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Features of tumor heterogeneity in regional metastasis of breast cancer

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Abstract

The review looked at the issues of tumor heterogeneity in breast cancer. Tumor heterogeneity is classified according to the main feature demonstrating regional differences within a tumor (for example, heterogeneity of clinical manifestations, histological heterogeneity, heterogeneity of protein expression, etc.) and by tumor regions (differences between primary tumors and metastases, differences between cell clones within a single tumor node, etc.). Temporal heterogeneity is also distinguished, which manifests itself in the clonal evolution of tumor cells. The review covers the heterogeneity in the expression of four biomarkers from the "gold standard" for immunohistochemical staining of breast cancer: estrogen receptors, progesterone receptors, Her2/neu and Ki67 in primary tumor tissue and regional metastases. According to various studies, discordance in estrogen receptor status of primary tumor cells and metastases was observed with a frequency of 4 to 62%, progesterone receptors — from 12 to 54%, Her2/neu — from 0 to 24%, Ki67 — from 4 to 39%. The results of studies of changes in the expression levels of individual markers in breast cancer metastases, as well as the heterogeneity of surrogate subtypes of tumor tissue in metastasis, are briefly described. Possible reasons for heterogeneity in the expression of key prognostic and predictive markers by primary tumor and metastatic cells, such as artificial factors at the preanalytic and analytic stages of the study, polyclonality of the primary tumor before metastasis, clonal evolution of tumor cells during metastasis, selection of tumor clones under the therapy are highlighted.

Keywords: review, breast cancer, metastasis, tumor heterogeneity, immunohistochemistry.

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Introduction. Methodological advances in recent years have significantly expanded our complex understanding of the morphology, pathophysiology, molecular biology, and genetics of breast cancer (BC). Tumor heterogeneity is presently one of the most pressing problems [1–4]. Differences in the morphological properties of tumor tissue as well as the coexistence of areas with different histological characteristics in the same patient are known throughout the history of microscopic examination of tumor lesions of any localization and have become established in the official classifications of diseases and the rules for the diagnosis statement.

Advances in the study of molecular and genetic characteristics of tumors and related aspects of carcinogenesis have led to the formation of contemporary complex concepts of tumor heterogeneity [5–7]. Accordingly, the following levels of tumor heterogeneity have been identified.

1. Heterogeneity of clinical manifestations.
2. Heterogeneity of radiation imaging.
3. Macroscopic heterogeneity.
4. Microscopic (histological) heterogeneity.
5. Heterogeneity of protein expression.
6. Genetic heterogeneity.

In addition, tumor heterogeneity can be spatial or temporal.

Temporal tumor heterogeneity reflects the clonal evolution of cells during tumor development.

Spatial heterogeneity of the tumor is manifested in the following differences.

1. Differences between tumor foci in multicentric/multifocal growth.
2. Differences between the primary tumor and metastases.
3. Differences between the primary tumor and recurrence.
4. Differences between clones of cells in the same tumor.
5. Differences between individual cells within the same tumor.

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Problems associated with tumor heterogeneity, particularly in BC, are currently being actively investigated [8–10]. At the same time, genetic differences in clones of tumor cells are studied both within the primary tumor and between clones of cells of primary and metastatic tumors, and differences in the expression levels of protein biomarkers are compared, as a rule, between the tissues of primary and metastatic tumors [11–14].

The gold standard of BC research includes immunohistochemical assessment of expression levels of estrogen receptors (ER), progesterone receptors (PR), Her2/neu oncoprotein, and the Ki67 proliferation marker in the primary tumor tissue to assess prognosis and prescribe personalized therapy. The importance of each of these markers in clinical decision-making as well as their relationship with the key mechanisms of carcinogenesis have given rise to unremitting interest for over 20 years in the disparity between the levels of expression of each of them in the tissue of primary and metastatic tumors [15–18].

Understanding the mechanisms of changes in the expression levels of biomarkers included in the "gold standard" of BC research in the case of metastasis and the subsequent change in clinical guidelines for the diagnostics of this pathology will reduce the number of cases with an insufficient therapeutic effect of the prescribed drug regimens [19].

Differences in the cells of regional metastases and primary tumors in BC in terms of the expression level of clinically significant biomarkers have remained a subject of study since the 1970s. Thus, differences in ER concentrations in cells of primary and metastatic tumor foci in BC were investigated by Rosen et al. using biochemical methods. In this study, the frequency of differences in the ER statuses of the primary tumor and metastases of any localization was 38%, and the incidence of ER-positive metastatic tissue in the ER-negative tissue of the primary tumor and ER-negative metastatic tissue in the ER-positive tissue of the primary tumor did not demonstrate significant differences [20].

Over the subsequent years, studies regarding the correspondence of receptor status to sex steroid hormones and the Her2/neu oncoprotein in the tissue of primary and metastatic tumors in BC were published regularly [21–23].

Heterogeneity of ER in a tumor with regional metastasis of BC. When studying the change in ER status, most studies have shown the existence of a certain proportion of BC cases where the ER status of the metastatic tissue and the primary tumor tissue do not coincide [24, 25].

A meta-analysis that combined the results of 33 studies (4200 cases) of differences between the ER statuses of primary and metastatic tumors, performed by Aurilio et al. presented the combined value of the proportion of cases with differences in the ER statuses of the primary tumor and metastases, equal to 0.2 [95% confidence interval (CI) 0.16–0.25], and the same indicator when only regional metastases were included in the study was 0.16 (95% CI 0.11–0.22). At the same time, the proportion of cases with ER-negative status of metastatic tissue with ER-positive status of the primary tumor tissue was 0.24 (95% CI 0.18–0.31), and the proportion of cases with ER-positive status of metastatic tissue and ER-negative status of the primary tumor was 0.14 (95% CI 0.09–0.20). The significance of differences in the proportions obtained was confirmed statistically [1].

