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Companion diagnostics (CDx) – potential to reveal a specific, efficacious therapy for a breast cancer

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ABSTRACT

Precision medicine is a practice related to the individualization of diagnosis and treatment so that the right dose of the right drug can be used at the right time in a given patient. This allows you to shorten diagnosis time, avoid side effects, increase treatment efficacy, improve clinical outcomes, and reduce healthcare costs. Companion diagnostics (CDx) concerns research aimed at identifying and testing optimal biomarkers useful in diagnostics and qualifying a patient for a specific treatment method. Identification of biomarkers is also important in the context of developing new targets and/or new therapies. CDx was tested for oncology applications. Among these, the most targeted drug development efforts include breast cancer (BC), which is the most commonly diagnosed cancer in women (approximately 2.1 million new cases annually; 1/4 of all cancers in women). Over the past 10 years, the incidence of breast cancer in women has increased by more than 20%. Comprehensive breast cancer control covers prevention, early detection, diagnosis, treatment, rehabilitation, and palliative care. Much progress has been made in the diagnosis and treatment of BC. However, it remains a multi-faceted disease that exhibits heterogeneity within the same tumor or different neoplasms, and a variable course of the disease. Expanding knowledge through genetic, proteomic, and metabolomic research on the molecular processes that make up the etiology of cancer allowed for the identification of specific tumor features and the development of targeted therapies against tumors with specific molecular features. Efficient diagnostics or the possibility of predicting the patient's response to treatment is an important goal of modern PM. The concept of co-development of drugs and diagnostics or companion diagnostics (CDx) has emerged, which is now the new horizon of cancer care.

INTRODUCTION

Personalized medicine (PM) is a concept concerning the use of knowledge on the molecular and genetic determinants of diseases to optimize diagnostics and treatment consisting in administering the right dose of a precisely selected drug depending on the individual needs of the patient, at the right time. In the practice of personalized medicine, special emphasis is placed on such attributes of therapy as the type of patient, drug, or dosage. Taking this into account, it can be said that conventional therapy is imprecise – it is based on generalized data on complex populations of patients with a given disease entity, treated in a specific way. Therapy determined in this way often shows lower-than-expected effectiveness. It has long been known that different patients react differently to the same drug and its components. For example, 33% of patients suffering from depression do not respond to antidepressants, and as many as 75% of cancer patients do not respond positively to the same prescribed pharmaceutical. Such patients may not only not benefit from the use but are also at risk of adverse effects. The US Adverse Event Reporting System (FDA Adverse Events Reporting System (FAERS) recorded 5.4 million reports and over a million deaths due to adverse drug reactions second reaction (ADR) in the last 10 years.

Awareness of genetic predisposition to the occurrence of a particular disease contributes significantly to taking actions aimed at diagnosis or implementation of therapy. For example, over 86% of patients who are aware of the genetic predisposition to familial hypercholesterolemia adhere to the prescribed therapy for up to 2 years while having a positive attitude toward the treatment process. Personalization of therapy – in a way that takes into account the patient's genetic characteristics – allows to reduce healthcare costs, and the number of trials and errors in prognosis, while improving clinical outcomes (Frost, 2020).

Personalized medicine is increasingly used in many fields of medicine, including psychiatry, neurology, and cardiology, as well as in oncology – including, among others, in the diagnosis, prevention, and treatment of breast cancer (WHO, 2021).

SEARCH STRATEGY AND SELECTION CRITERIA

A systematic review of the literature was conducted following the Preferred Reporting Items for Systematic reviews like Pubmed. Only 14 articles have been used.

RESULTS OF REVIEW

BREAST CANCER: DEFINITION, EPIDEMIOLOGY, TREATMENT

Breast cancer (BC) is the most common cancer in women. It is formed in the cells lining the ducts (85%) or lobules (15%) of the glandular tissue of the breast. Initially, the cancerous growth is confined to the duct or lobule (*in situ*) without causing symptoms. At this stage, it has minimal potential to spread. However, cancers *in situ* (stage 0) can progress and infiltrate the surrounding breast tissue (invasive breast cancer) and then spread to nearby lymph nodes, forming regional metastases or to other organs in the body, resulting in distant metastases. Extensive metastasis is the most common cause of death (WHO, 2021).

