fixed paraffin-embedded tissue

The abovementioned new therapy concepts based on PARP inhibitors target tumors with germline mutated genes as well as somatically mutated genes. Testing for hereditary mutations in *BRCA* genes from blood samples is common in genetics laboratories and clinical pathology departments. However, this approach is insufficient to meet the requirements of patients with somatic *BRCA* mutations, who are eligible for a PARP inhibitor therapy. Testing *BRCA* mutational status on formalin-fixed paraffin-embedded (FFPE) tissue samples as starting material for NGS thus permits the determination of somatic as well as hereditary mutations

utilizing tissue that is taken routinely during tumor surgery. Following routine histopathologic assessment the samples are stored in pathology departments and are easily available for further molecular pathological testing (28). FFPE tissue is one of the most widely practiced methods for clinical sample preservation and archiving. It is estimated that, worldwide, over a billion FFPE tissue samples are being stored in numerous hospitals, tissue banks, and research laboratories. While standard for histopathology and microscopic investigation (hematoxylin and eosin (H&E) staining, immunohistochemistry, and tissue microarray), FFPE samples pose a major challenge for molecular pathologists, because nucleic acids are heavily modified and trapped by extensive protein-nucleic acid and protein-protein cross linking. Historically, FFPE samples were not considered to be a viable source for molecular analyses. However, with appropriate protease digestion, it is possible to release microgram amounts of DNA and RNA from FFPE samples. The purified nucleic acids, although highly fragmented, are suitable for a variety of downstream genomic and gene expression analyses, such as polymerase chain reaction (PCR), quantitative reverse transcription PCR (qRT-PCR), microarray, array comparative genomic hybridization (CGH), microRNA, methylation profiling, and NGS. Several commercial kits are currently available for FFPE extraction (29). This approach of using FFPE material is of significant importance for local networks of smaller hospitals providing an opportunity to achieve diagnostic independence (30, 31).

To date, only a total of about 13 papers have been published dealing with this issue (28, 32, 33). To evaluate the feasibility of detection of mutations in *BRCA* genes for routine applications in a local Lower Austria hospital network, we have examined *BRCA1* and *BRCA2* genes in four high-grade ovarian and fallopian tube carcinomas and in one case of non-cancerous ovarian tissue, using a commercially available gene panel on a NGS platform on FFPE tissue specimens and on matched blood samples.

1.4 Variants of unknown significance

Members of the International Agency for Research on Cancer (IARC) Working Group on Unclassified Genetic Variants published a paper in 2008 proposing a classification system that more clearly conveys information about the relevance of a variant of unknown significance (VUS) to clinical practice including cancer risk assessment. This system encompasses variants that are definitely pathogenic and definitely neutral as well as those (currently) of unknown clinical significance. The classification system classifies VUS into five categories:

Class 1 corresponds to the qualitative classification "Not Pathogenic" or "No Clinical Significance". Assigning a variant to Class 1 rules out a major clinically significant effect of that variant on cancer risk. The major advance of the proposed classification system is the creation of Class 2 and Class 4, "Likely Not Pathogenic/Little Clinical Significance" and "Likely Pathogenic" with a consistent definition of the likelihood of pathogenicity of 5% and 95%, respectively. The IARC working group differentiated these two groups of variants from Class 3 ("Uncertain") in order to differentiate variants for which there is really too little information to make any recommendation (Class 3) from those for which there is significant, but not irrefutable, evidence against (Class 2) or for (Class 4) pathogenicity. Class 5 defines a sequent variant as „Definitely Pathogenic" (34).

2 Material and Methods

2.1 Patients

The present study investigated three patients with high-grade ovarian carcinomas and one patient with fallopian tube carcinoma, all diagnosed using WHO diagnosis criteria (35). All patients (mean age 53.5±11.3 years, median 57.5 years) underwent tumor debulking surgery between 2010 and 2017 at the Department of Gynecology and Obstetrics of the Hospital of Korneuburg. Three patients were diagnosed with stage IIIC cancer and one with stage I cancer, according to FIGO staging system (36). One sample of non-cancerous ovarian tissue was taken from a patient (patient 4) who underwent prophylactic bilateral salpingo-oophorectomy (PBSO) due to HBOC. In this patient a germline *BRCA1* mutation (c.904delG) had

been already identified previously and the sample served as positive control. Blood samples of the patients were also harvested and served as control. In addition to FFPE material cancer tissue was fixed using PaxGene Tissue Systems (PreAnalytiX, Qiagen) at the time of surgery of patient 5.

2.2 Ethics

The FFPE samples were acquired from the Institute of Pathology of the Hospital of Mistelbach after obtaining consent of patients for genetic analysis. Blood samples were harvested following consent of patients after mandatory genetic counseling according to Austrian laws.

2.3 DNA isolation and qualification

Microscopic examination of FFPE samples showed a tumor cell portion of a minimum of 80%. DNA extraction was performed from 3x10 µm FFPE sections on a QIAcube using the GeneRead DNA FFPE Kit (Qiagen).

2.4 DNA quantification and genomic quality number (GQN)

The total amounts of DNA were specified on a Qubit 3.0 Fluorometer (ThermoFisher) using the Quant-iT dsDNA BR Assay Kit. Per patient 4x50 ng of DNA were used for NGS analysis. DNA quality was verified on a Fragment Analyzer (Advanced Analytical Technologies) using a Standard Sensitivity Genomic DNA Analysis Kit and PROSize Software 2.0. GQN were determined using a cutoff of 150 base pairs.

2.5 BRCA mutation analysis by NGS

BRCA mutation analysis was performed on a MiSeqDx (Illumina) sequencer using the BRCA Tumor MASTR Plus Dx (Multiplikom, Agilent Technologies) gene panel. BRCA Tumor MASTR Plus Dx is a CE-IVD-labeled molecular diagnostic assay for the identification of 100% of activating mutations (single nucleotide variants, deletions, insertions, indels, splice variants) in the coding regions of the *BRCA1* and *BRCA2* genes. The assay uses multiplex PCR amplification of specific genomic targets to make a DNA library for the NGS device. In a first step, all coding regions of *BRCA1* and *BRCA2* are amplified using 181 amplicons divided over 4 separate multiplex PCR amplification reactions. In a second step, a

universal PCR is performed to tag the amplicons with drMID Dx for Illumina NGS systems. The assay is compatible with both blood- and FFPE-derived DNA (37-39). Copy number variation (CNV) analysis is limited to blood derived DNA, i.e. detection of somatic CNV and large rearrangements is not feasible. Additionally, inactivation of *BRCA* genes by promoter methylation occuring in up to 15% in ovarian cancers cannot be recorded.

2.6 Quality control and bioinformatics

The quality of DNA sequenzing runs was verified using the Sequence Analysis Viewer Software (Illumina v.2.1.8), MASTR Reporter (Multiplicom v.1.0.2) and FastQC Software (v.0.11.4). Data analysis, including alignment to the GRCh38 human reference genome and variant calling were carried out using the MiSeq Reporter (Illumina v.2.6.2), Variant Studio Software (Illumina v. 3.0) and MASTR Reporter (Multiplicom v.1.0.2). Results were verified using SeqNext Software (JSI Medical Systems v.4.3.1 build 506). CNV analysis of DNA derived from blood was performed using the CNV modul of SeqNext Software. Clinical evaluation of mutations was supported by the ClinVar database and varSEAK Software (JSI Medical Systems).

