

## Genetics and Oncology (part 2.)

# Fundamentals of personalised medicine in the treatment of breast and ovarian cancer

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Individualisation of medical management based on prognostic and predictive markers (personalised medicine) allows customisation of prophylaxis and optimisation of treatment by increasing its efficiency and minimisation of adverse effects. In the case of breast cancer, therapy selection is still based on histopathology and immunohistochemical assessment including analysis of estrogen receptor (ER) expression, progesterone receptor (PgR) expression and over-expression or amplification of receptor tyrosine kinase erbB-2 gene (*ERBB2* aka *HER2*). An additional role, facilitating decision on application or waiver of chemotherapy in early breast cancer, may be played by panels assessing gene expression within tDNA (tumour DNA, i.e. DNA isolated from tumour cells) and evaluation of concentration of uPA (urokinase-type plasminogen activator) and PAI-1 (plasminogen activator inhibitor type 1) in tumour cells. Growing hope surrounds the new, targeted therapies, including: inhibitors of CDK 4/6 (cyclin-dependant kinases 4 and 6), mTOR inhibitors (rapamycin's mammalian target), inhibitors of poly(ADP-ribose)polymerase(PARP) or inhibitors of PI3K (phosphatidylinositol-4,5-bisphosphate 3-kinases). For ovarian cancer, treatment selection is based on assessment of the histopathologic type, malignancy degree, FIGO classification and platinum sensitivity of the tumour. However, the increasing use of PARP inhibitors and angiogenesis inhibitors is noteworthy. In the context of personalised medicine for both these cancers, an important element involves also individualisation of prophylactic and therapeutic recommendations in carriers of germline mutations associated with hereditary cancer syndromes.

**Key words:** personalised medicine, breast cancer, ovarian cancer, predictive tests, prognostic tests, germline mutations

### Introduction to personalised medicine

Personalised therapies are currently among the most notable trends in medicine, especially in management of cancer patients. Progress in genetics and molecular pathology allowed selection of a number of biomarkers of patient-specific status. Their analysis enables selection of an optimal, individually tailored procedure. The said biomarkers can be diagnostic

(helpful in precise diagnosis), prognostic (allowing estimation of the probable course of the disease in terms of recurrence risk), and predictive (allowing prediction of the likely response to particular therapies, and therefore helpful in selecting personalised therapy). Normally, biological material from the tumour is used for marker evaluation which may be carried out at the level of genetic changes (using appropriately se-

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lected cytogenetic and/or molecular tests) as well as protein changes (usually using immunohistochemical methods). The concept of personalised medicine in oncology is very broadly defined and it includes choosing patient-specific treatment, taking into account both individual prophylaxis and the type, time and sequence of therapy, as well as doses of drugs used. Management adapted to the needs of an individual patient is aimed at increasing the effectiveness of prophylaxis and therapy and at reducing the frequency and intensity of side effects [1].

The following review presents basics of personalised medicine as applied in breast cancer and ovarian cancer patients, considering especially guidelines of the European Society for Medical Oncology (ESMO).

### Genetic profile of breast and ovarian cancer

The basis of the neoplastic transformation process lies in mutations. Their accumulation leads to genetic instability within neoplastic cells.

Most cancers, including most breast cancers (70–75%) and most ovarian cancers (75–90%) are sporadic in nature and develop as a consequence of accumulated somatic mutations, which are non-hereditary changes acquired during the individual's life and limited to the genome of neoplasm's cells. Presence of somatic mutations is thus limited to tDNA – DNA isolated from tumour cells. Characteristically, sporadic neoplasms are usually diagnosed in older age patients with no family history of cancer.

Some cancers, including 15–20% of breast cancers, are familial. In these cases, their origin is characterised by aggregation of neoplasms of a specific type among members of a family. In patients with familial neoplasms, multi-gene variations are observed in constitutive genome, which increase susceptibility to environmental cancerogenic factors. Therefore, familial neoplasms develop as a consequence of combined effect of constitutive genetic susceptibility and adverse environmental factors, which together lead to occurrence of mutations related to neoplastic transformation. However, the complexity and limited penetrance of constitutional variants do not allow their application as markers that would unequivocally define the individual risk of developing a cancer.

Hereditary neoplasms (developing on the basis of inherited mutation) are relatively rare e.g. hereditary breast cancer accounts for 5–10% of all breast cancer cases, and hereditary ovarian cancer for 10–25% of all ovarian cancer cases. They are characterised by unique clinical features. A suspicion of a hereditary cancer should be raised with such clinical features as: atypical cancer, young age of diagnosis (e.g. pre-menopausal breast cancer), multifocal and/or bilateral lesions, occurrence of two or more primary neoplasms in an individual or occurrence of neoplasms of the same spectrum in several members of a family. In patients with hereditary breast and/or

ovarian cancer, observations confirmed an increased lifetime risk of development of not only those cancers, but also other neoplasms of the spectrum characteristic of the syndrome in question. A hereditary cancer is associated with carrying a specific germline mutation, i.e. a hereditary, congenital mutation of a single gene which is present in all cells of the body, and therefore identified both in tDNA tests and on DNA isolated from cells outside the tumour (e.g. peripheral blood lymphocytes, saliva cells, oral mucosa cells, fibroblasts). Identifying people with hereditary breast and/or ovarian cancer is important not only because of the individualisation of prophylactic and therapeutic recommendations for such patients, but also considering the necessity to provide genetic counselling to other family members [1].

### Breast cancer – individualisation of therapy based on histological and immunohistochemical classification

Selection of treatment for patients with breast cancer is still based on histopathology and immunochemistry of the tumour.

Decisions concerning targeted therapy are mostly based on the tumour's biological profile. In the case of breast cancer, this profile refers to the immunohistochemical (IHC) analysis of expression of the estrogen receptor (ER), expression of the progesterone receptor (PgR) and overexpression of the human epidermal growth factor type 2 receptor (HER2) or (if this assessment is equivocal) analysis of amplification of receptor tyrosine kinase erbB-2 aka HER2 receptor gene (*ERBB2* aka *HER2*). The above biomarkers are diagnostic, prognostic and predictive, too (tab. I).

According to ESMO recommendations, examination of HER2 status should conform to standards of the American Society of Clinical Oncology – College of American Pathologists (ASCO-CAP). Additionally, amplification of *HER2* gene may be analysed by *in situ* hybridisation (ISH) or fluorescence *in situ* hybridisation (FISH), usually applied as an additional test in cases of equivocal immunochemistry results (+2) [2].

Each patient with an invasive breast cancer should have ER, PgR and HER2 status assessed, optimally in the biopsy specimen [2, 3]. In cases of equivocal or triple negative receptor status of the biopsy specimen, additionally post-operative material should be immunohistochemically tested. Furthermore, HER2 status should be re-evaluated in post-operative material in cases in which a test of the biopsy specimen revealed G1 ER+, PgR+, HER2+ NST breast cancer, as well as for selected cases of specific-type breast cancer. In all of the above situations, the postoperative results should be considered final [2]. ESMO also recommends that in cases of advanced breast cancer in the metastatic stage, at least one IHC assessment on biological material from a metastatic focus should be performed to assess the biological profile, which may be different from that of the primary tumour [4].

**Table I.** Individualisation of systemic treatment of breast cancer depending on the tumour's biological profile

Classification	ER	PgR	HER-2	Prognosis	Systemic treatment [2–6]
luminal A	+	+	–	good	<ol style="list-style-type: none"> <li>Hormone therapy 5–10 years: <ul style="list-style-type: none"> <li>estrogen receptor blockers like tamoxifen, toremifene, fulvestrant and/or</li> <li>aromatase inhibitors like anastrozole, letrozole, exemestane.</li> </ul> </li> <li>Chemotherapy in cases of T3 and/or involvement of 4 lymph nodes.</li> <li>CDK 4/6 inhibitors (palbociclib, ribociclib, abemaciclib) or mTOR inhibitors (everolimus) in cases of advanced cancer.</li> <li>PI3K inhibitors (alpalisib) with fulvestrant in the next line of treatment (after hormone therapy) in patients with advanced breast cancer and <i>PIK3CA</i> mutation in tDNA.</li> <li>PARP inhibitors (olaparib or talazaparib) in the next line of treatment (after anthracycline and taxanes) in patients with a germline <i>BRCA1</i> or <i>BRCA2</i> mutation in cases of advanced cancer.</li> </ol>
luminal B HER2-negative	+	–	–	moderate- ly good	<ol style="list-style-type: none"> <li>Hormone therapy 5–10 years: <ul style="list-style-type: none"> <li>estrogen receptor blockers like tamoxifen, toremifene, fulvestrant and/or</li> <li>aromatase inhibitors like anastrozole, letrozole, exemestane</li> </ul> </li> <li>Chemotherapy.</li> <li>Anti-HER2 therapy in HER2-positive cases: <ul style="list-style-type: none"> <li>monoclonal antibodies such as trastuzumab, pertuzumab, T-DM1 and/or</li> <li>kinase inhibitors such as lapatinib, neratinib, tucatinib.</li> </ul> </li> <li>CDK 4/6 inhibitors (palbociclib, ribociclib, abemaciclib) or mTOR inhibitors (everolimus) in cases of advanced HER2-negative luminal B cancer.</li> <li>PI3K inhibitors (alpalisib) with fulvestrant in the next line of treatment (after hormone therapy) in patients with advanced HER2-negative luminal B breast cancer and <i>PIK3CA</i> mutation in tDNA.</li> <li>PARP inhibitors (olaparib or talazaparib) in the next line of treatment (after anthracycline and taxanes) in patients with a germline <i>BRCA1</i> or <i>BRCA2</i> mutation in cases of HER2-negative advanced cancer.</li> </ol>
luminal B HER2-positive	+	+/-	+		
non-luminal HER2- positive	–	–	+	moderate- ly severe	<ol style="list-style-type: none"> <li>Anti-HER2 therapy in HER2 positive cases: <ul style="list-style-type: none"> <li>monoclonal antibodies such as trastuzumab, pertuzumab, T-DM1 and/or</li> <li>kinase inhibitors such as lapatinib, neratinib, tucatinib.</li> </ul> </li> <li>Chemotherapy.</li> </ol>
triple negative (TNBC)	–	–	–	severe	<ol style="list-style-type: none"> <li>Chemotherapy (considering platinum derivatives, among others).</li> <li>PARP inhibitors (olaparib or talazaparib) in the next line of treatment (after anthracycline and taxanes) in patients with a germline <i>BRCA1</i> or <i>BRCA2</i> mutation in cases of advanced cancer.</li> </ol>