When analyzing the available sources, the frequency of cases with a change in the ER status in BC metastasis ranged from 4% to 62% of all cases with metastases [26–28]. No studies were revealed, demonstrating the complete absence of changes in the ER status in regional metastasis of BC.

Heterogeneity of PR in a tumor with regional metastasis of BC. Studies focused on the change in the status of PR in regional BC metastasis began to be performed later than studies of the inconsistency of ER status. The first publication found on this subject was published in 1983 by Holdaway and Bowditch, who revealed an incidence of discrepancy between the PR statuses of primary and metastatic tumors of 35%, with the incidence of the cases of PR-negative metastases in PR-positive tissue of the primary tumor over the incidence of cases with a change in the opposite direction [29].

Similarly to the studies of ER, the concentration of PR in tumor cells was assessed by biochemical methods until the early 1990s, when immunohistochemical examination of tumor tissue became the generally accepted method of determining the PR status of BC [30]. The proportion of discrepancies in the PR status of the tissues of primary and metastatic tumors in most studies exceeds the same indicator for ER [31–33]. Thus, the above-considered meta-analysis by Aurilio et al. based on the material of 24 studies and including 2739 patients, presents the combined value of the proportion of cases with differences in PR status between the primary tumor and metastases, equal to 0.33 (95% CI 0.29–0.38). For regional metastases considered separately, this indicator was 0.26 (95% CI 0.21–0.32). The proportion of cases with PR-negative metastases and a PR-positive primary tumor was 0.46 (95% CI 0.37–0.55) and was significantly higher than the proportion of cases with PR-positive metastatic tissue and PR-negative primary tumor tissue, which was 0.15 (95% CI 0.12–0.17) [1].

The frequency of changes in the PR status during metastasis, in accordance with the results of available publications, ranged from 12% to 54% among all cases of metastatic BC [30, 34]. The available sources included no works in which authors did not reveal changes in PR status in BC metastasis.

Heterogeneity of the Her2/neu oncoprotein in a tumor with regional metastasis of BC. The presence of changes in the Her2/neu status of BC cells during metastasis remains a subject of discussion among researchers. The study by Lacroix et al. was the first work found comparing the expression levels of Her2/neu in primary tumor cells and regional metastases. In this work, the researchers compared the expression levels of the Her2/neu oncoprotein and the amplification of the Erbb2 gene encoding it in the tissue of primary and metastatic tumors and concluded that the parameters studied are preserved during regional metastasis [35].

Later, works were published that both refuted and confirmed the existence of changes in the Her2/neu status of BC tumor tissue during metastasis [36–43]. Among the studies reviewed, the highest frequency of changes in the Her2/neu status of tumor tissue during metastasis was found by Regitnig et al. and was 0.24 (95% CI 0.07–0.5) [44]. The proportion of changes in Her2/neu status in BC metastasis was given in a meta-analysis by Aurilio et al. who combined the material of 2987 cases from 31 studies, and the result amounted to 0.08 (95% CI 0.06–0.10) [1].

In contrast to the determination of the ER and PR statuses based on the results of immunohistochemical examination of tumor tissue, in the study of the discrepancies between the Her2/neu statuses of the primary tumor and metastases, in some cases, the *in situ* hybridization method was also used, enabling assessment of the amplification of the Erbb2 gene encoding the Her2/neu protein [45–47].

Heterogeneity of the Ki67 proliferation marker in the tumor with regional metastasis of BC. The publication of studies on the heterogeneity of expression of the proliferation marker Ki67 began later than similar studies on the heterogeneity of ER, PR, and Her2/neu expression, which is associated with the later inclusion of the assessment of proliferative status according to the level of Ki67 expression in the gold standard of BC diagnostics. The threshold value of the proportion of Ki67-positive tumor cells that separates cases of BC with high and low proliferation levels changed over time. At present, the threshold recommended for diagnostics is considered to be 20% of stained tumor cells; however, the issue of introducing two threshold values is discussed, providing for cases with low (≤10% of tumor cells stained), intermediate (10% to 30% of tumor cells stained), and high (≥30% of tumor cells stained) levels of proliferative activity of BC [48, 49]. At the same time, a threshold value of 20% of stained tumor cells remains recommended for diagnostics.

The earliest study found on the change in the proliferative status of a tumor during metastasis was conducted in 2002 by Buxant et al. who registered a significant increase in the expression level of Ki67 in metastatic tissue (29.8 ± 12.2%) compared with the primary tumor tissue (21.8 ± 9.8%) [50]. Later studies confirmed the existence of a certain group of cases where the Ki67 status of the tumor tissue changes with a frequency of 4.4% to 38.8% [51, 52].

In the studies reviewed, no significant differences were revealed in the frequency of cases with a high proliferative status of metastatic tissue with a low proliferative status of the primary tumor tissue and the frequency of cases with a reverse change in Ki67 status during metastasis. At the same time, in several works studying the changes in the Ki67 expression level in BC tissue during metastasis, a significant increase in the proliferative activity of the metastatic tissue was established, compared with the primary tumor tissue [31, 50, 53].

Various approaches to the study of immunophenotypic heterogeneity of BC in regional metastasis. The high predictive value of immunohistochemical examination of the expression level of ER, PR, Her2/neu oncoprotein, and the proliferation marker Ki67 in BC tissue is inextricably associated with the subsequent assessment of the status of each of these markers. The subsequent adoption of therapeutic decisions is based precisely on information about the positive or negative status of each of the cell receptors and the high or low proliferative status of the tumor, determined by immunohistochemical staining of the tissue section with antibodies to Ki67.