3 Results

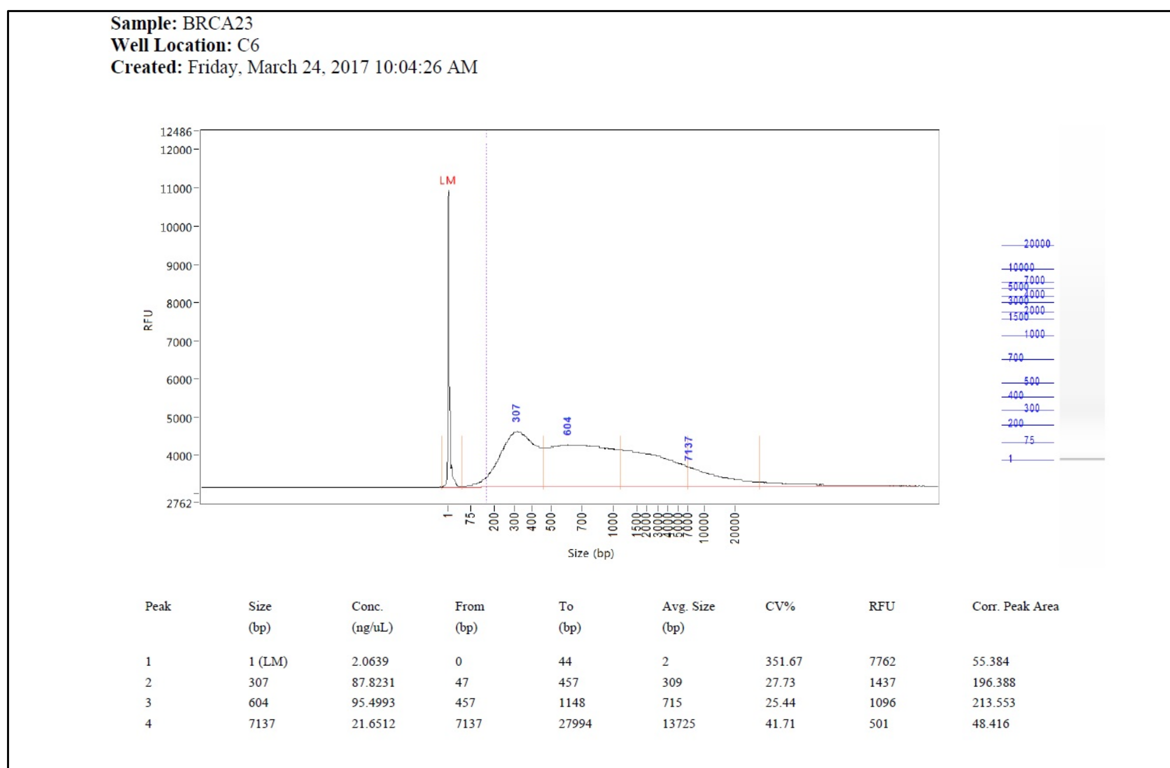
3.1 Total DNA quantities, DNA quality and GQN

DNA from FFPE- and blood samples was successfully amplified in PCR. Total amounts of DNA and GQN of patients are shown in table 1. Quality for both FFPE tissue and blood derived DNA of patient 1 is shown in figures 1 and 2. DNA quality of DNA derived from FFPE tissue, blood and PaxGene Tissue Systems fixed tissue of patient 5 is shown in figures 3 to 5.

Table 1. Total amounts of DNA (nanograms) and GQN of patients

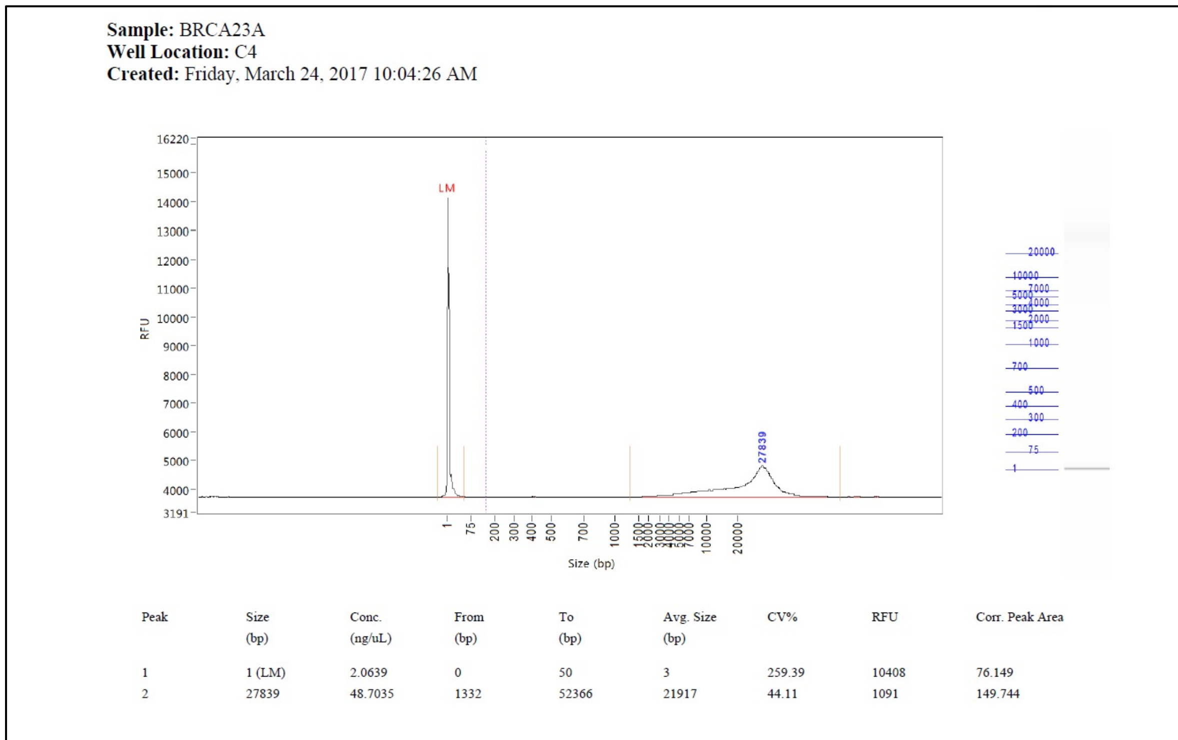
Patient	FFPE Amount DNA/GQN	Whole blood Amount DNA/GQN	PaxGene Tissue Amount DNA/GQN
1	4560/9.9	4620/10	-
2	498/10	8860/9.9	-
3	5140/9.3	7010/9.5	-
4	n.a.	n.a.	-
5	7240/10	602/10	1546/9.9

