Whole Exome Analysis to Select Targeted Therapies for Patients with Metastatic Breast or Advanced Gynecological Cancer -A Feasibility Study-

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Abstract

In this feasibility study we applied next generation sequencing (NGS) based whole exome sequencing (WES) of tumor tissue and peripheral blood of patients with metastatic breast (MBC, n = 44) or advanced gynecological cancer (AGC, n = 8). The purpose was to select targeted therapies according to “ESMO Scale for Clinical Actionability of molecular Targets (ESCAT)”. Data interpretation was further supported by a browser-based Treatment Decision Support platform (MH Guide™, Molecular Health, Heidelberg, Germany).

We identified 25 genomic alterations (GAs) with ESCAT LoE I or II in 18/32 MBC patients, which were available for evaluation: three copy number gains in ERBB2, two gBRCA1, two gBRCA2, six PIK3CA, one ESR1, three PTEN, one AKT1 and two ERBB2 mutations. In addition, five samples displayed MSI-H. In AGC we found one somatic BRCA1 mutation and one patient with a MSI-H endometrial cancer out of seven evaluable patients.

Resulting treatment options were discussed in the molecular tumor board and could be recommended in a small but relevant proportion of patients with MBC (7/18). However, WES still is a technical challenge with sometimes long processing times and high costs. With a customized panel (38 genes), we want to shorten time for analyses and optimize selection of targeted therapies.

Introduction

Metastatic breast (MBC) and advanced gynecological cancer (AGC) are still incurable diseases [1]. Routine therapy options are limited or accompanied by relevant side effects. Therefore, targeted therapy to optimize treatment adherence and outcome has emerged as the preferred approach in past years.

Alongside the decoding of the human genome „precision medicine” moved into the focus and became a reachable goal. The molecular genetic profile of a tumor, a metastatic lesion or even germline status of a patient can give a valuable direction for personalized therapy options [2]. The exome contains all genetic information which remains after transcription into and splicing of RNA (mature RNA) and thus represents the coding genome. Whole exome sequencing (WES) by identifying genomic alterations (GAs) can therefore give crucial insights into the cellular features which cause and drive cancer development and progression, respectively, and may inform about potential therapeutic strategies [3]. GAs detected by next generation sequencing (NGS) include mutations (single nucleotide variants such as missense, nonsense, splice-site mutations) as well as copy number variations (CNV). Identifying cancer-causing and driving genes activated due to somatic CNV partly also led to specific therapeutic approaches [3].

The first targeted therapy for BReast CAncer Gene (BRCA)-mutated ovarian cancer was approved in 2014 being the Poly-ADP-Ribose-Polymerase (PARP)-Inhibitor olaparib. In patients with relapsed, platinum-sensitive, high-grade serous ovarian cancer olaparib significantly improved progression-free survival [4], which represents a milestone in the systemic treatment. Meanwhile, further PARP-Inhibitors were developed to treat ovarian cancer (niraparib [5] and rucaparib [6]) or approval was gained for gBRCA-mutated breast cancer (olaparib and talazoparib [7, 8]), as well as for BRCA-mutated metastatic castration-resistant prostate [9] and pancreatic cancer [10] (olaparib). Therefore, in individual cases targeted therapies approved in other tumor types may also be an option for off-label use. Other GAs provide hints for ineffectiveness or toxicity of certain
therapies. There is for example good evidence for mutations in retinoblastoma 1 (Rb1) causing resistance of CDK4/6 inhibitors in MBC patients [11, 12]. Moreover since 2020 it is recommended to test patients for lack of dihydropyrimidine dehydrogenase (DPD) before starting a therapy with fluorouracil or with the related medicines such as capecitabine [13]. The enzyme DPD, which is deficient in a significant proportion of the general population, is needed to break down fluorouracil. Accumulation of fluorouracil in the blood can lead to severe and life-threatening side effects [13].

However, obtaining information of molecular genetic properties to choose a targeted therapy is still technically challenging and clinical variant interpretation is not trivial. Further the actual challenge of personalized medicine is to combine the clinical information of a specific patient and the molecular properties of its tumor with the existing biomedical knowledge to offer a personalized treatment.

To get a comprehensive picture of a patient’s GAs we present a feasibility study that explores both the somatic (tumor/metastatic tissues) and germline (white blood cells) mutational status. Therefore, we performed WES of patients with MBC or AGC and recommended individualized targeted therapy accordingly.

Results

GAs detected by WES in patients with MBC

Out of the total number of 44 screened MBC patients 32 were available for data evaluation; 21 patients harbored hormone receptor positive (HR+) disease, five presented with HER2+ MBC and six with triple negative breast cancer (TNBC). In total we detected 481 GAs in 77 different genes. This comprises 253 mutations, 223 CNVs and five samples showed high microsatellite instability (MSI-H). Most alterations were found in the XPC gene (n = 22), followed by the genes FCGR3A (n = 19) and MTHFR (n = 17) as well as CYP2C19 (n = 17), which were exclusively mutations. We detected a minimum of three and a maximum of 32 GAs per patient (median 16.5, mean 15.7).