Due to the pronounced clinical orientation of most of the published studies, the main interest for their authors was the phenomenon of differences in the status of biomarkers in the tissue of primary and metastatic tumors in BC, as well as the influence of changes in the receptor and proliferative status during metastasis on the choice of a treatment approach and prognostic indicators [54, 55]. Changes in the expression levels of ER, PR, Her2/neu, and Ki67 during BC metastasis, demonstrating the biological patterns of tumor progression, are not widely covered in the available literature.

Falck et al. (2013) presented data on the absence of significant differences in the expression levels of ER and Ki67 and a decrease in the PR expression

level when comparing these biomarkers in the tissue of regional metastases and primary tumor in BC [56]. At the same time, no works were revealed containing a comprehensive methodically uniform description and analysis of the relationships between changes in the expression levels of ER, PR, Her2/neu, and Ki67 as well as the mechanisms of carcinogenesis associated with these changes.

Immunohistochemical analysis of tumor tissue in BC serves as a surrogate method for determining the molecular genetic subtype, since various combinations of positive and negative statuses of ER, PR, and Her2/neu and high and low Ki67 status correspond significantly to the true molecular genetic subtypes of BC, determined using multigene signatures [48, 57].

The phenotypic heterogeneity of BC, manifested in a change in the surrogate tumor subtype during metastasis, has been described in several publications. The frequency of changes in the molecular biological subtype reported in these studies ranged from 11% to 32% among the investigated cases of BC with regional metastases [58]. The statistical significance of the differences (McNemar–Bowker test of asymmetry) in the directions of changes in the tumor subtype during regional metastasis was revealed for the tissue of the primary tumor belonging to the luminal A subtype of BC (prognostically the most favorable subtype). In the cases of this subgroup, where changes in the subtype occurred, the metastatic tissue showed signs of more aggressive molecular subtypes. In the study of BC cases with any molecular subtype of the primary tumor, the direction of changes in the subtype during metastasis was not statistically confirmed [56].

Causes and clinical significance of heterogeneity of primary and metastatic tumors in BC. According to several researchers, tumor heterogeneity and differences in cell clones currently represent the most probable explanations for the insufficient efficacy of anticancer therapy in BC [59, 60]. However, this position cannot be considered generally accepted, since many authors associate the differences detected not with biological phenomena but with technical errors or limitations of the methods. The literature describes four groups of possible causes of the discrepancy between the receptor and proliferative status of metastatic tissue and primary tumors in BC.

1. *Errors or limitations of methods at the preanalytical and analytical stages of the analysis of histological material*. After collecting a BC tissue sample during surgery or during trephine biopsy, the results of subsequent immunohistochemical studies may turn out to be false due to cold ischemia, insufficient or excessive fixation, the use of fixatives not recommended by the manufacturer of immunohistochemical reagents, heat treatment to accelerate fixation, or violation of the recommended volumes and timing of the fixative replacement.

Suboptimal performance of the staining protocol, the use of low-quality regents, and errors in the technique setting can also lead to false results of immunohistochemical studies of tumor tissue. Thus, differences in the processing conditions of the primary tumor material and regional metastases can result in differences being recorded in the expression levels of the biomarkers studied.

At the analytical stage, insufficient standardization of methods for assessing staining results or non-compliance with recommendations for assessing staining results may lead to a discrepancy between the results of immunohistochemical studies of the tissue of primary and metastatic tumors [61].

2. *Tumor polyclonality preceding metastasis*. In some cases, the level of expression of biomarkers, which is assessed by immunohistochemical examination of BC, differs between areas of the primary tumor. In cases where the primary tumor material is collected using a trephine biopsy, its volume is limited and the cells coincident with the immunophenotype with the cells of metastases may not enter the section.

The metastasis of cells representing a clone with different properties from those clones to which most cells of the primary tumor belong and from those that form its immunophenotype is also possible. In this case, as a result of the multiplication of metastatic cells, the level of expression of biomarkers in its tissue will be different from that of the primary tumor [39, 62].

3. *Evolution of cells in the process of tumor development*. Changes in the genetic profile of BC cells occur due to mutational and other changes in the expression of individual genes. The change in the immunophenotype in such cases is associated with the acquisition by the cell of the ability to form metastases [39].

4. *Clonal selection of tumor cells under pressure of therapy*. Hormone therapy, which acts on tumor cells expressing receptors for sex steroid hormones, suppresses the development of the corresponding clones and does not act on those tumor clones in the development of which the signaling pathways associated with ER and PR are not involved. Tumor clones, which are not affected by hormone therapy, gain an advantage in development and ensure the emergence of metastases. Targeted anti-Her2/neu therapy similarly suppresses clones of Her2/neu-positive cells, giving an advantage to clones with negative Her2/neu status of tumor cells that are involved in metastasis [61].

The discrepancy between the receptor and proliferative statuses of the primary tumor and metastases in BC, as shown in several studies, has prognostic and predictive significance. Many studies have revealed worsening prognosis in cases with a change in the status of ER, PR, or Her2/neu in tumor cells during metastasis, regardless of the direction of changes [63] or in the appearance of ER-, PR-, or Her2/neu-negative metastases with a positive primary tumor [52, 64]. In addition, the available literature presents data on the absence of influence of the discrepancy in the status of key biomarkers between the tissues of primary and metastatic tumors in BC on disease prognosis [65].