Treatment of breast cancer often consists of a combination of several therapeutic strategies: surgical removal, radiotherapy, and pharmacotherapy (hormone therapy, chemotherapy, and/or targeted biological therapy) used for the spread of cancer from the breast tumor through the blood (WHO, 2021).

There are 2,1 million new cases of the disease recorded annually in the world. In 2020 2.3 million women were diagnosed with breast cancer; 685,000 deaths were related to this cancer. According to data collected at the end of 2020, there were 7.8 million women worldwide who had been diagnosed with breast cancer in the last 5 years. Worldwide, cancer is the largest contributor to the loss of disability-adjusted life years (DALYs) among women with cancer. Breast cancer occurs in every country in the world, regardless of age after puberty. The risk of developing this cancer increases with age. Survival improved in the 1980s in countries that implemented early detection programs combined with various treatment regimens to eradicate invasive diseases (WHO, 2021). Over the last decade, the incidence of breast cancer in low- and middle-income countries has increased by more than 20%. A lower proportion of patients diagnosed with advanced disease in low- and middle-income countries, LMICs), is listed in High-Income countries, HIC). HIC has achieved significant reductions in breast cancer mortality, mainly due to heavy investment in research and advances in early detection and treatment. Known risk factors contributing to the increase in the incidence of breast cancer include changes in reproductive patterns (childbirth, breastfeeding), a sedentary lifestyle, and an unhealthy diet. Cancer control includes prevention, early detection, diagnosis, treatment, rehabilitation, and palliative care. The modern standard of oncological care is characterized by a multidisciplinary team approach. Educational activities developed for healthcare professionals, especially in primary care, can improve diagnosis, treatment, and outcomes and increase the number of appropriate referrals. The involvement of patient support organizations in ensuring adherence to post-operative care from the moment of diagnosis can promote the continuity of treatment. Multidisciplinary cancer care is effective. Oncology councils have the potential to help overcome diagnostic and management barriers in resource-constrained environments where specialists may be less available. An alternative is to partner with regional and private academic centers to run remote cancer councils, allowing for increased access to multidisciplinary expertise. Molecular oncology is increasingly becoming part of the standard of care, and molecular cancer teams are expected to become as important as site-specific cancer teams today (Alvarado, 2021). When a multidisciplinary approach is unavailable, patients first consult their GP. Only then does a surgical consultation follow. The presence of the tumor is confirmed histopathologically, which precedes the establishment of a treatment plan by a clinical oncologist or surgeon. Early detection of cancer improves patient outcomes because it affects the range of treatment options and contributes to extending the life and improving its quality (WHO, 2021). In addition, early identification of predispositions and genetic conditions may help in taking preventive measures and influence the early start of treatment in the event of a diagnosis. It is worth mentioning that a patient in the advanced stage of the disease generates much higher healthcare costs than a patient in the first stage. Thus, the earlier the diagnosis is made, the more likely it is that treatment will be started at an early stage, where the cancer is more amenable to treatment. Early detection of the disease also helps to prevent early mortality. High-quality mammography examinations, their proper targeting, and their frequency allow for achieving optimal benefits in screening programs (Alvarado, 2021). An integrated approach to the treatment of breast cancer patients can help mitigate the adverse effects of treatment and improve survival. This is most easily achieved by disseminating education to clinicians, who then pass on recommendations to their patients (Pelosci, 2022). Treatment of breast cancer can be very

successful, with a survival rate of 90% or more, especially if the disease is diagnosed early. While all breast cancers used to be treated surgically by mastectomy (total removal of the breast), a mastectomy may now be required if a large tumor is found. A lumpectomy or partial mastectomy is a procedure in which only the tumor and surrounding healthy tissue are removed from the breast. In these cases, radiotherapy to the breast is generally required to minimize the risk of recurrence. In the case of invasive cancer, lymph nodes are removed during cancer surgery. Procedures on smaller lymph nodes, i.e. 'sentinel node biopsies', with relatively few complications, are preferred. Breast cancer chemotherapy does not require hospitalization in the absence of complications. In the early stages, irradiation (radiotherapy) may prevent the need for a mastectomy. For late-stage cancer, radiation therapy can reduce the risk of cancer coming back, even after a mastectomy. In advanced breast cancer, in certain circumstances, radiotherapy can reduce the chance of dying from the disease. The effectiveness of breast cancer therapy depends on the correct course of treatment (WHO, 2021).