Figure 1. Patient 1: quality of FFPE tissue derived DNA



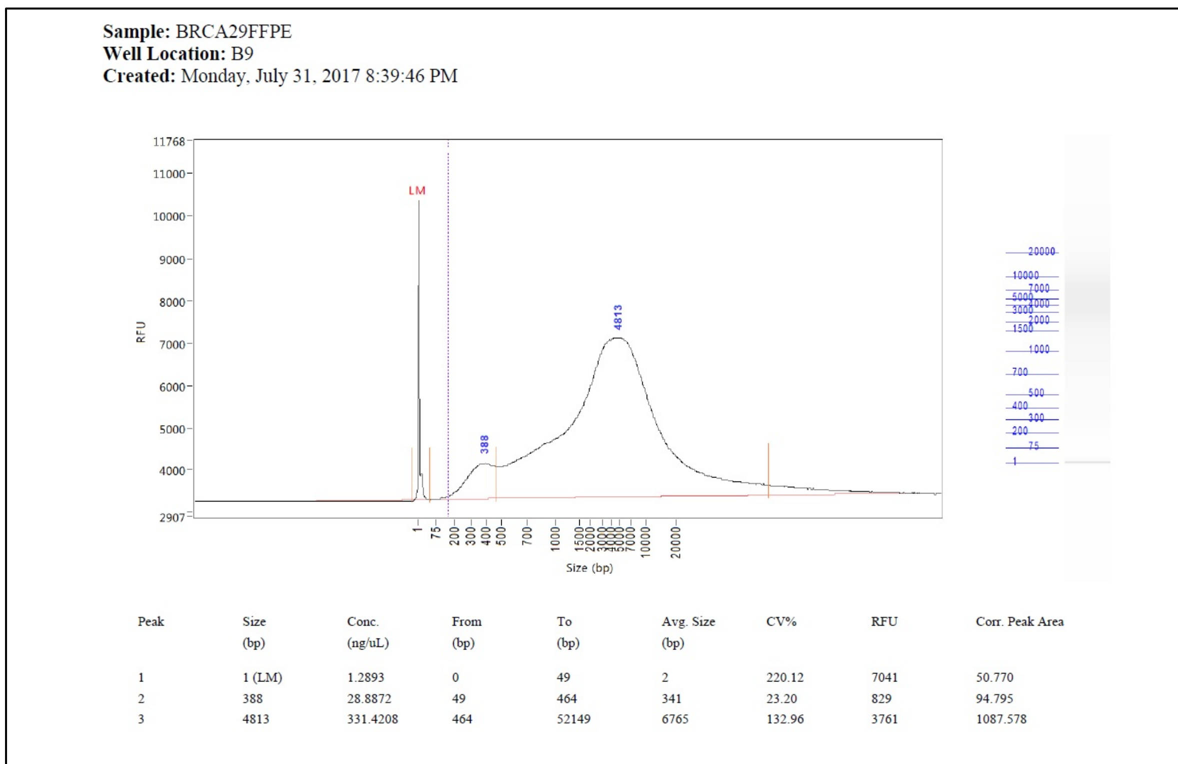
Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	CV%	RFU	Corr. Peak Area
1	1 (LM)	2.0639	0	44	2	351.67	7762	55.384
2	307	87.8231	47	457	309	27.73	1437	196.388
3	604	95.4993	457	1148	715	25.44	1096	213.553
4	7137	21.6512	7137	27994	13725	41.71	501	48.416

Figure 2. Patient 1: quality of blood derived DNA



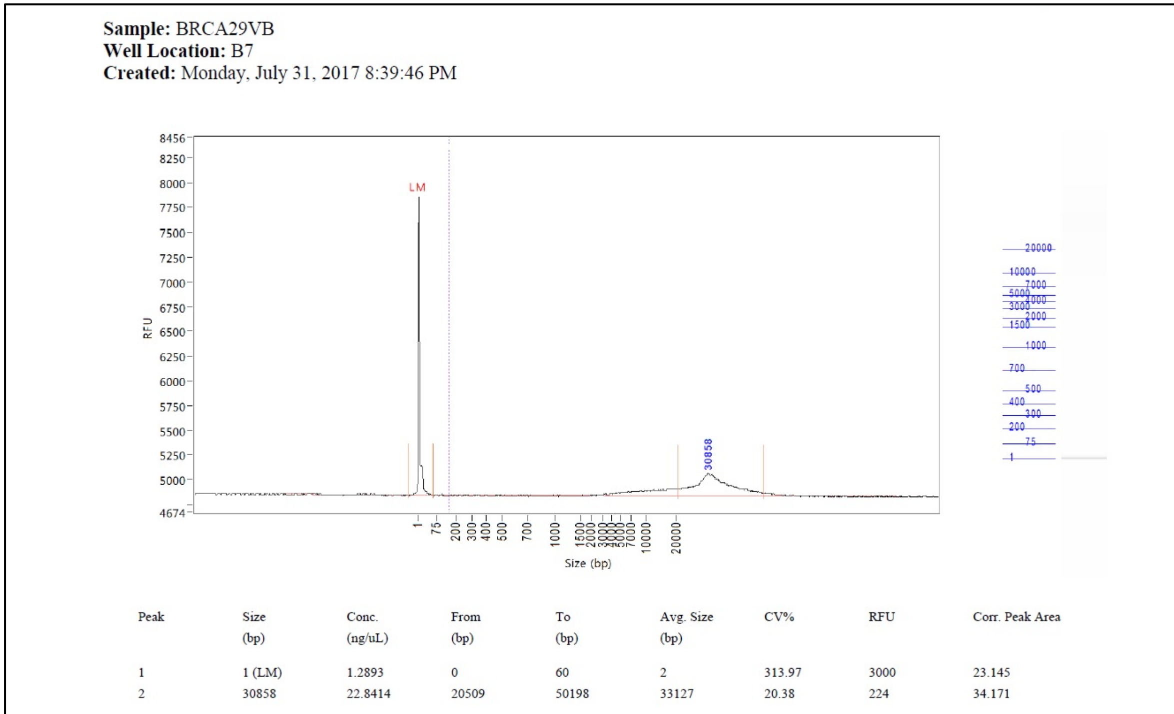
Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	CV%	RFU	Corr. Peak Area
1	1 (LM)	2.0639	0	50	3	259.39	10408	76.149
2	27839	48.7035	1332	52366	21917	44.11	1091	149.744

Figure 3. Patient 5: quality of FFPE tissue derived DNA



Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	CV%	RFU	Corr. Peak Area
1	1 (LM)	1.2893	0	49	2	220.12	7041	50.770
2	388	28.8872	49	464	341	23.20	829	94.795
3	4813	331.4208	464	52149	6765	132.96	3761	1087.578

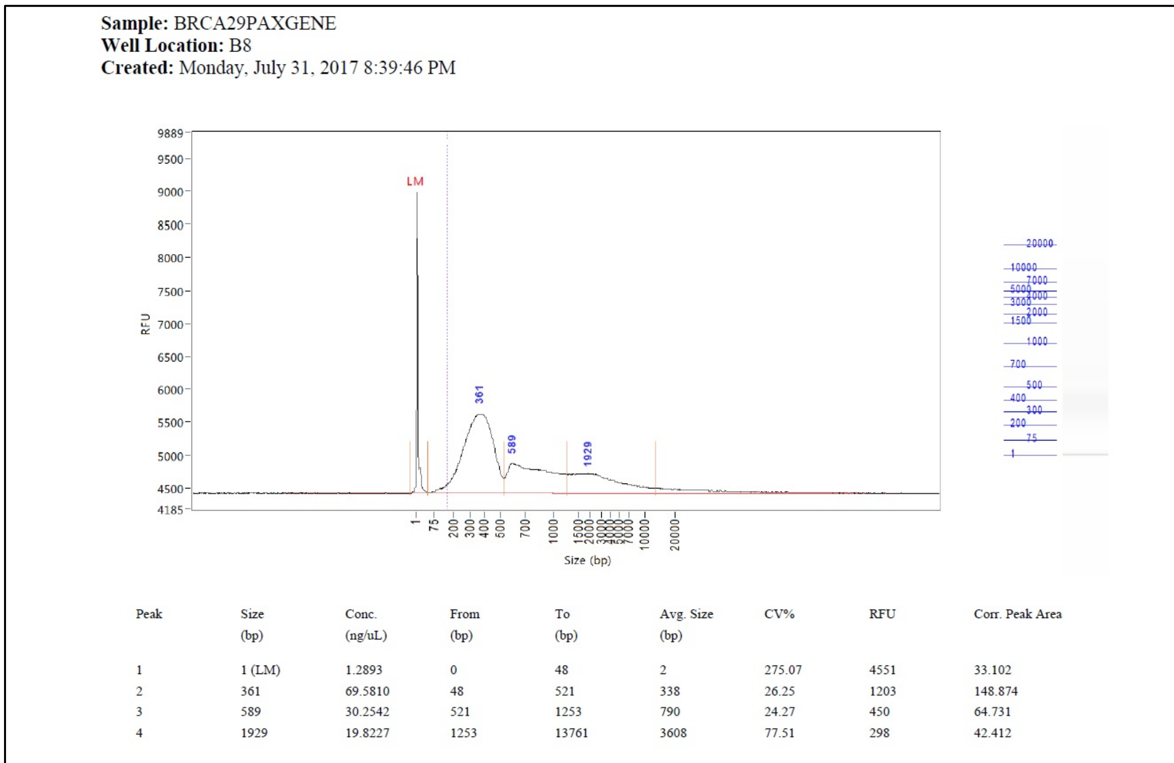
Figure 4. Patient 5: quality of blood derived DNA