## Breast cancer – therapy individualisation based on molecular changes and ancillary markers

### PI3K inhibitors

A lot of hope is now associated with PI3K inhibitors (inhibitors of phosphatidylinositol-4,5-bisphosphate 3-kinases), such as alpelisib, which are new targeted therapeutical substances applied in the next-line treatment (after hormonal anti-estrogen therapy) in patients with advanced ER-positive, HER2-negative breast cancer displaying presence of *PIK3CA* gene mutation (catalytic subunit of alpha-phosphatidylinositol-4,5-bisphosphate 3-kinase) in tDNA. In these cases, it is recommended to use the PI3K inhibitor in combination with fulvestrant [5]. Importantly, in tDNA of luminal breast cancers, mutation of *PIK3CA* gene is the most frequent molecular lesion, so a large group of women with luminal breast cancer would benefit from application of the targeted therapy using PI3K inhibitors [5, 6].

### PARP inhibitors

The latest ESMO recommendations suggest also possibility to apply PARP inhibitors (poly (ADP-ribose) polymerase – PARP) – olaparib or talazaparib – in the next-line treatment (after

anthracycline and taxanes) in patients with germline mutation of *BRCA1* or *BRCA2* gene (mutation responsible for hereditary breast cancer and ovarian cancer syndrome – table II) and diagnosed triple negative advanced breast cancer or advanced luminal HER2-negative breast cancer [4].

### Gene expression panels

In the targeted therapy of breast cancer, gene expression panels on tDNA can be applied, too: MammaPrint (Agendia, Amsterdam, The Netherlands), Oncotype DX (Genomic Health, Redwood City, CA, USA), Prosigna (PAM 50, NanoString Technologies, Seattle, WA, USA), Endopredict (Myriad Genetics SaltLake City, UT, USA), Breast Cancer Index (Biotheranostics, Inc., San Diego, CA, USA). The above tests assess expression of selected – usually several dozen – genes related to processes of proliferation, angiogenesis, metastasis and others, allowing determination of the specific expression profile of the tumour. According to the ESMO recommendations, gene expression panels are used as a supplementary prognostic marker (enabling estimation of the course of the neoplastic disease and the risk of metastasis) and a predictive marker mainly in cases of early ER-positive and HER2-negative breast cancer, without

nodal involvement or with involvement of up to 3 lymph nodes. In such cases, results of the discussed panels play an auxiliary role in equivocal situations, where application or withdrawal of chemotherapy is considered [2, 3].

### **Assessment of uPA and PAI-1 concentrations in tumour cells**

Tests to assess uPA (urokinase-type plasminogen activator) and PAI-1 (plasminogen activator inhibitor-1) concentrations in tumour cells have similar application in individualisation of breast cancer therapy as gene expression panels. The test is based on the ELISA technique, and it requires a fresh and unfixed or freshly frozen tumour specimen. According to ESMO recommendations, the test should be considered primarily in cases of early breast cancer without lymph node involvement. High concentrations of uPA and /or PAI-1 are considered unfavourable prognostic markers suggesting high risk of recurrence and indicating that adjuvant chemotherapy is advisable [2, 3].

### **Broad-panel tDNA molecular testing**

Next generation sequencing (NGS) allows analysis of the whole exome (whole-exome sequencing – WES) or even the whole genome (whole genome sequencing – WGS) of the tumour, raising hopes for application of new targeted therapies. Broad-panel molecular tests showed that the most frequent molecular changes in cancer cells include gene mutations in *PIK3CA*, *TP53*, *GATA3*, *PTEN*, *AKT1*, *CDH1*, *ARID1B*, *CASP8*, *BRCA1*, *RB1*, *MLL3*, *MAP3K1*, *MAP3K13*, *NCOR1*, *SMARCD1*, *CDKN1B*, *TBX3*, *RUNX1*, *CBFB*, *AFF2*, *PIK3R1*, *PTPN22*, *PTPRD*, *NF1*, *SF3B1* and *CCND3*, as well as copy number variants (CNV) in *PIK3CA*, *ERBB2*, *TP53*, *MAP2K4*, *MLL3*, *CDKN2A*, *PTEN* and *RB1* [6]. Comprehensive genomic profiling (CGG) enables establishment of a molecular classification of breast cancers based on changes in individual signalling pathways, such as PI3K / AKT / mTOR pathway (molecular target for such therapeutical substances as everolimus, temsirolimus, alpelisib), double-strand DNA break repair in genes *BRCA1* / *BRCA2* / *PALB2* (their mutations are a good predictive factor for PARP inhibitors), estrogen receptor ER pathway, cell cycle regulatory pathway *CCND1* / *CDK4* / *RB1* (molecular target for palbociclib, ribociclib, abemaciclib), growth factors *ERBB2* / *EGFR* / *FGFR1* (molecular target for such therapeutic substances as trastuzumab, pertuzumab, afatinib, lapatinib, neratinib, pazopanib, ponatinib) [7]. Thus, detection of changes in the cellular pathways identified in breast cancer allows assumptions concerning potential efficacy of therapies targeted at them. There are commercial broad-panel genetic tests available on the market, offering sequencing of many genes within tDNA, both in the form of WES and WGS tests of the tumour genome, as well as gene panels selected for a given tumour. Their analysis is potentially important in the context of developing targeted therapies. However, ESMO recommendations do not provide for routine application of

broad-panel molecular tests of tDNA, due to their currently limited use in clinical practice in patients with breast cancer.

### **Hereditary breast cancer**

There is a specific dimension of personalised medicine applied in breast cancer which concerns carriers of mutations typical for hereditary cancer syndromes including breast cancer. Breast cancer appears in the spectrum of many hereditary cancer syndromes caused by germline mutations of such genes as: *BRCA1* and *BRCA2* (HBOC hereditary breast and ovarian cancer), *CHEK2*, *PALB2*, *TP53* (Li-Fraumeni syndrome), *ATM*, *PTEN* (Cowden syndrome), *CDH1* (hereditary diffuse gastric cancer) or *STK11* (Peutz-Jeghers syndrome). The role of mutations of individual genes in aetiopathogenesis of the hereditary breast cancer varies depending on the studied ethnic group, but most hereditary breast cancers are associated with germline mutation of *BRCA1* or *BRCA2* gene responsible for the hereditary breast and ovarian cancer syndrome [8, 9].

Currently in Poland, module I of the National Cancer Control Programme of the Ministry of Health (Narodowy Program Zwalczenia Chorób Nowotworowych Ministerstwa Zdrowia – NPZChN MZ) recommends for all breast cancer patients genetic counselling. Genetic testing to assess critical hereditary mutation is recommended for selected group of patients, in whom the hereditary form of disease has been suspected on the basis of pedigree analysis. The recommendations concern the presence of five germline mutations of *BRCA1* gene which are the most common in the Polish population (c.5266dupC, c.181T>G, c.4035del, c.66\_67AG, c.3695\_3699GTAAA), two selected germline mutations of *PALB2* gene (c.509\_510del, c.168\_171TTGT) and three selected germline mutations of *CHEK2* gene (c.1100del, del5395, c.444+1G>A). This range of molecular diagnostics has been developed based on the specificity of the Polish population, with dominating carriers of one of the five founder mutations of *BRCA1* gene, which account for aetiology of approximately 64% of BRCA-dependent hereditary breast cancers [10].