ESCAT Level of evidence (LoE) I or II in MBC patients

According to ESMO recommendations 25 GAs from 18 patients belong to ESCAT LoE I or II [14] (see Fig. 1).

Most of the patients (12/18; 67%) had one actionable GA, five had two therapeutic options and one patient had three GAs with two possible therapy recommendations. The detected alterations of Level IA were: three copy number gains (CNG) in ERBB2, two BRCA1, two BRCA2 and six PIK3CA mutations. MSI-H as a molecular target of level 1C was identified in five samples. One ESR1 mutation, three PTEN mutations, one AKT1 mutation and two ERBB2 mutations were grouped in Level II A+B. Table 1 shows the detected mutations in detail and the clinical consequences which were taken.
Table 1
GAs of our MBC patients with LoE I or II according to ESMO/ESCAT and clinical consequences drawn. Table adapted from [14]

<table>
<thead>
<tr>
<th>Alterations</th>
<th>LoE</th>
<th>GA in MBC patient</th>
<th>Variant</th>
<th>MBC Subtype</th>
<th>Same patient</th>
<th>Targeted Therapy Option</th>
<th>Consequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERBB2 amplification</td>
<td>IA</td>
<td>ERBB2 (CNG)</td>
<td>copy number: 4</td>
<td>HR+</td>
<td>♣</td>
<td>HER2-targeted</td>
<td>3</td>
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<tr>
<td>ERBB2 (CNG)</td>
<td></td>
<td>copy number: 4</td>
<td>HR+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERBB2 (CNG)</td>
<td></td>
<td>copy number: 7</td>
<td>HER2+</td>
<td></td>
<td></td>
<td></td>
<td>1, 2 and 3</td>
</tr>
<tr>
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<td>IA</td>
<td>BRCA1 p.R1645fs</td>
<td>c.4932_4933dup</td>
<td>HR+</td>
<td>♥</td>
<td>Olaparib</td>
<td>1 and 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BRCA1 p.Q1756fs</td>
<td>c.5266dup</td>
<td>HER2+</td>
<td>♦</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BRCA2 p.S2835*</td>
<td>c.8504C &gt; G</td>
<td>HR+</td>
<td>♠</td>
<td></td>
<td>1 and 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BRCA2 p.S1271*</td>
<td>c.3812C &gt; G</td>
<td>HR+</td>
<td>♠</td>
<td></td>
<td>1 and 2</td>
</tr>
<tr>
<td>PIK3CA mutation</td>
<td>IA</td>
<td>PIK3CA p.H1047R</td>
<td>c.3140A &gt; G</td>
<td>HR+</td>
<td>$</td>
<td>Alpelisib</td>
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<tr>
<td></td>
<td></td>
<td>PIK3CA p.H1047R</td>
<td>c.3140A &gt; G</td>
<td>HR+</td>
<td>♣</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PIK3CA p.H1047R</td>
<td>c.3140A &gt; G</td>
<td>HR+</td>
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<tr>
<td></td>
<td></td>
<td>PIK3CA p.H1047R</td>
<td>c.3140A &gt; G</td>
<td>HR+</td>
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<td>#</td>
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<tr>
<td></td>
<td></td>
<td>PIK3CA p.E545K</td>
<td>c.1633G &gt; A</td>
<td>HR+</td>
<td>∞</td>
<td></td>
<td>3</td>
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<tr>
<td></td>
<td></td>
<td>PIK3CA p.E545K</td>
<td>c.1633G &gt; A</td>
<td>HR+</td>
<td>Ω</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Microsatellite instability (MSI)</td>
<td>IC</td>
<td>MSI-H</td>
<td>Instability score: 0,37</td>
<td>HR+</td>
<td>♦</td>
<td>Pembrolizumab</td>
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<tr>
<td></td>
<td></td>
<td>MSI-H</td>
<td>Instability score: 0,35</td>
<td>HR+</td>
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<td></td>
<td></td>
<td>MSI-H</td>
<td>Instability score: 0,48</td>
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<tr>
<td></td>
<td></td>
<td>MSI-H</td>
<td>Instability score: 0,31</td>
<td>HER2+</td>
<td>♦</td>
<td></td>
<td>#</td>
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<td>Alterations</td>
<td>LoE</td>
<td>GA in MBC patient</td>
<td>Variant</td>
<td>MBC Subtype</td>
<td>Same patient</td>
<td>Targeted Therapy Option</td>
<td>Consequences</td>
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<td>ESR1 mutation</td>
<td>IIA</td>
<td>ESR1 p.Y537C</td>
<td>c.1610A &gt; G</td>
<td>HR+</td>
<td></td>
<td></td>
<td>5</td>
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<tr>
<td>PTEN mutation</td>
<td>IIA</td>
<td>PTEN p.Y225fs</td>
<td>c.673dupT</td>
<td>HR+</td>
<td>~</td>
<td>capivasertib plus fulvestrant</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PTEN p.T319fs</td>
<td>c.955dupA</td>
<td>HR+</td>
<td>♠</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PTEN p.R130*</td>
<td>c.388C &gt; G</td>
<td>TNBC</td>
<td>Θ</td>
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<td>#</td>
</tr>
<tr>
<td>AKT1 mutation</td>
<td>IIB</td>
<td>AKT1 p.E17K</td>
<td>c.49G &gt; A</td>
<td>HR+</td>
<td>•</td>
<td></td>
<td>#</td>
</tr>
<tr>
<td>ERBB2 mutation</td>
<td>IIB</td>
<td>ERBB2 p.V777L</td>
<td>c.2329G &gt; T</td>
<td>HR+</td>
<td>Y</td>
<td>Neratinib</td>
<td>#</td>
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<tr>
<td></td>
<td></td>
<td>ERBB2 p.V842I</td>
<td>c.2524G &gt; A</td>
<td>HR+</td>
<td>Y</td>
<td></td>
<td>#</td>
</tr>
</tbody>
</table>

