MASTERARBEIT / MASTER'S THESIS

Titel der Masterarbeit / Title of the Master's Thesis

„Impact of dual blockade with subcutaneous Pertuzumab/Trastuzumab on HER2-positive breast cancer in Republic of Srpska"

verfasst von / submitted by
Marija Nikic

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Hvala!

Abstract

INTRODUCTION: Breast cancer is the most frequently diagnosed malignant disease in the world, with 500-600 people diagnosed annually in Republic of Srpska. Dual blockade with trastuzumab and pertuzumab leads to increased antiproliferative activity in breast cancer. Subcutaneous fixed-dose combination of these two monoclonal antibodies has a potential in improving the quality of patients' lives.

OBJECTIVES: Analysis of HER2 population and therapies used in Republic of Srpska from year 2019 to 2023.

METHODOLOGY: The analysis included patients with early stage HER2-positive breast cancer. A retrospective analysis was conducted in the period from 2019 to 2023 at the Oncology Clinic of the University Clinical Center of Republic of Srpska in Banja Luka.

RESULTS: Among 129 patients analysed from 2019 until 2022, 127 were women and 2 were male. Most of the patients received a combination of chemotherapy and dual blockade with pertuzumab and trastuzumab applied intravenously (63 patients). Out of 122 patients who have received adjuvant treatment, 41 received therapy with dual HER2 blockade, 37 patients received only mono HER2 blockade and 18 received therapy without HER2 blockade. In 2023, subcutaneous application was initiated in Republic of Srpska. From March the 1st until the 30th of September 2023, 55 patients (all women) were observed. Out of these 55 patients, 14 were started on Phesgo therapy (neoadjuvant), and another 9 patients were successively initiated within the following six months. Following the surgery and complete regression, the patients continued with the same therapy. However, 9 patients were candidates for subcutaneous formulation but have received T + P intravenously, 5 patients are on waiting list for this formulation, and there are currently 14 patients still on the waiting list for subcutaneous dual blockade.

CONCLUSION: 28 patients have an unmet need for the use of the drug Phesgo in the neo/adjuvant setting that would significantly improve their satisfaction and quality of life.

Kurzfassung

HINTERGRUND: Brustkrebs ist die am häufigsten diagnostizierte bösartige Erkrankung der Welt. Jährlich wird in der Republika Srpska 500–600 Menschen diagnostiziert. Eine Doppelblockade mit Trastuzumab und Pertuzumab führt zu einer erhöhten antiproliferativen Aktivität bei Brustkrebs. Die subkutane Kombination dieser beiden monoklonalen Antikörper mit fester Dosis kann die Lebensqualität der Patienten verbessern.

ZIELE: Analyse der HER2-Population und die eingesetzte Therapien in der Republika Srpska im Zeitraum 2019 bis 2023.

METHODEN: Die Analyse umfasste Patientinnen mit HER2-positivem Brustkrebs im Frühstadium. Eine retrospektive Analyse wurde im Zeitraum von 2019 bis 2023 in der Onkologieklinik des Universitätsklinikums der Republika Srpska in Banja Luka durchgeführt.

ERGEBNISSE: Unter den 129 Patienten, die von 2019 bis 2022 analysiert wurden, waren 127 Frauen und 2 männlich. Die meisten Patienten erhielten eine Kombination aus Chemotherapie und dualer Blockade mit intravenös verabreichtem Pertuzumab und Trastuzumab (63 Patienten). Von den 122 Patientinnen, die eine adjuvante Behandlung erhielten, erhielten 41 eine Therapie mit dualer HER2-Blockade, 37 Patientinnen nur eine Mono-HER2-Blockade und 18 eine Therapie ohne HER2-Blockade. Im Jahr 2023 wurde in der Republika Srpska mit der subkutanen Anwendung begonnen. Vom 1. März bis 30. September 2023 wurden 55 Patienten (alle Frauen) beobachtet. Von diesen 55 Patienten wurde bei 14 mit der Phesgo-Therapie (neoadjuvant) begonnen, bei weiteren 9 Patienten wurde innerhalb der folgenden sechs Monate sukzessive damit begonnen. Nach der Operation und vollständiger Rückbildung setzten die Patienten die gleiche Therapie fort. Allerdings waren 9 Patienten Kandidaten für eine subkutane Formulierung, haben aber T + P intravenös erhalten, 5 Patienten stehen auf der Warteliste für diese Formulierung und derzeit stehen noch 14 Patienten auf der Warteliste für eine subkutane Doppelblockade.

SCHLUSSFOLGERUNG: 28 Patienten haben einen ungedeckten Bedarf an der Verwendung des Medikaments Phesgo im neo-adjuvanten Setting, das ihre Zufriedenheit und Lebensqualität deutlich verbessern würde.

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INTRODUCTION

Breast cancer is the most frequently diagnosed malignant disease and the most frequent cause of death from malignant diseases among women. Around 2million patients are diagnosed with breast cancer each year, resulting in a total of 684,996 deaths. Approximately every 15 seconds, one woman is diagnosed with breast cancer (Ferlay et al., 2018).

In Bosnia and Herzegovina, according to GLOBOCAN data from 2020, approximately 1,554 new cases have been diagnosed. In Republic of Srpska, 500-600 cases of breast cancer are diagnosed annually. Around 18% of all early breast cancer cases show elevated expression of HER2 receptor (Sung et al., 2021). These HER2-positive breast cancers are related with an especially violent course of disease and high rates of disease recurrence.

The treatment and prognosis of HER2+ breast cancer patients were fundamentally changed by the discovery of Herceptin (trastuzumab), a humanized monoclonal antibody with targeted anti-HER2 activity. The usage of Herceptin (trastuzumab) in the early stages of HER2+ breast cancer treatment (starting from the neoadjuvant i.e., preoperative stage and continuing in the adjuvant/postoperative treatment) led to an improvement in therapy response rate, a reduction in risk of relapse and disease progression, and has almost doubled the rate of pCR - complete pathological response (Gianni et al., 2010). The addition of the drug PERJETA (pertuzumab) to the combination of Herceptin and chemotherapy further improved outcomes in the neoadjuvant stage, including pCR (Gianni et al., 2012; Schneeweiß et al., 2018). Perjeta (pertuzumab) is also a humanized monoclonal antibody that, when combined with Herceptin, enables complete blockade of the HER2 signaling pathway, which ultimately results in greater anti-tumor activity.

Pertuzumab and trastuzumab are monoclonal IgG1 antibodies, both humanized and recombinant, that target transmembrane HER2 glycoprotein, which has intrinsic tyrosine kinase activity. These antibodies bind to different epitopes of HER2 without competitive activity, and possess correlative mechanisms that interfere with HER2 signaling, leading to increased antiproliferative activity when administered in combination. A fixed dose subcutaneous combination of trastuzumab and pertuzumab includes 2 monoclonal antibodies, with recombinant human hyaluronidase. The target population for the use of the combination of fixed subcutaneous dose of trastuzumab and pertuzumab is the same as for the combination of Perjeta + Herceptin with potential in improving the quality patients' lives.

Historical overview of breast cancer treatment

Over time, the diagnosis and treatment of breast cancer has undergone numerous changes, and its origins can be traced back to oldest known surgical treatise on trauma, Edwin Smith's papyrus. Attitudes have changed from those that surgery can worsen the outcome of treatment to giving preference to neoadjuvant therapy in certain circumstances. It became clear that the disease is not local but systemic, and that the treatment is complex and cannot be treated by one doctor (specialty) alone. The very surgical approach to treatment has changed over the centuries in accordance with new achievements and the understanding of the complexity of diseases. Halsted's operation, which was extensive, was the method of choice for more than a century, and then came modified mastectomies as well as post-surgery and breast reconstruction.

In contrast, the Greek physician Leonid, who was skilled in surgical techniques, advocated the removal of tumorous changes. He described the appearance of breast with cancer, the retraction of the nipple and the skin, and it is assumed that the first person to remove a breast because of cancer, was him (Sakorafas & Safioleas, 2009).

In the eighteenth and nineteenth centuries, great progress was made in the breast cancer treatment. It is known that the lymph node involvement is a bad prognostic sign, and during the nineteenth century anesthesia and antisepsis as well as examinations of samples under a microscope were introduced. Various substances were used in the treatment of breast cancer, such as arsenic and iron (Sakorafas & Safioleas, 2010a).

In the twentieth century, the use of radical procedures was slowly fading away, with the appearance of new diagnostic methods such as mammography and new therapeutic options such as chemotherapy. It was suddenly possible to prove a hereditary component of cancer. Large, randomized clinical trials were being conducted and awareness of the importance of early detection and implementation of screening programs began to surge.

The question of whether radiation can contribute to healing was raised. The first controlled clinical study was started in Milan in 1973 under the leadership of Umberto Veronesi and then in the large NSABP study in 1976. Both studies proved that the posterior procedure followed by radiation of the breast tissue has the same outcome as radical mastectomy, but that the cosmetic effect is more beautiful, and the psychological condition of the patient is better. Radiation become an effective treatment in local control for the preponderance of early breast cancers (stage I and II) and is the standard found today in all leading international guidelines.

In this century, there were scientific breakthroughs regarding the research of cell growth factors, signaling pathways within the cell and genetic mutations that can be targeted and used for treatment. Surgery, radiotherapy, pathology with immunohistology, genetics, targeted therapy as well as traditional cytostatic use, are all a part of multidisciplinary approach to this problem. However, the final chapter on the treatment and cure for breast cancer, regardless of the stage of the disease, has not yet been written. Perhaps we are living in an era when it is being written, and perhaps the future will look at this period as another significant step towards the ultimate goal - curing breast cancer in each woman individually. After all, cancer is for each woman individually a disease for itself (Sakorafas & Safioleas, 2010b).

Risk factors for the development of breast cancer

The exact cause of breast cancer emergence is still not well-known, but some of the risk factors are older age, genetics, influence of female reproductive hormones, environmental conditions, food habits, presence of benign breast disease etc. In the time to come, new risk factors will certainly be defined. Women who have a family history of breast cancer, or who have already had surgery for cancer in one breast, or those randomly diagnosed with proliferation with atypia (precancer) on pathohistological findings, are more likely to get breast cancer. At an increased risk are also women from higher socioeconomic classes, as well as women who did not give birth or gave birth late after the age of 30, as well as those with a long reproductive period (early first period, before the age of 11 or 12 or late menopause start after the age of 55). Based on such epidemiological observations, high-risk groups can be identified.

Classification of risk factors

1) High-risk factors (3 times or higher growth)
    a) Women after the age of 40
    b) Former cancer in one breast (particularly before menopause)
    c) Family breast carcinoma
    d) Hyperplasia with atypia
    e) Women with proliferative breast disease with atypical hyperplasia have a greater risk for the development and occurrence of breast cancer (5x increase)
    f) Women who have not given birth or those who had their first pregnancy after the age of 31 have a 3-4 times elevated risk of breast carcinoma compared to women younger than 18 who have had their first child.

g) LCIS (lobular carcinoma in situ) increases the potential of developing breast carcinoma by 30%
h) Key risks in men for the development of breast cancer - Klinefert syndrome, gynecomastia, and the presence of breast cancer in the family

2) Medium risk factors (1,2-1,5 times increase)
   a) characteristics of the menstrual cycle (early or late menarche)
   b) carcinoma of the ovary, uterine fundus, or colon
   c) oral estrogens in women
   d) diabetes mellitus
   e) use of alcoholic beverages
   f) radiation exposure during adolescence
3) Factors that reduce the risk of developing breast cancer
   a) Asian ancestors
   b) pregnancy before the age of 18
   c) menopause that occurs before the age of 45
   d) surgical castration before the age of 37 (Guest, n.d.)

Prevention measures

Early detection of breast cancer is done by physical examination and mammography - screening mammography, which is somewhat different from regularly scheduled examinations. The most important goal of screening is to reduce mortality. Taking into account the nature of the disease, at present there is no primary method of breast cancer prevention in the world, but great efforts and funds are being invested in secondary prevention, i.e. the earlier detection of the disease, before the cancer has spread from the place of origin of the so-called in situ cancer, and even more ideal is the detection of precancerous changes, because early removal of such changes prevents the development and return of the disease. The bottom line is that women receive an invitation to come for a mammogram. After the imaging, the woman returns home. The radiologist will look at the image and if he has any doubts, the patient is referred to a further program of diagnosis and confirmation of the disease. Screening mammography is a generally accepted way of early detection of this disease in the world. Women over 40 are candidates for screening mammography. Younger women are only given a mammogram if they are at high risk for developing the disease. Men can also suffer from this disease, and if necessary, they

can also get a mammogram. Usually, if there is no screening program, after the age of 50, every woman should have a mammogram at least once every two years.

It is also necessary to implement education programs for women to perform breast self-examination. After turning 20, once a month, every woman should preform a breast self-examination. Self-examination is done after menstrual bleeding because the breasts are the least painful and tense then. For women who are in menopause, the woman should determine one date a month when she will do a breast self-examination.

