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Clinical possibilities of HER2-positive breast cancer diagnosis using alternative scaffold proteins

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ABSRTACT

HER2-positive breast cancer occurs in 15–20% of breast cancer patients and is associated primarily with a poor prognosis of the disease and the need for highly specific targeted therapy. Despite the clinical importance of determining HER2/neu, traditional diagnostic methods have their disadvantages and require the study of new additional research techniques.

The information presented in this review makes it possible to consider current trends in the radionuclide diagnosis of HER2-positive breast cancer using the latest class of alternative scaffold proteins and to consider various aspects of their use in clinical practice.

Keywords: breast cancer, radionuclide diagnosis, alternative scaffold proteins, HER2/neu

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Клинические возможности диагностики HER2-позитивного рака молочной железы с применением альтернативных каркасных белков

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РЕЗЮМЕ

HER2-позитивный рак молочной железы (РМЖ) встречается у 15–20% пациенток с РМЖ и ассоциируется прежде всего с неблагоприятным прогнозом заболевания и необходимостью назначения высокоспецифичной таргетной терапии. Несмотря на клиническую важность определения рецептора эпидермального фактора роста 2-го типа, существующие диагностические методики являются несовершенными и требуют изучения новых дополнительных методов исследования.

Представленные в обзоре данные позволяют рассмотреть современные тенденции в радионуклидной диагностике HER2-позитивного РМЖ с применением новейшего класса «нацеливающих» модулей (альтернативных каркасных белков), а также демонстрируют различные аспекты их использования в клинической практике.

Ключевые слова: рак молочной железы, радионуклидная диагностика, альтернативные каркасные белки, DARPinG3, HER2/neu

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INTRODUCTION

Human epidermal growth factor receptor 2 (HER2 / neu) is one of the most well-described molecular targets on the surface of tumor cells belonging to the receptor tyrosine kinase family, the epidermal growth factor receptor subfamily [1]. The highest expression of HER2 / neu is most often observed in patients with breast cancer (15–20% of cases) and associated with an unfavorable prognosis of the disease and its aggressive course [2]. In addition, according to clinical recommendations, overexpression of HER2 / neu requires targeted therapy, including mandatory prescription of such drugs as trastuzumab (herceptin), lapatinib, pertuzumab, and trastuzumab emtanzine (T-DM1) [3, 4].

Targeted therapy is highly specific and determines the need for careful selection of candidates for the treatment. Currently, several FDA-approved (U.S. Food and Drug Association) techniques are used to determine the status of HER2 / neu, which include

immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH). According to the American Society of Clinical Oncology (ASCO), category 0 and 1+ cases are considered negative, and category 3+ cases are considered positive (as of 2018). Controversial category 2+ cases require FISH; amplification is considered positive if an average number of copies for the *ERBB2* gene and an average number of chromosome 17 centromeres in a cell are more than 2.2 [5].

An obvious disadvantage of IHC, despite availability and relative cheapness, is significant influence of many factors on the results, including the method of sample preparation (the duration of fixation and the fixator used), the characteristics of the antibodies used, the qualifications of the staff, and the interpretation of the results (mainly category 2+ cases) [6]. FISH remains a very reliable method for assessing amplification of the *ERBB2* gene, however, it takes nine times longer (36 vs 4 hours) and costs several times more compared with standard IHC. Moreover, FISH requires expensive equipment for detecting and recognizing signals and highly qualified staff for interpreting results [7].

One of the main disadvantages of the study is a need for invasive manipulations to obtain material for diagnosis, as well as inability to simultaneously assess tumor spread in a patient and analyze molecular characteristics of tumor sites before specific treatment [8]. The latter is especially relevant in terms of possible differences in HER2 / neu expression in a primary tumor and regional lymph nodes and distant metastases, which can occur in 6–48% of cases, according to various studies [9].

The problem of intratumoral heterogeneity, which occurs in 40% of breast cancer cases and is characterized by lower rates of relapse-free survival and effective targeted therapy with trastuzumab also remains unresolved. All this dictates the need for development of a new additional diagnostic technique to optimize the diagnosis in breast cancer patients [10].

CLINICAL STUDIES ON THE DIAGNOSIS OF HER2-POSITIVE BREAST CANCER USING ALTERNATIVE SCAFFOLD PROTEINS

In recent years, targeted radionuclide methods have been studied to identify malignancies. They have a number of significant advantages, such as non-invasiveness with a possibility of repeated studies; runtime assessment of marker expression with ongoing treatment; simultaneous visualization of the whole patient's body with an assessment of HER / neu expression in the primary tumor and metastatic sites [11], as well as improvement of diagnostic equipment, manifested through development of devices combining modules for radionuclide studies and anatomical visualization of metastatic sites (computed tomography and magnetic resonance imaging) [12].