PERSONALIZED MEDICINE IN BREAST CANCER

In recent years, tremendous progress has been made in the treatment of breast cancer. Personalized medicine plays an increasingly important role in cancer prevention, diagnosis, treatment, and prognosis (Jackson, 2015). This cancer is a multifaceted disease that exhibits heterogeneity and fluctuating course. Knowledge about the molecular processes that contribute to the etiology of cancer is increasing. This allows the identification of specific features of the tumor and the development of targeted therapies targeted at specific cancers with specific molecular characteristics. Therefore, the goal of personalized medicine is the ability to predict the individual response to specific therapy and adjust the method of treatment to the individual characteristics of the patient and the characteristics of cancer cells. The proven effectiveness of targeted therapies in various tumors suggests that personalized medicine should be promoted as giving the best results.

ACCOMPANYING DIAGNOSIS – DEFINITION, BENEFITS DIAGNOSIS, AND TREATMENT

In oncology, the concepts of drug development in conjunction with companion diagnostics based on cancer-derived DNA tests are important (companion diagnostic, CDx), isolated from peripheral blood. Such concepts fit into pharmacogenomics. This, in turn, is based on the use of a person's genomic structure to predict the response to a drug or to tailor therapy specifically for a given patient. CDx is described as a medical device (often an *in vitro device*) that provides the information necessary for the safe and effective use of an appropriate drug (Alvarado, 2021) or biological product (U.S. Food & Drug Administration, 2021; Frost, 2020). CDx is understood and applied according to specific genomic, and molecular findings identifying patients likely to respond to targeted therapy and stratifying patients according to the molecular profile of a particular disease (Alvarado, 2021). Several CDx tests exist for oncology applications. In 2017, at least 387 targeted drugs were developed or marketed in oncology in the United States. Among these, the most targeted actions were those for breast cancer, non-small cell lung cancer, and colorectal cancer. CDx also improves the selection of participants for clinical trials (Frost, 2020).

The difficulty in obtaining tissue samples is a current challenge for accurate and timely oncology diagnostics. Therefore, liquid biopsy is becoming an increasingly used diagnostic tool. The undoubted advantage of this solution is minimal invasiveness and the fact that this method directly detects circulating tumor cells, cell-free circulating tumor DNA (ctDNA), or extracellular vesicles. This undoubtedly promotes accurate diagnosis (Frost, 2020). The use of accompanying diagnostics allows you to control the growing health expenses. This is due to the possibility of limiting the use of drugs only to those whose effects will be beneficial while reducing the costs associated with side effects. CDx has a positive effect on patient safety by reducing the frequency of invasive procedures. CDx can also improve the predictability of the oncology drug development process, as it enables better selection of the target population and lower research costs. The added value throughout the process is the ability to collect useful data (Alvarado, 2021).

MOLECULAR TESTS IN ACCOMPANYING DIAGNOSTICS

Hybridization *in situ* (*In situ hybridization*, ISH), quantitative real-time polymerase chain reaction (qRT PCR), and immunohistochemistry (IHC) are used to diagnose or detect the relevant disease biomarker. Next-generation sequencing, real-time single-molecule DNA sequencing, digital pathology, and quantitative histopathology have particularly influenced the development of CDx. Quantitative histopathology and digital pathology are medical imaging-based diagnostic approaches. For example, they measure protein biomarkers