And as the most basic prevention measure, we should mention lifestyle changes: no alcohol, regular physical activity, reduced obesity in post-menopause, reduced fat intake, advise breastfeeding, usage of vitamins A, C, E and D etc.

Unfortunately, no number of preventive measures can guarantee that cancer will not appear, but you should certainly work to ensure that there are as few chances as possible of it ever appearing (Schrijvers et al., 2008).

Different types of breast cancer

Breast cancer is not one disease and can be divided in different types. Today, given the great heterogeneity of the tumor itself, it is considered that no two women have the same breast cancer. With the introduction of molecular and genetic testing, a wide range of different entities is obtained. The doctor uses numerous pathological characteristics and examines biological behavior of the tumor when deciding on the choice of treatment. On the other hand, one must also consider the patient condition as well as their comorbidities. Due to all the above, prognosis for each patient differs, although the arrival of new technologies allows for a more accurate disease prognosis. For decades, the most important prognostic factors were tumor size, status of axillary lymph nodes, nuclear grade and pathohistological tumor type. Then comes the discovery of estrogen and progesterone receptors, and soon also the epidermal growth factor receptor - HER2, that are also predictive factors because they represent targets for a certain type of therapy (Nagini, 2017).

There are many divisions and categorizations of breast cancer, but the main division is into three types:

- Tripple negative breast cancer (TNBC)
- Hormone-dependent breast cancer
- HER2-positive breast cancer

Tripple negative breast cancer (TNBC)

Carcinomas in which estrogen and progesterone receptors are not expressed, as well as HER2 receptors, bear the collective name of triple-negative breast cancer and account for about 15% of all malignant breast tumors. These tumors are most often high-grade, they often involve the lymph nodes, they often occur in young patients and in African American women. These cancers often result in the removal of metastases, especially visceral ones, and as a result, women with this disease have a shorter life span compared to those with other types of breast cancer. They also show a high prevalence of embryonic BRCA mutation. Compared to other types of breast cancer, TNBC has a BRCA 1/2 mutation in 15-20%. Genetic testing guidelines include TNBC subtype as an independent criterion for the syndrome of hereditary breast and/or ovarian cancer, recommending counseling and testing for all TNBC patients younger than 60 years regardless of family history. Although it is labeled as one subtype of breast cancer, this is fundamentally incorrect because this is a very heterogeneous group of cancers and can be divided into ≥ 6 molecular subtypes. In recent years, achievements were made in revealing the biological diversity of TNBC and associated gene expression pathways according to specific molecular subtypes with potential therapeutic significance (Almansour, 2022).

This cancer type is challenging to treat and has worse prognosis compared to other subtypes of breast cancer. It has a higher probability of early recurrence and death. Chemotherapy is still the first line of standard treatment, but in some patients, it can be combined with treatment based on anti-PD-L1. The current FDA approval of the combination of atezolizumab with nab-paclitaxel, for the initial treatment of PD-L1+TNBC, promises future options for patients and potential improvement of treatment outcomes. This combination is authorized for women who were diagnosed with locally advanced, unresectable, or triple-negative metastatic breast carcinoma who show first-line PD-L1 expression (Schmid et al., 2018). Recently, another promising Check point inhibitor – Pembrolizumab was approved, in metastatic, as well as and neo-adjuvant setting (Dudley et al., 2016).

Hormone-dependent breast cancer

A key step towards a better understanding of the effects of hormones on cancer growth occurred with the discovery of receptors for steroid hormones. Steroid receptors are tissue-specific proteins to which the corresponding steroid hormones bind. The hormone-receptor complex binds to DNA, after which gene expression is induced, that is, the synthesis of specific proteins. Steroid receptors include proteins that bind to estrogens, progestins, androgens, and glucocorticoids. Estrogens play a key role among them, which is why the basic principle of hormone therapy is to lower the levels of these regulatory hormones. Structurally, estrogen receptors come in two isoforms, ERα and ERβ, and are produced by two different genes. It is assumed that ERα is important for the normal development of the mammary gland and at the same time is highly present in premalignant changes in the breast. In contrast, ERβ may serve to prevent the stimulatory effects of ERα, and high expression is correlated with good response to anti-estrogen therapy (Chan et al., 2016).

Unlike estrogen receptors, progesterone receptors are the product of one gene, although they come in two isoforms: PR-A and PR-B. The relationship between the two isoforms is essential for proper lobuloalveolar development and proper structure and function of the glandular part of the breast. The role and relationship of these two isoforms has not been fully clarified, so overexpression of PR-A may be associated with resistance to tamoxifen, and an increase in the PR-B isoform is related to higher risk of breast cancer (Li et al., 2022).

The main advantages of hormonal treatment are selectivity, good efficiency, low toxicity, and low price, while the disadvantage is the limitation of application only to hormone-dependent tumors.

A prerequisite for the effect of hormones on breast cancer is its hormonal dependence, that is, the expression of estrogen and/or progesterone receptors. Therefore, the concentration of estrogen and progesterone receptors must be determined for each breast cancer. About 60-70% of breast tumors are hormonally dependent. From 1896 to the present day, various procedures for the hormonal treatment of breast cancer have been developed, which include surgical and radiotherapy methods, the use of pharmacological doses of androgens, progestogens and estrogens, and the use of inhibitors of the production and effect of estrogen. This last group consists of aromatase inhibitors, antiestrogens, antigestagens and luteinizing hormone releasing hormone-agonists (McAndrew & Finn, 2020).

HER2-positive breast cancer

HER2 receptor structure

In addition to the hormone receptor status, the pathologist also submits the HER2 receptor status. It stands for human epidermal growth receptor 2. This receptor was discovered in 1962 and it is a 185kDa transmembrane glycoprotein which consists of 53 amino acids (Lv et al., 2016). Her2/neu is encoded by the c-erbB2 gene, which stands for cellular avian erythroblastosis homologue B2 (Turzynski, n.d.).

It is a membrane receptor that can be over-exposed on the surface of tumor cells. The receptor is one of the tyrosine kinase receptors of EGFR (extracellular growth factor) family, where the intracellular domain has an enzymatic activity that couples phosphate groups to tyrosine residues of the intracellular effector molecules (tyrosine kinase activity). Extracellular domain has an amino terminal where ligands would be binding.

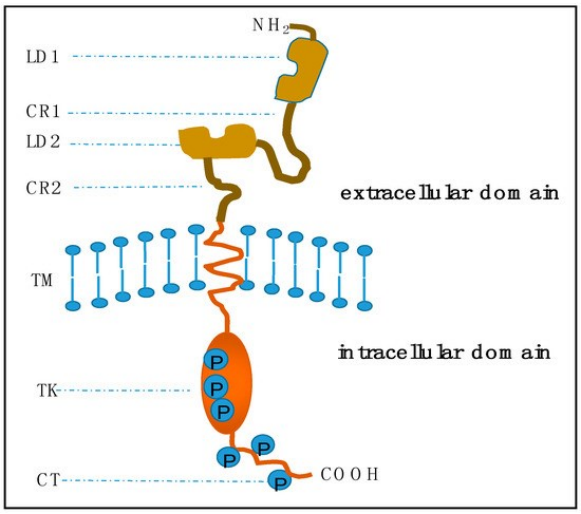


Figure 1. HER2 receptor structure (Lv et al., 2016) based on the work of (Moasser, 2007)

Extracellular domain consists of LD1 and LD2, which are domains that bind ligands, and CR1 and CR2 regions with cysteine. Tyrosine kinase activity of intracellular domain is provided with TK (tyrosine kinase) region. Intracellular domain ends with CT carboxy terminal tail. Both regions are filled with phosphorylation sites. Single transmembrane spanning sequence between the domains is the TM region. This figure is revised based on the review of the oncogene human epidermal growth factor 2 (Lv et al., 2016).

HER2 Signaling Pathway

Compared to other receptors of EGFR family, HER2 does not have receptor specific ligands. HER2 kinase catalytic activity is considered one of the most potent as it has a lot of dimerization partners, and it has an important role in cellular process regulation (Graus-Porta et al., 1997; Tzahar et al., 1996).

Among the EGFR receptor pairs, HER2+HER3 heterodimer complex is the most signaling one (Issekutz et al., 2011; Moens et al., 2013)

Signal pathway begins when ligand binds on the extracellular domain of the receptors. It induces the HER2 + EGFR family receptor dimerization, followed by a series of signalling pathways. Different pathways lead to series of different events, one of them being the tumor proliferation, which takes an important role in development of cancer cells. The most important pathway is PI3K-phosphatidyl inositol 3-kinase, as well as MAP-mitogen activated protein (Tai et al., 2010; Yarden & Sliwkowski, 2001).

At the end of these events, DNA synthesis and cell division are stimulated. Therefore, this factor promotes DNA synthesis and division in both normal and tumor cells, and its overexpression is often present in epithelial tumors.

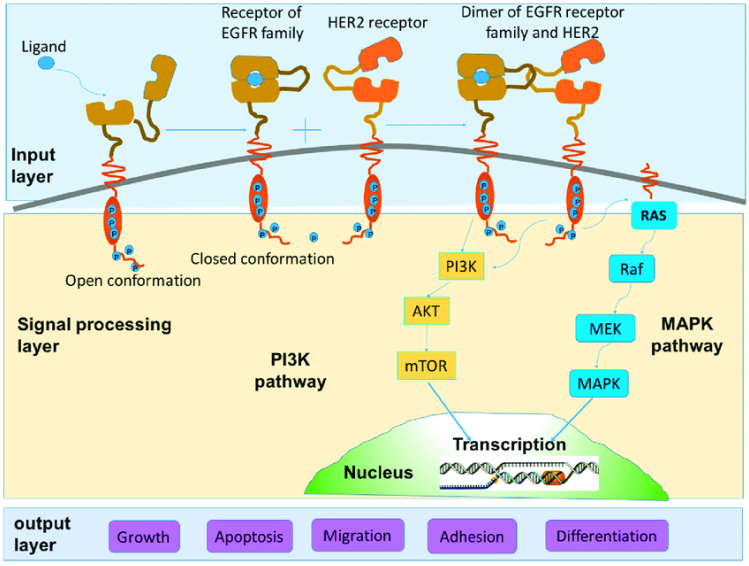


Figure 2. Schematic of HER2 signaling pathway (Lv et al., 2016) The scheme is modified based on two works contributed by (Yarden & Sliwkowski, 2001) and (Tai et al., 2010), respectively.

In normal cells, HER2 would be degraded by enzymes or recycled after they fulfill their role in a cellular process, but the problem occurs when HER2 is overexpressed. The receptors cannot

be deactivated so they continue to send the signals that lead to tumor cell growth, that later becomes uncontrollable (Rubin & Yarden, 2001a).

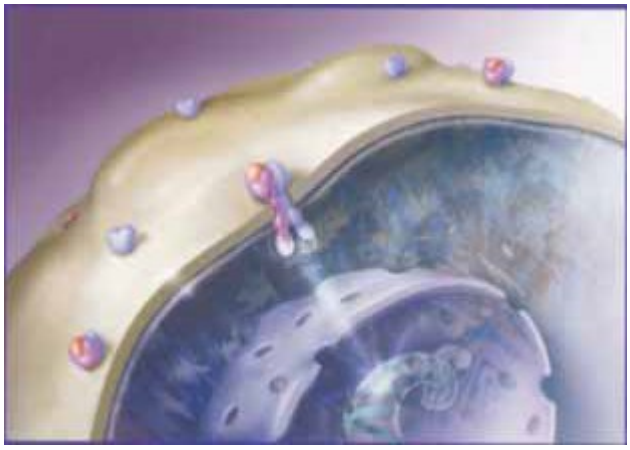


Figure 3. HER2 receptor-normal expression (Gojković, 2021)

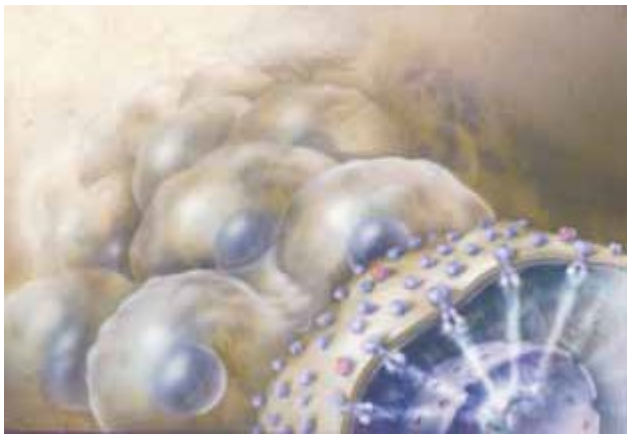


Figure 4. HER2 receptor overexpressed (Gojković, 2021)

Detection of HER2 in clinical tumor samples

Numerous methods have been used in the detection of HER2 amplification on DNA level (Southern blot, fluorescent in situ hybridization, PCR-based techniques), on transcriptional level (Northern blot, RT-PCR) and on protein level. In situ techniques are generally more sensitive in detecting HER2 gene amplification or protein overexpression. The most widespread method for spotting overexpression of HER2 is immunohistochemistry, which detects the protein amount. The problem with this approach is that there are many primary antibodies in clinical use, each of which binds a different epitope; these antibodies also have different sensitivity and specificity, and different values when distinguishing between tumors with and without overexpression. Therefore, HER2/neu detection is not standardized well due to

alteration of the gene, and while this strategy is globally available, portion of these tests are not optimal. 1 (Western blot, IHC or ELISA for detection of the soluble HER2 protein. Consequently, it is challenging to determine whether a sample that was HER2 positive in one laboratory, will also be detected to be positive in another laboratory.