In cases of hereditary history (tab. II) module I of the NPZChN MZ Programme recommends expanded molecular diagnostics, including sequencing of *BRCA1* and *BRCA2* genes, currently by the next generation sequencing (NGS) technique. However, this test, too, has some limitations. It is not recommended for analysing large rearrangements (deletions and duplications), which account for up to 10% of mutations identified in *BRCA1* and *BRCA2* genes [11]. Their occurrence should be verified by another method such as MLPA technique (multiplex ligation-dependent probe amplification). Furthermore, sequencing entire gene-coding sequences yields a lot of information requiring diligent bio-IT analysis for verification of clinical significance of the identified variations. Identifying variations is a complex process, requiring advanced *in silico* analysis, assessment of the variation's frequency in the general population, access to available databases, such as: ClinVar, dbSNP, Breast

Cancer Information Core, Varsome, 1000GP, Consensus PathDB, Gene Ontology, GWAS, OMIM, UniProt or HGMD, etc. The currently applied classification recommended by the American College of Medical Genetics and Genomics (ACMG) provides for five classes of pathogenic effect of variations:

- non-pathogenic variant (class 1),
- possibly non-pathogenic variant (class 2),
- variant of unknown clinical significance (VUS, class 3),
- possibly pathogenic variant (class 4),
- pathogenic variant (class 5) [12].

Class 4 and Class 5 variations are considered to be mutations, i.e. changes of clinical significance. The analysis of germline VUS variants remains a major challenge in genetic counselling. Therefore, it is recommended that prophylactic and therapeutic recommendations should be based on history and clinical analysis. Further – considering progress of knowledge on molecular changes and constant updating of databases – it is stressed that the identified variation should be consulted again after 2–3 years.

The variability of clinical significance of individual variations within a studied gene can be observed in the case of *CHEK2* gene: its shortened protein or frameshift variants have a far more significant impact on neoplasm risk than missens variations. Therefore, individual medical recommendations should be based not only on the gene where the mutation is found, but also on the type of change identified.

Identification of a germline mutation is a molecular confirmation of a specific hereditary cancer syndrome. However, due to the limitations of genetic testing presented above, non-detection of mutations in the tested range does not allow for clear exclusion of the suspected hereditary cancer syndrome. The result of a genetic test should therefore be supported by specialised genetic counselling, and individual medical recommendations should take into account not only results of molecular tests, but also clinical and history evaluation.

Patients with family and clinical history suggestive of hereditary form of disease who do not have mutations revealed in sequencing of *BRCA1* and *BRCA2* remain a challenge. Hereditary breast and ovarian cancer (HBOC), while dominant, is not the only syndrome of hereditary predisposition to cancers with breast cancer in the spectrum. In some of the other, rarer syndromes, there are associated characteristic signs and symptoms, such as specific family history, macrocephaly (Cowden syndrome) or typical changes in skin and mucosa (Peutz-Jeghers syndrome), facilitating identification of a specific suspicion and referring to targeted genetic testing. In non-specific cases, the only option is to consider broad-panel genetic NGS testing, which allows sequencing of many genes associated with many hereditary cancer syndromes within a single test. There are many commercial wide-panel tests available on the market, differing in the scope of the studied genes. They may take into account both genes of high penetrance (in the case of occurrence of a germline mutation, they increase the risk of

developing neoplasms from a given spectrum very strongly, even fivefold) and of moderate penetrance (increasing the risk of developing neoplasms from a given spectrum about 2–5 times in the case of occurrence of a germline mutation).

Carriers of germline mutations should be informed about the risk of occurrence of the mutation in their relatives. Genetic counselling should cover not only people with hereditary breast cancer, but also selected members of their families. Individualisation of medical procedures applies to all mutation carriers (including those diagnosed with another cancer from the spectrum of a given cancer syndrome, as well as those without cancer diagnosis), and also families with cancer in which no causative mutation was detected.

Detailed characteristics of the most frequent hereditary cancer syndromes of spectrum including breast cancer, and therapeutic and prophylactic recommendations for mutation carriers considering ESMO guidelines and module I of the NPZChN Programme are shown in table II.

### Ovarian cancer – classification

In patients with diagnosed ovarian cancer prognosis of the course of disease and potential response to applied therapy depends on the neoplasm's histopathology type, tumour grading, four-stage FIGO classification and tumour's platinum sensitivity.

According to the classification of the World Health Organisation (WHO), epithelial ovarian tumours include:

- serous type (about 80% of cases),
- endometrioid type (about 10% of cases),
- clear-cell type (about 5% of cases),
- mucous type,
- transitional epithelial tumours (Brenner tumour),
- mixed type,
- undifferentiated type,
- unclassified type [20].

Additionally, there is a separate group of borderline epithelial tumours of the ovary, accounting for 10–15% of ovarian tumours and characterised by equivocal histopathology, which doesn't allow their identification as either malignant or non-malignant ovarian neoplasms. Serous borderline tumours are the most frequent, followed by mucous and endometrioid types [20].

Apart from the standard classification, there is also another division of epithelial ovarian tumours, considering jointly: etiopathogenetic factors, histopathological type, histologic malignancy stage, molecular changes, response to chemotherapy and prognosis. This division distinguishes the following types:

- type 1 ovarian cancer, characterised by low histological malignancy, a more stable course and frequent mutations of *KRAS*, *BRAF*, *ERBB2*, *PTEN*, *PIK3CA* and *ARID1A* genes in the genetic material of the tumour (*ARID1A* mutations are particularly frequently identified in cases of endometrioid and clear cell carcinoma). Type 1 ovarian cancer includes low-grade serous, endometrioid, mucous and clear-cell

**Table II.** Selected hereditary cancer syndromes with breast cancer in the spectrum - characteristics and therapeutic and prophylactic management

Hereditary cancer syndrome	Genes in which germline mutations are present	Indications for genetic testing	Risk of breast cancer in carriers of the mutation	Other neoplasms in the spectrum of increased risk and associated symptoms	Recommendations
<b>Hereditary breast and ovarian cancer (HBOC)</b> [8, 11]	<i>BRCA1</i>	<p>According to ESMO<sup>1</sup>:</p> <p>a) genetic testing for HBOC should be considered in families with:</p> <ul style="list-style-type: none"> <li>breast cancer ≤50 years of age</li> <li>triple negative TNBC breast cancer</li> <li>ipsilateral and/or contralateral breast cancer</li> <li>male breast cancer</li> <li>breast cancer in an Ashkenazi woman</li> <li>two breast cancers among first / second / third degree relatives</li> <li>three breast cancers among first / second / third degree relatives, regardless of age</li> </ul> <p>b) optimal scope of the test includes sequencing and evaluation of large rearrangements (deletions/duplications) of <i>BRCA1</i> and <i>BRCA2</i> genes</p> <p>c) in families with identified specific <i>BRCA1/2</i> mutation, an occurrence of hereditary mutation should be verified in the carrier's family, above all first-degree relatives.</p> <p>According to module 1 of the NPZChN MZ Programme <sup>2</sup>:</p> <p>a) analysis of occurrence of five mutation of <i>BRCA1</i> gene which are the most common in the Polish population (c.5266dupC, c.181T &gt; G, c.4035del, c.66_67AG, c.3695_3699GTAAA) in:</p> <ul style="list-style-type: none"> <li>any person with diagnosed with breast cancer (including DCIS, male breast cancer)</li> <li>any person with ovarian cancer (including peritoneal cancer and fallopian tube cancer)</li> <li>in families with breast and/or ovarian cancer, in cases where the person with cancer is unavailable for examination, the analysis should be performed in the closest relatives (optimally first or second degree)</li> </ul> <p>b) sequencing of <i>BRCA1</i> and <i>BRCA2</i> genes:</p> <ul style="list-style-type: none"> <li>only in people diagnosed with breast and/or ovarian cancer</li> <li>only in cases where 5 most common mutations in the Polish population have been excluded</li> <li>only if: <ul style="list-style-type: none"> <li>the patient has both breast cancer and ovarian cancer, including the first diagnosis before the age of 50</li> <li>the patient has had bilateral breast cancer, including the first diagnosis before the age of 50</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>risk of developing breast cancer in carriers up to approx. 87%</li> <li>risk of developing contralateral breast cancer in carriers up to approx. 83%</li> <li>risk of developing breast cancer in male carriers up to approx. 1%</li> </ul>	<ul style="list-style-type: none"> <li>risk of developing ovarian cancer up to approx. 63%</li> <li>risk of developing prostate cancer up to approx. 8.5%</li> <li>risk of developing pancreatic cancer up to approx. 3%</li> </ul>	<ul style="list-style-type: none"> <li>each carrier should perform monthly self-palpation of the breasts</li> <li>each carrier is recommended to breastfeed for a long time and to abandon/limit hormone replacement therapy (HRT)</li> <li>each carrier is recommended to have a medical palpation of the breasts and breast imaging every 6 months (the age of beginning the tests depends on the family and clinical assessment, but should not be later than 25 years of age); MRI alternating with ultrasound (up to 30 years of age) or mammography (after 30 years of age)</li> <li>chemoprevention with tamoxifen may be considered in any carrier</li> <li>each carrier may consider a prophylactic bilateral mastectomy, optimally with simultaneous reconstruction in carriers who developed breast cancer; breast conserving surgery should be abandoned in favour of mastectomy, possibly with prophylactic contralateral mastectomy, ideally with simultaneous reconstruction in carriers who have developed triple-negative or luminal type breast cancer that progresses despite anti-estrogen therapy; PARP inhibitors (olaparib or talazoparib) should be considered as the next-line treatment (after anthracycline and taxanes)</li> <li>each carrier from 30 years of age is recommended to have a TV-USG of the small pelvis and assessment of serum CA125 concentration every 6–12 months</li> <li>in each carrier, prophylactic, bilateral adnexectomy is recommended, optimally at the age of 35–40 years, after completion of procreation plans</li> <li>carriers diagnosed with ovarian cancer are expected to respond well to platinum derivatives and PARP inhibitors</li> <li>each male carrier should conduct regular breast self-examination, and from 30–35 years of age annual medical palpation of the breast is recommended (especially in carriers of the <i>BRCA2</i> mutation)</li> <li>each male carrier (especially with <i>BRCA2</i> mutation) may consider annual screening for prostate cancer starting from 40–45 years of age</li> </ul>
	<i>BRCA2</i>		<ul style="list-style-type: none"> <li>risk of developing breast cancer in carriers up to approx. 84%</li> <li>risk of developing contralateral breast cancer in carriers up to approx. 62%</li> <li>risk of developing breast cancer in male carriers up to approx. 9%</li> </ul>	<ul style="list-style-type: none"> <li>risk of developing ovarian cancer up to approx. 27%</li> <li>risk of developing prostate cancer up to approx. 20%</li> <li>risk of developing pancreatic cancer up to approx. 7%</li> <li>a discreetly increased risk of developing melanoma (of the skin and/or eyeball)</li> </ul>	