Consequences: 1 = none, known before, 2 = Therapy already taken, 3 = not suitable regarding medical history, 4 = high toxicity expected, 5 = the general health at the time the genetic test result was available did not allow the implementation, # recommended; ♠♦♥♣♠♦♥♣♦♥♣♦♥♣♦♥♣ indicating the same patient (see also Table 2).

MSI instability scores above MANTIS cutoff threshold 0.3 [40]

Only one out of five patients with HER2+ MBC (HER2 status according to clinical routine assessment) harbored a CNG in ERBB2, based on NGS CNV analysis. Contrarily, CNG in ERBB2, based on NGS CNV analysis, were reported in two of the patients with HR+/HER2- tumor. Further, in one patient diagnosed with HR+/HER2- tumors we detected two somatic ERBB2 mutations (ERBB2 p.V777L and ERBB2 p.V842I).

In the HR+/HER2- subgroup one patient was carrier of a gBRCA1 mutation (BRCA1 p.R1645fs) and two patients were identified with a gBRCA2 mutation (BRCA2 p.S2835*, BRCA2 p.S1271*), respectively. Interestingly the patient with gBRCA1 mutation additionally had a somatic BRCA2 mutation (BRCA2 p.S3250*, ESCAT LoE IIIA) and one patient with a gBRCA2 mutation (BRCA2 p.S2835*) also had an PTEN mutation (PTEN p.T319fs). Further, one gBRCA1 mutation (BRCA1 p.Q1756fs) was found in a patient with HER2+ MBC. No gBRCA mutations were seen in the subgroup of TNBC patients. All detected gBRCA mutations were also confirmed by routine genetic analysis due to young age or family history according the criteria for genetic counselling in Germany [15].

All spotted PIK3CA mutations (four times PIK3CA p.H1047R and twice PIK3CA p.E545K) were seen in the subgroup of HR+ patients (29% of HR+ patients).
MSI-H tumors were detected in all subgroups (three times HR+, once HER2 + and TNBC respectively).

The *ESR1* p.Y537C and the *AKT1* p.E17K mutation and two of the three *PTEN* mutations (*p.Y225fs* and *p.T319fs*) were found in HR + MBC patients. One *PTEN* nonsense mutation (*p.R130*) was detected in a TNBC patient.

**ESCAT LoE III or IV in MBC**

We detected 20 GAs grouped in LoE III or IV according to ESCAT. In Level IIIA we detected two somatic *BRCA2* mutations (*p.S3250*; *p.S368*) in the subgroup of HR+/HER2- patients and one somatic *BRCA2* mutation (*p.E1879fs*) in a HER2 + tumor. In LoE IV we found one *ARID1A*, one *ATM*, five *CDH1*, two *NF1* and seven *TP53* mutations, as well as one *MYC CNG* (for detail see Table 2).
Table 2
GAs of our MBC patients with LoE III and IV according to ESMO/ESCAT. ♥ ∞ * ◊ § & ~ Ω Θ indicating the same patient (see also Table 1).