Sample: BRCA29VB
Well Location: B7
Created: Monday, July 31, 2017 8:39:46 PM

Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	CV%	RFU	Corr. Peak Area
1	1 (LM)	1.2893	0	60	2	313.97	3000	23.145
2	30858	22.8414	20509	50198	33127	20.38	224	34.171

Figure 5. Patient 5: quality of PaxGene Tissue Systems tissue derived DNA



Sample: BRCA29PAXGENE
Well Location: B8
Created: Monday, July 31, 2017 8:39:46 PM

Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	CV%	RFU	Corr. Peak Area
1	1 (LM)	1.2893	0	48	2	275.07	4551	33.102
2	361	69.5810	48	521	338	26.25	1203	148.874
3	589	30.2542	521	1253	790	24.27	450	64.731
4	1929	19.8227	1253	13761	3608	77.51	298	42.412

3.2 NGS targeted sequencing

Results of NGS sequencing are shown in table 2. The minimum sequencing depth was 500-fold, with a coverage of regions-of-interest (ROI) of 100%.

Table 2. Variants in *BRCA1* and *BRCA2* analyzed by NGS of five patients

Patient	BRCA1	BRCA2	Mutation type	Germline-somatic	dbSNP ID	ClinVar class
3	-	c.4420A>G p.Lys1474Glu	Missense	Germline	rs766719443	Uncertain significance
4	c.904delG p.Ala302Leufs	-		Germline	rs273903793	Pathogenic

3.3 Pathogenic mutation

A pathogenic mutation in *BRCA1* (c.904delG) was found in one (patient 4) of the five (20%) patients. This mutation was a frameshift and is registered as pathogenic in the ClinVar database by four submitters. As shown by analysis of DNA from FFPE tissue and patient's blood and as recorded in the ClinVar database the *BRCA1* mutation is germline. The pathogenic mutation had been already identified earlier from the patient's blood by an independent genetics laboratory and data served as positive control in this study.

Figure 6. Detection of *BRCA1* mutation c.904delG using SeqNext Software

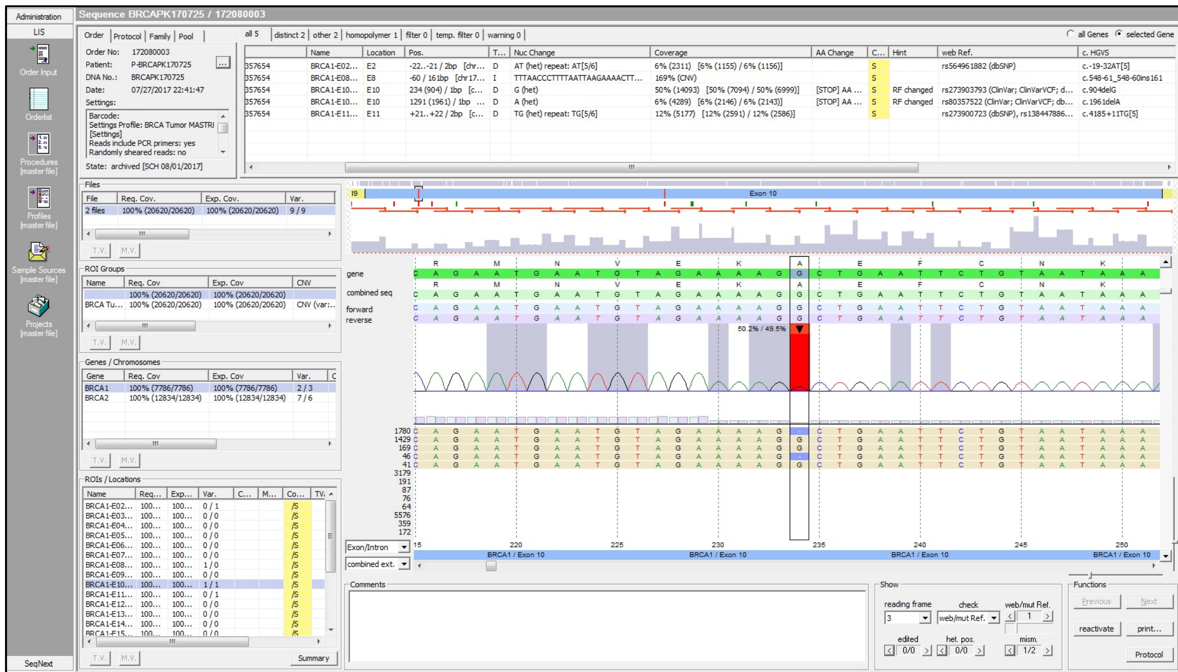
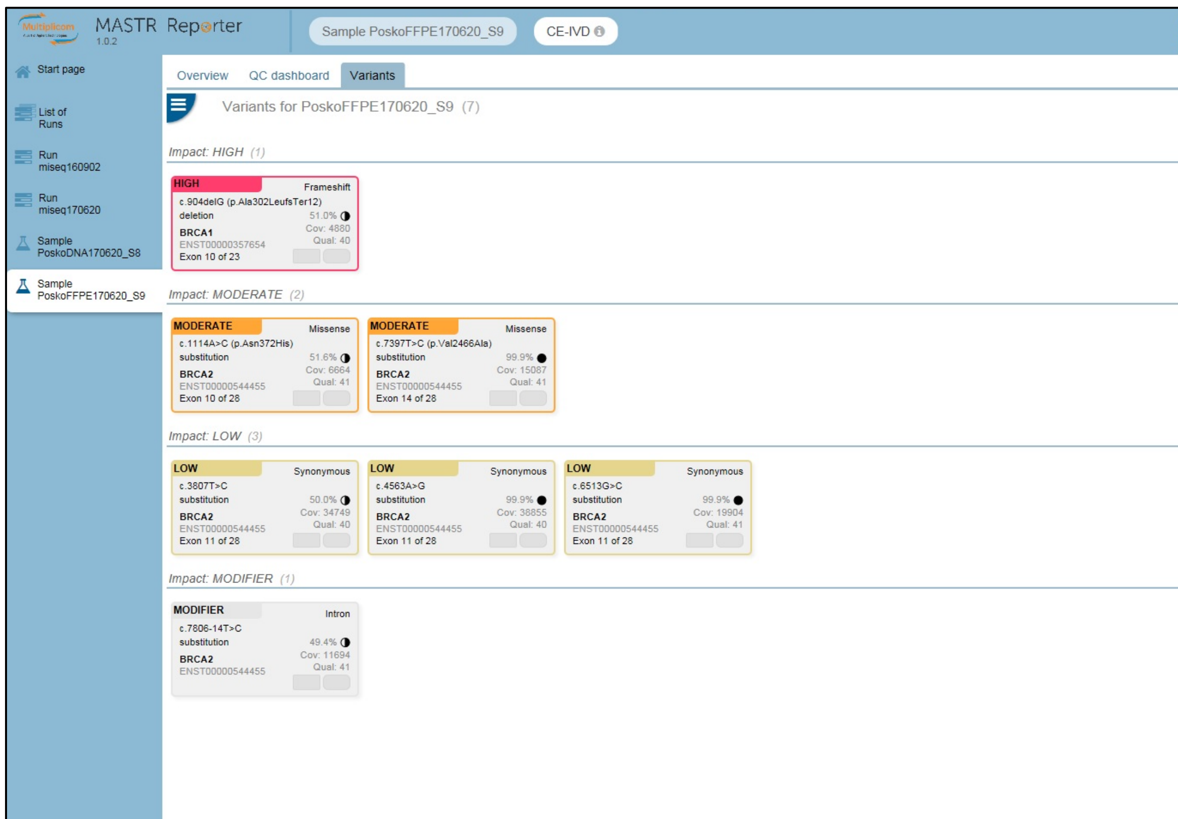