Tumor samples that have been fixated with formalin and later preserved in paraffin result in degradation of epitope, meaning that the sensitivity of the sample would later be irretrievable in assembled clinical material. As a solution to this case, some assays operate with antigen loss replacement technique that involves cooking the tumor sample with heat, as well as microwave radiation, so that epitopes can be rehabilitated, which are lost during processing and storage of the tissue (Fornier et al., 2002). This can cause new problems, because normal tissue and malignancies without HER2 overexpression often contain some physiological level of HER2 expression. Antigen replacement techniques can increase the signal of this low level of HER2 protein, resulting in a false positive interpretation of HER2 overexpression (Wolff et al., 2007).

While the ELISA assay, designed to detect soluble HER2 protein in serum samples, is potentially useful clinically for monitoring the course of disease in cases with HER2-positive advanced breast carcinoma, it is not practical for early-stage breast cancer, as soluble HER2 protein can usually be found only in the serum of patients with a high risk and a high level of overexpression of HER2, which is the case in metastatic disease. In patients with small tumors, this assay is often negative even though there is overexpression of HER2 at the tissue level.

Perhaps the most accurate technique currently available for detection of HER-2/neu alteration of the gene is FISH-fluorescence in situ hybridization (Wolff et al., 2007). In this essay, a fluorescently labeled genomic DNA probe, which contains the HER-2/neu gene and its flanking sequences, enables the hybridization of tumor cell DNA within a thin section of the tumor under the microscope. A second DNA probe, specific for the centromere of chromosome 17 and marked with a different color than the HER2 probe, is used to distinguish between true HER-2/neu gene amplification and chromosome 17 fertility. Using this technique, the quantitative number of HER-2/neu gene copies per centromere of chromosome 17 can be determined (Sapino et al., 2013). One theoretical shortcoming of this essay is the inability to detect single copy overexpressor. In such cases, there is overexpression of HER2 protein in the absence of HER-2/neu gene amplification. However, the frequency of overexpression of a single copy of HER-2/neu in breast cancers is estimated to be less than 5% of all overexpressing tumors; therefore, the advantage of FISH in terms of sensitivity and specificity outweighs its disadvantage in terms of diagnostic accuracy.

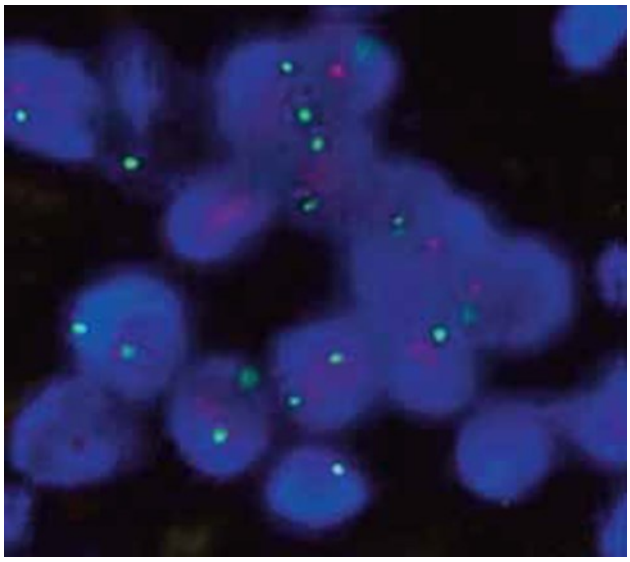


Figure 5. Normal number of HER2 genes detected with FISH method (Gojković, 2021)

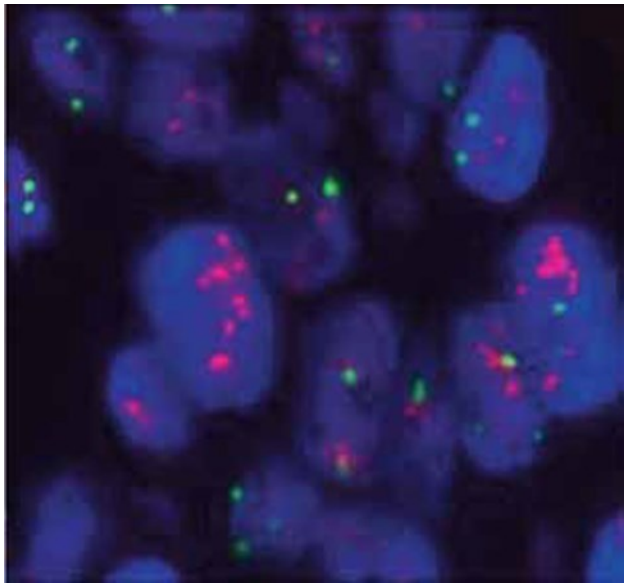


Figure 6. Amplified number of HER2 genes detected with FISH method (Gojković, 2021)

It is important to remember that overexpression of HER2 correlates well with numerous other established prognostic factors and clinical parameters of breast cancer. Therefore, the clinician can assess the possibility of HER2 overexpression in the appropriate sample based on these clinical parameters. If the result of a certain HER2 diagnostic assay is not consistent with the clinical picture, it is necessary to use a different methodology, such as FISH, to retest the sample to confirm or reject the first result.

Overexpression of this protein is found in 15-20% of all cases of breast cancers, often related to poorly differentiated cancers and with hormone-negative cancers. According to the guidelines from 2013, HER2 positive tumors are considered to be those tumors which in >10%

of the tumor cells that are invasive show extreme and complete staining of the membrane (IHH 3+ staining) or are amplified (HER2/CEP17 ratio, using ISH, in at least 20 tumor cells cell, >2.2), or have an average number of copies of the HER2 gene >6 signals (Zhang et al., 2020).

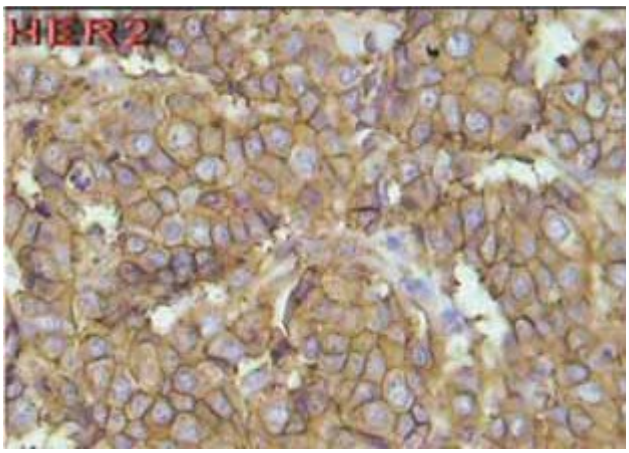


Figure 7. Almost all cells are with reaction 3+ (Gojković, 2021)

During the IHC analysis, the pathologist receives a result that is 2+ and then it is necessary to perform in situ hybridization to determine whether there is amplification of the HER2 gene.

Additional diagnostic tests enable the selection of patients into groups according to specific mutations (molecular type) of the tumor, and thus determine the optimal therapy. Testing for HER2 is recommended for all newly diagnosed primary cancers, metastatic cancers, as well as for newly occurring recurrent cancers.

Primary tumor tissue can be obtained by needle core biopsy, incisional or excisional surgical procedure. Metastatic cancer tissue can be obtained by biopsy of the chest wall of regional lymph nodes or visceral organs. Initial determination of receptors by immunohistochemistry (IHC) can be done on needle CORE biopsies of the breast.

It is advisable to perform a repeat biopsy if the initial result of HER2 testing on a needle biopsy is questionable due to some differences.

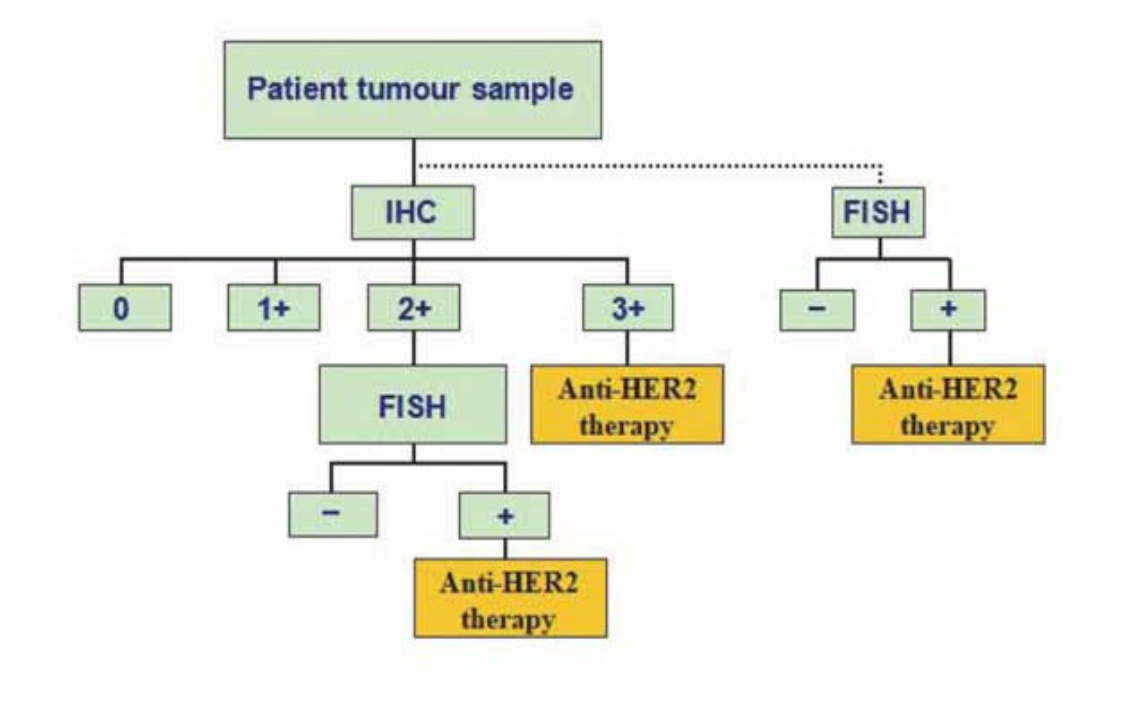


Figure 8. Algorithm of HER2 testing (Gojković, 2021)

Treatment of HER2- positive breast cancer

History of HER2-positive breast cancer treatment

The treatment of HER2+ breast cancer has seen revolutionary progress since 1987, when overexpression of HER2 was first linked to a particularly aggressive form of breast cancer (Slamon et al., 1987). Already in 1990, trastuzumab (Herceptin) was created, which was labeled as "potentially useful for the treatment of humans". This monoclonal antibody targets the extracellular part of the HER2 receptor, and represents a so-called directed, targeted therapy that is aimed at treating cancer (Delaney, 1999). In 1998, the FDA (U.S. Food and Drug Administration) approved Herceptin in the treatment of HER2+ advanced and metastatic breast cancer, following the groundbreaking results of the 2nd study. For the first time, patients have hope. And in 2000, the same registration was approved by the EMA (European medicine agency (Carter et al., 2000). In 1998, the HercepTest was approved, the first test that allowed doctors to assess which patients would benefit from anti-HER2 therapy (Jørgensen et al., 2021). Further progress in anti-HER2 treatment was seen in 2005 after the presentation of the results of five clinical studies. The efficacy of trastuzumab in early HER2+ breast cancer was investigated. After the publication of the study, the FDA, through an accelerated procedure, approved the

registration of trastuzumab for the adjuvant therapy of early HER2-positive breast carcinoma, and it was soon approved by the EMA. Trastuzumab was registered in Bosnia and Herzegovina in 2006. Since 2006, the drug has been available in Bosnia and Herzegovina for patients that have been diagnosed with metastatic HER2 positive breast cancer, and the indication for adjuvant therapy was soon approved. With the arrival of the results of clinical studies for the use of trastuzumab with pertuzumab in non-adjuvant treatment, both drugs were approved as non-adjuvant therapy of early HER2 positive breast cancer in Bosnia and Herzegovina.