Hereditary cancer syndrome	Genes in which germline mutations are present	Indications for genetic testing	Risk of breast cancer in carriers of the mutation	Other neoplasms in the spectrum of increased risk and associated symptoms	Recommendations
	<ul style="list-style-type: none"> <li>the patient has been diagnosed with breast cancer or ovarian cancer and has a first and / or second degree relative who was diagnosed with breast and/or ovarian cancer, at least one of these cases before the age of 50</li> <li>the patient was diagnosed with breast cancer before the age of 50 or ovarian cancer at any age, and in addition first and/or second grade relatives were diagnosed with male breast cancer and/or ovarian cancer</li> <li>in families with identified specific <i>BRCA1/2</i> mutation, an occurrence of hereditary mutation should be verified in the carrier's family, above all first-degree relatives</li> <li>if a specific <i>BRCA1/2</i> mutation is identified in DNA from tumour cells (tDNA), its presence should be analysed on DNA isolated from outside the tumour cells (blood, saliva, oral swab, skin biopsy specimen) to assess the nature of the mutation (somatic, i.e. non-hereditary or germline, i.e. hereditary).</li> </ul>	<ul style="list-style-type: none"> <li>the patient has been diagnosed with breast cancer or ovarian cancer and has a first and / or second degree relative who was diagnosed with breast and/or ovarian cancer, at least one of these cases before the age of 50</li> <li>the patient was diagnosed with breast cancer before the age of 50 or ovarian cancer at any age, and in addition first and/or second grade relatives were diagnosed with male breast cancer and/or ovarian cancer</li> <li>in families with identified specific <i>BRCA1/2</i> mutation, an occurrence of hereditary mutation should be verified in the carrier's family, above all first-degree relatives</li> <li>if a specific <i>BRCA1/2</i> mutation is identified in DNA from tumour cells (tDNA), its presence should be analysed on DNA isolated from outside the tumour cells (blood, saliva, oral swab, skin biopsy specimen) to assess the nature of the mutation (somatic, i.e. non-hereditary or germline, i.e. hereditary).</li> </ul>	<ul style="list-style-type: none"> <li>each carrier should perform monthly self-palpation of the breasts</li> <li>each carrier is recommended to have a medical palpation of the breasts and breast imaging every 6 months (the age of beginning the tests depends on the family and clinical assessment, but should not be later than 20-25 years of age): MRI alternating with ultrasound (up to 30 years of age) or mammography (after 30 years of age)</li> <li>each carrier may consider bilateral prophylactic mastectomy, optimally with simultaneous reconstruction, but no such recommendations are included in module I of NPZChN MZ Programme</li> <li>the person with identified mutation should be notified that if she gets pregnant by a carrier of <i>PALB2</i> mutation, the risk of giving birth to a child with Fanconi anaemia type N is 25%</li> </ul>	<ul style="list-style-type: none"> <li>in all carriers of the <i>BRCA2</i> mutation, annual dermatological and ophthalmological testing for melanoma may be considered, especially in the presence of this tumour in relatives</li> <li>in any carrier of the <i>BRCA2</i> mutation, especially in cases with a family history of pancreatic cancer, annual pancreatic cancer screening (EUS or MRI) may be considered, starting from the age of 50 or 10 years earlier than the youngest family history of pancreatic cancer</li> </ul>	<ul style="list-style-type: none"> <li>each carrier should perform monthly self-palpation of the breasts</li> <li>each carrier is recommended to have a medical palpation of the breasts and breast imaging every 6 months (the age of beginning the tests depends on the family and clinical assessment, but should not be later than 20-25 years of age): MRI alternating with ultrasound (up to 30 years of age) or mammography (after 30 years of age), but no recommendation for breast MRI in module I of NPZChN MZ Programme<sup>2</sup></li> <li>additionally recommends annual ultrasound of the thyroid gland</li> </ul>
<b>germline mutations of <i>PALB2</i></b> [8, 11, 13]	<p><i>PALB2</i></p> <p>According to module I of the NPZChN MZ Programme<sup>2</sup>: two selected mutations of <i>PALB2</i> (c.509_510del, c.168_171TTGT) should be analysed:</p> <ol style="list-style-type: none"> <li>in any person with breast cancer</li> <li>in the case when the person diagnosed with breast cancer is unavailable for examination and the family has been diagnosed with: <ul style="list-style-type: none"> <li>bilateral breast cancer</li> <li>breast cancer before 40 years of age</li> <li>male breast cancer</li> </ul> </li> <li>2 cases of breast cancer and/or ovarian cancer in people who are first/second degree relatives.</li> </ol>	<ul style="list-style-type: none"> <li>increased risk of developing pancreatic cancer</li> <li>increased risk of developing breast cancer in male carriers</li> </ul>	<ul style="list-style-type: none"> <li>risk of developing breast cancer in carriers up to approx. 58%</li> </ul>	<ul style="list-style-type: none"> <li>risk of developing prostate cancer</li> <li>risk of developing papillary thyroid cancer</li> </ul> <p><i>The spectrum and risk of cancer development largely depend on the type of mutation found. Literature data are ambiguous for many CHEK2 variants.</i></p>	<ul style="list-style-type: none"> <li>each carrier should perform monthly self-palpation of the breasts</li> <li>each carrier is recommended to have a medical palpation of the breasts and breast imaging every 6 months (the age of beginning the tests depends on the family and clinical assessment, but should not be later than 20-25 years of age): MRI alternating with ultrasound (up to 30 years of age) or mammography (after 30 years of age), but no recommendation for breast MRI in module I of NPZChN MZ Programme<sup>2</sup></li> <li>additionally recommends annual ultrasound of the thyroid gland</li> </ul>
<b>germline mutations of <i>CHEK2</i></b> [8, 11]	<p><i>CHEK2</i></p> <p>According to module I of the NPZChN MZ Programme<sup>2</sup>: three selected mutations of <i>CHEK2</i> (c.1100del, del5395, c.444+1G &gt; A) should be analysed:</p> <ol style="list-style-type: none"> <li>in any person with breast cancer</li> <li>in the case when the person diagnosed with breast cancer is unavailable for examination and the family has been diagnosed with: <ul style="list-style-type: none"> <li>bilateral breast cancer</li> <li>breast cancer before 40 years of age</li> <li>male breast cancer</li> </ul> </li> <li>2 cases of breast cancer and/or ovarian cancer in people who are first/second degree relatives.</li> </ol>	<ul style="list-style-type: none"> <li>risk of developing prostate cancer</li> <li>risk of developing papillary thyroid cancer</li> </ul> <p><i>The spectrum and risk of cancer development largely depend on the type of mutation found. Literature data are ambiguous for many CHEK2 variants.</i></p>	<ul style="list-style-type: none"> <li>risk of developing breast cancer up to approx. 39%</li> </ul> <p><i>The spectrum and risk of cancer development largely depend on the type of mutation found. Literature data are ambiguous for many CHEK2 variants.</i></p>	<ul style="list-style-type: none"> <li>risk of developing prostate cancer</li> <li>risk of developing papillary thyroid cancer</li> </ul> <p><i>The spectrum and risk of cancer development largely depend on the type of mutation found. Literature data are ambiguous for many CHEK2 variants.</i></p>	<ul style="list-style-type: none"> <li>each carrier should perform monthly self-palpation of the breasts</li> <li>each carrier is recommended to have a medical palpation of the breasts and breast imaging every 6 months (the age of beginning the tests depends on the family and clinical assessment, but should not be later than 20-25 years of age): MRI alternating with ultrasound (up to 30 years of age) or mammography (after 30 years of age), but no recommendation for breast MRI in module I of NPZChN MZ Programme<sup>2</sup></li> <li>additionally recommends annual ultrasound of the thyroid gland</li> </ul>