Conclusion. Insufficient volume and inconsistency of data on the existence, causes, and biological mechanisms of changes in receptor and proliferative status during cancer metastasis as well as the lack of a unified standpoint among experts on the clinical significance of this phenomenon does not enable the inclusion of a mandatory comprehensive assessment of the levels of expression of ER, PR, Her2/neu, and Ki67 in tissue metastases in clinical guidelines [66–69]. Recently, however, the inclusion of immunohistochemical examination of the status of key biomarkers in metastatic BC tissue in clinical guidelines as an additional study [70] or a mandatory study of Her2/neu status alone has begun [71]. In 2020, the question was raised about the introduction of a comparative study of a primary tumor and metastasis in the detection of metastatic lymph nodes in the clinical guidelines of the Russian Society of Oncomammologists [72].

Thus, there is no consensus among researchers about the mechanisms of tumor heterogeneity and the patterns of changes in the expression levels of the receptor apparatus of BC cells during metastasis. Most studies do not question the phenomenon of differences in the statuses and levels of expression of ER, PR, Her2/neu, and Ki67 in the tissue of primary and metastatic tumors [73]. At the same time, in some works, the preferential direction of changes in the statuses and levels of expression of various components of the receptor apparatus of BC cells during regional metastasis was not revealed, while the results of those works in which authors described the patterns of such changes are contradictory. The lack of a unified standpoint on this issue indicates the need to continue research on this subject.

Despite the extensive information about the intracellular mechanisms associated with tumor metastasis, the amount of factual material describing the participation of various components of the receptor apparatus of cells in BC metastasis remains extremely scanty.

When signaling pathways associated with ER, PR, and Her2/neu are implemented in tumor cells, the molecules are mutually influential. At the same time, the patterns of their interaction in BC metastasis remain uninvestigated.

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REFERENCES

1. Aurilio G., Disalvatore D., Pruneri G., Bagnardi V., Viale G., Curigliano G., Adamoli L., Munzone E., Sciandivasci A., De Vita F., Goldhirsch A., Nole F. A meta-analysis of oestrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 discordance between primary breast cancer and metastases. *Eur. J. Cancer*. 2014; 50 (2): 277–289. DOI: 10.1016/j.ejca.2013.10.004.

2. Konyshev K.V., Sazonov S.V. Changes in expression of immunohistochemical markers in regional breast cancer etastases. *Arkhiv patologii*. 2020; 82 (4): 19–26. (In Russ.) DOI: 10.17116/patol20208204119.

3. Erofeeva L.M., Mnikhovich M.V. Related characteristic of lymphoid infiltration in invasive cancer of breast of the different molecular-biological subtypes. *Byulleten' eksperimental'noy biologii i meditsiny*. 2021; 171 (1): 80–85. (In Russ.) DOI: 10.47056/0365-9615-2021-171-1-80-85.

4. Mnikhovich M.V., Midiber K.Yu., Gallyamova A.R., Erzieva A.G., Luchinin V.V., Anistratov S.V., Shilov A.V. Immunohistochemical evaluation of cadherin-catenin complex expression in breast cancer. *Zhurnal anatomii i gistopatologii*. 2017; 6 (1): 63–68. (In Russ.) DOI: 10.18499/2225-7357-2017-6-1-63-68.

5. Alekseenko I.V., Kuzmich A.I., Pleshkan V.V., Tyulkina D.V., Zinovyeva M.V., Kostina M.B., Sverdlov E.D. The cause of cancer mutations: improvable bad life or inevitable stochastic replication errors? *Molecular biology*. 2016; 50 (6): 799–811. (In Russ.) DOI: 10.1134/S0026893316060030.

6. Zardavas D., Irrthum A., Swanton C., Piccart M. Clinical management of breast cancer heterogeneity. *Nature Rev. Clin. Oncol*. 2015; 12 (7): 381–394. DOI: 10.1038/nrclinonc.2015.73.

7. Sagakyants A.B., Ulyanova E.P., Shulgina O.G., Zlatnik E.Yu., Novikova I.A., Karnaukhov N.S., Kuznetsova M.A., Vaschenko L.N., Petriashvili I.R., Lysenko E.P. Expression of tumor stem cell markers in regional and distant breast cancer metastases. *Modern Problems of Science and Education*. 2020; (4): 128. (In Russ.) DOI: 10.17513/spno.30045.

8. Kolyadina I.V., Andreeva Yu.Yu., Frank G.A., Poddubnaya I.V. Role of biological heterogeneity in recurrent and metastatic breast cancer. *Arkhiv patologii*. 2018; 80 (6): 62–67. (In Russ.) DOI: 10.17116/patol20188006162.

9. Konyshev K.V., Sazonov S.V. Her2-positive subtypes of the primary tumor in breast cancer often have heterogeneous metastases, whereas the triple-negative subtype of the tumor remains stable when metastasized. *Vestnik ural'skoy meditsinskoy akademicheskoy nauki*. 2020; 17 (4): 285–289. (In Russ.) DOI: 10.22138/2500-0918-2020-17-4-285-289.

10. Konyshev K.V., Sazonov S.V., Kazantseva N.V. Changes of the luminal A subtype of breast cancer in local metastasis. *Virchows Archiv.* 2020; 477 (Suppl. 1): S226. DOI: 10.1007/s00428-020-02938-x.

11. Konyshev K.V., Sazonov S.V. Study of her2/neu status of locoregionally metastasizing breast cancer cells in the cases with equivocal (2+) level of her2/neu oncoprotein expression in primary tumor tissue. *Vestnik ural'skoy meditsinskoy akademicheskoy nauki.* 2018; 15 (1): 48–54. (In Russ.) DOI: 10.22138/2500-0918-2018-15-1-48-54.