<table>
<thead>
<tr>
<th>Alterations</th>
<th>LoE</th>
<th>GA in MBC patient</th>
<th>Variant</th>
<th>Subtyp</th>
<th>Same patient</th>
</tr>
</thead>
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<tr>
<td>Somatic BRCA 1/2 mutation</td>
<td>IIA</td>
<td>BRCA2</td>
<td>c.9749C &gt; G</td>
<td>HR+</td>
<td>♥</td>
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<tr>
<td></td>
<td></td>
<td>p.S3250*</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>BRCA2</td>
<td>c.1103C &gt; A</td>
<td>HR+</td>
<td>∞</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p.S368*</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>BRCA2</td>
<td>c.5636_5676del</td>
<td>HER2+</td>
<td>*</td>
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<tr>
<td></td>
<td></td>
<td>p.E1879fs</td>
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<tr>
<td>ARID1A/B</td>
<td>IVA</td>
<td>ARID1A</td>
<td>c.3988C &gt; T</td>
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</tr>
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<td></td>
<td></td>
<td>p.Q1330*</td>
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<td></td>
<td>TNBC</td>
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<tr>
<td>ATM (som/g mutation)</td>
<td>IVA</td>
<td>ATM</td>
<td>c.8509G &gt; T</td>
<td>HR+</td>
<td>◊</td>
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<tr>
<td></td>
<td></td>
<td>p.E2837*</td>
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<tr>
<td>CDH1 mutation</td>
<td>IVA</td>
<td>CDH1</td>
<td>c.67C &gt; T</td>
<td>HR+</td>
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<tr>
<td></td>
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<td>p.Q23*</td>
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<tr>
<td></td>
<td></td>
<td>CDH1</td>
<td>c.2629G &gt; T</td>
<td>HR+</td>
<td>§</td>
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<tr>
<td></td>
<td></td>
<td>p.G877*</td>
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<tr>
<td></td>
<td></td>
<td>CDH1</td>
<td>c.2552C &gt; A</td>
<td>HR+</td>
<td>∞</td>
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<tr>
<td></td>
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<td>p.S851*</td>
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<tr>
<td></td>
<td></td>
<td>CDH1</td>
<td>c.1397_1398del</td>
<td>HR+</td>
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<tr>
<td></td>
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<td>p.L466fs</td>
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<td></td>
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<td>CDH1</td>
<td>c.476del</td>
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<td>p.P159fs</td>
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<tr>
<td>MYC</td>
<td>IVA</td>
<td>MYC (CNG)</td>
<td>Copy number 6</td>
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<td>NF1 mutation</td>
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<td>NF1</td>
<td>c.4925_4926insCGTGGCTCAAA</td>
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<tr>
<td></td>
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<td>p.D16444fs</td>
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<td></td>
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<td>NF1</td>
<td>c.8059_8060del</td>
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<td>p.S2687fs</td>
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<tr>
<td>TP53 mutation</td>
<td>IVA</td>
<td>TP53</td>
<td>c.637C &gt; T</td>
<td>HR+</td>
<td>♥</td>
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<tr>
<td></td>
<td></td>
<td>p.R213*</td>
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<td></td>
<td></td>
<td>TP53</td>
<td>c.742C &gt; T</td>
<td>HR+</td>
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<td>p.R248W</td>
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<td></td>
<td></td>
<td>TP53</td>
<td>c.881_885del</td>
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<td></td>
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<td>p.E294fs</td>
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<td></td>
<td></td>
<td>TP53</td>
<td>c.853G &gt; A</td>
<td>HR+</td>
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<td>p.E285K</td>
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<td></td>
<td></td>
<td>TP53</td>
<td>c.610G &gt; T</td>
<td>HER2+</td>
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<tr>
<td></td>
<td></td>
<td>p.E204*</td>
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</tr>
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</table>
### GAs (including actionable) detected by WES in patients with AGC

From 8 patients with AGC selected 7 were available for analysis. We found 53 GAs in 28 different genes (see Fig. 2) with a minimum of two and a maximum of 24 GAs per patient. As actionable GAs we detected one somatic *BRCA1* mutation in a patient with ovarian cancer and one patient had a MSI-H endometrial cancer.

### Copy number variations in known onco-targets

The following known onco-targets [16] with copy number loss were detected in our MBC patients: *RB1* (n = 5), *PTEN* (n = 4), *CDKN2B* (n = 2), *NF1* (n = 5), *SMAD4* (n = 1), *BRCA1* (n = 9), *BRCA2* (n = 4). CNG were identified for *ERBB2* (n = 3, as already described), *EGFR* (n = 1), *MYC* (n = 1), *PIK3CA* (n = 7), *FGFR1* (n = 5), *FGFR2* (n = 3), *KRAS* (n = 5), *CCND1* (n = 4), *MET* (n = 3), and *CDK6* (n = 4). In AGC we detected one *FGFR1* CNG in a patient with endometrial cancer.