Over the past decade, a new class of "targeting" modules called alternative scaffold proteins or scaffolds has become very popular. It meets all the requirements for optimal radionuclide delivery to tumor cells [13]. The advantages of targeted molecules include significantly smaller sizes compared with a standard antibody, which increases penetration of the substance into the tumor; stable structure; additional functionalization and expression in the bacterial system, which contributes to low production costs; high thermal stability, contributing to long-term storage at room temperature, as well as a possibility of direct chemical synthesis [14]. Currently, 3 scaffolds have been clinically tested in the diagnosis of HER2-positive breast cancer: affibodies, ADAPTs, and DARPins.

Affibodies. Affibody molecules are three tightly packed alpha helices stabilized by a hydrophobic core. They consist of 58 amino acids with a molecular weight of 6–7 kDa and have a small size. The largest number of studies on affibodies are based on their high affinity to the HER2 / neu receptors [15]. In the phase I clinical trial of ^{111}In-ABY-025 (^{111}In, half-life of 2.8 days), including patients with locally advanced and metastatic breast cancer (5 patients with HER2 / neu overexpression and 2 patients without receptor expression), J. Sorensen et al. demonstrated the safety of ^{111}In-ABY-025 affibody molecule and the possibility of differentiating a primary tumor and metastatic sites depending on the HER2 / neu status [16]. However, this study demonstrated a limited ability to visualize small tumor sites in HER2-positive patients, which was probably due to the low resolution of single-photon emission computed tomography (SPECT / CT). The obtained data determined the beginning of the study on ^{68}Ga-ABY-025 (^{68}Ga, half-life of 68 minutes) already for positron emission computed tomography (PET / CT).

The phase I clinical trial of ^{68}Ga-ABY-025 demonstrated the safety of the affibody molecule in 8 patients with metastatic breast cancer and stressed the importance of the dosage. So, using 78 μg of the protein resulted in statistically higher accumulation of ^{68}Ga-ABY-025 in the liver and kidneys compared with accumulation resulting from the use of 427 μg of

protein [17]. The study of ^{68}Ga-ABY-025 by J. Sorensen et al. involving 16 patients with metastatic breast cancer (12 patients with overexpression of HER2 / neu; 4 patients without it) showed not only the possibility of visualizing metastases to regional lymph nodes and distant organs and tissues in all cases, but also the possibility of their exact differentiation depending on the HER2 / neu expression [18].

One of the findings in the study was a clinical observation of a breast cancer patient with a HER2-negative breast tumor and liver metastasis with HER2 / neu overexpression, which were detected by ^{68}Ga-ABY-025 and confirmed by immunohistochemistry of the biopsy material from the identified tumor sites (Fig. 1).

In an additional analysis of ^{111}In-ABY-025 and ^{68}Ga-ABY-025 involving 23 patients with metastatic breast cancer, D. Sandberg et al. also determined that the spleen was the best reference organ, while the tumor-to-spleen ratio reached 100% accuracy in the differentiation of tumor nodules depending on the HER2 / neu status 4 hours after the injection according to PET data and 24 hours after the injection according to SPECT [19].

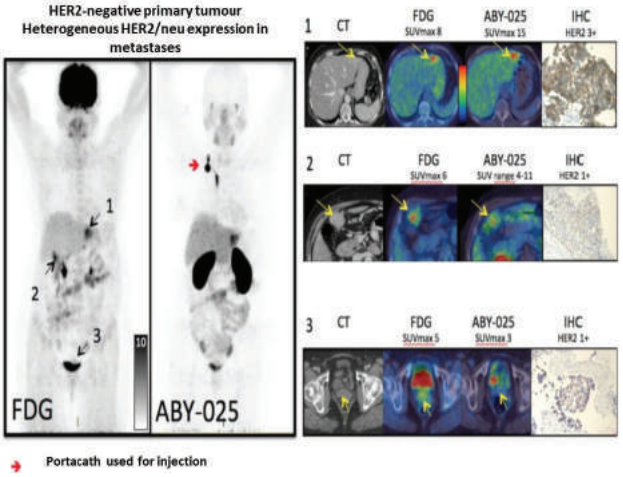

Fig 1. HER2-negative primary breast tumor patient with HER2-positive metastases in left liver lobe according to PET / CT findings using ^{68}Ga-ABY-025

ADAPTs (ABD-Derived Affinity Proteins). The molecules were developed using a 46-amino acid framework derived from an albumin binding domain (ABD), which spontaneously folds into a three-spiral structure and is independent of disulfide bridges [20]. The HER2 / neu-specific ADAPT6 molecule was chosen because of its high affinity (1 nM) and rapid removal from the bloodstream due to weak binding to albumin, which was reflected by the results of preclinical studies [21].