Neoadjuvant chemotherapy (NACT)

Neoadjuvant (preoperative, induction) chemotherapy is systemic drug therapy that begins before surgical treatment. In a high percentage, NACT achieves a reduction in the tumor mass and can convert an inoperable tumor process into an operable one or increase the chances for a posterior surgical procedure as well as axillary dissection that is less radical, and degrade the morbidity related to surgical breast procedures, and together with other risk factors can help in predicting prognosis. If possible, anti-HER2 therapy needs to start before the surgery to raise the chance of attaining a pCR-pathologic complete response (Untch et al., 2011). Today, anti-HER therapy is the standard of care while treating early HER2+ breast carcinoma, as well as locally advanced cancer. Treatment should begin immediately, omitting anthracyclines. If anti-HER2 therapy is prescribed together with anthracyclines, it significantly increases cardiotoxicity. Neoadjuvant therapy is usually started with anthracyclines, especially in countries with low resources, where the HER2 test results require a longer wait period. In order to start treatment as soon as possible, multidisciplinary teams often decide to start the treatment of HER2+ breast cancer with AC (anthracycline/cyclophosphamide) until the results are available. After four cycles of AC, treatment continues with the use of combined chemotherapy with taxans and with anti-HER2 or two anti-HER2 drugs combined and with taxanes.

Important goals and responses we get from neoadjuvant treatments are:

- Direct assessment of drug effectiveness
- Faster introduction of effective drugs into clinical use
- Better understanding of tumor biology
- Searching for biomarker predictors (tissue, blood)
- Development of radiological techniques

The most frequently used protocol in the neoadjuvant stage of HER2 positive breast cancer treatment is the pertuzumab + trastuzumab combination with different chemotherapy protocols (Cardoso et al., 2019).

Adjuvant therapy

The main goal of adjuvant (postoperative) therapy is the removal/destruction of micro metastases, which are known to be left behind after surgery, and thus prevent the recurrence of the disease. As with neoadjuvant and adjuvant therapy, the possibility of healing should be provided, especially if it is an early stage of the disease. If this is not achieved, after some time, metastases will appear and then the disease becomes incurable, although with newer therapeutic procedures, personalized drugs, metastatic disease can be controlled for a very long time and in rare cases complete regressions can be achieved. HER2+ breast cancer is an aggressive tumor, and treatment with trastuzumab/pertuzumab should start soon after surgery, that is, within three to six weeks after surgery. Anti-HER2 therapy is administered for a total of one year.

Pertuzumab/trastuzumab combination in neo/adjuvant treatment of HER2+ breast cancer

Anti-HER therapy resistance

Trastuzumab has transformed, in a positive sense, the treatment of HER2-positive breast cancer, but many patients still die from recurrent disease. Although the HER2 receptor is recognized as a therapeutic target, it has become clear that there are certain mechanisms of resistance to the applied target therapy. It is known that there are four forms of HER2 receptor tyrosine kinase, and they are important for the growth and development of human cells. All four receptors belong to the receptor family of epidermal growth factor, have analogous structures, and can pair with the same or another HER receptor. Ligand binding causes HER receptors to associate in pairs in a process called dimerization. Dimerization and then autophosphorylation must occur to initiate the signaling pathway to the interior of the cell. Pairs can be formed by two identical receptors (homodimers) or two different ones (heterodimers), and HER2 is the favored partner for other receptors in HER family for dimerization (Rubin & Yarden, 2001b).

Figure 10. Homo- and heterodimers (Gojković, 2021)

The ability to heterodimerize can be one of the reasons for resistance to anti-HER therapy. Other mechanisms of resistance that may arise because:

- Changed binding to the ErbB2 receptor
- Shortened noncellular domain (p95 ErbB2)
- Other proteins that can bind such as MUC 4
- Mutations in receptor
- Transition to alterative ways of tumor regulations such as IGFR pathway

Reasons for dual targeting of HER2

HER2 overexpression occurs in 18-20% of breast cancers. Dimers containing HER2 induce potent mitogenic signaling resulting in cell survival and proliferation. HER2-positive breast cancers are stimulated with both ligand-independent and ligand-dependent HER2-HER3 complexes. Simply put, although trastuzumab binds to the HER2 receptor and thus blocks the transmission of signals to the nucleus, another domain remains free that can pair with the HER3 receptor and cause intracellular tyrosine kinase phosphorylation and start the signaling cascade to the nucleus again. Therefore, dual blockade of these complexes should result in greater clinical activity than when trastuzumab is administered alone. HER2:HER3 is the most frequent and potent oncogenic dimer in HER2-positive breast cancer] (Agus et al., 2002). Dimerization of HER2:HER3 launches numerous signaling pathways, one of them being elevated proliferation of tumor cells. Dual blockade is shown in the figure, and a new monoclonal antibody that binds to the second domain is pertuzumab. Pertuzumab and trastuzumab approach different domains on HER2 and bind on them. Doing so, they complement their mechanism of action.

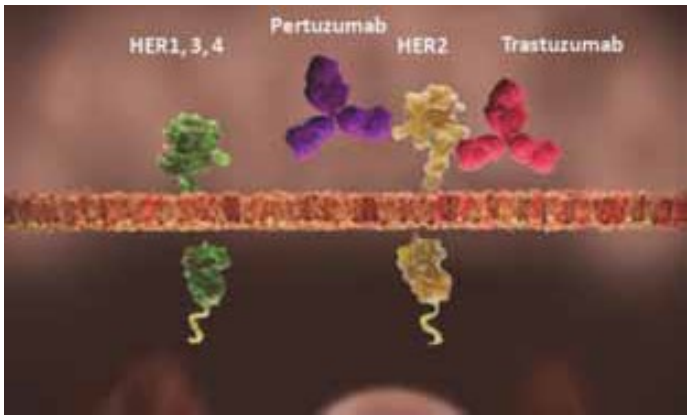


Figure 9. Pertuzumab and trastuzumab binding to different domains of the HER2 receptor (Gojković, 2021)

The essence of the action of dual blockade lies in the fact that pertuzumab and trastuzumab bind to different domains of HER2: Pertuzumab binds to sub-domain II and inhibits ligand-dependent signaling. Trastuzumab binds to sub-domain IV and inhibits ligand-independent intracellular signaling (Nahta et al., 2004). Thanks to the dual blockade, the scale of response to neo/adjuvant therapy is very high. Various studies, like Neosphere (Gianni et al., 2012) and TRYPHAENA (Schneeweiß et al., 2018) in neoadjuvant treatment, and APHINITY in adjuvant setting, examined and confirmed the effectiveness of dual blockade (Von Minckwitz et al., 2017).

Subcutaneous vs intravenous application

Dual blockade in adjuvant and neoadjuvant treatment lasts one year. Treatments are repeated every 21 days, so the total number of cycles is 18. The very fact that it is necessary to administer drugs every 21 days via intravenous access reduces the quality of life. The application of an intravenous dual block requires about 9.5 hours – more than one full working day, spent on the ward for both patients and staff. Preparation of intravenous therapy requires about 22 minutes per treatment, and on average, hospital staff spend about 45 minutes preparing a double block per application. These demands on time and resources not only affect treatment capacity but also the overall well-being of overworked staff, cited as a major cause of stress and poor mental health. Each scheduled IV. therapy for HER2+ breast cancer requires up to 40 minutes of medical staff time including oncologists, nurses, pharmacists, and pharmacy technicians. Drug preparation takes 50% of the time, which is almost equal to the time required for drug administration and observation. The estimated time saving is an average of 5 hours per patient,

i.e., per treatment, which can potentially be increased if two treatments occur simultaneously (Jackisch et al., 2022).

For this reason, the subcutaneous formulation of trastuzumab was designed, and later the subcutaneous formulation of trastuzumab + pertuzumab known under the market name Phesgo® was introduced. The use of recombinant humane hyaluronidase, as an auxiliary substance, enabled the SC administration of the drug (Phesgo & EPAR, 2021).

Figure 11. Complications of intravenous drug administration (Gojković, 2021)

Human hyaluronidase and endoglycosidase are used for subcutaneous distribution of pertuzumab and trastuzumab. The role of hyaluronidase is to improve the dispersal and absorption of drugs being co-administered subcutaneously, which allows larger amounts of the drug to be administered. Also, compared to other subcutaneous administration forms that have a reservoir with only 1mL volume, here it is possible for larger amounts of drugs to be administered, allowing the amount of dosage up to 15 mL volume.

Hyaluronidase, based on preclinical studies, depolymerizes hyaluronan, and in doing so, increases the permeability of tissue beneath the skin, also known as subcutaneous tissue. In the applied combination of a fixed subcutaneous dose of trastuzumab and pertuzumab, hyaluronidase acts temporarily and locally. Within 24 to 48 hours, the permeability of the tissue returns to the initial state, which is allowed through reversible activity of hyaluronidase. Animal studies have displayed that hyaluronidase accelerates the trastuzumab absorption into the systemic circulation (US Patent Application for PERTUZUMAB PLUS TRASTUZUMAB FIXED DOSE COMBINATION Patent Application, 2021).

Figure 12. Activity of human recombinant hyaluronidase (Gojković, 2021)

The target population for the use of the combination of a fixed subcutaneous dose of trastuzumab and pertuzumab is the same as for the combination of chemotherapy with intravenous trastuzumab + pertuzumab in HER2-positive breast carcinoma. The combination of a fixed subcutaneous dose of trastuzumab and pertuzumab was the best option for improving the care of patients within the institutions where they are treated. Based on the experience of subcutaneous administration of trastuzumab, the use of the subcutaneous combination would shorten the time the patient spends while receiving IV infusions in the hospital by about 90%. It is expected that by reducing the time a patient spends in the day hospital, patient flow would be improved, allowing for greater access to patients and shortening the waiting list (Kümmel et al., 2021)

PHESGO

Phesgo is a fixed combination of two monoclonal antibodies Pertuzumab + Trastuzumab that is administered subcutaneously. In 2020, the medication was first approved by FDA (Office of the Commissioner, 2020), and later EMA (Ema, 2023) and since then it's been used in European countries as part of the anti-HER therapy. It's only been on the market for three years, but it's proven to have more advantages than intravenous application, especially in terms of patient care and their quality of life.

Indications

- Neoadjuvant treatment of early HER2-positive breast carcinoma in patients with increased risk of recurrence (e.g., lymph node involvement or tumor size >2 cm).
- Adjuvant treatment of early HER2-positive breast carcinoma in patients with a increased risk of recurrence (with minimally affected lymph nodes regardless of HR status), without prior neoadjuvant treatment.

- Adjuvant treatment after neoadjuvant of early HER2-positive breast carcinoma in patients who have achieved a complete pathological response (eg continuation of neoadjuvant treatment).
- First line of treatment of patients with HER2 positive metastatic breast cancer (Phesgo & EPAR, 2021).

Dosage

Patients must have a HER2-positive tumor in order to be treated with PHESGO. The recommended dosing of Phesgo in early and metastatic breast cancer is a loading dose of 1200mg pertuzumab/600mg trastuzumab and a preservation dose of 600mg pertuzumab/600mg trastuzumab that is given every three weeks. Subcutaneous injection of loading dose takes 8 minutes, while the injection of preservation dose takes less time, approximately 5 minutes. Observation time of preservation dose is also shorter, only 15 minutes, compared to 30 minutes of observation of the loading dose (Phesgo & EPAR, 2021).

Pharmacokinetic properties

Pharmacokinetic results for the primary outcome measure: C_{trough} of pertuzumab after cycle 7 (ie, before the cycle 8 dose), indicated non-inferiority of pertuzumab in Phesgo (geometric mean of 88.7 μg/ml) compared to pertuzumab for intravenous administration (geometric mean of 72.4 μg/ml), with a geometric mean ratio of 1.22, 90% CI: 1.14 - 1.31 (Phesgo & EPAR, 2021).

Pharmacokinetic results for the secondary outcome measure: C_{trough} of trastuzumab after cycle 7 (ie, before the cycle 8 dose), indicated non-inferiority of trastuzumab in Phesgo (geometric mean of 57.5 μg/ml) compared to trastuzumab for intravenous administration (geometric mean of 43.2 μg/ml), with a geometric mean ratio of 1.33 , 90% CI: 1.24 - 1.43 (Phesgo & EPAR, 2021).