### GAs indicating ineffectiveness or safety concerns according to MH Guide®

In addition to the actionable targets, we have also identified 92 GAs in 25 different genes that indicate ineffectiveness of a particular drug in patients with MBC (mutations n = 22, MSI-H n = 5, CNV n = 65). From a clinical point of view [11, 17, 18] the most relevant GAs were: *ESR1* (mutation, n = 1; as already described), *RB1* (mutation, n = 2), *CCNE1* (CNG, n = 5), *FGFR1* (CNG, n = 5) and *PIK3CA* (mutation, n = 6; as stated before). Other GAs of clinical interest due to possible ineffectiveness [11, 19–22] detected in our collective are: *NF1* (CNL, n = 5), *AR* (CNG, n = 4), *CDK6* (CNG, n = 4), *MET* (CNG, n = 3), *ERBB2* (mutation, n = 2; as specified above), *LRP1B* (CNL, n = 2) and *FGFR2* (CNG, n = 1) (see Fig. 3).

The *RB1* mutations (*RB1*p.L199fs and *RB1*p.E184) and three of the *CCNE1* CNG were seen in the HR+/HER2- subgroup which may cause failure of CDK4/6 treatment. Further *ESR1* mutation and *FGFR1* CNG can be associated with endocrine resistance and was also altered exclusively in this subgroup. However, *PIK3CA* mutations (as mentioned above), which may indicate trastuzumab resistance, were also detected exclusively in the HR+/HER2- subgroup.

Toxicity concerns were raised for 214 GAs in 22 different genes, with mutations in *XPC* (n = 22), *MTHFR* (n = 17), *CYP2C19* (n = 17) and *CYP2D6* (n = 16) being the most altered ones (see Fig. 4).
A DPD mutation was identified in four patients (two HR+ and two TNBC), which is highly important from a clinical perspective as it increases the likelihood of relevant adverse events during therapy with fluorouracil. Further we detected three EGFR mutations, which may be a driver of tumorigenesis in breast cancer and/or may indicate therapy resistance since GAs have been found to develop under drug pressure [23].

In AGC ineffectiveness was stated only in four GAs (twice KRAS mutation, once CYP2D6 mutation and once FGFR1 CNG). Toxicity concerns arose in 34 GAs (exclusively mutations) regarding 12 different genes with ABCC2 (n = 6), CYP2C8 (n = 6), ERCC2 (n = 5) and MTHFR (n = 4) being most affected (independent of tumor subtype; see Fig. 2).

Discussion

Here we present a feasibility study performing NGS-based WES in patients with MBC or AGC. We have established a working procedure for patient recruitment, sample collection, collaboration between different institutions (Department of Gynecology and Obstetrics, Department of Pathology and Cancer Genetics Laboratory) and discussion of the results in a molecular tumor board. Due to the availability of new targeted therapies whose indications are based on GAs, there is an increasing need for genetic analyses at an early stage of metastatic/advanced breast or gynecological cancer. Because of tumor heterogeneity and changes during carcinogenesis or the metastatic process, several analyses during the course of disease may even be useful and necessary (e.g. regarding a somatic PIK3CA mutation) [24, 25]. In 2017 the FDA approved two comprehensive mid-size panels for genetic testing in cancer (Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) and FoundationOne CDx) which addressed the unmet need for precision oncology [26]. Nevertheless, it is a question of time, costs, and technical equipment to be able to perform genetic tests and, above all, to make them available for clinical use. For instance, the relatively high dropout rate (25%) of our patient population due to sample errors (too little tumor tissue or insufficient DNA quality) is in line with data published by another research group [27]. For clinical decision-making it is further necessary to know the patient’s medical history, tumor subtype, possible secondary diagnoses or concomitant therapies, and allergies. These are undoubtedly essential and must be included in the discussion of treatment decisions in a molecular tumor board.

We identified 481 GAs in patients with MBC and 53 in patients with AGC. These include CNV, mutations and MSI-H. According to the 2019 ESMO criteria for MBC, 25 GAs were classified as ESCAT level I-II. In 7/18 patients with MBC (39%), we were able to recommend targeted therapy according to ESCAT level I-II [28] with one patient having two options and one patient having two GAs leading to the same therapy recommendation. Precisely we suggested four times pembrolizumab if MSI-H was detected, once alpelisib for a PIK3CA mutation, once capivasertib plus fulvestrant if there was a PTEN loss-of-function mutation, once capivasertib plus fulvestrant for an AKT mutation and after reevaluation of the “likely pathogenic” ERBB2 mutations we could recommend neratinib in one patient diagnosed as HER2- MBC. In six patients, the possible targeted therapy was not suitable due to the individual medical history. This means that concomitant diseases did not allow the administration of the matching drug, or the concomitant medication did not fulfil the approval. Once, the high probability of side effects and the patient’s advanced age spoke against with the optional targeted therapy. Unfortunately, the general health of six patients at the time the genetic test result was available did not allow the implementation of potential targeted therapy. In three patients no additional
therapeutic recommendations were made after WES, mainly because appropriate targeted therapies were already in use based on findings of routine diagnostic (HER2-targeted therapies or olaparib). However, this also means that we recognize these findings in clinical routine and do not miss these GAs (see Table 1).