Hereditary cancer syndrome	Genes in which germline mutations are present	Indications for genetic testing	Risk of breast cancer in carriers of the mutation	Other neoplasms in the spectrum of increased risk and associated symptoms	Recommendations
<b>Li-Fraumeni Syndrome (LFS)</b> [8, 14]	<i>TP53</i>	<p>Sequencing of <i>TP53</i> gene is recommended if:</p> <ol style="list-style-type: none"> <li>Chompret criteria are met: <ul style="list-style-type: none"> <li>diagnosed breast cancer in a patient aged <math>\leq 30</math></li> <li>aged <math>\leq 45</math> and at least one relative in first/second degree with diagnosed malignant neoplasm in LFS spectrum (excluding breast cancer if in the original patient) at the age of <math>\leq 55</math> or in a multifocal form</li> <li>diagnosed multiple primary malignancies (except multiple primary breast cancer lesions), of which at least 2 belong to the LFS spectrum and the first diagnosis occurred at the age <math>\leq 45</math></li> <li>diagnosed rare malignant neoplasm typical of LFS, such as: adrenal cortex cancer, choroid plexus cancer, anaplastic embryonal rhabdomyosarcoma</li> </ul> </li> <li>The patient was diagnosed with hypodiploid acute lymphoblastic leukaemia (ALL) before 21 years of age.</li> <li>Findings in tumour cell studies: <ul style="list-style-type: none"> <li>presence of <i>TP53</i> mutation of allele frequency approaching 50% or higher in tDNA</li> <li>absent or decreased expression of TP53 in IHC tests</li> </ul> </li> </ol>	<ul style="list-style-type: none"> <li>risk of breast cancer development in carriers up to approx. 54% [14] – 79% [8] (usually premenopausal breast cancers)</li> </ul>	<ul style="list-style-type: none"> <li>notably young age of oncological diagnoses (including <math>&lt; 18</math> years of age) and risk of multifocal primary cancers in a single patient</li> <li>neoplastic disease develops in at least 90% of female carriers and 70% of male carriers of the <i>TP53</i> mutation</li> <li>risk of developing a soft tissue sarcoma up to approx. 27%</li> <li>risk of developing osteosarcoma up to approx. 16%</li> <li>risk of developing a malignant tumour of the CNS up to approx. 14% (especially <i>glioblastoma</i>, <i>astrocytoma</i>)</li> <li>risk of developing adrenal cortex cancer (ACC) up to approx. 13%</li> <li>risk of developing leukaemia (especially ALL, AML, MDS) up to approx. 4%</li> <li>risk of developing lymphoma up to approx. 2%</li> <li>risk of developing colorectal cancer (up to approx. 3–8%) and gastric cancer</li> <li>risk of developing melanoma</li> </ul>	<ul style="list-style-type: none"> <li>each carrier should perform monthly self-palpation of the breasts</li> <li>in each carrier from 20 years of age medical palpation of the breasts is recommended every 6–12 months</li> <li>in each carrier from 20 years of age annual breast MRI is recommended</li> <li>in carriers who developed breast cancer, breast conserving surgery should be abandoned in favour of mastectomy, possibly with prophylactic contralateral mastectomy, ideally with simultaneous reconstruction</li> <li>each carrier may consider a prophylactic bilateral mastectomy, optimally with simultaneous reconstruction in all carriers from 25 years of age gastroduodenoscopy and colonoscopy is recommended (at least every 5 years, the endoscopic image determines the frequency of the examination)</li> <li>annual neurological examination is recommended for all carriers and consideration of annual whole-body MRI and six-monthly blood counts</li> <li>in all carriers, ultrasound of the abdominal cavity and small pelvis may be considered: every 3–4 months until the age of 18 and every year after the age of 18</li> <li>annual dermatological examination is recommended for all carriers</li> <li>X-ray and ionizing tests and therapies should be abandoned (or limited) in all carriers</li> </ul>
<b>Cowden syndrome</b> [8, 15]	<i>PTEN</i>	<p>performance of <i>PTEN</i> genetic testing is recommended in patients with a Cleveland Clinic score (CC score) of at least 10; the scale considers assessment of occurrence of malignant cancers (including breast, endometrium, thyroid, renal cancer) and non-malignant tumours and non-neoplastic lesions typical for Cowden syndrome spectrum.</p> <p><a href="https://www.lernerccf.org/gmi/ccscore/">https://www.lernerccf.org/gmi/ccscore/</a></p>	<ul style="list-style-type: none"> <li>risk of developing breast cancer in female carriers up to approx. 50%, according to some sources up to approx. 85%</li> </ul>	<ul style="list-style-type: none"> <li>risk of developing thyroid cancer (especially follicular cancer) up to approx. 35%</li> <li>risk of developing renal cancer (especially papillary cancer) up to approx. 35%</li> <li>risk of developing endometrial cancer up to approx. 28%</li> <li>risk of developing colorectal cancer up to approx. 9%</li> <li>risk of developing melanoma up to approx. 5%</li> <li>very frequent occurrence on non-malignant tumours:</li> </ul>	<ul style="list-style-type: none"> <li>each carrier should perform monthly self-palpation of the breasts</li> <li>in each carrier from 20–25 years of age medical palpation of the breasts is recommended every 6–12 months</li> <li>in each carrier from 30 years of age annual breast MRI and/or mammography is recommended</li> <li>in each carrier from 30–35 years of age annual TV-USG with endometrial biopsy is recommended (unless a hysterectomy has been previously performed)</li> <li>each carrier may consider a prophylactic bilateral mastectomy, optimally with simultaneous reconstruction in all carriers, annual dermatological control and annual ultrasound of the thyroid gland are recommended</li> </ul>



Hereditary cancer syndrome	Genes in which germline mutations are present	Indications for genetic testing	Risk of breast cancer in carriers of the mutation	Other neoplasms in the spectrum of increased risk and associated symptoms	Recommendations
<p><b>germline ATM mutations</b> [8, 16]</p>	<p>ATM</p>	<ul style="list-style-type: none"> <li>– risk of developing breast cancer in carriers up to approx. 52%</li> </ul>	<ul style="list-style-type: none"> <li>– carriers are also likely to have a moderately increased risk of developing gastric and colorectal cancer</li> </ul>	<ul style="list-style-type: none"> <li>– gastrointestinal polyps (hamartomatous, juvenile adenomas), fibrocystic breast dysplasia, nodular goitre and/or thyroid adenomas, uterine fibroids, vascular malformations</li> <li>– frequent coexistence of dermal and mucosal lesions; trichilemmoma lesions of the face, skin papillomatosis, oral papillomas, acral keratosis, freckle lesions on the skin of the penis, lipomas and skin fibromas</li> <li>– macrocephaly</li> <li>– possible signs in the autism spectrum and psychomotor retardation in childhood</li> <li>– possible coexistence of Lhermitte-Ducros disease (LDD), i.e. dysplastic cerebellar ganglion</li> </ul>	<ul style="list-style-type: none"> <li>– in all carriers, colonoscopy is recommended from the age of 35, frequency of the examination depends on the endoscopic image</li> <li>– in all carriers from 40 years of age annual renal CT or MRI is recommended</li> </ul>
<p><b>hereditary diffuse gastric cancer</b> [8, 17, 18]</p>	<p>CDH1</p>	<p>sequencing with the analysis of rearrangement (deletion / duplication) of <i>CDH1</i> gene is recommended in the following cases:</p> <ul style="list-style-type: none"> <li>– diagnosed diffuse gastric cancer at any age and at least one relative in the first/second degree with diagnosed any gastric cancer</li> <li>– diffuse gastric cancer diagnosed in the patient or a relative in the first/second degree before the age of 40</li> <li>– the patient or family member has had both diffuse gastric cancer and lobular breast cancer, and at least one diagnosis was made before the age of 50.</li> </ul>	<ul style="list-style-type: none"> <li>– risk of developing lobular breast cancer in carriers up to approx. 42%</li> <li>– risk of developing diffuse gastric cancer up to approx. 70% in men and up to approx. 56% in women</li> </ul>	<ul style="list-style-type: none"> <li>– risk of developing diffuse gastric cancer up to approx. 70% in men and up to approx. 56% in women</li> </ul>	<ul style="list-style-type: none"> <li>– each carrier should perform monthly self-palpation of the breasts</li> <li>– each carrier is recommended to have a medical palpation of the breasts every 6–12 months and annual MRI of the breasts (no clear recommendations as to the age to start the tests, probably no later than 25)</li> <li>– X-ray and ionizing tests and therapies should be abandoned (or limited) in all carriers</li> <li>– the person with identified mutation should be notified that if she gets pregnant by a carrier of ATM mutation, the risk of giving birth to a child with ataxia-telangiectasia is 25%</li> <li>– each carrier should perform monthly self-palpation of the breasts</li> <li>– in each carrier from 20 years of age medical palpation of the breasts every 6 months is recommended and also regular breast imaging: annual MRI (from 20 years of age) alternating with annual mammography (additionally from 30 years of age)</li> <li>– each female carrier may consider a prophylactic bilateral mastectomy, optimally with simultaneous reconstruction in each adult male carrier, it is recommended to consider prophylactic gastrectomy, performed optimally between 20 and 30 years of age</li> </ul>