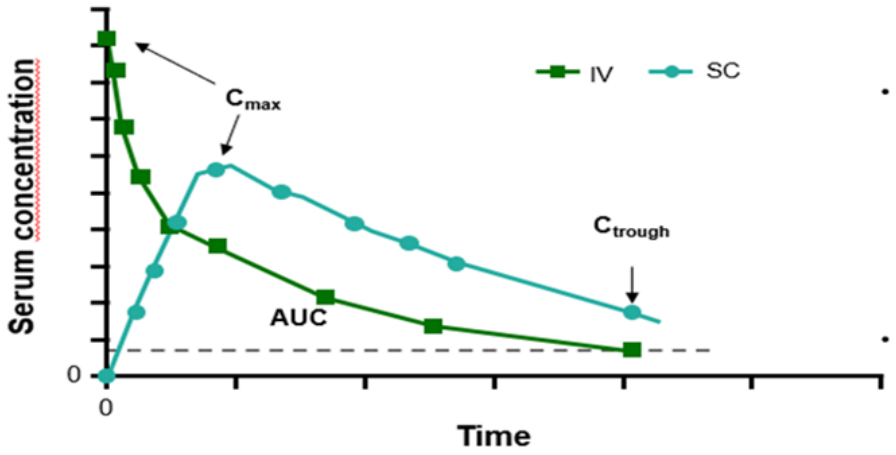


Figure 13. Serum concentration of intravenous vs subcutaneous form of application

C_{trough} = trough concentration of drug in plasma/serum; concentration measured at the end of the dosing interval related to the method of application, associated with clinical outcomes

AUC = Area under the curve provides information on exposure during the therapeutic cycle (how much drug remains in the body and for how long) It can be correlated with the Ctrough value

C_{max} = maximum (peak) drug concentration in plasma/serum

C_{max} after IV administration is not subject to distribution and elimination effects, compared to C_{max} after SC administration which requires time for absorption and is subject to elimination effects before reaching the bloodstream.

Absorption

The median maximum serum concentration (Cmax) of pertuzumab in Phesgo was 157 µg/ml, and the time to reach the maximum concentration (Tmax) was 3.82 days. According to the population pharmacokinetic analysis, the absolute bioavailability was 0.712, and the first-order absorption rate (Ka) was 0.348 (1/day).

The median Cmax of trastuzumab in Phesgo was 114 µg/ml, and the Tmax was 3.84 days. According to population pharmacokinetic analysis, absolute bioavailability was 0.771, and Ka 0.404 1/day (Phesgo & EPAR, 2021).

Distribution

According to population pharmacokinetic analysis, in a typical patient, the volume of distribution of pertuzumab in Phesgo to the central compartment (Vc) was 2.77 liters.

According to population pharmacokinetic analysis, in a typical patient the Vc of subcutaneously administered trastuzumab was 2.91 liters (Phesgo & EPAR, 2021).

Biotransformation

No direct studies of the metabolism of Phesgo have been performed. Antibodies are primarily removed by catabolism (Phesgo & EPAR, 2021).

Elimination

The elimination half-life (t1/2) was approximately 24.3 days and the clearance of pertuzumab in Phesgo was 0.163 l/day, and. The clearance of trastuzumab in Phesgo was 0.111 l/day. It is estimated that trastuzumab concentrations will reach < 1 μg/ml (approximately 3% of the Cmin,ss value predicted for the population, i.e. a washout of approximately 97%) in at least 95% of patients 7 months after the last dose (Phesgo & EPAR, 2021).

Clinical evidence

FeDeriCa is a pivotal, non-inferiority phase III pharmacokinetic study coducent in 500 patients with early HER2+ breast carcinoma. The primary objective of the study was to assess the safety and efficacy of PHESGO therapy compared to intravenous application of pertuzumab + trastuzumab. Patients received either intravenous pertuzumab + trastuzumab in neoadjuvant setting or fixed subcutaneous pertuzumab + trastuzumab PHESGO every 3 weeks. Concurrently taxane were administered during 4 cycles of the therapy. After the surgery patients received additional 14 cycles of anti HER therapy. All patients were women, the median age was 50 years (Tan et al., 2021).

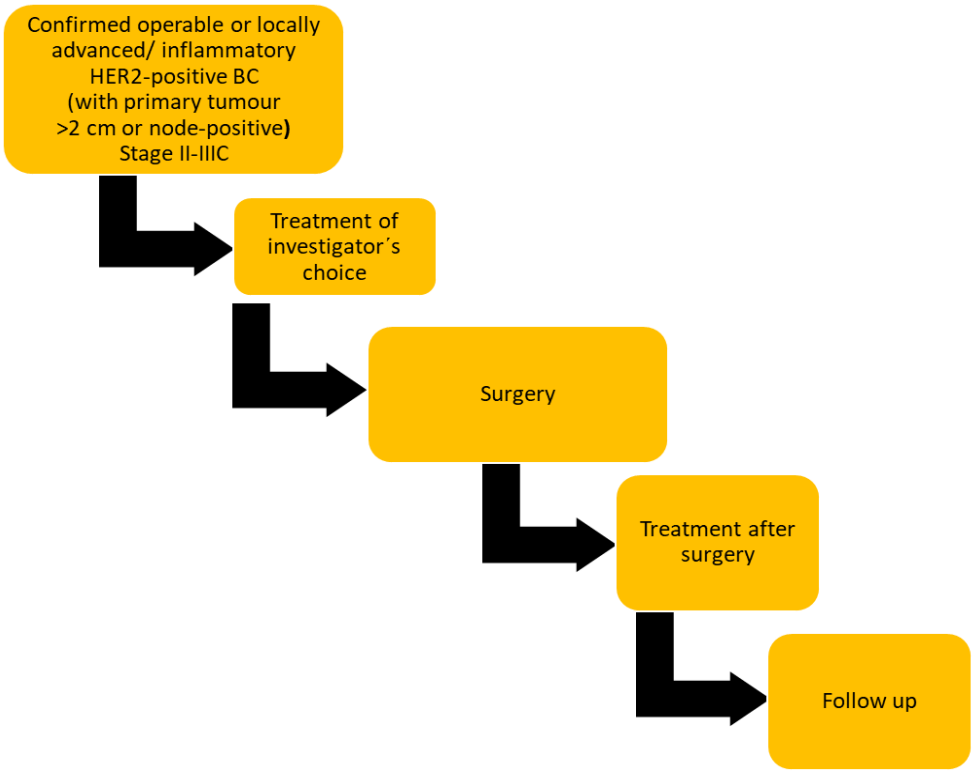
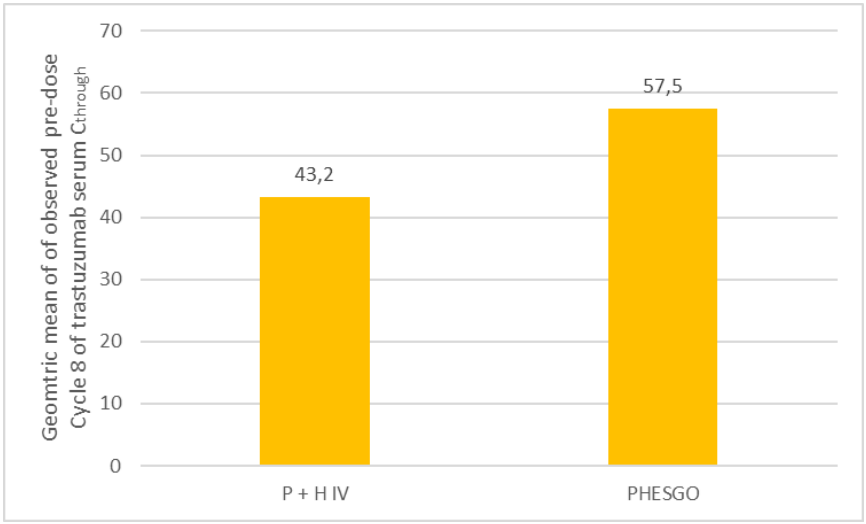


Figure 14: Schematic stages of process and design of study FeDeriCa

Primary endpoint of the study was met. Cycle 7 (pre-dose Cycle 8) pertuzumab serum $C_{through}$ within PHESGO was non-inferior to pertuzumab IV.



Graph 1: Cycle 7 (pre-dose Cycle 8) pertuzumab serum Cthrough compared to PHESGO

Based on the data regarding the side effects, FeDeriCa study has proven that there is no significant difference in side effects between the two application forms (Tan et al., 2021).

	Intravenous group (n=252)		Fixed-dose combination group (n=248)	
	Grade 1 or 2	Grade 3 or 4	Grade 1 or 2	Grade 3 or 4
Headache	48(19%)	2(1%)	36(15%)	0
Nausea	150(60%)	4(2%)	142(57%)	5(2%)
Diarrhoea	134(53%)	12(4%)	133(54%)	17(7%)
Injection site reaction	2(1%)	0	32(13%)	0
Hot Flush	26(10%)	0	19(8%)	0
Alopecia	177(70%)	1(<1%)	190(77%)	1(<1%)
Cough	30(12%)	0	32(13%)	1(<1%)

Table 2: Side effects reported by patients in FeDeriCA phase III study (Tan et al., 2021)

PHranceSCa is an open-label, randomized phase II study conducted in 160 patients that have been diagnosed with early HER2+ breast carcinoma and that have completed neoadjuvant treatment with intravenous pertuzumab + trastuzumab, surgery and chemotherapy. The primary objective of the study was to assess preference of the patients after the 6th cycle of adjuvant treatment for PHESGO. In the adjuvant setting, patients received either 3 cycles of PHESGO followed by 3 cycles of intravenous pertuzumab + trastuzumab or cycles of intravenous pertuzumab + trastuzumab followed by 3 cycles of PHESGO. Preservation doses were used for ensuing administrations or delayed doses less than 6 weeks. All patients were women, the median age was 49 years (O'Shaughnessy et al., 2021).

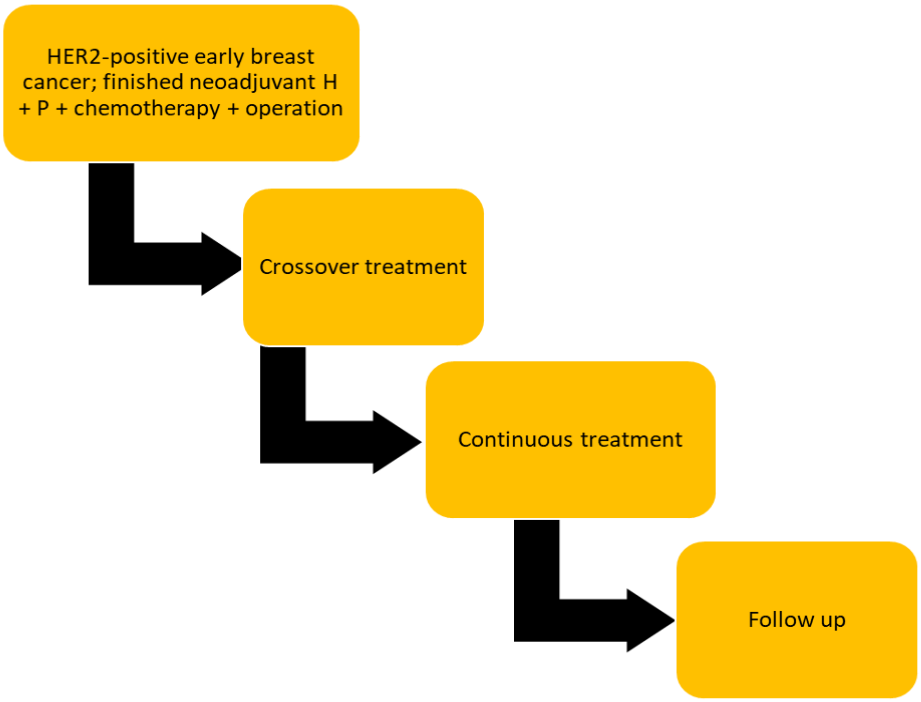


Figure 15: Schematic stages of process and design of study PHranceSCa

The fixed-dose combination of SC trastuzumab and pertuzumab has diverse dosing volume and instructions of administration than intravenous pertuzumab, intravenous trastuzumab, and subcutaneous trastuzumab when administered alone (O'Shaughnessy et al., 2021).

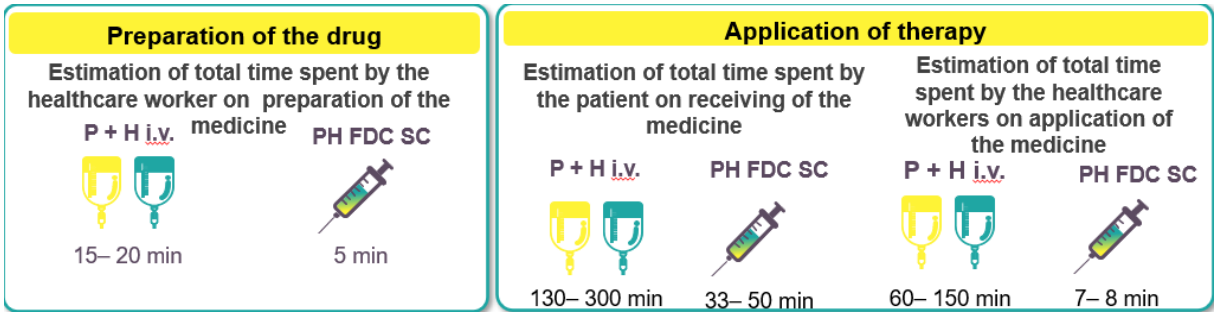
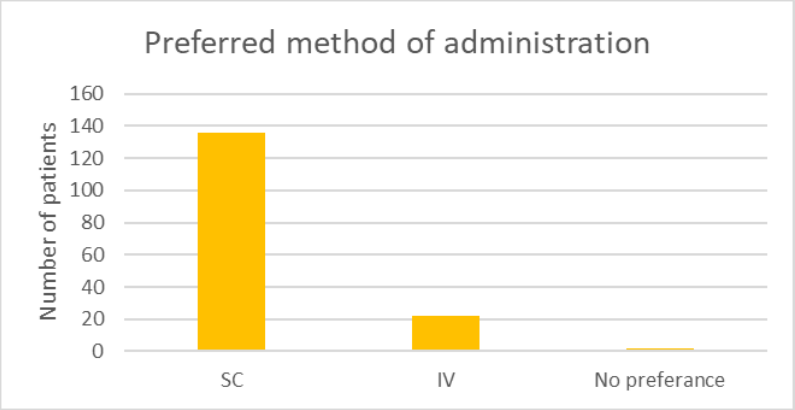


Figure 16: Comparison of preparation of the drug and application of therapy between Perjeta + Herceptin intravenous vs. Phesgo subcutaneous

85% of patients favored PHESGO over intravenous pertuzumab + trastuzumab, mentioning prevailing reason to be less amount of time spent in the clinic.