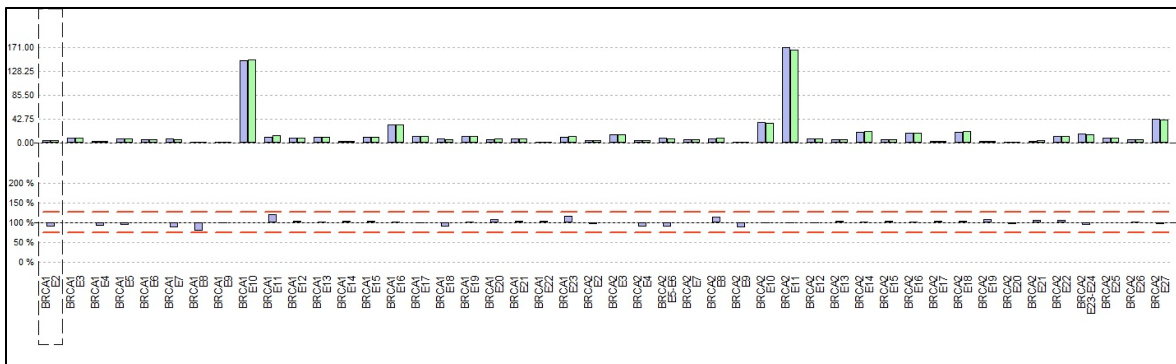
Figure 7. Assessment of *BRCA1* mutation c.904delG using MASTR Reporter Software



3.4 Additional variant

An additional variant in Exon 11 of *BRCA2* (c.4420A>G, p.Lys1474Glu, allele frequencies blood = 50% and FFPE tissue = 55%, respectively) was found in one (patient 3) of the five (20%) patients. This variant was a missense and is not recorded in the ARUP Laboratories BRCA2, BIC, BRCA Share, ClinVar, COSMIC, dbSNP and LOVD BRCA database. In the VarSome database the variant (chr13-32912912-A-G) is recorded with conflicting predictions of pathogenicity with predictions ranking from polymorphism (MutationTaster) to damaging (Provean). DANN score is 0.9916, suggesting the variant to be damaging (40). According to the Ensembl Variant Effector Predictor the variant is a Class 3 VUS with moderate impact on the gene function (34, 41). As shown by analysis of DNA from FFPE tissue and patient's blood the *BRCA2* variant is germline. Figure 8 shows CNV analysis performed using the CNV modul of SeqNext Software.

Figure 8. CNV analysis using the CNV modul of SeqNext Software



3.5 Polymorphisms

Polymorphisms detected are shown in tables 3 to 6 and were the same in DNA derived from FFPE tissue, patients' blood, and PaxGene Tissue Systems fixed material (patient 5). Patient 4 served merely as positive control for the detection of the pathogenic mutation (c.904delG) and no further analysis of polymorphisms was performed. All listed polymorphisms are classified as benign according to the ClinVar database.

Table 3. Polymorphisms detected in patient 1

Patient 1

NM_007294.3(BRCA1):c.442-34C>T: Allelfrequenzen EDTA-Vollblut=50%, FFPE-Tumor=84%; Populationsallelfrequenz Europa: 0.12500000; Reference SNP (refSNP) Cluster Report rs799923, ClinVar Variation ID 125864, Klassifizierung: benign
NM_000059.3(BRCA2):c.-26G>A: Allelfrequenzen EDTA-Vollblut=52%, FFPE-Tumor=38%; Populationsallelfrequenz Europa: 0.21570000; Reference SNP (refSNP) Cluster Report rs1799943, ClinVar Variation ID 125965, Klassifizierung: benign
NM_000059.3(BRCA2):c.3396A>G (p.Lys1132=): Allelfrequenzen EDTA-Vollblut=50%, FFPE-Tumor=38%; Populationsallelfrequenz Europa: 0.27530003; Reference SNP (refSNP) Cluster Report rs1801406, ClinVar Variation ID 126010, Klassifizierung: benign
NM_000059.3(BRCA2):c.3807T>C (p.Val1269=): Allelfrequenzen EDTA-Vollblut=49%, FFPE-Tumor=61%; Populationsallelfrequenz Europa: 0.19980000; Reference SNP (refSNP) Cluster Report rs543304, ClinVar Variation ID 126022, Klassifizierung: benign
NM_000059.3(BRCA2):c.4563A>G (p.Leu1521=): Allelfrequenzen EDTA-Vollblut=100%, FFPE-Tumor=100%; Populationsallelfrequenz Europa: 0.99900001; Reference SNP (refSNP) Cluster Report rs206075, ClinVar Variation ID 132779, Klassifizierung: benign
NM_000059.3(BRCA2):c.6513G>C (p.Val2171=): Allelfrequenzen EDTA-Vollblut=100%, FFPE-Tumor=100%; Populationsallelfrequenz Europa: 0.99174529; Reference SNP (refSNP) Cluster Report rs206076, ClinVar Variation ID 132780, Klassifizierung: benign
NM_000059.3(BRCA2):c.7242A>G (p.Ser2414=): Allelfrequenzen EDTA-Vollblut=49%, FFPE-Tumor=37%; Populationsallelfrequenz Europa: 0.20870000; Reference SNP (refSNP) Cluster Report rs1799955, ClinVar Variation ID 126133, Klassifizierung: benign
NM_000059.3(BRCA2):c.7397C>T (p.Ala2466Val): Allelfrequenzen EDTA-Vollblut=100%, FFPE-Tumor=100%; Populationsallelfrequenz Europa: 0.99900001; Reference SNP (refSNP) Cluster Report rs169547, ClinVar Variation ID 52317, Klassifizierung: benign
NM_000059.3(BRCA2):c.7806-14T>C: Allelfrequenzen EDTA-Vollblut=50%, FFPE-Tumor=38%; Populationsallelfrequenz Europa: 0.51789999; Reference SNP (refSNP) Cluster Report rs9534262, ClinVar Variation ID 126158, Klassifizierung: benign