in a tissue sample, which are identified and quantified using an automated fluorescence-based imaging platform (Frost, 2020). The combination of a study aimed at assessing the HER2 predictor factor (human epidermal growth factor receptor 2) in patients diagnosed with breast cancer, CDx, and trastuzumab treatment is an example of the successful implementation of CDx in Los Angeles (LA). This combination is cost-effective for healthcare systems and improves patient survival. The therapeutic use of molecular biomarkers is based on their detection and quantification. However, there are problems associated with this. For example, IHC testing is the standard method for detecting tumor biomarkers due to its ease of use, accessibility, use of routine microscopy, and ability to archive stained slides. However, in LA, IHC validation processes are not standardized and quality control is not common practice in laboratories. *In situ* nucleic acid hybridization (ISH) techniques are only available in highly specialized laboratories and institutes. They consist in attaching a labeled polynucleotide to complementary DNA sequences in the cell. The development of biosimilar tests requires a long validation process, which increases the cost of the tests and extends the delay in the availability of tests (Alvarado, 2021).

DIAGNOSTICS ACCOMPANYING BREAST CANCER

In breast cancer, therapeutic decisions must take into account both the biological subtypes of the tumors and their molecular and genetic characteristics; it is important to consider the risk of recurrence in patients with early breast cancer. Current methods for determining the risk of recurrence in this patient population are based on staging and are carried out according to standard clinical and pathological features of the disease. In particular, these features may not reflect the full risk of recurrence in people with early breast cancer (Hurvitz, 2022).

RECEPTORS: ESTROGEN AND PROGESTERONE

Cancers that express the estrogen receptor (ER) and/or the progesterone receptor (PR) are likely to respond to endocrine therapies (hormones) involving substances such as tamoxifen or aromatase inhibitors. These drugs are taken by mouth for 5-10 years and reduce the chance of recurrence of these ' hormone-positive ' tumors by almost half. Endocrine therapies may cause menopausal symptoms but are generally well tolerated. Cancers that do not express ER or PR are "hormone receptor negative" and require chemotherapy treatment unless the cancer is very small (WHO, 2021).

HER-2

Breast cancers can independently overexpress a molecule called the HER-2/ neu oncogene. "HER-2 positive" tumors are amenable to therapy with targeted biological drugs, e.g. trastuzumab. These biological agents are very effective but also expensive. Targeted biological therapies are often combined with chemotherapy to increase the effectiveness of killing cancer cells (WHO, 2021).

The success stories of trastuzumab and endocrine therapy in patients with HER2-positive and HR-positive BC show the potential of PM in the treatment of cancer. The current list of FDA-approved CDx remains almost exclusively based on molecular targets. In BC, most are based on protein detection with IHC. The over-expression of HER2 and the presence of the HER2 protein on the cell surface make HER2 an ideal molecule for use in targeted therapy. Trastuzumab was the first FDA-approved biologic for the treatment of HER2-positive BC. The use of the drug improves survival in 50% of neoadjuvant, adjuvant, and metastatic cases (Alvarado, 2021). HER2 testing is recommended for all primary, metastatic, and recurrent BC due to its high prognostic and predictive value. There are currently seven U.S. Food and Drug Administration (FDA) approved CDx kits to detect HER2 in BC *in vitro*.

For the FD A-approved HER2 breast cancer diagnostic test that also uses IHC CDx. To reduce interlaboratory variability, the American Society of Clinical Oncology (ASCO) in collaboration with the College of American Pathologists (CAP) has published recommendations and guidelines for HER2 IHC staging and scoring (Alvarado, 2021).

HERCEPTEST IN RESEARCH CLINICAL CONCERNING CANCER BREASTS (HERA, CLEOPATRA, EMILIA, ETC.)

The development of a drug that inhibits the proliferation of cancerous cells was one of the most important projects implemented in the 20th century. This anti-cancer drug is trastuzumab. The challenge for the development of trastuzumab concerned the selection of the right group of patients who are likely to respond

to the drug. In this case, the availability of a robust, accurate, and reliable test to detect HER2 overexpression in tumor cells was crucial.