Hereditary cancer syndrome	Genes in which germline mutations are present	Indications for genetic testing	Risk of breast cancer in carriers of the mutation	Other neoplasms in the spectrum of increased risk and associated symptoms	Recommendations
					<ul style="list-style-type: none"> <li>in carriers who do not decide to undergo prophylactic gastrectomy, it is optimal to perform high-resolution chromoendoscopy using indigo carmine and the Cambridge protocol (starting 5–10 years younger than the youngest diagnosis in the family, examination repeated every 6–12 months)</li> <li>in every carrier from the age of 40 it is recommended to perform a colonoscopy examination at least every 5 years (frequency of the examination is determined by the endoscopic image)</li> </ul>
<b>Peutz-Jeghers Syndrome (PJS)</b> [8, 18, 19]	<i>STK11</i>	Sequencing of <i>STK11</i> gene is recommended for patients with: <ul style="list-style-type: none"> <li>identified presence of <math>\geq 2</math> hamartomatous polyps in the GI tract, confirmed by histopathology</li> <li>identified presence of at least 1 hamartomatous polyp of the gastrointestinal tract and the family history indicative of PJS</li> <li>identified presence of at least 1 hamartomatous polyp and presence of dermal and mucosal discoloration spots typical of PJS</li> <li>identified presence of dermal and mucosal discolorations typical of PJS and family history suggesting</li> </ul>	<ul style="list-style-type: none"> <li>risk of developing breast cancer in carriers up to approx. 54%</li> </ul>	<ul style="list-style-type: none"> <li>presence of multiple polyps (usually hamartomatous) of the GI tract</li> <li>risk of developing colorectal cancer up to approx. 39%</li> <li>risk of developing pancreatic cancer up to 36%</li> <li>risk of developing gastric cancer up to approx. 29%</li> <li>risk of developing small intestine cancer up to approx. 13%</li> <li>risk of development of ovarian tumour –SCITAT (sex cord tumours with annular tubules) up to approx. 21%</li> <li>risk of developing a malignant cervical adenoma up to approx. 10%</li> <li>risk of developing endometrial cancer up to approx. 9%</li> <li>risk of developing Sertoli cell tumour of the testicle up to approx. 9%</li> <li>risk of developing lung cancer up to approx. 17%</li> <li>frequently associated dermal and mucosal discoloration: around lips, eyes, nose and on the oral mucosa, around the perineum and on fingers</li> </ul>	<ul style="list-style-type: none"> <li>each carrier should perform monthly self-palpation of the breasts</li> <li>in each carrier from 20–25 years of age medical palpation of the breasts every 6 months is recommended and also regular breast imaging: annual MRI (from 20–25 years of age) alternating with annual mammography (additionally from 30 years of age)</li> <li>each female carrier may consider a prophylactic bilateral mastectomy, optimally with simultaneous reconstruction in each carrier imaging of the upper GI tract and small intestine (gastroscopy/MR endoscopy/capsule endoscopy) and colonoscopy are recommended at the age of 8 and then:           <ul style="list-style-type: none"> <li>every 2–3 years in the case of polypoid lesions identified at the age of 8</li> <li>at 18 years of age in the case of no polypoid lesions identified at the age of 8 and then from 18 years of age every 2–3 years</li> </ul> </li> <li>in girls, ultrasound of the pelvis minor with ovary evaluation is recommended from childhood until the age of 12</li> <li>in each adult carrier, annual TV-USG examination and annual evaluation of serum CA125 concentration are recommended</li> <li>in each carrier from 30 years of age annual screening for pancreatic cancer (EUS or MRI-MRCP) is recommended in boys, annual examination of the testicles (palpation and possibly ultrasound) is recommended, from childhood until approx. 12 years of age</li> </ul>

<sup>1</sup>ESMO – European Society for Medical Oncology

<sup>2</sup>National Cancer Control Programme of the Ministry of Health

ovarian cancers, malignant Brenner tumours, and borderline epithelial tumours;

- type 2 ovarian cancer, characterised by high histological malignancy, aggressive course and metastatic tendency, poor prognosis and frequent *TP53* mutations (very common in high-grade serous ovarian cancer), *BRCA1* and *BRCA2* mutations (any of the above identified in approximately 20% of type 2 ovarian cancers) in genetic material of the tumour. Type 2 ovarian cancer includes serous or endometrioid high-grade ovarian cancers, mixed-type tumours and undifferentiated tumours. Characteristically, in the most common cases of type 2 ovarian cancers, i.e. serous tumours and those of high histological malignancy, a specific etiopathogenetic mechanism is suggested, with the neoplastic process starting within the hyphae of the fallopian tube [20].

## Ovarian cancer – individualisation of therapy

### **Histopathological classification vs. response to chemotherapy**

The mainstay of treatment in patients with ovarian cancer is radical surgery and adjuvant chemotherapy, usually applying platinum derivatives (carboplatin, cisplatin) in combination with paclitaxel. In further treatment lines, depending on platinum sensitivity of the tumour, it is possible to apply platinum preparations and paclitaxel, traditional or pegylated liposomal doxorubicin (PLD), topotecan, gemcitabine and trabectedin [20].

Predictive markers of response to the classic chemotherapy regimen include histopathological type and degree of malignancy of ovarian cancer. Low effectiveness of standard chemotherapy protocols based on platinum compounds is observed in cases of serous ovarian tumours of low histological malignancy and clear-cell ovarian cancer [20].

### **PARP inhibitors**

In systemic treatment of patients with ovarian cancer, poly-(ADP-ribose) polymerase (PARP) inhibitors such as olaparib, niraparib and rucaparib are becoming increasingly important. The effect of these substances relies on inducing double-strand DNA breaks in neoplasm cells, leading to interruption of the cell cycle and death of the cancer cells. Therefore, the best effects of treatment with PARP inhibitors are achieved in the presence of the *BRCA1* or *BRCA2* mutation in the tDNA, because in such tumours DNA break repair is impaired by homologous recombination deficiency (HRD) and dependence of the repair process on the mechanisms related to PARP polymerases. Consequently, routine sequencing of both genes on tDNA isolated from post-operative material, cell block or possibly biopsy specimen has been introduced to the diagnostic process. Until recently, inclusion of PARP inhibitors in ovarian cancer therapy depended on presence of the pathogenic variants

(class 5) or probably pathogenic variants (class 4) of *BRCA1* or *BRCA2* in tDNA.

DNA break repair failure due to homologous recombination deficiency may occur also because of other molecular changes than *BRCA1* or *BRCA2* mutation and further clinical trials showed that therapeutic effect of application of PARP inhibitors was observed in general in cases of ovarian cancer with evidence of homologous recombination deficiency. There are commercial tests on the market that enable assessment of the homologous gene recombination deficiency and the resulting genomic instability in tumour cells. These tests are based on the measurement of loss of heterozygosity (LOH), telomeric allelic imbalance (TAI) and damage to chromosomal structure (large scale state transitions – LST), among other parameters. However, the tests are notably expensive. Furthermore, the latest clinical trial results show that PARP inhibitor exhibit efficacy which is also significant, although lower, in the group of patients with ovarian cancer without *BRCA1* and *BRCA2* mutations in tDNA, and even without evidence of HRD [20].

Current recommendations provide for administration of any PARP inhibitor in patients with recurrent platinum-sensitive ovarian cancer of high grade of malignancy, regardless of mutational status of *BRCA1* and *BRCA2* in tDNA in the case of supportive treatment after administration of chemotherapy based on platinum compounds, as well as in patients with advanced (FIGO grades III and IV), platinum-sensitive ovarian cancer of high grade of malignancy with known *BRCA1* or *BRCA2* mutation in tDNA in supportive treatment after administration of chemotherapy based on platinum compounds.

There have also been recommendations concerning consideration of rucaparib monotherapy as next-line treatment in patients with ovarian cancer and known *BRCA1/2* mutation in tDNA, who have counterindications to chemotherapy with platinum derivatives [20].

### **Inhibitors of angiogenesis**

Enhanced angiogenesis is one of the pathomechanisms leading to increasing mass of ovarian cancer. Therefore, angiogenesis inhibitors such as bevacizumab, a monoclonal antibody directed against vascular endothelial growth factor (VEGF) are considered in the treatment of ovarian cancer. Current ESMO recommendations provide for application of bevacizumab in first-line treatment along with paclitaxel and carboplatin in patients with advanced ovarian cancer (FIGO stage IV and FIGO stage III after suboptimal cytoreduction with residual lesions exceeding 1 cm) in an adjuvant and supportive scheme for one year and in cases of recurrence in patients with platinum-sensitive ovarian cancer, who have not received bevacizumab as first-line treatment [20].