12. Konyshev K.V., Sazonov S.V. Changes of oestrogen receptor, progesterone receptor and Her2/neu statuses of localmetastases compared with primary tumour in breast cancer. *Virchows Archiv.* 2019; 475 (Suppl. 1): S84. DOI: 10.1007/s00428-019-02631-8.

13. Sazonov S.V., Konyshev K.V. Relationship of Her2/neu and oestrogen receptor changes in local metastases compared with primary tumour in breast cancer patients with equivocal (2+) Her2/neu expression level in primary tumour. *Virchows Archiv.* 2019; 475 (Suppl. 1): S91. DOI: 10.1007/s00428-019-02631-8.

14. Kolyadina I.V., Poddubnaya I.V., Van de Velde C.J.H., Kuppen P.J.K., Frank G.A., Komov D.V., Karseladze A.I., Bastiaannet E., Dekker-Ensink N.G. Immunological heterogeneity of stage I breast cancer: biological, population-based and prognostic value (international cooperation experience). *Sovremennaya onkologiya.* 2015; 17 (1): 30–38. (In Russ.)

15. Carlsson J., Nordgren H., Sjöström J., Wester K., Villman K., Bengtsson N.O., Ostenstad B., Lundqvist H., Blomqvist C. HER2 expression in breast cancer primary tumours and corresponding metastases. Original data and literature review. *Brit. J. Cancer.* 2004; 90 (12): 2344–2348. DOI: 10.1038/sj.bjc.6601881.

16. Cui X., Schiff R., Arpino G., Osborne C.K., Lee A.V. Biology of progesterone receptor loss in breast cancer and its implications for endocrine therapy. *J. Clin. Oncol.* 2005; 23 (30): 7721–7735. DOI: 10.1200/JCO.2005.09.004.

17. Konyshev K.V., Brilliant A.A., Sazonov S.V. Ki67 level in primary tumor and locoregional metastases of breast cancer. *Ural'skiy meditsinskiy zhurnal.* 2015; 130 (7): 51–55. (In Russ.)

18. Konyshev K.V., Brilliant A.A., Sazonov S.V. Change of estrogen receptor expression in breast cancer cells in locoregional metastases. *Vestnik ural'skoy meditsinskoy akademicheskoy nauki.* 2015; (2): 4–6. (In Russ.)

19. Pectasides D., Gaglia A., Arapantoni-Dadioti P., Bobota A., Valavanis C., Kostopoulou V., Mylonakis N., Karabelis A., Pectasides M., Economopoulos T. HER-2/neu status of primary breast cancer and corresponding metastatic sites in patients with advanced breast cancer treated with trastuzumab-based therapy. *Anticancer Res.* 2006; 26 (1B): 647–653. PMID: 16739334.

20. Rosen P.P., Menendez-Botet C.J., Urban J.A., Fracchia A., Schwartz M.K. Estrogen receptor protein (ERP) in multiple tumor specimens from individual patients with breast cancer. *Cancer.* 1977; 39 (5): 2194–2200. DOI: 10.1002/1097-0142(197705)39:5<2194::AID-CNCR2820390537>3.0.CO;2-Y.

21. Liedtke C., Broglio K., Moulder S., Hsu L., Kau S.W., Symmans W.F., Albarracin C., Meric-Bernstam F., Woodward W., Theriault R.L., Kiesel L., Hortobagyi G.N., Pusztai L., Gonzalez-Angulo A.M. Prognostic impact of discordance between triple-receptor measurements in primary and recurrent breast cancer. *Ann. Oncol.* 2009; 20 (12): 1953–1958. DOI: 10.1093/annonc/mdp263.

22. Aitken S.J., Thomas J.S., Langdon S.P., Harrison D.J., Faratian D. Quantitative analysis of changes in ER, PR and HER2 expression in primary breast cancer and paired nodal metastases. *Ann. Oncol.* 2010; 21 (6): 1254–1261. DOI: 10.1093/annonc/mdp427.

23. Dawood S., Broglio K., Buzdar A.U., Hortobagyi G.N., Giordano S.H. Prognosis of women with metastatic breast cancer by HER2 status and trastuzumab treatment: An institutional-based review. *J. Clin. Oncol.* 2010; 28 (1): 92–98. DOI: 10.1200/JCO.2008.19.9844.

24. Hoefnagel L.D.C., van de Vijver M.J., van Slooten H.-J., Wesseling P., Wesseling J., Westenend P.J., Bart J., Seldenrijk C.A., Nagtegaal I.D., Oudejans J., van der Valk P., van der Groep P., de Vries E.G.E., van der Wall E., van Diest P.J. Receptor conversion in distant breast cancer metastases. *Breast Cancer Res.* 2010; 12: R75. DOI: 10.1186/bcr2645.

25. Thompson A.M., Jordan L.B., Quinlan P., Anderson E., Skene A., Dewar J.A., Purdie C.A. Prospective comparison of switches in biomarker status between primary and recurrent breast cancer: the Breast Recurrence In Tissues Study (BRITS). *Breast Cancer Res.* 2010; 12: R92. DOI: 10.1186/bcr2771.

26. Gataullin I.G., Khasanov A.I., Khusainova G.N., Petrov S.V., Khasanov R.Sh. Rationale for optimal treatment of patients with metastatic breast cancer. *Kubanskiy nauchnyy meditsinskiy vestnik.* 2010; (9): 55–58. (In Russ.)

27. Van Agthoven T., Timmermans M., Dorssers L.C.J., Henzen-Logmans S.C. Expression of estrogen, progesterone and epidermal growth factor receptors in primary and metastatic breast cancer. *Intern. J. Cancer.* 1995; 63 (6): 790–793. DOI: 10.1002/ijc.2910630607.