In the clinical development of trastuzumab, the CTA (Clinical Trial Assistant), which was developed by Genentech, was used to select HER2-positive patients. It was only in the phase III trial with trastuzumab that the new IHC test, HercepTest ™, was optimized. HercepTest ™ was designed and developed by Dako. During the final development phase of HercepTest ™, a comparative study was conducted between HercepTest ™ and the CTA test. The goal was to indicate agreement between the two tests. In 1998, HercepTest ™ and the trastuzumab Test were simultaneously approved. HercepTest ™ has been used to screen cancer patients in several major breast cancer clinical trials. In these trials, HercepTest ™ has been used in the clinical stages of adjuvant, neoadjuvant, and metastatic therapy, and for many different types of HER2-targeted therapies. The HercepTest ™ test was also used, among others, in the selection of HER2-positive gastric cancer patients in the ToGA pathway. HercepTest ™ is considered the first companion diagnostic to be approved by the FDA. Over 20 years of using HercepTest ™ have documented the clinical relevance of this diagnostic test (Trost, 2021).

FUTURE DIAGNOSTICS ACCOMPANYING BREAST CANCER

In the oncology literature, theses were indicated that factors such as the patient's age, expression of ER, PR, and HER2 receptors, histological type of cancer and its diameter, degree of histological malignancy or the condition of the regionally located lymph nodes of the patient do not provide sufficient information necessary to plan individually tailored post-operative therapy in the case of breast cancer.

In 2006, an article was published presenting three modern diagnostic tests: CellSearchTM, OncotypeDxTM, and GeneSearchTM. The CellSearch test detects cancer cells circulating in the body of a patient with stage IV breast cancer. The analysis performed with this test helps to determine the prognosis of patients' progression-free survival and overall survival and is more effective than previous standards of care, which included diagnostic imaging studies. The sensitivity of the CellSearch test enables the detection of single cancer cells – it can detect even one cell in a volume of 7.5 ml of a patient's blood. The specificity of the CellSearch test is 99.99%. In studies on a group of patients with cancer progression confirmed by radiological imaging, survival was three times longer among patients with a circulating cancer cell level below 5 than among patients with a circulating cancer cell level above 5 – cells were detected using the CellSearch test. The CellSearch test includes an immunomagnetic technique and identifies circulating tumor-derived tumor cells as EpCAM +, CD45–, and cytokeratin 8,18 and/or 19+.

The OncotypeDX test provides additional data in the form of a score indicating the risk of cancer recurrence. The test allows us to identify the risk of recurrence 10 years ahead (in the case of patients with early-detected breast cancer, at the stage without metastases in regional lymph nodes and positive ER receptors). The impetus for the development of the OncotypeDX test was the analysis of 250 genes, followed by the selection of 21 genes based on the results of reverse transcription polymerase chain reaction (RT-PCR) studies (samples for testing from patients participating in research conducted by the National Surgical Adjuvant Breast and Bowel Project (NSABP) (Kołacińska, 2007).

The OncotypeDx test is therefore a 21-gen test that evaluates in a way quantitative the likelihood of breast cancer recurrence. In this way, it supports planning the optimal treatment of patients. In addition, it was noted that among patients included in the NSABP B-20 study, OncotypeDx allowed to correctly predict the effectiveness of adjuvant chemotherapy) (Kołacińska, 2007).

The third of the modern tests – GeneSearch – is designed to detect clinically significant (more than 0.2 mm in size) breast cancer metastases in the material from the removed lymph nodes as soon as possible using two markers: mammaglobin tested using the RT-PCR method and cytocreatine19. The results of the GeneSearch test allow you to make the right decision regarding the performance of total axillary lymphadenectomy, as well as to assess the advancement of the neoplastic disease. The GeneSearch test has become an alternative to the currently used intraoperative methods for examining lymph nodes. A study of 416 patients showed a higher sensitivity of GeneSearch compared to histopathological examinations of imprint biopsy and frozen sections. The GeneSearch test enables very good identification of metastases in cases of lobular breast cancer) (Kołacińska, 2007).