14% of patients favored the use intravenous pertuzumab + trastuzumab, mentioning prevailing reason to be comfort during administration of the drug (O'Shaughnessy et al., 2021).



Graph 2: Preferred method of administration by patients in study PHranceSCa

According to the results of clinical study PHranceSCa, side effects of subcutaneous Phesgo formulation compared to intravenous pertuzumab + trastuzumab application were proven to be similar and with no significant difference (O'Shaughnessy et al., 2021).

	P + H i.v. → PH FDC SC		**PH FDC SC → P + H i.v.**	
	P + H i.v. cycles 1– 3 (n=80)	PH FDC SC cycles 4– 6 (n= 80)	PH FDC SC cycles 1– 3 (n=80)	P+ H i.v. cycles 4– 6 (n= 80)
Diarrhoea	12(15%)	7(9%)	6(7,5)	4(5%)
Fatigue	5(6%)	4(5%)	5(6%)	0
Hot Flush	6(7,5%)	4(5%)	5(6%)	0
Injection site reaction	0	12 (15%)	24 (30%)	0
Radiation scin injury	17 (12%)	7 (9%)	10(12,5%)	10(12,5%)

Table 3: Side effects reported by patients in PHranceSCa phase II study (O'Shaughnessy et al., 2021)

Study objectives

The first objective is to analyse the HER2 population in Republic of Srpska from January the 1st of 2019 to 30th September of 2023.

The second objective is to analyse the application of subcutaneous application in the period from March the 1st of 2023 to 30th of September of 2023 in Republic of Srpska.

The third objective is to comment on the subcutaneous application form of these drugs, and how the hospitals will benefit from it in the future for years to come, given that Republic of Srpska ordered the subcutaneous application form for the first time in March 2023, and with that, stepped in the next milestone of anti HER2 treatment.

Methodology

The analysis included 129 patients from 2019 to 2022, and 55 patients from January until September 2023 with early stage HER2-positive breast cancer. A retrospective analysis was conducted in a time frame from 2019 to 2023 in the Oncology Clinic of the University Clinical Center of the Republic of Srpska in Banja Luka. The patients were from all over Republic of Srpska and all of them were presented to the breast tumor multidisciplinary board. The data was collected from consultant findings, medical history and pathohistological findings. It includes treated patients, as well as those on the waiting list, who, due to lack of medication, were not able to receive the needed treatment immediately. The medical documentation was reviewed, and the following data was analyzed:

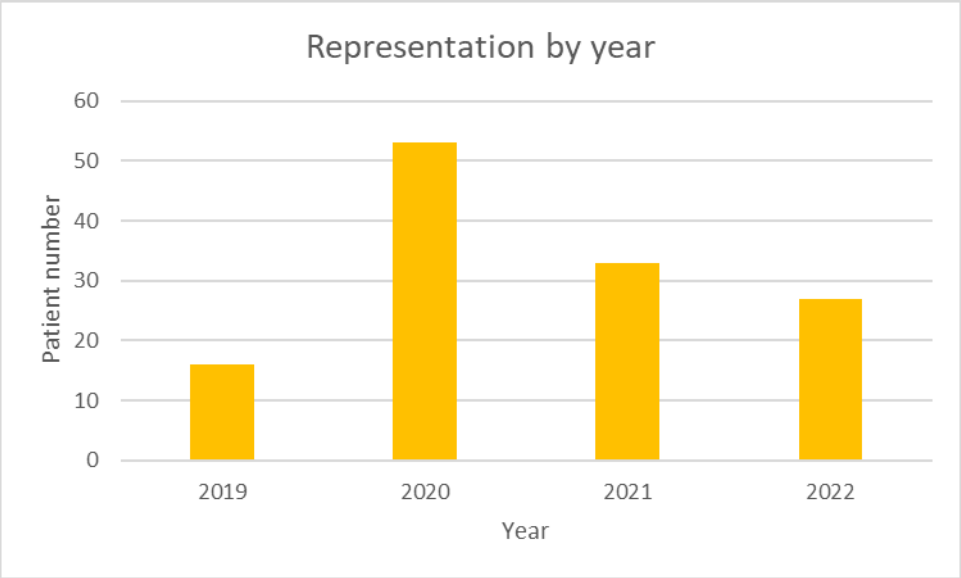
- Age
- Sex
- Menopausal status of female patients
- Tumor type
- Tumor grade
- Disease stage
- Hormone receptor status
- Applied neo/adjuvant chemotherapy.

The download and use of this data is approved by the general director, as well as the Ethics Committee of the University Clinical Center of the Republic of Srpska.

The health insurance fund of Republic of Srpska provided information about drugs pertuzumab, trastuzumab, trastuzumab emtansine and the combination of trastuzumab and pertuzumab, including the list of approved indications in the Republic of Srpska which were published in the official gazette of Republic of Srpska (Sluzbeni glasnik RS) number 111/15 published on 31.12.2015, as well as data on the amount of pertuzumab from 2017 and trastuzumab from 2012, that has been acquired in Republic of Srpska, and the ordered amount of subcutaneous formulation Pertuzumab and Trastuzumab for years 2023 and 2024.

Results

Number of analysed HER2-positive patients in Republic of Srpska between 2019 and 2022 was 129, with 16 patients in 2019, 53 in 2020, 33 in 2021 and 27 in 2022 (Graph 3).



Graph 3: Representation by year

Only 2 patients were male, the rest being women.

Average patient age was 54, with the youngest patient being 25, and the oldest 84. In 2019, average age was 54, the youngest patient was 39 while the oldest was 78. In 2020, average age was 52, the youngest patient was 28 while the oldest was 84. In 2021, average age was 60, the youngest patient was 25 while the oldest was 81. Lastly, in 2022, average age was 51, the youngest patient was 37, while the oldest patient was 79 years old.

75 patients had invasive carcinoma of no special type (NST), which is the most usual type of tumor. 47 patients had ductal tumor, and only 2 lobular types. 4 patients had other types of tumor, which are not that common (Table 3).

			Year of the disease				
			2019	2020	2021	2022	Total
Tumor type	NST	Count	0	32	20	23	75
		% within year of disease	0.0%	61.5%	60.6%	85.2%	58.6%
	Ductal	Count	16	17	11	3	47
		% within year of disease	100.0%	32.7%	33.3%	11.1%	36.7%
	Lobular	Count	0	1	0	1	2
		% within year of disease	0.0%	1.9%	0.0%	3.7%	1.6%
	Other	Count	0	2	2	0	4
		% within year of disease	0.0%	3.8%	6.1%	0.0%	3.1%
Total		Count	16	52	33	27	128
		% within year of disease	100.0%	100.0%	100.0%	100.0%	100.0%

Table 3: Tumor type of each analyzed year and in total

Only 6 patients had grade 1 tumor, which is the least dangerous, while 80 patients had progressed grade 2 tumor. 39 patients had the most invasive grade 3 tumor (Table 6).

			Year of the disease				
			2019	2020	2021	2022	Total
Grade	1	Count	0	4	1	1	6
		% within year of disease	0.0%	7.7%	3.0%	3.8%	4.8%
	2	Count	8	33	22	17	80
		% within year of disease	57.1%	63.5%	66.7%	65.4%	64.0%
	3	Count	6	15	10	8	39
		% within year of disease	42.9%	28.8%	30.3%	30.8%	31.2%
Total		Count	14	52	33	26	125
		% within year of disease	100.0%	100.0%	100.0%	100.0%	100.0%

Table 4: Tumor grade of each analyzed year and in total

Stage of the disease is determined by many factors, such as the size of the tumor and lymph node status. If the tumor is smaller than 1cm, then the neoadjuvant therapy is not given, because the tumor will immediately be surgically removed. In other cases, neoadjuvant treatment, chemotherapy, surgery, and later adjuvant treatment are the usual treatments. There is also stage

IV where the tumor has metastasized, and neoadjuvant treatment is also not an option in this case. The most common stage is II. (Table 5).

			Year of the disease				Total
			2019	2020	2021	2022	
Stage of disease	1	Count	4	9	5	4	22
		% within year of disease	28.6%	17.3%	16.1%	15.4%	17.9%
	2	Count	7	29	23	19	78
		% within year of disease	50.0%	55.8%	74.2%	73.1%	63.4%
	3	Count	3	14	3	3	23
		% within year of disease	21.4%	26.9%	9.7%	11.5%	18.7%
Total		Count	14	52	31	26	123
		% within year of disease	100.0%	100.0%	100.0%	100.0%	100.0%

Table 5: Stage of the disease of each analyzed year and in total

81 patients had positive hormone receptor status, meaning they were candidates for adjuvant hormone treatment (Table 6).

			Year of the disease				Total
			2019	2020	2021	2022	
HR status	Negative	Count	7	15	13	13	48
		% within year of disease	43.8%	28.3%	39.4%	48.1%	37.2%
	Positive	Count	9	38	20	14	81
		% within year of disease	56.3%	71.7%	60.6%	51.9%	62.8%
Total		Count	16	53	33	27	129
		% within year of disease	100.0%	100.0%	100.0%	100.0%	100.0%

Table 6: Hormone receptor status of each analyzed year and in total

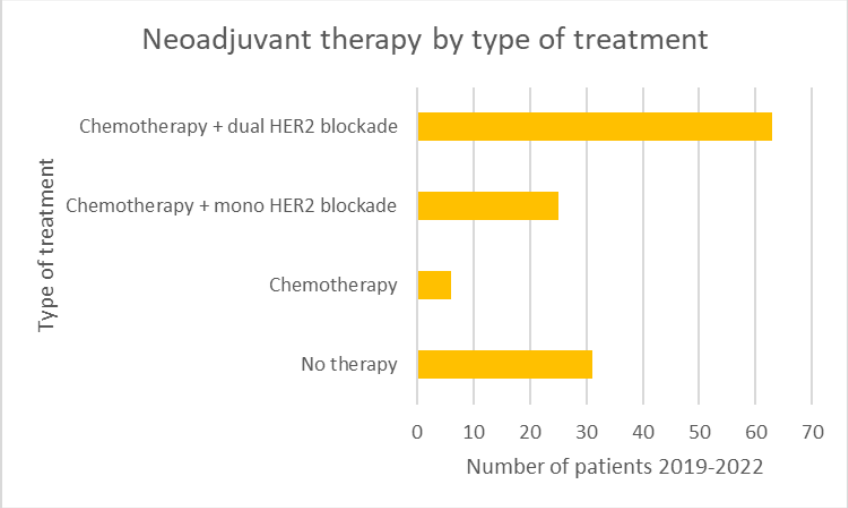
Different neoadjuvant chemotherapy protocols were applied, and 31 patients did not receive neoadjuvant treatment, because they were not suitable candidates. 2 patients were given 4 cycles of anthracyclines, 2 patients received anthracyclines in combination with taxanes, while 25 patients were given anthracyclines in combination with taxanes and trastuzumab (Herceptin). Most patients, 56 of them, were given a combination of anthracyclines and taxanes with dual blockade T + P. Only 1 patient received capecitabine, because she was not a candidate

for dual blockade due to heart problems and other comorbidities. 2 younger patients, under the age of 30, received docetaxel, carboplatin, and dual blockade T + P combination, because of higher grade tumor. 5 patients with lower stadium of disease received taxanes with dual blockade T + P, therefore anthracyclines were excluded (Table 7).