### **Hereditary ovarian cancer**

Personalised medicine in cases of ovarian cancer, similarly to breast cancer, also extends to an individual approach in

**Table III.** Selected hereditary cancer syndromes with ovarian cancer in the spectrum – characteristics and therapeutic and prophylactic management

Hereditary cancer syndrome	Genes in which germline mutations are present	Indications for genetic testing	Risk of ovarian cancer in carriers of the mutation	Other neoplasms in the spectrum of increased risk and associated symptoms	Recommendations
<b>breast and ovarian cancer (HBOC)</b> [8, 11]	<i>BRCA1</i>	<p>according to ESMO<sup>1</sup>:</p> <p>a) genetic testing for HBOC should be considered in families with:</p> <ul style="list-style-type: none"> <li>– ovarian cancer</li> <li>– breast cancer <math>\leq 50</math> years of age</li> <li>– triple negative TNBC breast cancer</li> <li>– ipsilateral and/or contralateral breast cancer</li> <li>– male breast cancer</li> <li>– breast cancer in an Ashkenazi woman</li> <li>– two breast cancers among first/second/third degree relatives</li> <li>– three breast cancers among first/second/third degree relatives, regardless of age</li> <li>– pancreatic cancer and/or prostate cancer of Gleason score <math>\geq 7</math> and diagnosed breast cancer and/or ovarian cancer</li> </ul> <p>b) optimal scope of the test includes sequencing and evaluation of large rearrangements (deletions/duplications) of <i>BRCA1</i> and <i>BRCA2</i> genes</p> <p>c) in families with identified specific <i>BRCA1/2</i> mutation, an occurrence of hereditary mutation should be verified in the carrier's family, above all first-degree relatives</p>	<ul style="list-style-type: none"> <li>– risk of developing ovarian cancer up to approx. 63%</li> <li>– risk of developing breast cancer in carriers up to approx. 87%</li> <li>– risk of developing breast cancer in male carriers up to approx. 1%</li> <li>– risk of developing prostate cancer in carriers up to approx. 8.5%</li> <li>– risk of developing pancreatic cancer up to approx. 3%</li> </ul>	<ul style="list-style-type: none"> <li>– each carrier from 30 years of age is recommended to have a TV-USG of the small pelvis and assessment of serum CA125 concentration every 6–12 months</li> <li>– in each carrier, prophylactic, bilateral adnexectomy is recommended, optimally at the age of 35–40 years, after completion of procreation plans</li> <li>– carriers diagnosed with ovarian cancer are expected to respond well to platinum derivatives and PARP inhibitors</li> <li>– each carrier should perform monthly self-palpation of the breasts</li> <li>– each carrier is recommended to breastfeed for a long time and to abandon/limit hormone replacement therapy (HRT)</li> <li>– each carrier is recommended to have a medical palpation of the breasts and breast imaging every 6 months (the age of beginning the tests depends on the family and clinical assessment, but should not be later than 25 years of age); MRI alternating with ultrasound (up to 30 years of age) or mammography (after 30 years of age)</li> <li>– in carriers who developed breast cancer, breast conserving surgery should be abandoned in favor of mastectomy, possibly with prophylactic contralateral mastectomy, ideally with simultaneous reconstruction</li> <li>– each female carrier may consider a prophylactic bilateral mastectomy, optimally with simultaneous reconstruction</li> <li>– chemoprevention with tamoxifen may be considered in any carrier</li> <li>– in carriers who have developed triple-negative or luminal type breast cancer that progresses despite anti-estrogen therapy, PARP inhibitors (olaparib or talazaparib) should be considered as the next-line treatment (after anthracycline and taxanes)</li> </ul>	
		<i>BRCA2</i>	<p>according to module I of the NPZChN MZ Programme<sup>2</sup>:</p> <p>a) analysis of occurrence of five mutations of <i>BRCA1</i> gene which are the most common in the Polish population (c.5266dupC, c.181T &gt; G, c.4035del, c.66_67AG, c.3695_3699GTAAA) in:</p> <ul style="list-style-type: none"> <li>– any person with breast cancer (including DCIS, male breast cancer)</li> <li>– any person with ovarian cancer (including peritoneal cancer and fallopian tube cancer)</li> <li>– in families with breast and/or ovarian cancer, in cases where the person with cancer is unavailable for examination, the analysis should be performed in the closest relatives (optimally first or second degree)</li> </ul> <p>b) sequencing of <i>BRCA1</i> and <i>BRCA2</i> genes:</p> <ul style="list-style-type: none"> <li>– only in people diagnosed with breast and/or ovarian cancer</li> <li>– only in cases where 5 most common mutations in the Polish population have been excluded</li> <li>– only if:</li> <li>• the patient has both breast and ovarian cancer, with the first diagnosis before the age of 50</li> <li>• the patient has had bilateral breast cancer, with the first diagnosis before the age of 50</li> <li>• the patient has been diagnosed with breast cancer or ovarian cancer and has a first and/or second degree relative who was diagnosed with breast and/or ovarian cancer, at least one of these cases before the age of 50</li> </ul>	<ul style="list-style-type: none"> <li>– risk of developing ovarian cancer up to approx. 27%</li> <li>– risk of developing breast cancer in carriers up to approx. 84%</li> <li>– risk of developing breast cancer in male carriers up to approx. 9%</li> <li>– risk of developing prostate cancer in carriers up to approx. 20%</li> <li>– risk of developing pancreatic cancer up to approx. 7%</li> <li>– a discreetly increased risk of developing melanoma (of the skin and/or eyeball)</li> </ul>	<ul style="list-style-type: none"> <li>– each carrier from 30 years of age is recommended to have a TV-USG of the small pelvis and assessment of serum CA125 concentration every 6–12 months</li> <li>– in each carrier, prophylactic, bilateral adnexectomy is recommended, optimally at the age of 35–40 years, after completion of procreation plans</li> <li>– carriers diagnosed with ovarian cancer are expected to respond well to platinum derivatives and PARP inhibitors</li> <li>– each carrier should perform monthly self-palpation of the breasts</li> <li>– each carrier is recommended to breastfeed for a long time and to abandon/limit hormone replacement therapy (HRT)</li> <li>– each carrier is recommended to have a medical palpation of the breasts and breast imaging every 6 months (the age of beginning the tests depends on the family and clinical assessment, but should not be later than 25 years of age); MRI alternating with ultrasound (up to 30 years of age) or mammography (after 30 years of age)</li> <li>– in carriers who developed breast cancer, breast conserving surgery should be abandoned in favor of mastectomy, possibly with prophylactic contralateral mastectomy, ideally with simultaneous reconstruction</li> <li>– each female carrier may consider a prophylactic bilateral mastectomy, optimally with simultaneous reconstruction</li> <li>– chemoprevention with tamoxifen may be considered in any carrier</li> <li>– in carriers who have developed triple-negative or luminal type breast cancer that progresses despite anti-estrogen therapy, PARP inhibitors (olaparib or talazaparib) should be considered as the next-line treatment (after anthracycline and taxanes)</li> </ul>