The differences in HER2 status detected by NGS (ERBB2 CNG) compared to immunohistochemistry or FISH could be explained by tumor heterogeneity [29, 30] or by changes in HER2 status during the course of the disease [31], which are common phenomena in MBC. ERBB2 mutations occur in approximately 2% of patients with breast cancer and could expand treatment options to targeted HER2 therapy in patients with HER2-PT. In our collective, we detected two likely pathogenic ERBB2 mutations (6%), which is significantly more compared to the current literature [32]. This may be explained mainly due to our small number of patients. Hempel et al. detected an ERBB2 amplification by NGS in nine out of 41 patients with advanced breast cancer (out of which seven had a HER2 + tumor by immunohistochemistry and/or in situ hybridization) and ERBB2 mutation in two patients. They conclude that a threshold must be defined on basis of CNV to allow a better interpretation of NGS based amplification analysis. Further, performing NGS analyses they could recommend promising treatment options according ESCAT Level I in 58.5% of their patients (ERBB2 mutation n = 9, PIK3CA mutation n = 14, MSI n = 1) [33].

At our AGC patients the somatic BRCA1 mutation in one patient with ovarian cancer was also known before and PARP-Inhibitor already administrated. The patient with endometrial cancer and MSI-H unfortunately died before the findings were known. Sawada et al. detected in 19 out of 20 patients actionable GAs in gynecological cancer. Out of these seven received a genotype-matched therapy with a response rate of 43% (one patient with a PTEN mutation received everolimus, another patient having a TSC2 mutation was treated with everolimus and letrozole, and the third patient had a BRIP1 mutation which was treated with olaparib) [34]. Consequently, according to our results and those of others, targeted therapy based on molecular findings is appropriate for a significant proportion of patients [35]. In this context, the selection of suitable patients and definition of targets are crucial [27, 33, 36, 37].

Due to the late stage of disease or irrelevance for the current treatment option none of the ineffectivity or safety concerns based on GAs changed our clinical therapy decision. However, in earlier stage of disease the detected GAs may give valuable hints concerning possible ineffectiveness or relevant toxicity of certain therapies. Similar to ESCAT a validated classification of these GAs is highly needed for clinical routine. According to the experts, due to its high clinical relevance, the ESR1 mutation, for example, although indicating endocrine resistance, was included in ESCAT.

Focus of our feasibility study was to identify possibly druggable molecular targets. Besides, using WES we further detected several CNV mainly in MBC patients which until now have no suitable therapeutic agent or at least there is no sufficient clinical data. Their relevance stems from the fact that the expression level of a gene strongly correlates with its copy number [16, 38]. Somatic CNV typically arise during carcinogenesis and, if resulting in the deletion of tumor suppressor genes or the amplification of oncogenes, they are usually pathogenic [16].

Although we were able to implement the NGS based diagnostic workflow for patients with MBC or AGC in our center, several limitations must be considered. Different tumor cell clones harboring different somatic
mutations can divide a cancer into several subgroups (heterogeneity). In this context, the prognosis and response to treatment can be very unique for each clone [16]. In addition, the genomic profile of biopsy tissues provides a picture that is limited to only a single point in space and time. This may lead to an under-representation of intratumor heterogeneity [39], limiting the predictive value of a single tissue biopsy as in our study. Other limitations of our work are, first, the heterogeneous patient population recruited in routine clinical practice without randomization, follow-up, or survival data. Secondly, missing or very late tissue samples of varying quality and highly variable time points in the disease course and thirdly, the long processing time.

In line with ESMO recommendations, our data show that large gene panels and routine use of NGS result in few clinically significant responders. Nevertheless, the patient and physician may decide together to perform a large gene panel if the patient is informed that the likelihood of benefit is rather low. According to ESMO, the use of off-label drugs matched to GAs is only recommended when an access program and decision-making process are available [35].

Consequently, we have established a customized targeted therapy panel involving 38 genes (see supplement data). In this way, we can ensure time- and cost-effective results of possible actionable GAs accompanied by a promptly case presentation in the molecular tumor board.

**Conclusion**

In this feasibility study we demonstrate that WES using NGS for patients with MBC or AGC is technically possible and feasible. However, in oncology practice there are no recommendations from scientific societies about its use in daily routine [35]. Due to the low detection rate of truly actionable GAs leading to therapeutic consequences genetic testing is recommended only for a selected patient collective. In this context the molecular tumor board is an essential tool to choose appropriate patients and discuss the results. The identification of these molecular alterations among many possible targets and especially among the various alterations of the same target, is crucial. ESCAT provides a useful continuously updated tool for classifying GAs according to their clinical relevance which assists clinicians and molecular tumor boards in delivering accurate and individualized indications for the patients [28]. The browser-based Treatment Decision Support platform MH Guide™ can further help interpret the abundance of genetic data. Still there are challenges in daily clinical routine regarding partly long processing time and high costs with no coverage by the health care insurances. Consequently, the current low number of approved treatment options led us to implement a customized targeted therapy panel focusing on 38 known druggable target genes to optimize analysis time, reducing costs and to increase informativity and impact on therapeutic decision making.