BRCA1 AND BRCA2

Epidemiological and molecular studies have shown a relationship between the risk of breast and ovarian cancer and *BRCA1* and *BRCA2 gene mutations*. This association has been demonstrated and confirmed in a variety of patient groups of different ethnic backgrounds.

The BRCA1 gene encodes a protein that plays an essential role in repairing damaged DNA and regulating the cell cycle during cell division. As a result of mutations in this gene, excessive, uncontrolled cell division and the development of cancer can occur. Mutations in the *BRCA1 gene* increase the likelihood of developing breast, ovarian, prostate, and colorectal cancer. They are inherited in an autosomal dominant fashion and can be passed down from generation to generation.

Women with mutations in the *BRCA1 gene* have an over 80% risk of breast cancer and a 40% risk of ovarian cancer. Cancers associated with the *BRCA1 mutation* account for approximately 10-15% of all breast cancer cases. *BRCA1* mutation carriers also have an increased risk of the fallopian tube and peritoneal cancer, which is about 10%.

BRCA2 encodes a protein that plays an essential role in repairing damaged DNA and regulating the cell cycle. Mutations in this gene can also lead to uncontrolled cell division and tumor development. Mutations are associated with an increased risk of breast, ovarian and gastrointestinal cancers – stomach, colon, and pancreas, in both women and men. It is estimated that mutations in the *BRCA2 gene* increase the risk of breast cancer to 56% and 27% of ovarian cancer.

Identification of the relationship between *BRCA1* and *BRCA2 mutations and* the risk of breast cancer is of great importance in the diagnosis, prevention of cancer, and epidemiological studies (Wang, 2012).

TP53

The TP53 gene is located on chromosome 17. It consists of 10 introns and 11 exons. The product resulting from the translation of the *TP53 gene* is a phosphoprotein, which functions as the main tumor suppressor in the cells of the human body. Phosphoprotein is a transcription factor composed of domains characteristic of known transcription activators. Among these domains, the following can be distinguished: the N-terminal, the C-terminal domain, and the domain responsible for binding to the DNA strand, which performs their respective functions. Currently, at least 12 p53 protein isoforms are known numerous studies are conducted (Guimaraes, 2002). *TP53 gene* mutates in most types of human cancer. It is one of the most frequently analyzed genes in oncology research. The tp53 protein is responsible for tumor growth suppression by regulating cellular repair mechanisms. Loss of tp53 suppressive capacity is caused by autosomal dominant inheritance of *TP53 mutations.* People with *TP53 mutations* show increased susceptibility to cancer, mainly: soft tissue sarcoma, osteosarcoma, breast cancer, adrenal cortex cancer, leukemia, or brain tumors. The p53 protein plays a particularly important role in cells exposed to carcinogens and oncogenic changes (Guimaraes, 2002., Zając, 2015). Thus, the rapid clonal expansion of cells with mutations of the *TP53 gene* may be the initiation of the process of tumor development.

Mutations in the *TP53 gene* are found in nearly 50% of solid tumors. It has been noted that hematological malignancies are less likely to show the presence of mutations within this gene. In the population of patients suffering from hematological malignancies, *TP53* mutations and 17p mutations covering the entire p arm of chromosome 17 are associated with resistance to standard chemotherapy and, consequently, with poor prognosis. Over the last few years, the therapies offered to patients with the *TP53 mutation* are considered to be one of the main challenges facing modern hematology. Mutations in the *TP53 gene* are of great importance in the prognosis and selection of appropriate treatment in patients with chronic lymphocytic leukemia (CCL) (Zając, 2015). Clinical trials of various drugs are underway, which have a chance to directly influence and regulate the activity of the p53 protein or otherwise force the activation of cell cycle regulatory mechanisms. The discovery of such drugs could improve the currently poor prognosis of patients with the *TP53 gene aberration*.