Table 4. Polymorphisms detected in patient 2

Patient 2

NM_007294.3(BRCA1):c.548-58delT: Allelfrequenzen EDTA-Vollblut=51%, FFPE-Tumor=52%; Populationsallelfrequenz Europa: 0.35290; Reference SNP (refSNP) Cluster Report rs8176144, ClinVar Variation ID 125889, Klassifizierung: benign
NM_007294.3(BRCA1):c.2082C>T (p.Ser694=): Allelfrequenzen EDTA-Vollblut=52%, FFPE-Tumor=50%; Populationsallelfrequenz Europa: 0.30019999; Reference SNP (refSNP) Cluster Report rs1799949, ClinVar Variation ID 125536, Klassifizierung: benign
NM_007294.3(BRCA1):c.2311T>C (p.Leu771=): Allelfrequenzen EDTA-Vollblut=49%, FFPE-Tumor=50%; Populationsallelfrequenz Europa: 0.35290000; Reference SNP (refSNP) Cluster Report rs16940, ClinVar Variation ID 125554, Klassifizierung: benign
NM_007294.3(BRCA1):c.2612C>T (p.Pro871Leu): Allelfrequenzen EDTA-Vollblut=49%, FFPE-Tumor=50%; Populationsallelfrequenz Europa: 0.36280000; Reference SNP (refSNP) Cluster Report rs799917, ClinVar Variation ID 41812, Klassifizierung: benign
NM_007294.3(BRCA1):c.3113A>G (p.Glu1038Gly): Allelfrequenzen EDTA-Vollblut=49%, FFPE-Tumor=49%; Populationsallelfrequenz Europa: 0.35690001; Reference SNP (refSNP) Cluster Report rs16941, ClinVar Variation ID 41815, Klassifizierung: benign
NM_007294.3(BRCA1):c.3548A>G (p.Lys1183Arg): Allelfrequenzen EDTA-Vollblut=51%, FFPE-Tumor=51%; Populationsallelfrequenz Europa: 0.35490000; Reference SNP (refSNP) Cluster Report rs16942, ClinVar Variation ID 41818, Klassifizierung: benign
NM_007294.3(BRCA1):c.4308T>C (p.Ser1436=): Allelfrequenzen EDTA-Vollblut=49%, FFPE-Tumor=50%; Populationsallelfrequenz Europa: 0.35789999; Reference SNP (refSNP) Cluster Report rs1060915, ClinVar Variation ID 125703, Klassifizierung: benign
NM_007294.3(BRCA1):c.4837A>G (p.Ser1613Gly): Allelfrequenzen EDTA-Vollblut=51%, FFPE-Tumor=50%; Populationsallelfrequenz Europa: 0.35980001; Reference SNP (refSNP) Cluster Report rs1799966, ClinVar Variation ID 41827, Klassifizierung: benign
NM_007294.3(BRCA1):c.5152+66G>A: Allelfrequenzen EDTA-Vollblut=51%, FFPE-Tumor=50%; Populationsallelfrequenz Europa: 0.35980001; Reference SNP (refSNP) Cluster Report rs3092994, ClinVar Variation ID 55428, Klassifizierung: benign
NM_000059.3(BRCA2):c.-26G>A: Allelfrequenzen EDTA-Vollblut=51%, FFPE-Tumor=49%; Populationsallelfrequenz Europa: 0.21570000; Reference SNP (refSNP) Cluster Report

rs1799943, ClinVar Variation ID 125965, Klassifizierung: benign
NM_000059.3(BRCA2):c.3396A>G (p.Lys1132=): Allelfrequenzen EDTA-Vollblut=51%, FFPE-Tumor=49%; Populationsallelfrequenz Europa: 0.27530003; Reference SNP (refSNP) Cluster Report rs1801406, ClinVar Variation ID 126010, Klassifizierung: benign
NM_000059.3(BRCA2):c.3807T>C (p.Val1269=): Allelfrequenzen EDTA-Vollblut=51%, FFPE-Tumor=50%; Populationsallelfrequenz Europa: 0.19980000; Reference SNP (refSNP) Cluster Report rs543304, ClinVar Variation ID 126022, Klassifizierung: benign
NM_000059.3(BRCA2):c.4563A>G (p.Leu1521=): Allelfrequenzen EDTA-Vollblut=100%, FFPE-Tumor=100%; Populationsallelfrequenz Europa: 0.99900001; Reference SNP (refSNP) Cluster Report rs206075, ClinVar Variation ID 132779, Klassifizierung: benign
NM_000059.3(BRCA2):c.6513G>C (p.Val2171=): Allelfrequenzen EDTA-Vollblut=100%, FFPE-Tumor=100%; Populationsallelfrequenz Europa: 0.99174529; Reference SNP (refSNP) Cluster Report rs206076, ClinVar Variation ID 132780, Klassifizierung: benign
NM_000059.3(BRCA2):c.7397C>T (p.Ala2466Val): Allelfrequenzen EDTA-Vollblut=100%, FFPE-Tumor=100%; Populationsallelfrequenz Europa: 0.99900001; Reference SNP (refSNP) Cluster Report rs169547, ClinVar Variation ID 52317, Klassifizierung: benign

Table 5. Polymorphisms detected in patient 3

Patient 3

NM_007294.3(BRCA1):c.2082C>T (p.Ser694=): Allelfrequenzen EDTA-Vollblut=50%, FFPE-Tumor=10%; Global MAF:A=0.3365/1685; Reference SNP (refSNP) Cluster Report rs1799949; ClinVar Variation ID 125536, Klassifizierung: benign
NM_007294.3(BRCA1):c.2311T>C (p.Leu771=): Allelfrequenzen EDTA-Vollblut=51%, FFPE-Tumor=9%; Populationsallelfrequenz Europa: 0.35290000; Reference SNP (refSNP) Cluster Report rs16940, ClinVar Variation ID 125554, Klassifizierung: benign
NM_007294.3(BRCA1):c.2612C>T (p.Pro871Leu): Allelfrequenzen EDTA-Vollblut=51%, FFPE-Tumor=10%; Populationsallelfrequenz Europa: 0.500; Reference SNP (refSNP) Cluster Report 799917, ClinVar Variation ID 41812, Klassifizierung: benign
NM_007294.3(BRCA1):c.3113A>G (p.Glu1038Gly): Allelfrequenzen EDTA-Vollblut=50%, FFPE-Tumor=9%; Populationsallelfrequenz Europa: 0.35690001; Reference SNP (refSNP) Cluster Report rs16941, ClinVar Variation ID 41815, Klassifizierung: benign
NM_007294.3(BRCA1):c.3548A>G (p.Lys1183Arg): Allelfrequenzen EDTA-Vollblut=51%, FFPE-Tumor=9%; Populationsallelfrequenz Europa: 0.35490000; Reference SNP (refSNP) Cluster Report rs16942, ClinVar Variation ID 41818, Klassifizierung: benign
NM_007294.3(BRCA1):c.4308T>C (p.Ser1436=): Allelfrequenzen EDTA-Vollblut=49%, FFPE-Tumor=10%; Populationsallelfrequenz Europa: 0.35789999; Reference SNP (refSNP) Cluster Report rs1060915, ClinVar Variation ID 125703, Klassifizierung: benign
NM_007294.3(BRCA1):c.4837A>G (p.Ser1613Gly): Allelfrequenzen EDTA-Vollblut=50%, FFPE-Tumor=8%; Populationsallelfrequenz Europa: 0.35980001; Reference SNP (refSNP) Cluster Report rs1799966, ClinVar Variation ID 41827, Klassifizierung: benign
NM_007294.3(BRCA1):c.4956G>A (p.Met1652Ile): Allelfrequenzen EDTA-Vollblut=50%, FFPE-Tumor=7%; Populationsallelfrequenz Europa: 0.01590000; Reference SNP (refSNP) Cluster Report rs1799967, ClinVar Variation ID 41830, Klassifizierung: benign
NM_000059.3(BRCA2):c.978C>A (p.Ser326Arg): Allelfrequenzen EDTA-Vollblut=50%, FFPE-Tumor=42%; Populationsallelfrequenz Europa: 0.00100000; Reference SNP (refSNP) Cluster Report rs28897706, ClinVar Variation ID 52898, Klassifizierung: benign
NM_000059.3(BRCA2):c.1114A>C (p.Asn372His): Allelfrequenzen EDTA-Vollblut=100%, FFPE-Tumor=100%; Populationsallelfrequenz Europa: 0.35555556; Reference SNP (refSNP) Cluster Report rs144848, ClinVar Variation ID 9329, Klassifizierung: benign