28. Amir E., Ooi W.S., Simmons C., Kahn H., Christakis M., Popovic S., Kalina M., Chesney A., Singh G., Clemons M. Discordance between receptor status in primary and metastatic breast cancer: An exploratory study of bone and bone marrow biopsies. *Clin. Oncol.* 2008; 20 (10): 763–768. DOI: 10.1016/j.clon.2008.08.005.

29. Holdaway I.M., Bowditch J.V. Variation in receptor status between primary and metastatic breast cancer. *Cancer.* 1983; 52 (3): 479–485. DOI: 10.1002/1097-0142(19830801)52:3<479::AID-CNCR2820520317>3.0.CO;2-O.

30. Hammond M.E.H., Hayes D.F., Dowsett M., Allred D.C., Hagerty K.L., Badve S., Fitzgibbons P.L., Francis G., Goldstein N.S., Hayes M., Hicks D.G., Lester S., Love R., Mangu P.B., McShane L., Miller K., Osborne C.K., Paik S., Perlmutter J., Rhodes A., Sasano H., Schwartz J.N., Sweep F.G.C., Taube S., Torlakovic E.E., Valenstein P., Viale G., Visscher D., Wheeler T., Williams R.B., Wittliff J.L., Wolff A.C. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *Arch. Pathol. Lab. Med.* 2010; 134 (6): 907–1101. DOI: 10.1200/JCO.2009.25.6529.

31. Nishimura R., Osako T., Okumura Y., Tashima R., Toyozumi Y., Arima N. Changes in the ER, PgR, HER2, p53 and Ki-67 biological markers between primary and recurrent breast cancer: Discordance rates and prognosis. *World J. Surg. Oncol.* 2011; 9: 131. DOI: 10.1186/1477-7819-9-131.

32. Bogina G., Bortesi L., Marconi M., Venturini M., Lunardi G., Coati F., Massocco A., Manfrin E., Pegoraro C., Zamboni G. Comparison of hormonal receptor and HER-2 status between breast primary tumours and relapsing tumours: Clinical implications of progesterone receptor loss. *Virchows Arch.* 2011; 459 (1): 1–10. DOI: 10.1007/s00428-011-1097-7.

33. Idirisinghe P.K., Thike A.A., Cheok P.Y., Tse G.M.-K., Lui P.C.-W., Fook-Chong S., Wong N.S., Tan P.H. Hormone receptor and c-ERBB2 status in distant metastatic and locally recurrent breast cancer: pathologic correlations and clinical significance. *Am. J. Clin. Pathol*. 2010; 133 (3): 416–429. DOI: 10.1309/AJCPJ57FLLJRXKPV.

34. Sari E., Guler G., Hayran M., Gullu I., Altundag K., Ozisik Y. Comparative study of the immunohistochemical detection of hormone receptor status and HER-2 expression in primary and paired recurrent/metastatic lesions of patients with breast cancer. *Med. Oncol*. 2011; 28 (1): 57–63. DOI: 10.1007/s12032-010-9418-2.

35. Lacroix H., Iglehart J.D., Skinner M.A., Kraus M.H. Overexpression of erbB-2 or EGF receptor proteins present in early stage mammary carcinoma is detected simultaneously in matched primary tumors and regional metastases. *Oncogene*. 1989; 4 (2): 145–151. PMID: 2564657.

36. Tapia C., Savic S., Wagner U., Schönegg R., Novotny H., Grilli B., Herzog M., Barascud A.D., Zlobec I., Cathomas G., Terracciano L., Feichter G., Bubendorf L. HER2 gene status in primary breast cancers and matched distant metastases. *Breast Cancer Res.* 2007; 9 (3): R31. DOI: 10.1186/bcr1676.

37. Vincent-Salomon A., Pierga J.-Y., Couturier J., D'Enghien C.D., Nos C., Sigal-Zafrani B., Lae M., Fréneaux P., Diéras V., Thiéry J-P., Sastre-Garau X. HER2 status of bone marrow micrometastasis and their corresponding primary tumours in a pilot study of 27 cases: A possible tool for anti-HER2 therapy management? *British J. Cancer*. 2007; 96 (4): 654–659. DOI: 10.1038/sj.bjc.6603584.

38. Cho E.Y., Han J.J., Choi Y.-L., Kim K.-M., Oh Y.L. Comparison of Her-2, EGFR and cyclin D1 in primary breast cancer and paired metastatic lymph nodes: An immunohistochemical and chromogenic in situ hybridization study. *J. Korean Med. Sci*. 2008; 23 (6): 1053–1061. DOI: 10.3346/jkms.2008.23.6.1053.

39. Pusztai L., Viale G., Kelly C.M., Hudis C.A. Estrogen and HER-2 receptor discordance between primary breast cancer and metastasis. *Oncologist*. 2010; 15 (11): 1164–1168. DOI: 10.1634/theoncologist.2010-0059.

40. Aoyama K., Kamio T., Nishikawa T., Kameoka S. A comparison of HER2/neu gene amplification and its protein overexpression between primary breast cancer and metastatic lymph nodes. *Japanese J. Clin. Oncol*. 2010; 40 (7): 613–619. DOI: 10.1093/jjco/hyq019.

41. Bozzetti C., Negri F.V., Lagrasta C.A., Crafa P., Bassano C., Tamagnini I., Gardini G., Nizzoli R., Leonardi F., Gasparro D., Camisa R., Cavalli S., Capelli S., Silini E.M., Ardizzoni A. Comparison of HER2 status in primary and paired metastatic sites of gastric carcinoma. *British J. Cancer*. 2011; 104 (9): 1372–1376. DOI: 10.1038/bjc.2011.121.