GENES INVOLVED IN CELL CYCLE INHIBITION

The cell cycle consists of the interphase (G_1, S, and G_2 phases), the mitotic phase (mitosis and cytokinesis), and the G_0 phase; which is related to DNA synthesis. Products of various genes from at least three families, such as Cip/Kip, Ink4, and pRb protein families, function as inhibitors of DNA synthesis. They inhibit the

entry of the cell into the S phase of the cell cycle. Ink4 proteins achieve the cell cycle inhibition effect by antagonizing the activation and formation of cyclin (D-CDK4) complexes. Cip/Kip inhibitors achieve their inhibition effect by inhibiting kinases (CDK2). These kinases, in turn, participate in pRb inactivation and, in addition, similarly to cyclin E, probably play other pRb -independent roles. The coordination of the actions of the three classes of proteins mentioned here remains to be explored (Van 't Veer, 2002).

GENE ECPRESSION PROFILING

An important goal of research using molecular techniques, the results of which may have clinical significance, is to link the expression of specific genes with a specific, known cell phenotype. Studies using multiplex molecular probes coupled with fluorescent markers, combined with computer analysis of the results, are of great importance, which allows for the simultaneous visualization of the expression of many genes with high resolution, both in time and space, in single cells of the human body.

MOLECULES INBOLVED IN THE INHIBITION OF IMMUNE CHECKPOINTS

The discovery of immunological checkpoints, such as CTLA-4 or PD-1, had a key impact on the development of new methods of cancer immunotherapy. Initially, these molecules were shown to play an important role in the mechanism of apoptosis and T-cell activation.

The CTLA-4 molecule mainly affects T lymphocytes in the early activation phase within the lymph nodes and acts as a "switch", contributing to the reduction of cytotoxic T lymphocyte activity and limiting autoimmune reactions. Studies conducted on mice deficient in PD-1 or CTLA-4 molecules develop autoimmune-like diseases. These diseases appear quickly after birth and are fatal in cases of CTLA-4 deficiency (if the deficiency is related to PD-1, the symptoms appear much later).

Subsequently, preclinical studies were conducted, the results of which showed the significant role of molecules such as CTLA-4 and PD-1 in maintaining immune tolerance against tumor cells in the periphery.

The development of PD-1 and CTLA-4 blocking methods based on the use of monoclonal antibodies made it possible to restore the normal anti-cancer immune response with the participation of cytotoxic T lymphocytes. So it was amazing that single molecules with PD-1 or CTLA-4 blocking activity showed effective anticancer activity. These discoveries revolutionized cancer immunotherapy. Thanks to the use of such molecules, after many years, for the first time, it was possible to extend the overall survival time of patients with melanoma (Haanen, 2015). Currently, methods of cancer immunotherapy, based on the use of molecules that block immune checkpoints, are used in the treatment of several other cancers, including breast, lung, colon, ovarian, and renal cell carcinoma. Clinical trials are underway, which will probably result in extending the scope of this type of therapy to other types of cancer.

DISCUSSION AND A SHORT CONCLUSION

In recent years, thanks to the implemented projects and clinical trials, it has been possible to design, implement and obtain funding for many effective and innovative methods of cancer therapy. The aim of the actions taken in the field of developing innovative methods of oncological treatment should be to reduce the global mortality rate due to breast cancer by 2.5% per year, thus avoiding 2.5 million deaths due to breast cancer worldwide in the years 2020-2040. Reducing global breast cancer mortality by 2.5% per year would avoid 25% of breast cancer deaths by 2030 and 40% by 2040 among women under 70. The three pillars leading to the achievement of these goals are:

- health promotion for early detection;
- timely diagnosis;
- comprehensive treatment of breast cancer.

Prompt diagnosis can be linked to successful cancer treatment, which in many cases requires specialist oncology care. CDx activities are important, as they provide many therapeutic and prognostic benefits for BC patients. Survival of breast cancer patients for at least 5 years after diagnosis ranges from more than 90% in high-income countries to 66% in India and 40% in South Africa. The developed methods of early detection and treatment of BC have proven effective in high-income countries and should be implemented for use in resource-constrained countries where only some standard diagnostic and therapeutic tools with limited effectiveness are available. In the era of PM, optimal results can be obtained by using the latest methods of diagnosis and treatment, including CDx tests.

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