NM_000059.3(BRCA2):c.4563A>G (p.Leu1521=): Allelfrequenzen EDTA-Vollblut=100%, FFPE-Tumor=100%; Populationsallelfrequenz Europa: 0.99900001; Reference SNP (refSNP) Cluster Report rs206075, ClinVar Variation ID 132779, Klassifizierung: benign
NM_000059.3(BRCA2):c.6513G>C (p.Val2171=): Allelfrequenzen EDTA-Vollblut=100%, FFPE-Tumor=100%; Populationsallelfrequenz Europa: 0.99174529; Reference SNP (refSNP) Cluster Report rs206076, ClinVar Variation ID 132780, Klassifizierung: benign
NM_000059.3(BRCA2):c.7397C>T (p.Ala2466Val): Allelfrequenzen EDTA-Vollblut=100%, FFPE-Tumor=100%; Populationsallelfrequenz Europa: 0.99900001; Reference SNP (refSNP) Cluster Report rs169547, ClinVar Variation ID 52317, Klassifizierung: benign
NM_000059.3(BRCA2):c.7806-14T>C: Allelfrequenzen EDTA-Vollblut=50%, FFPE-Tumor=56%; Populationsallelfrequenz Europa: 0.51789999; Reference SNP (refSNP) Cluster Report rs9534262, ClinVar Variation ID 126158, Klassifizierung: benign

Table 6. Polymorphisms detected in patient 5

Patient 5

NM_007294.3(BRCA1):c.2082C>T (p.Ser694=): Allelfrequenzen EDTA-Vollblut=100%, PAX-Tumor=100%, FFPE-Tumor=99,9%; Global MAF:A=0.3365/1685; Reference SNP (refSNP) Cluster Report rs1799949; ClinVar Variation ID 125536, Klassifizierung: benign

NM_007294.3(BRCA1):c.2311T>C (p.Leu771=): Allelfrequenzen EDTA-Vollblut=100%, PAX-Tumor=100%, FFPE-Tumor=99,9%; Populationsallelfrequenz Europa: 0.35290000; Reference SNP (refSNP) Cluster Report rs16940, ClinVar Variation ID 125554, Klassifizierung: benign

NM_007294.3(BRCA1):c.2612C>T (p.Pro871Leu): Allelfrequenzen EDTA-Vollblut=100%, PAX-Tumor=100%, FFPE-Tumor=99,9%; Populationsallelfrequenz Europa: 0.500; Reference SNP (refSNP) Cluster Report 799917, ClinVar Variation ID 41812, Klassifizierung: benign

NM_007294.3(BRCA1):c.3113A>G (p.Glu1038Gly): Allelfrequenzen EDTA-Vollblut=100%, PAX-Tumor=100%, FFPE-Tumor=99,9%; Populationsallelfrequenz Europa: 0.35690001; Reference SNP (refSNP) Cluster Report rs16941, ClinVar Variation ID 41815, Klassifizierung: benign

NM_007294.3(BRCA1):c.3548A>G (p.Lys1183Arg): Allelfrequenzen EDTA-Vollblut=100%, PAX-Tumor=100%, FFPE-Tumor=99,9%; Populationsallelfrequenz Europa: 0.35490000; Reference SNP (refSNP) Cluster Report rs16942, ClinVar Variation ID 41818, Klassifizierung: benign

NM_007294.3(BRCA1):c.4308T>C (p.Ser1436=): Allelfrequenzen EDTA-Vollblut=100%, PAX-Tumor=100%, FFPE-Tumor=99,9%; Populationsallelfrequenz Europa: 0.35789999; Reference SNP (refSNP) Cluster Report rs1060915, ClinVar Variation ID 125703, Klassifizierung: benign

NM_007294.3(BRCA1):c.4837A>G (p.Ser1613Gly): Allelfrequenzen EDTA-Vollblut=100%, PAX-Tumor=100%, FFPE-Tumor=99,9%; Populationsallelfrequenz Europa: 0.35980001; Reference SNP (refSNP) Cluster Report rs1799966, ClinVar Variation ID 41827, Klassifizierung: benign

NM_000059.3(BRCA2):c.3807T>C (p.Val1269=): Allelfrequenzen EDTA-Vollblut=100%, PAX-Tumor=100%, FFPE-Tumor=100%; Populationsallelfrequenz Europa: 0.19980000; Reference SNP (refSNP) Cluster Report rs543304, ClinVar Variation ID 126022, Klassifizierung: benign

NM_000059.3(BRCA2):c.4563A>G (p.Leu1521=): Allelfrequenzen EDTA-Vollblut=100%, PAX-Tumor=100%, FFPE-Tumor=100%; Populationsallelfrequenz Europa: 0.99900001; Reference SNP (refSNP) Cluster Report rs206075, ClinVar Variation ID 132779, Klassifizierung: benign

NM_000059.3(BRCA2):c.6513G>C (p.Val2171=): Allelfrequenzen

EDTA-Vollblut=100%, PAX-Tumor=100% FFPE-Tumor=100%; Populationsallelfrequenz Europa: 0.99174529; Reference SNP (refSNP) Cluster Report rs206076, ClinVar Variation ID 132780, Klassifizierung: benign

NM_000059.3(BRCA2):c.7397C>T (p.Ala2466Val): Allelfrequenzen EDTA-Vollblut=100%, PAX-Tumor=100%, FFPE-Tumor=100%; Populationsallelfrequenz Europa: 0.99900001; Reference SNP (refSNP) Cluster Report rs169547, ClinVar Variation ID 52317, Klassifizierung: benign

4 Discussion

The detection of mutations in *BRCA1* and *BRCA2* genes in patients with ovarian cancer is of high importance. It is crucial for the detection of hereditary cancer syndromes like HBOC and for decisions regarding therapies with PARP inhibitors. Recently published work shows that fewer than one in five individuals with a history of breast cancer or ovarian cancer meeting select National Cancer Comprehensive Network (NCCN) criteria have undergone genetic testing (42, 43). It is estimated that in Austria the situation regarding genetic testing is comparable to the situation in the U.S.

Approximately 44% of ovarian cancer patients without a corresponding case of cancer in their maternal or paternal lines have been shown to have a heriditary mutation in *BRCA1* or *BRCA2* genes (15). Treatment response to platinum based chemotherapy is better for ovarian cancer patients who have mutations in *BRCA* genes. Additionally, these patients have the opportunity to benefit from a PARP inhibitor therapy. Thus it is of clinical importance to track down both germline and somatic mutations in *BRCA* genes in patients with ovarian cancer. Up to now mainly DNA from patients' blood is analyzed for the presence of heriditary *BRCA* mutations. By this means patients with HBOC are identified with the option of being included in an early detection programme versus the option of having prophylactic surgery. To track down germline and somatic *BRCA* mutations in tumor tissue to identify patients eligible to benefit from a PARP inhibitor therapy DNA from tumor samples has to be analyzed (28). These facts underline on the one hand the importance of broadening genetic counseling and testing in patients with HBOC and on the other hand the need of establishing and providing a

structure for testing for *BRCA1* and *BRCA2* hereditary and somatic mutations from a readily available material that is routinely taken during surgery for ovarian cancer. This is of particular interest for smaller hospital networks to achieve diagnostic independence from remote genetics laboratories and to save costs.