42. Chang H.J., Han S.-W., Oh D.-Y., Im S.-A., Jeon Y.K., Park I.A., Han W., Noh D.-Y., Bang Y.-J., Kim T.-Y. Discordant human epidermal growth factor receptor 2 and hormone receptor status in primary and metastatic breast cancer and response to trastuzumab. *Japanese J. Clin. Oncol*. 2011; 41 (5): 593–599. DOI: 10.1093/jjco/hyr020.

43. Fabi A., Di Benedetto A., Metro G., Perracchio L., Nisticò C., Di Filippo F., Ercolani C., Ferretti G., Melucci E., Buglioni S., Sperduti I., Papaldo P., Cognetti F., Mottolese M. HER2 protein and gene variation between primary and metastatic breast cancer: Significance and impact on patient care. *Clin. Cancer Res*. 2011; 17 (7): 2055–2064. DOI: 10.1158/1078-0432.CCR-10-1920.

44. Regitnig P., Schippinger W., Lindbauer M., Samonigg H., Lax S.F. Change of HER-2/neu status in a subset of distant metastases from breast carcinomas. *J. Pathol*. 2004; 203 (4): 918–926. DOI: 10.1002/path.1592.

45. Xiang J., Pan X., Xu J., Fu X., Wu D., Zhang Y., Shen L., Wei Q. Human epidermal growth factor receptor 2 protein expression between primary breast cancer and paired asynchronous local-regional recurrences. *Experim. Therap. Med*. 2011; 2 (6): 1187–1191. DOI: 10.3892/etm.2011.335.

46. Xiao C., Gong Y., Han E.Y., Gonzalez-Angulo A.M., Sneige N. Stability of HER2-positive status in breast carcinoma: A comparison between primary and paired metastatic tumors with regard to the possible impact of intervening trastuzumab treatment. *Ann. Oncol*. 2011; 22 (7): 1547–1553. DOI: 10.1093/annonc/mdq623.

47. Zurrida S., Montagna E., Naninato P., Colleoni M., Goldhirsch A. Receptor status (ER, PgR and HER2) discordance between primary tumor and locoregional recurrence in breast cancer. *Ann. Oncol*. 2011; 22 (2): 479–480. DOI: 10.1093/annonc/mdq688.

48. Goldhirsch A., Winer E.P., Coates A.S., Gelber R.D., Piccart-Gebhart M., Thürlimann B. Personalizing the treatment of women with early breast cancer: Highlights of the st gallen international expert consensus on the primary therapy of early breast Cancer 2013. *Ann. Oncol*. 2013; 24 (9): 2206–2223. DOI: 10.1093/annonc/mdt303.

49. Coates A.S., Winer E.P., Goldhirsch A., Gelber R.D., Gnant M., Piccart-Gebhart M. Tailoring therapies — improving the management of early breast cancer: St GallenInternational Expert Consensus on the Primary Therapy of Early Breast Cancer 2015. *Ann. Oncol*. 2015; 26 (8): 1533–1546. DOI: 10.1093/annonc/mdv221.

50. Buxant F., Anaf V., Simon P., Fayt I., Noël J.C. Ki-67 immunostaining activity is higher in positive axillary lymph nodes than in the primary breast tumor. *Breast Cancer Res. Treat*. 2002; 75 (1): 1–3. DOI: 10.1023/A:1016504129183.

51. D'Andrea M.R., Limiti M.R., Bari M., Zambenedetti P., Montagutti A., Ricci F., Pappagallo G.L., Sartori D., Vinante O., Mingazzini P.L. Correlation between genetic and biological aspects in primary non-metastatic breast cancers and corresponding synchronous axillary lymph node metastasis. *Breast Cancer Res. Treat*. 2007; 101 (3): 279–284. DOI: 10.1007/s10549-006-9300-2.

52. Ibrahim T., Farolfi A., Scarpi E., Mercatali L. Hormonal receptor, human epidermal growth factor receptor-2, and Ki67 discordance between primary breast cancer and paired metastases: Clinical impact. *Oncology*. 2013; 84 (3): 150–157. DOI: 10.1159/000345795.

53. Paksoy S.E., Tasdelen I., Balaban A.S., Ozkaya G., Tolunay S. A comparison of Ki67 proliferative index in primary tumor and axillary metastatic lymph nodes with length of survival in patients with breast cancer. *Bratislavské lekárske listy*. 2013; 114 (11): 645–649. DOI: 10.4149/bll_2013_138.

54. Rossi S., Basso M., Strippoli A., Dadduzio V., Cerchiaro E., Barile R., D'Argento E., Cassano A., Schinzari G., Barone C. Hormone receptor status and HER2 expression in primary breast cancer compared with synchronous axillary metastases or recurrent metastatic disease. *Clin. Breast Cancer*. 2015; 15 (5): 307–312. DOI: 10.1016/j.clbc.2015.03.010.

55. Raica M., Cîmpean A.M., Ceausu R.A., Fulga V., Nica C., Rudico L., Saptefrati L. Hormone receptors and HER2 expression in primary breast carcinoma and corresponding lymph node metastasis: Do we need both? *Anticancer Res*. 2014; 34 (3): 1435–1440. PMID: 24596391.

56. Falck A.K., Bendahl P.O., Chebil G., Olsson H., Fernö M., Rydén L. Biomarker expression and St Gallen

molecular subtype classification in primary tumours, synchronous lymph node metastases and asynchronous relapses in primary breast cancer patients with 10 years' follow-up. *Breast Cancer Res. Treat*. 2013; 140 (1): 93–104. DOI: 10.1007/s10549-013-2617-8.

57. Pak D.D., Rasskazova E.A., Danilova T.V. Subtypes of breast cancer. *Opukholi zhenskoy reproduktivnoy sistemy*. 2012; (3–4): 13–19. (In Russ.)