Hereditary cancer syndrome	Genes in which germline mutations are present	Indications for genetic testing	Risk of ovarian cancer in carriers of the mutation	Other neoplasms in the spectrum of increased risk and associated symptoms	Recommendations
<p><b>hereditary non-polyposis colorectal cancer (HNPCC, Lynch syndrome)</b> [8, 18, 22]</p>	<p><i>MLH1, MSH2, MSH6, EPCAM, PMS2</i></p>	<p>the patient was diagnosed with breast cancer before the age of 50 or ovarian cancer at any age, and in addition first and/or second grade relatives were diagnosed with male breast cancer and/or ovarian cancer in families with identified specific <i>BRCA1/2</i> mutation, hereditary mutation should be verified in the carrier's family, above all first-degree relatives if a specific <i>BRCA1/2</i> mutation is identified in DNA from tumor cells (tDNA), its presence should be analysed on DNA isolated from outside the tumor cells (blood, saliva, oral swab, skin biopsy specimen) to assess the nature of the mutation (somatic, i.e. non-hereditary or germline, i.e. hereditary)</p>	<p>– risk of developing ovarian cancer up to approx. 20%</p> <p>The risk of developing particular tumours depends on the gene in which the germline mutation occurred</p>	<p>– risk of developing colorectal cancer up to approx. 74%</p> <p>– risk of developing endometrial cancer up to approx. 54%</p> <p>– risk of developing gastric cancer up to approx. 18%</p> <p>– risk of developing small intestine cancer up to approx. 12%</p> <p>– risk of developing bile duct cancer up to approx. 6%</p> <p>– risk of developing urinary tract (urothelial) cancer up to approx. 25%</p> <p>– risk of developing a CNS tumor (so-called Turcot's syndrome) up to approx. 6%</p> <p>– additionally, in carriers of the <i>MLH1</i> or <i>MSH2</i> mutation:</p> <ul style="list-style-type: none"> <li>• increased risk of developing prostate cancer to approx. 30%</li> <li>• increased risk of developing breast cancer to approx. 18%</li> </ul> <p>– increased risk of developing pancreatic cancer to approx. 9%</p> <ul style="list-style-type: none"> <li>• increased risk of developing sebaceous gland tumor (called Muir-Torre syndrome) to approx. 9%</li> </ul>	<p>– each male carrier should conduct regular breast self-examination, and from 30–35 years of age annual medical palpation of the breast is recommended (especially in carriers of the <i>BRCA2</i> mutation)</p> <p>– each male carrier (especially with <i>BRCA2</i> mutation) may consider annual screening for prostate cancer starting from 40–45 years of age</p> <p>– in all carriers of the <i>BRCA2</i> mutation, annual dermatological and ophthalmological testing for melanoma may be considered, especially in cases of presence of this tumor in relatives</p> <p>– annual colonoscopy is recommended for each carrier:</p> <ul style="list-style-type: none"> <li>• starting from 20–25 years of age in carriers of the <i>MLH1</i> and <i>MSH2</i> mutations</li> <li>• starting from 30–35 years of age in carriers of the <i>MSH6, PMS2</i> and <i>EPCAM</i> mutations</li> </ul> <p>– in the case of diagnosed colorectal cancer, subtotal colectomy is recommended followed by endoscopic examinations of the preserved part of the large intestine at least every 2 years</p> <p>– in each carrier from 30–35 years of age gastroduodenoscopy every 1–3 years should be considered</p> <p>– in each female carrier from 30–35 years of age annual TV-USG with endometrial biopsy is recommended (unless a hysterectomy has been previously performed) and further an annual evaluation of serum CA-125 concentration</p> <p>– each female carrier may consider prophylactic bilateral adnexectomy with hysterectomy after the completion of procreation plans (optimally around 35–40 years of age)</p> <p>– annual neurological examination is recommended for each carrier</p>
		<p>according to ESMO recommendations<sup>1</sup>:</p> <p>a) in each case of colorectal cancer, the following tests should be performed: IHC studies to analyse expression of <i>MLH1, MSH2, MSH6</i> and <i>PMS2</i> proteins and/or microsatellite instability (MSI) on tumour cells; and a genetic test (sequencing and evaluation of large gene rearrangement associated with HNPCC) only in cases of abnormal IHC and/or MSI results</p> <p>b) in cases where tumour cells and/or the person diagnosed with colorectal cancer are unavailable for testing, a genetic test (sequencing and evaluation of large gene rearrangements associated with HNPCC) should be performed, if at least Bethesda criteria are met:</p> <ul style="list-style-type: none"> <li>– colorectal cancer diagnosed before the age of 50</li> <li>– the patient has at least 2 malignant neoplasms from the HNPCC spectrum</li> <li>– colorectal cancer diagnosed before 60 years of age and displaying the histological features typical of high microsatellite instability</li> <li>– a patient with colorectal cancer, who has at least 1 first-degree relative with malignant neoplasm of the HNPCC spectrum, and at least one of these diagnoses was made before the age of 50</li> <li>– a patient with colorectal cancer, who has at least 2 first/second-grade relatives with malignant neoplasm from the HNPCC spectrum, regardless of the age of diagnosis</li> </ul> <p>according to module II of the NPZChN MZ Programme<sup>2</sup>:</p> <p>a) in cases with Bethesda criteria met, IHC test should be performed analysing <i>MLH1, MSH2, MSH6</i> and <i>PMS2</i> proteins expression on cells of the intestine cancer and if results are out of normal range, diagnostics should include a genetic test verifying HNPCC-related genes, too</p> <p>b) in cases where tumour cells and/or the person diagnosed with colorectal cancer are unavailable for testing, a genetic test analysing HNPCC-related genes should be performed, if at least Bethesda criteria are met</p>			

Hereditary cancer syndrome	Genes in which germline mutations are present	Indications for genetic testing	Risk of ovarian cancer in carriers of the mutation	Other neoplasms in the spectrum of increased risk and associated symptoms	Recommendations
<b>RAD51 germline mutations</b> [23]	RAD51C RAD51D		<ul style="list-style-type: none"> <li>moderately increased risk of developing ovarian cancer:               <ul style="list-style-type: none"> <li>for RAD51C OR mutation approx. 5%</li> <li>for mutation RAD51D OR approx. 7%</li> </ul> </li> </ul>	<p><i>The risk of developing particular tumours depends on the gene in which the germline mutation occurred.</i></p> <ul style="list-style-type: none"> <li>currently, there are no sufficient data concerning increased risk of development of other cancers</li> </ul>	<ul style="list-style-type: none"> <li>each carrier may consider prophylactic bilateral adnexectomy after the age of 45</li> </ul>
<b>BRIP1 germline mutations</b> [23]	BRIP1		<ul style="list-style-type: none"> <li>moderately increased risk of developing ovarian cancer: OR approx. 4%</li> </ul>	<ul style="list-style-type: none"> <li>currently, there are no sufficient data concerning increased risk of development of other cancers</li> </ul>	<ul style="list-style-type: none"> <li>each carrier may consider prophylactic bilateral adnexectomy after the age of 45</li> </ul>

<sup>1</sup>ESMO – European Society for Medical Oncology

<sup>2</sup>National Cancer Control Programme of the Ministry of Health

prophylaxis and therapy for carriers of germline mutations associated with hereditary cancer syndromes with ovarian cancer in their spectrum. As in the case of hereditary breast cancers, most hereditary ovarian cancers are associated with carrying germline *BRCA1* or *BRCA2* gene mutation, which account for the hereditary breast and ovarian cancer syndrome. However, there is also high risk of development of the ovarian cancer associated with mutations of *MLH1*, *MSH2*, *MSH6*, *EPCAM*, *PMS2* genes, responsible for the *hereditary non-polyposis colorectal cancer* (HNPCC) or germline mutations in *BRIP1*, *RAD51C* or *RAD51D* genes (tab. III) [8, 21].

Currently, in patients with ovarian cancer, due to the growing importance of PARP inhibitors in therapy, it is recommended to start molecular diagnostics with sequencing of the *BRCA1* and *BRCA2* genes on tDNA, which can be isolated from both postoperative and biopsy material as well as from a tissue block. The results of the sequencing are then subjected to bioinformatic processing and thorough analysis in order to assess the clinical significance of the identified variants. Presence of *BRCA1* or *BRCA2* mutation (variations of class 5 and 4) in tDNA is a good predictive marker, which indicates probably high effectiveness of PARP inhibitors in therapy. Additionally, identification of *BRCA1* or *BRCA2* mutation in tDNA requires verification of the nature of the detected change (germline mutation which is hereditary or somatic mutation which is non-hereditary) through analysis of its occurrence in DNA isolated from cells outside the neoplasm. Interpretation of VUS variants (class 3, variants of unknown clinical significance) remains a challenge, both in terms of doubtful predictive value with respect to PARP inhibitors, and unknown effect of germline VUS on neoplasia risk.

Nevertheless, apart from patients with identified mutation in tDNA, genetic counselling should cover patients with no identified mutation in tDNA and those who did not have molecular testing on tDNA performed. Each patient with ovarian cancer referred for genetic counselling has their history and clinical analysis examined and differential diagnostics is performed considering hereditary breast-ovarian cancer (HBOC) and hereditary nonpolyposis colorectal cancer (HNPCC aka Lynch syndrome). On the other hand, patients who did not have *BRCA1* and *BRCA2* genes sequenced in tDNA, are referred to genetic testing of constitutive genome for 5 founder mutations of *BRCA1* gene. In the case of patients with family history, the testing is then expanded to include *BRCA1* and *BRCA2* gene sequencing. And only this is a basis for individual prophylactic and therapeutic recommendations (tab. III). In ovarian cancer patients, especially with family history of cancer who did not have mutations identified in *BRCA1* and *BRCA2* gene sequencing, broad-panel, commercial NGS testing may be considered, as it allows for sequencing many genes associated with many hereditary cancer syndromes within a single test. However, each genetic test has its limitations and if no mutation is found within the tested range, it does not unequivocally

exclude the suspected hereditary cancer syndrome. Therefore, the final recommendations should be formulated based on a comprehensive analysis considering molecular testing results, as well as family and clinical assessment.

After diagnosis of a germline mutation, the patient should be informed of the risk of the mutation in the family. It is also necessary to provide genetic counselling not only to patients with inherited ovarian cancer, but to selected members of their families, too. Individualisation of medical recommendations applies to all mutation carriers (including those diagnosed with another cancer from the spectrum of a given cancer syndrome, as well as those without cancer diagnosis), and also families with cancer in which no causative mutation was detected.

Detailed characteristics of the most frequent hereditary cancer syndromes of spectrum including ovarian cancer, and therapeutic and prophylactic recommendations for mutation carriers considering ESMO guidelines and modules I and II of the NPZChN Programme are shown in table III.

## Conclusion

Personalised medicine is increasingly applied in prophylaxis and treatment of breast and ovarian cancers. Application of individually tailored therapies based on immunochemical and molecular markers increases the patients' chances to avoid adverse effects, to prolong survival and progression-free survival.

Increasing access to molecular broad-panel and whole-genome testing allows identification of individual pathomechanisms that lead to neoplastic transformation within the tumour as well as to metastases, and this knowledge gives hope for application of potentially effective molecularly targeted therapies. Also, identification of patients with hereditary breast cancer and/or hereditary ovarian cancer enables development of individualised prophylactic and therapeutic recommendations for the patients, and for members of their families, too. However, this approach to diagnosing breast or ovarian cancer requires comprehensive assessment of the patient and multi-specialist, coordinated care by oncologist, surgeon, gynaecologist, geneticist, pathologist and laboratory diagnostician.

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