The analysis of *BRCA1* and *BRCA2* genes on FFPE material is difficult due to the denaturated DNA extracted from these tissue samples. In the present study we used a commercially available gene panel and NGS on DNA obtained from FFPE tumor samples to determine the usability of this material in medical routine operations of our local hospital network. The analysis of three high grade serous ovarian carcinomas, one fallopian tube carcinoma and one non-cancerous sample of ovarian tissue of a patient with HBOS identified one (20%) pathogenic mutation in *BRCA1* and one (20%) Class 3 VUS in *BRCA2*. As shown by analysis of DNA from matched patients' blood both the *BRCA1* mutation and the *BRCA2* VUS were germline. Although in this study the number of patients is limited it could be demonstrated that the detection of mutations and VUS in both FFPE material and matched blood is feasible using the BRCA Tumor MASTR Plus Dx gene panel performed on a NGS platform. Additionally, the analysis of polymorphisms from FFPE tissue and matched blood showed reliable results.

The scope of results obtained is limited by the small number of patients included in the assessment and the fact that only one mutation and one VUS were detected, both of whom were germline. Furthermore, the analysis of CNV was limited to blood derived DNA. Therefore the detection of somatic CNV and large rearrangements, respectively, from FFPE tumor tissue was not feasible. Possible inactivation of *BRCA1* and *BRCA2* genes by promotor methylation occuring in about 15% of ovarian carcinomas were not recorded.

Interestingly, the patient with the Class 3 VUS had her intial diagnosis of stage IIIC high grade ovarian cancer at the age of 69 in September, 2010. After debulking surgery she received six cycles of carboplatin and paclitaxel chemotherapy starting November, 2010. At first platinum sensitive recurrence a second six cycles of carboplatin and paclitaxel chemotherapy in combination with Avastin were given beginning June, 2012. At second platinum sensitive relapse she received six cycles of topotecan starting August, 2014 and subsequently six cycles of

trabectedin and doxorubicin hydrochloride chemotherapy starting August, 2015. It can be speculated that the relatively good course of ovarian cancer in our patient is due to an impairment of the *BRCA1* or *BRCA2* gene, an idea supported by the detection of a germline Class 3 VUS in BRCA2 that has not been described up to now (44). According to recommendations published by Plon et al. Class 3 variants carry sufficient uncertainty such that no clinical predictive testing should be done on other relatives and further data are needed before any action is undertaken based on the test result. It is also possible that Class 3 variants represent variants with intermediate clinical effects or low penetrance alleles. Further data are needed to verify this hypothesis. Overall, given the uncertainty, clinical advice for carriers of a Class 3 variant and their family members should depend on the pattern of cancer in the family, tumor histology, etc. rather than the presence or absence of the variant (34).

However, tracing the maternal line of the patient revealed no history of breast or ovarian cancer. Tracing the paternal line was not possible because our patient's father passed away when she was still a baby and no further contact was maintained with her father's relatives. If there were a history of breast or ovarian cancer in the patient's paternal line it might be an indication that the detected VUS in *BRCA2* equates to a pathogenic mutation. However, the finding will be discussed in the interdisciplinary tumor board to deal with the issue whether the administration of a PARP inhibitor is appropriate or not.

Breast tumors are fort he most part driven by estrogen. It is suggested that the hormonal removal by bilateral ovarectomy inhibits the development of breast tumors (45). Additionally, prophylactic ovarectomy prevents both ovarian cancer and breast cancer. Moreover, harbouring pathogenic *BRCA* mutations increases the risk of developing fallopian tube and peritoneal carcinoma. Rebbeck et al. published data of a large, retrospective analysis of 551 *BRCA* carriers, PBSO was found to have reduced the risk of ovarian cancer by 96% and breast cancer by 53% at a mean follow-up of 9 years (46). Kauff et al. published similar findings observed in a prospective study of 170 *BRCA* carriers. During a mean follow-up of 2 years, the incidence of ovarian or peritoneal carcinoma and breast cancer was significantly greater amongst those women who selected surveillance than amongst those who chose to undergo PBSO (47). In a multicentre prospective

study, the same investigators found that, during a 3-year follow-up, PBSO was associated with 85% reduction in *BRCA1*-associated gynecologic cancer risk and 72% reduction in *BRCA2*-associated breast cancer risk. Although protection against *BRCA1*-associated breast cancer and *BRCA2*-associated gynecologic cancer was suggested, neither effect reached statistical significance. The authors postulate that the protection conferred by PBSO against breast and gynecologic cancers may differ between the carriers of *BRCA1* and *BRCA2* mutations (48).

Rebbeck et al. carried out a meta-analysis of ten studies which investigated breast or gynecologic cancer outcomes in *BRCA1/2* mutation carriers who had undergone PBSO and concluded that PBSO was associated with a statistically significant reduction in breast cancer risk amongst these mutation carriers. Interestingly, similar risk reductions were found in *BRCA1* mutation and in *BRCA2* mutation carriers. Furthermore, PBSO was strongly associated with reductions in the risk of *BRCA1/2*-associated breast, ovarian, and fallopian tube cancers, and should provide guidance to women in planning cancer risk-reduction strategies (49). Another prospective study by Domchek et al. with a short-term follow-up shows that PBSO was associated with a 90% reduction in breast cancer-specific mortality, a 95% reduction in gynecologic cancer-specific mortality, and a 76% reduction in overall mortality (50). This demonstrates the benefit of PBSO in patients carrying mutations in *BRCA* genes especially since PBSO is a laparoscopic and hence minimally invasive surgical intervention (51, 52).

The HBOC patient carrying the pathogenic germline mutation in *BRCA1* gene detected by a human genetics laboratory earlier decided to have prophylactic surgery and PBSO was performed in our hospital in March, 2017. Subsequent analysis of DNA from the non-cancerous FFPE ovarian tissue of the patient showed the same deleterious mutation c.904delG in *BRCA1* gene demonstrating the validity of our analysis.

In summary, our study shows that a commercially available gene panel (BRCA Tumor MASTR Plus Dx CE-IVD, Multiplicom, Agilent Technologies) on a NGS platform efficiently detects germline mutations, VUS and polymorphisms in *BRCA1* and *BRCA2* genes using DNA from both FFPE tumor specimens and matched blood samples. These findings proof this method sufficient for routine use in our

local hospital network. The major limitation of the study is that it was not able to show that it is efficient to detect somatic mutations in *BRCA1* and *BRCA2* genes from FFPE material. This is due to the very limited number of available patients where possibly no one harboured a somatic mutation. A new approach is the *BRCA* analysis using formalin-free solutions for the simultaneous preservation of histomorphology and biomolecules and the purification of high-quality RNA, DNA, and miRNA. Fixed tissue can be processed and embedded in paraffin similarly to formalin-fixed tissue (53, 54). However, further analyses on more tumor specimens of ovarian cancer patients are needed to find the test also working for somatic mutations. Using a new assay of the same vendor that will be due soon should overcome the inability of the currently used gene panel to detect somatic CNV and large rearrangements, respectively, from FFPE tumor tissue.

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