			Year of the disease				Total
			2019	2020	2021	2022	
Neoadjuvant therapy	No therapy	Count	2	16	6	7	31
		% within year of disease	14.3%	30.2%	18.2%	28.0%	24.8%
	AC protocol	Count	1	1	0	0	2
		% within year of disease	7.1%	1.9%	0.0%	0.0%	1.6%
	AC + taxanes	Count	0	2	0	0	2
		% within year of disease	0.0%	3.8%	0.0%	0.0%	1.6%
	AC + taxanes + trastuzumab	Count	11	12	2	0	25
		% within year of disease	78.6%	22.6%	6.1%	0.0%	20.0%
	AC + taxanes + trastuzumab + pertuzumab	Count	0	20	18	18	56
		% within year of disease	0.0%	37.7%	54.5%	72.0%	44.8%
	Capecitabine	Count	0	0	1	0	1
		% within year of disease	0.0%	0.0%	3.0%	0.0%	0.8%
	Docetaxel + carboplatin + trastuzumab + pertuzumab	Count	0	1	1	0	2
		% within year of disease	0.0%	1.9%	3.0%	0.0%	1.6%
	Taxanes + trastuzumab + pertuzumab	Count	0	1	4	0	5
		% within year of disease	0.0%	1.9%	12.1%	0.0%	4.0%
	FAC / FEC protocol	Count	0	0	1	0	1
		% within year of disease	0.0%	0.0%	3.0%	0.0%	0.8%
Total		Count	14	53	33	25	125
		% within year of disease	100.0%	100.0%	100.0%	100.0%	100.0%

Table 7: An overview of the use of neoadjuvant therapy of each analyzed year and in total

In Graph 4, neoadjuvant therapies were grouped. 63 of 125 patients received chemotherapy with dual HER2 blockade. It shows that dual blockade in combination with chemotherapy is the most common treatment choice.



Graph 4: Neoadjuvant therapy (by type of treatment) in total

			Neoadjuvant therapy by groups			
			Chemotherapy	Chemotherapy + mono HER2 blockade	Chemotherapy + dual HER2 blockade	Total
Pathological response	Response not achieved	Count	1	0	0	1
		% within Neoadjuvant therapy by groups	50.0%	0.0%	0.0%	1.3%
	Partial response	Count	1	15	26	42
		% within Neoadjuvant therapy by groups	50.0%	68.2%	51.0%	56.0%
	Complete response	Count	0	7	25	32
		% within Neoadjuvant therapy by groups	0.0%	31.8%	49.0%	42.7%
Total		Count	2	22	51	75
		% within Neoadjuvant therapy by groups	100.0%	100.0%	100.0%	100.0%

Table 8: Pathological response by therapy groups by year and in total

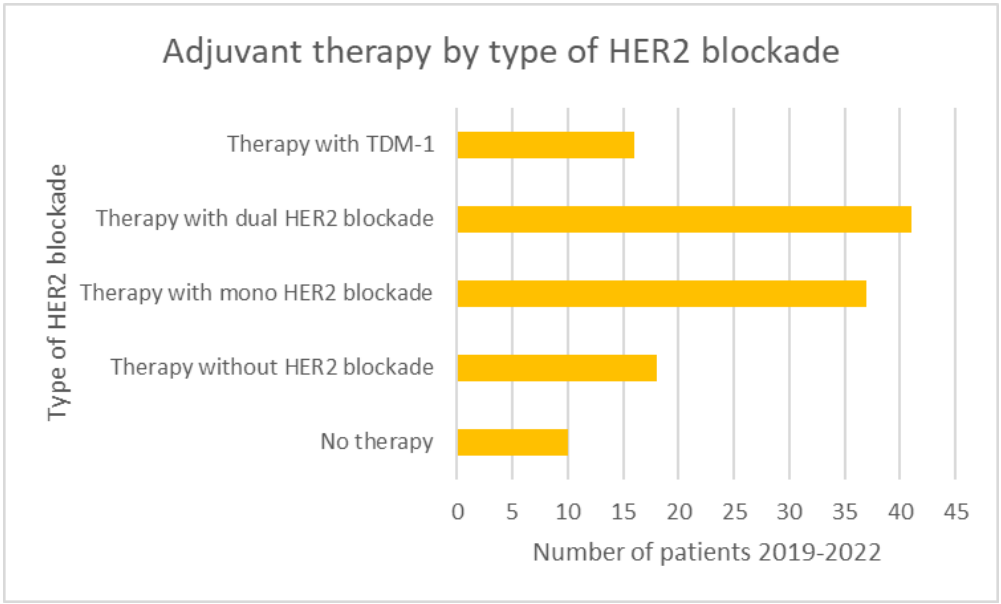
Out of 75 patients who received neoadjuvant treatment, 32 achieved complete response, and were therefore candidates for continuing therapy with dual blockade. 42 patients who achieved partial response were candidates for Kadcyla (trastuzumab emtansine). If Kadcyla was not available, they would continue therapy with dual blockade (Table 8).

			Year of the disease				Total
			2019	2020	2021	2022	
Adjuvant therapy by type of treatment	No therapy	Count	1	6	3	0	10
		% within year of disease	7.1%	11.5%	9.1%	0.0%	8.2%
	Chemotherapy	Count	13	1	1	3	18
		% within year of disease	92.9%	1.9%	3.0%	13.0%	14.8%
	Trastuzumab	Count	0	19	9	5	33
		% within year of disease	0.0%	36.5%	27.3%	21.7%	27.0%
	Trastuzumab + pertuzumab	Count	0	12	8	7	27
		% within year of disease	0.0%	23.1%	24.2%	30.4%	22.1%
	Trastuzumab emtansine	Count	0	6	7	3	16
		% within year of disease	0.0%	11.5%	21.2%	13.0%	13.1%
	Chemotherapy + mono HER2 blockade	Count	0	4	0	0	4
		% within year of disease	0.0%	7.7%	0.0%	0.0%	3.3%
	Chemotherapy + dual HER2 blockade	Count	0	4	5	5	14
		% within year of disease	0.0%	7.7%	15.2%	21.7%	11.5%
Total		Count	14	52	33	23	122
		% within year of disease	100.0%	100.0%	100.0%	100.0%	100.0%

Table 9: An overview of the use of adjuvant therapy (by type of treatment) by year and in total

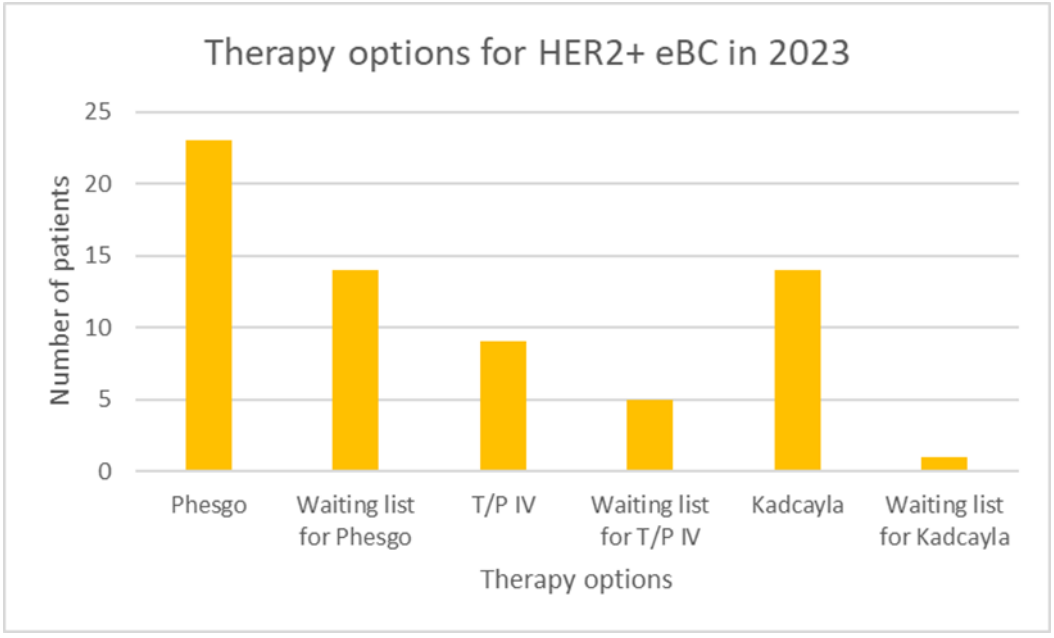
Out of 122 patients who received adjuvant treatment, 3 patients were given anthracyclines only, 4 of them received anthracyclines in combination with taxanes and trastuzumab, 7 received anthracyclines in combination with taxanes and dual blockade with P + T. 7 younger patients with higher grade tumor received combination of taxes with dual blockade P + T. 33 patients received only Trastuzumab, while 27 dual therapy P + T 16 patients received trastuzumab emtansine, therapy with TDM-1, which consists of Trastuzumab and DM1, a cytotoxic agent and it is applied if there is a tumor residual after surgery. Only 1 patient received capecitabine, because dual blockade was not an option due to other comorbidities and heart problems.

In adjuvant postoperative setting. as shown in Table 9, out of 122 patients, most received only trastuzumab (33 patients) because there was not enough pertuzumab for patients to receive, while 27 patients received dual therapy P + T. 14 patients underwent chemotherapy while receiving P + T, and 4 patients underwent chemotherapy but only received trastuzumab. 16 patients received trastuzumab emtasine, an upgraded version of trastuzumab. 18 patients underwent only chemotherapy. Graph 6 shows that 41 out of 122 patients received therapy with dual HER2 blockade, 37 patients were given only mono HER2 blockade and 18 received therapy without HER2 blockade.



Graph 6: An overview of the use of adjuvant therapy by type of HER2 blockade in total

In December 2022, the Health Insurance Fund of Republic of Srpska approved the use of Phesgo in neo/adjuvant setting (Sluzbeni glasnik RS) number 123/22, published on 20 December 2022, and in January 2023 the fund procured 335 pieces of Phesgo in a dosage form of 600mg/600mg in 10mL solution and 28 pieces in a dosage form of 1200mg/600mg in 15mL solution. The dosage form of 1200mg/600mg is the initial therapy, and then therapy is continued with the dosage form of 600mg/600mg. A total of 18 therapy cycles is applied at 3 week intervals. In the period from January 1st until the 30th of September 2023, a total of 55 women were diagnosed with HER2+ early breast cancer. 11 patients from 2022 were transferred to Phesgo therapy from intravenous P/T. Out of them, 14 patients started Phesgo therapy (neoadjuvant), and within the next six months another 9 patients were initiated. Following the surgery and achievement of complete regression, the patients continued the same therapy. The total number of patients receiving Phesgo as of September 30th of 2023 is 23. However, 9 patients were candidates for subcutaneous formulation but received T + P intravenously, 5 patients were on waiting list for IV formulation and there are currently 14 patients on the waiting list for subcutaneous dual blockade. TDM-1 was received by 14 female patients, which is given if the tumor remains after neoadjuvant therapy and surgery, and one is on the waiting list. There were no men in the observed period, and the average age of the female patients was 54, the youngest was 31, and the oldest was 78. The most frequent tumors were grade 2 and stage II.



Graph 7: An overview of therapy options for HER2+ patients with early breast cancer in 2023

Discussion

The optimal treatment for HER2+ breast cancer is chemotherapy in combination with biological therapy that targets the EGFR family of receptors. The discovery of trastuzumab, a monoclonal antibody that targets the HER2 receptor on the cell surface, which is significantly expressed in breast tumors, was a turning point in the treatment of this type of breast cancer. The first clinical studies from 1992 showed effectiveness in the treatment of metastatic HER2+ breast cancer, and then the same drug was tested in adjuvant treatment through 5 large randomized international studies. The results were impressive, and they were presented in May 2005 in ASCO meeting and in December 2005 in San Antonio Breast Cancer Symposium (Slamon et al., 2001). Since 2005, the FDA and then the EMA, approved the use of Herceptin in adjuvant treatment under an accelerated procedure. The drug was used in combination with chemotherapy, and after the completion of chemotherapy, only Herceptin was continued for a total of one year. The most serious side effect is cardiotoxicity, which is why heart ejection fraction control is required for three months. In addition to the extraordinary results that this drug showed, one out of four women would experience a return of the disease and it became clear that the HER2 receptor can pair with another domain of the HER3 receptor. This is how pertuzumab was subsequently designed. Pertuzumab is a monoclonal antibody that also binds to the HER2 receptor, but on a different domain than trastuzumab, and prevents the heterodimerization of HER2 and 3, with which the results of treatment, in combination with trastutumab, were significantly improved, which was proven in clinical studies that treated metastatic HER2 breast cancer. Given that this dual blockade achieved good results in the metastatic setting, its application in the neoadjuvant and adjuvant setting also began. This was proven through the clinical studies of Neosphere (Gianni et al., 2012) and TRYPHAENA (Schneeweiß et al., 2018), and for adjuvant treatment through the clinical study of Aphinity (Von Minckwitz et al., 2017)., and these results were published in 2017.