**Patients, Materials And Methods**

**Patients**

We identified patients with MBC and AGC in our weekly tumor conference. From 2017 to 2020 52 Patients (44 with MBC and 8 with AGC) signed written informed consent to participate in our feasibility study ”Fighting therapy resistance in patients with solid tumors” (approved by the Ethics Committee of the Medical Faculty of the Heinrich Heine University Düsseldorf; Ref-No: 5673). We hereby confirm that all research was performed in
accordance with relevant guidelines and regulations as well as informed consent was obtained from all
participants. Inclusion criteria were: metastatic patient with solid tumor; no standard therapy option;
Karnowsky-Index > 70%; expected life span > 6 months. Accordingly, our patient collective was heavily
pretreated with several lines of chemo- and/or endocrine therapies.

The HR status and HER2 amplification was determined according to German clinical routine
(immunohistochemistry and/or in situ hybridization analysis) and taken from our department of pathology or
external reports if tumor tissue was requested from outside. 26/44 (59%) had a HR+ (HER2 non amplified),
7/44 (16%) a HER2 positive (HER2+; HR+ or HR-) and 10/44 (23%) had a TNBC. They experienced bone and
visceral (n = 16), visceral only (n = 9), cerebral (n = 9), bone only (n = 1) or other metastatic lesions. The
patients with AGC suffered from ovarian (2/8), vulva/vaginal (3/8), endometrial (2/8) or cervical cancer (1/8).
Clinical patient data are shown in Table 3.

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>N = 52</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC Subtype</td>
<td></td>
</tr>
<tr>
<td>HR+, HER2-</td>
<td>26</td>
</tr>
<tr>
<td>HER2+, HR+ or HR-</td>
<td>7</td>
</tr>
<tr>
<td>TNBC</td>
<td>10</td>
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<td>missing</td>
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</tr>
<tr>
<td>Gyn Cancertype</td>
<td></td>
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<tr>
<td>Ovarian</td>
<td>2</td>
</tr>
<tr>
<td>Vulva/vaginal</td>
<td>3</td>
</tr>
<tr>
<td>Endometrial</td>
<td>2</td>
</tr>
<tr>
<td>Cervical</td>
<td>1</td>
</tr>
</tbody>
</table>

Due to insufficient DNA quality and quantity or failed sequencing procedure only 39 out of the recruited 52
patients (32 MBC, 7 AGC) were available for WES and data interpretation (Fig. 5).

Blood and tumor tissue

Peripheral blood was obtained by routine vain puncture (two 10ml EDTA container) after DNA isolation
samples were stored at -20°C until further use. Suitable tumor tissue for analyses was identified according to
patients’ history (preferable most recent metastatic lesion). The tissue was obtained in clinical routine
diagnostics. From archived FFPE tissues 5µm thick sections were prepared and slides were analyzed by
experienced pathologists to determine tumor content (at least 20%) by staining with hematoxylin/ eosin and
immunohistochemistry. We analyzed tumor tissue from breast, liver, lymph node, brain, skin, lung, or bone.
DNA extraction and whole-exome sequencing

DNA was purified from FFPE tissue with the GeneRead DNA FFPE Kit (Qiagen, Venlo, The Netherlands). DNA of matched blood and tumor tissue samples were sequenced using the Agilent Sure Select XT V7 or the Illumina IDT Exome Analysis Kit. The libraries were sequenced on a HiSeq 3000 system (Illumina).

Data Analysis

Anonymized NGS data were transferred via secure VPN channels to a server hosted by Molecular Health GmbH, Heidelberg, Germany. Data were analyzed with the CE-marked *in vitro* diagnostic software (IVD) MH GUIDE™ (MH Guide). The MH Guide Variant detection pipeline (MH Guide VDP) uses input sequencing data in (raw) FASTQ format, MH Guide requires for the analysis of paired exomes (WES) of somatic samples a minimum average real coverage of >200x (the average real coverage is defined as on-target coverage after removal of duplicate read pairs), in which at least 80% of the target region has >100x average real coverage in the tumor sample. SNVs and Indels that passed the quality filters fulfilled the following quality parameters: PHRED score > 28.5, coverage > 20x, allele frequency > 5%, population frequency < 1%. The raw sequencing data (reads) are aligned through the MH Guide VDP using standard (GRCh37, HG19) or proprietary population specific human reference genomes (MH PHREGs) based on data from the 1000 genomes project for alignment, using LoFreq (PMID: 23066108) for variant calling for SNVs and atlas and freebayes (https://arxiv.org/abs/1207.3907) for Indel calling. The MH Guide VDP provides all detected variants in VCF format for transcript and protein mapping by MH Guide. In paired analyses, all variants detected in the control sample are considered to be germline variants.