58. Falck A.-K., Fernö M., Bendahl P.-O., Rydén L. St Gallen molecular subtypes in primary breast cancer and matched lymph node metastases — aspects on distribution and prognosis for patients with luminal A tumours: Results from a prospective randomised trial. *BMC Cancer*. 2013; 13: 558. DOI: 10.1186/1471-2407-13-558.

59. Ufen M.-P., Kohne C.H., Wischneswky M., Wolters R., Novopashenny I., Fischer J., Constantinidou M., Possinger K., Regierer A.C. Metastatic breast cancer: are we treating the same patients as in the past? *Ann. Oncol*. 2013; 25 (1): 95–100. DOI: 10.1093/annonc/mdt429.

60. Aurilio G., Monfardini L., Rizzo S., Sciandivasci A., Preda L., Bagnardi V., Disalvatore D., Pruneri G., Munzone E., Della Vigna P., Renne G., Bellomi M., Curigliano G., Goldhirsch A., Nolè F. Discordant hormone receptor and human epidermal growth factor receptor 2 status in bone metastases compared to primary breast cancer. *Acta Oncologica*. 2013; 52: 1649–1656. DOI: 10.3109/0284186X.2012.754990.

61. Khasraw M., Brogi E., Seidman A.D. The need to examine metastatic tissue at the time of progression of breast cancer: is re-biopsy a necessity or a luxury? *Curr. Oncol. Rep*. 2011; 13 (1): 17–25. DOI: 10.1007/s11912-010-0137-9.

62. Zardavas D., Irrthum A., Swanton C., Piccart M. Clinical management of breast cancer heterogeneity. *Nature Rev. Clin. Oncol*. 2015; 12 (7): 381–394. DOI: 10.1038/nrclinonc.2015.73.

63. Niikura N., Liu J., Hayashi N., Mittendorf E.A., Gong Y., Palla S.L., Tokuda Y., Gonzalez-Angulo A.M., Hortobagyi G.N., Ueno N.T. Loss of human epidermal growth factor receptor 2 (HER2) expression in metastatic sites of HER2-overexpressing primary breast tumors. *J. Clin. Oncol*. 2012; 30 (6): 593–599. DOI: 10.1200/JCO.2010.33.8889.

64. Dieci M.V., Barbieri E., Piacentini F., Ficarra G., Bettelli S., Dominici M., Conte P.F., Guarneri V. Discordance in receptor status between primary and recurrent breast cancer has a prognostic impact: A single-institution analysis. *Ann. Oncol*. 2013; 24 (1): 101–108. DOI: 10.1093/annonc/mds248.

65. Amir E., Miller N., Geddie W., Freedman O., Kassam F., Simmons C., Oldfield M., Dranitsaris G., Tomlinson G., Laupacis A., Tannock I.F., Clemons M. Prospective study evaluating the impact of tissue confirmation of metastatic disease in patients with breast cancer. *J. Clin. Oncol*. 2012; 30 (6): 587–592. DOI: 10.1200/JCO.2010.33.5232.

66. Petrov S.V. The capabilities and limitations of immunohistochemical analysis in clinical oncology. *Ural'skiy meditsinskiy zhurnal*. 2014; (8): 5–9. (In Russ.)

67. Ieni A., Barresi V., Caltabiano R., Cascone A.M., Del Sordo R., Cabibi D., Zeppa P., Lanzafame S., Sidoni A., Franco V., Tuccari G. Discordance rate of HER2 status in primary breast carcinomas versus synchronous axillary lymph node metastases: A multicenter retrospective investigation. *Onco Targets Ther*. 2014; 7: 1267–1272. DOI: 10.2147/OTT.S65294.

68. Kaufman P.A., Bloom K.J., Burris H., Gralow J.R., Mayer M., Pegram M., Rugo H.S., Swain S.M., Yardley D.A., Chau M., Lalla D., Yoo B., Brammer M.G., Vogel C.L. Assessing the discordance rate between local and central HER2 testing in women with locally determined HER2-negative breast cancer. *Cancer*. 2014; 120 (17): 2657–2664. DOI: 10.1002/cncr.28710.

69. Sighoko D., Liu J., Hou N., Gustafson P., Huo D. Discordance in hormone receptor status among primary, metastatic, and second primary breast cancers: biological difference or misclassification? *Oncologist*. 2014; 19 (6): 592–601. DOI: 10.1634/theoncologist.2013-0427.

70. Cardoso F., Harbeck N., Fallowfield L., Kyriakides S., Senkus E. Locally recurrent or metastatic breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol*. 2012; 23 (Suppl. 7): VII11– VII19. DOI: 10.1093/annonc/mds232.

71. Wolff A.C., Hammond M.E.H., Hicks D.G., Dowsett M., McShane L.M., Allison K.H. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *Arch. Pathol. Lab. Med*. 2014; 138 (2): 241–256. DOI: 10.5858/arpa.2013-0953-SA.

72. *"Zolotoy standart" diagnostiki i lecheniya raka molochnoy zhelezy 2021*. (Golden standard of breast cancer diagnostics and threatment 2021.) M.: ABV-press. 2020; 23–28. (In Russ.)

73. Konyshev K.V., Sazonov S.V., Leont'ev S.L. *Izmenenie retseptornogo apparata kletok kartsinomy molochnoy zhelezy pri regionarnom metastazirovanii*. (Changes in the receptor apparatus of breast carcinoma cells during regional metastasis.) Yekaterinburg: Redaktsiya zhurnala "Vestnik Ural'skoy meditsinskoy akademicheskoy nauki". 2019; 88–92. (In Russ.)