The previously mentioned therapy required the use of an intravenous route. Therapy with monoclonal antibodies (mono or dual) was applied for a year, which made a total of 18 cycles (cycles were applied every three weeks). Intravenous access could lead to various side effects, such as pain at the injection site, redness and infection, rupture of blood vessels that were already damaged by previous chemotherapy, psychological stress, and other side effects. This resulted first in the design of the subcutaneous formulation of trastuzumab, and then in the

subcutaneous formulation of both T/P drugs in one syringe. Clinical trials FeDeriCa (Tan et al., 2021) and PHranceSCa (O'Shaughnessy et al., 2021) have shown that there is no difference in the bioavailability of the drug in the plasma, nor in the effectiveness of the dual blockade used in this way in the treatment of HER2 positive breast cancer, and no new side effects have been reported. On the contrary, there were no side effects associated with subcutaneous administration.

In the trial that lasted from 2019 to 2022, there was a total of 127 women and 2 men with HER2 + breast cancer who were presented to the multidisciplinary team of the oncology clinic. All patients with HER2+ breast cancer from Republic of Srpska were presented to this team. Descriptive statistics and analysis of the use of neoadjuvant and adjuvant therapy in these patients was discussed in the paper. Since the Health Insurance Fund of Republic of Srpska put PHESGO on the list of the fund's special program in December of 2023 and approved the adjuvant and neoadjuvant use in January 2023, a public procurement was carried out and the first patients for Phesgo therapy were initiated in March 2023. The research paper analyzed separately the patients who had HER2+ cancer from January 2019 to the end of September 2023.

The goal was to show the extent to which the need for subcutaneous formulation was unmet, for the therapy of choice of neo/adjuvant treatment of patients with HER2+ breast cancer. The subcutaneous formulation of dual blockade would significantly contribute to a better quality of life for the patients themselves on one hand, and significantly shorten the time of drug preparation by pharmacists, as well as application time by oncology nurses and oncologists. There is also no adjustment needed depending on the patient's body weight, thus avoiding errors in preparation. On the other hand, we must not forget the significant financial savings by applying the subcutaneous formulation compared to the intravenous one. One ampoule of Phesgo in Republic of Srpska is less expensive compared to intravenous administration of T+P. The financial calculation should also include the consumption of infusion solutions, transfusion systems, needles, disinfectants, cotton wool, leucoplasts, which must be used during intravenous administration, as well as the costs of destroying a large amount of waste. Another feature of the subcutaneous formulation is the possibility of application in home conditions that has already been tested and is practiced in some areas of Great Britain and China, (Lee, n.d.), as well as the possibility of application in the workplace. This is especially important for able-bodied women as well as those who are building a career. Due to the short administration of the drug itself, it is possible for them to take the drug during their lunch break and continue their

daily work duties. The plan also includes the possibility of administering the same medicine from the patient himself, that is, for the patient to receive a special device with already prepared medicines and instructions and apply the therapy herself. Otherwise, the therapy is applied subcutaneously in the thighs, with the fact that it is given alternately to the left and right thigh (Jackisch et al., 2022).

According to the analysis results, the largest number of breast cancers treated was in 2020. The most common ductal invasive breast cancer-NST was found in 122 women, which coincides with the data in literature (Tavassoli & Devilee, 2003). There were significantly fewer lobular and other types of tumors, which also matches numerous literary works. All tumors can be graded into grades 1, 2 and 3. The nuclear or histological grade describes the degree of tumor differentiation and is based on the pathologist's assessment of the size and shape of the nucleus, the number of mitoses, and the degree of tubule formation. The predictive role of this differentiation (1-3) is still not clear, and the "Nottingham" grading system (which is also used in this paper) seems to be the most acceptable due to its semiquantitative approach and is recommended by the American Association of Pathologists (Beslija & Vrbanec, 2014).

Grade 2 tumor is a broad category, while grades 1 and 3 show a good correlation with clinical parameters and the outcome of the disease. In the study, the largest number of patients had grade 2 (64%), and grade 3 (31.2%), which indicates a worse prognosis in contrast to grade 1, which was only 4.8% (Pappo et al., 2007). Based on the size of the tumor and the status of the lymph nodes in the axilla, the stage of the disease is determined. The lower the stage of the disease, the better the prognosis. Most often, the disease is detected in the second (63.4% in the study) and third stage of the disease (18.7% in the study), and a smaller number (17.9%) is in stage 1, when the prognosis of the disease is better and when there is a chance for healing (Isaacs, 2001; Maksimović et al., 2008).

The importance of hormone receptors in breast tumors is great. The first papers on this topic date back to 1997, and globally speaking, hormone-dependent cancers were considered to have a better prognosis. In the study, a significantly larger number (62.8%) of female patients had hormonal carcinoma, as a more favorable prognostic factor (Gojković, 2021).

In previous decades, after the surgical intervention for early breast cancer, adjuvant-protective chemotherapy was applied, and with the knowledge of the importance of HER2 receptor expression on the surface of tumor cells, trastuzumab was administered for one year. The goal of this therapy is to reduce the incidence of disease relapse.

Neoadjuvant therapy is applied with the aim of achieving a complete pathological response, and it is applied before surgery according to the already recommended guidelines, such as NCC (Gradishar et al., 2022) and ESMO (Cardoso et al., 2019). The most frequently applied chemotherapy protocols are four cycles of AC (anthracycline/cyclophosphamide), followed by four cycles of taxanes (paclitaxel or docetaxel) in combination with dual therapy if available. Four cycles of taxanes can be replaced by 12 weekly cycles of paclitaxel also combined with dual blockade. The choice of therapy in the patients presented in the paper depended on age (women younger than 35 most often received taxanes with dual blockade), tumor grade (a higher grade required more aggressive therapy, a combination of AC with taxanes), as well as comorbidities. Female patients most often received four cycles of AC, followed by therapy in combination with dual blockade, which is according to current guidelines NCC (Gradishar et al., 2022) and ESMO (Cardoso et al., 2019). After the completion of neoadjuvant therapy, patients are referred for surgery. The most important parameter for the decision on further treatment is the pathologist's report after the operation. A very important parameter is whether complete or partial or stable disease was achieved after neoadjuvant therapy. A complete pathological response represents the complete absence of both the tumor in the breast and in the regional lymph nodes. All patients who achieved a complete pathological response (pCR) have better long-term clinical outcomes, so it is considered that pCR is a surrogate market for survival in HER2+ patients, and failure to achieve pCR is a surrogate for a high risk of disease relapse despite the applied neoadjuvant therapy. Thanks to the possibility of using dual blockade in Republic of Srpska (42.7% of patients had a complete pathological response (pCR) and 56% a partial response), good treatment outcomes are expected. The results are in accordance with the results published in the large clinical studies of Neosphere (Gianni et al., 2012) and TRYPHAENA (Schneeweiß et al., 2018).

After the surgical treatment is completed and based on the pathologist's report on the response of the tumor to neoadjuvant therapy, the multidisciplinary team makes a decision whether to continue the post/neoadjuvant treatment. If pCR is achieved, the treatment continues with the use of biological therapy with T+P dual blockade. If the patient received 4 neoadjuvant cycles of dual blockade, she needs to receive another 14 cycles after the operation. If the pathologist reports that there is residual disease, according to the valid NCC (Gradishar et al., 2022), ESMO (Cardoso et al., 2019) and St-Gallen (Balić et al., 2023) guidelines, the use of TDM-1 is indicated. The largest number of patients (41 patients or 33.6%) continued to receive dual blockade in the period 2019-2022. Only trastuzumab was received by 27% or 33 patients who were candidates for dual blockade, but due to insufficient quantities of this drug in the RS, they

could not continue. It should be noted that the quantities of pertuzumab have been increased since 2019 and that the number of patients who received adequate therapy with dual blockade was higher in 2022 than in 2019. In the group of patients who received dual blockade, there are some patients who had residual disease and were candidates for TDM-1, but due to insufficient amounts of the drug, only 16 patients or 13.1% received adequate therapy with TDM-1, and the rest received dual blockage.

With the acquisition of Phesgo as a subcutaneous formulation of dual blockade in one ampoule, the application of the drug in adjuvant and neoadjuvant treatment in HER2+ breast cancer began. In the period from March the 1st until September the 30th of 2023, a total of 55 patients with HER2+ breast cancer, who were candidates for neoadjuvant therapy, were presented to the multidisciplinary team. As of March, patients were successively enrolled in therapy, and by the end of September 2023, the total number of patients receiving Phesgo was 23. In the same period, 9 patients received intravenous administration, five patients were on waiting list for this formulation, and fourteen patients were on the waiting list for Phesgo. Therefore, 28 patients had an unmet need for subcutaneous application. The advantages of the subcutaneous formulation were demonstrated in two large randomized, multicenter, international studies FeDeriCa (Tan et al., 2021) and PHranceSCa (O'Shaughnessy et al., 2021). According to the results of those studies, the effectiveness of subcutaneous application was the same as that of intravenous administration. It was also shown in the studies that the concentration of both drugs in plasma after 7th and before the application of 8th cycle with the subcutaneous formulation was the same as the concentration of both drugs if administered intravenously. Based on results published in Annal Oncology by Berinstein, pharmacokinetic (PK) parameters are important for understanding bioequivalence between IV and SC formulations. PK outcomes are a key focus of PHESGO drug development and are used to assess bioequivalence.

$C_{through}$ is the trough plasma/serum drug concentration, i.e., the concentration measured at the end of the dosing interval. C_{max} after IV administration is not subject to distribution and elimination effects, compared to C_{max} after SC administration which requires time for absorption and is subject to elimination effects before reaching the bloodstream without clear correlation with clinical outcomes (Berinstein et al., 1998).

PHranceSCa- an open-label, randomized, crossover phase II study examined patient preference for Phesgo versus IV P+T and this was the primary objective of this study. The secondary objective of the study is patient satisfaction and the patient's choice of formulation for continuation and transition from intravenous to subcutaneous formulation, as well as quality of

life. The study also examined the satisfaction of healthcare workers with the use of subcutaneous formulation, the safety of switching from intravenous to subcutaneous formulation, as well as the saving of time and resources. The study unequivocally showed the advantage of the SC formulation compared to the intravenous one according to all the stated objectives (Von Minckwitz et al., 2017). In the study, 85% of patients preferred subcutaneous administration of treatment, and 85% were satisfied with subcutaneous administration. 90% of patients stated that they had more than enough time to talk with a healthcare worker, as well as that they got more time to perform some other duties, which significantly improved the quality of life. Health workers stated that subcutaneous administration showed a significant saving of time, and that it required significantly fewer resources, compared to intravenous administration. Also, subcutaneous administration was well tolerated with a safety profile consistent with previous studies with IV formulations, and no new safety signals were observed with the subcutaneous formulation. The fixed formulation (subcutaneous) saved time for pharmacists because it was immediately ready for use (5 minutes) in contrast to the IV preparation, which requires an average of at least 20 minutes to prepare. The brevity of preparation of the subcutaneous formulation leaves more time for the pharmacist to dissolve other therapeutic protocols. Considering that this is a fixed dosage combination, the possibility of error when dosing trastuzumab and pertuzumab individually, when administered intravenously, is significantly reduced, and there is also less waste (O'Shaughnessy et al., 2021).

Considering that many patients did not receive the subcutaneous formulation due to limited quantities of the drug, the need for this drug in the Republic of Srpska would be for about 55 patients on an annual basis. Upon the result analysis, 23 patients have an unmet need for the use of the drug Phesgo in the neo/adjuvant setting, that would significantly improve their satisfaction and quality of life. On the other hand, adequate amounts of Phesgo would bring economic benefit to Republic of Srpska Health Insurance fund, and thus leave room for the procurement of other innovative drugs for other types of malignant diseases, or the eventual approval of an indication for metastatic HER2+ breast cancer, because these patients are condemned to long-term intravenous administration of dual blockade. Subcutaneous formulation would be significant for health personnel as well as pharmacists in a significantly shorter time, both for the preparation of the drug itself and for its applications, and all this would leave more time needed for talking with the patient.

Conclusion

1. Thanks to the use of monoclonal antibodies P+T for HER2-positive early breast cancer in the neo/adjuvant setting, the treatment outcomes of patients suffering from this disease have improved significantly.

2. Intravenous administration of the drug leads to unwanted effects at the site of application, requires a longer preparation time for the pharmacist, as well as a significantly longer time of therapy application for medical oncologist and oncology nurses.

3. The subcutaneous formulation of trastuzumab significantly improved the quality of life of patients and showed the same results in treatment. By adding another monoclonal antibody - pertuzumab (dual blockade), it further improved the treatment outcomes.

4. Subcutaneous formulation of dual blockade (Phesgo) further improved the quality of life of patients compared to intravenous administration, further reduced the preparation time for the pharmacist and the time of application for the medical oncologist and oncology nurse and brought significant financial savings to the health insurance fund of Republic of Srpska.

5. In Republic of Srpska, there is still an unmet need for a subcutaneous formulation of dual blockade, because a significant number of patients use the intravenous formulation, and some are on the waiting list for this drug. Unfortunately, some will not live to receive it at all, because the quantities of the drug are limited.

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