For MSI detection MH Guide™ uses the tool MANTIS (Microsatellite Analysis for Normal-Tumor Instability) to detect MSI biomarkers from FASTQ input [MANTIS tool available at: https://github.com/OSU-SRLab/MANTIS] [40]. If the stepwise difference is ≥ 0.3, an MSI-H biomarker is automatically added to the variants list. This threshold of 0.3 is based on validation of 40 TCGA (Cancer Genome Atlas) cases from three cancer entities.

Data interpretation

MH Guide™ system screens all genetic alterations identified against the reference information in the proprietary knowledge platform, Dataome. The core of this platform is a manually curated database with evidence-based biomarker information on peer-reviewed published evidence - the so-called clinical variant interpretations (CVIs).

Information captured during the curation process of the CVI's include I) The variant – i.e. the type of genomic aberration (e.g. SNV, Insertion, Deletion etc.); II) The drug or treatment used in the underlying published peer-reviewed evidence (preclinical studies and clinical trials) for which the data source is mainly PubMed; III) The effect of the variant on treatment - i.e. response, resistance or safety; IV) The quantity of effect – e.g. strong, medium, weak; V) The observation context (i.e. the disease/disease stage or model system); VI) A link to the underlying evidence and a grading of its reliability. Based on the information provided by the MH Guide™ platform a GA was classified as "effective" (potential target with therapeutic options), "ineffective" (GA with evidence of less effectiveness or ineffectiveness when certain drugs are applied) and "safety" (GA raising concerns about potential toxicity when administering some medication).
Evidence was further underpinned using the European Society for Medical Oncology (ESMO) classification “ESMO Scale for Clinical Actionability of molecular Targets (ESCAT)” [41]. Clinical decision on therapy options were based on these actual ESCAT evidence tier I and II [14]. In short:

ESCAT evidence tier

I: “alteration-drug match is associated with improved outcome in clinical trials”

II: “alteration-drug match is associated with antitumor activity, but magnitude of benefit is unknown”

III: “alteration-drug match suspected to improve outcome based on clinical trial data in other tumor type(s) or with similar molecular alteration”

IV: “pre-clinical evidence of actionability”

X: “lack of evidence for actionability”

**Abbreviations**

Advanced gynecological cancer (AGC)

BReast CAncer- Gene (BRCA)

Copy number gain (CNG)

Copy number variation (CNV)

Dihydropyrimidine dehydrogenase (DPD)

ESMO Scale for Clinical Actionability of molecular Targets (ESCAT)

Estrogen receptor (ER)

European Society for Medical Oncology (ESMO)

Genomic alteration (GA)

Germline (g)

Human epidermal growth factor receptor 2 (HER2)

Hormone receptor (HR)

Metastatic breast cancer (MBC)

Microsatellite instability (MSI)

Phosphoinositide-3-kinase (PI3K)
Poly-ADP-Ribose-Polymerase (PARP)

Primary tumor (PT)

Retinoblastoma 1 (Rb1)

Triple negative breast cancer (TNBC)

Whole-exome sequencing (WES)

**Declarations**

*Data Availability*

The sequencing data generated and analyzed during the current study will be deposited in the European Genome-phenome Archive prior to publication. Data will be made available to the handling editors and reviewers upon request. Please contact Bernadette Jaeger (Bernadette.Jaeger@med.uni-duesseldorf.de) who will share the datasets with permission from Tanja Fehm (Tanja.Fehm@med.uni-duesseldorf.de), Director of the Department of Obstetrics and Gynecology, University Hospital of the Heinrich-Heine University Duesseldorf, and responsible for ethical/legal issues as defined in the ethical approval of the study.

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*Additional Information*

Competing Interests Statement: The authors declare no potential conflicts of interest.

**References**


**Figures**

![Figure 1](image_url)

**Figure 1**

GAs in our MBC patients with potential therapeutical consequences (LoE I-II according to ESMO/ESCAT).
**Figure 2**

GAs detected in our patient collective with AGC.

**Figure 3**

GAs in our MBC patients with potential ineffectiveness concerning certain therapies.
Figure 4

GAs in our MBC patients with potential toxicity concerning certain therapies.

52 recruited patients
(44 MBC, 8 AGC)

51 patients suitable for WES
(43 MBC, 8 AGC)

41 patients analyzed
(34 MBC, 7 AGC)

39 patients for data interpretation
(32 MBC, 7 AGC)

Sample (tumor tissue) arrived too late
1 patient transferred for Panel-analyses in 2021

Excluded patient for WES
Sample never arrived/ unavailable or insufficient DNA quantity:
9 MBC, 1 AGC

Failed sequencing procedure:
2 MBC
Figure 5

Study cohort

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTableS1.pdf