In the phase I clinical trial of ^{99m}Tc-ADAPT6 (^{99m}Tc, half-life of 6.01 hours), including 22 breast cancer patients with different HER2 / neu expression in the primary tumor, three protein dosages were studied: 250, 500, and 1,000 μg. According to the results, ^{99m}Tc-ADAPT6 demonstrated good tolerability and the absence of changes in vital organs. The best difference between HER2-posivive and HER2-negative tumors was observed 2 hours after the injection of the protein at a dose of 500 μg with an average tumor-to-background ratio of 37±19 for HER2-positive tumors compared with 5±2 for HER2-negative tumors ($p < 0.05$, Mann – Whitney test). The difference between the groups in other time points was unreliable. The tumor-to-background ratio for HER2-positive breast tumors was significantly higher in patients who received 500 μg of the protein compared with the doses of 250 and 1,000 μg ($p < 0.05$, Mann – Whitney test). In addition, according to the study, a relatively low effective dose was determined for the patient when injecting 500 and 1,000 μg of the protein – 0.009 ± 0.002 and 0.010 ± 0.003 mSv / MBq, respectively, which was comparable with the data obtained in the study of other scaffold proteins (Fig. 2) [22].

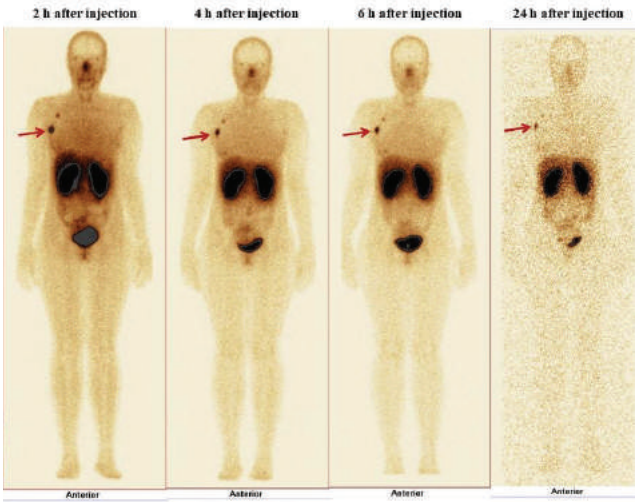

Fig. 2. Anterior projection of planar scintigraphy of the HER2-positive breast cancer patient 2, 4, 6, and 24 hours after the injection of 500 mμ ^{99m}Tc-ADAPT6 (arrows indicate tumor in the right breast)

In this study, one of the breast cancer patients was included with a HER2-positive tumor according to IHC of the biopsy material, however, after the ^{99m}Tc-ADAPT6 injection, a low tumor-to-background ratio

was revealed (1.33 2 hours after the injection of the protein at a dose of 500 μg). After IHC revision, the tumor status was changed to 2+, and FISH showed no amplification of the *ERBB2* gene. According to the results, the HER2-status of the breast tumor was changed to "negative", and targeted therapy was canceled (Fig. 3) [23].

Another clinical example of the use of ^{99m}Tc-ADAPT6 at a dose of 500 μg is detection of additional metastatic sites in the patient with HER2-positive breast cancer projected at the right 5th rib along the mid-clavicular line and at 8th–9th thoracic vertebrae. The revealed changes were not diagnosed by a standard bone scan and computed tomography of the chest, but were confirmed by magnetic resonance imaging (MRI) data (Fig.4) [24].

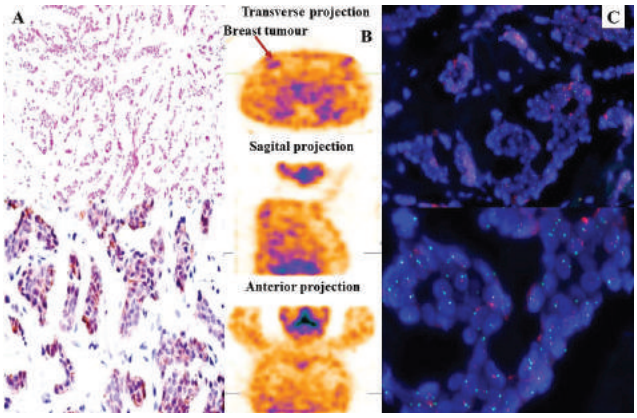

Fig. 3. Results of IHC, FISH, and radionuclide study with ^{99m}Tc-ADAPT6 at a dose of 500 μg in the breast cancer patient: *A* – IHC of the biopsy material (HER2/neu 2+ expression); *B* – radionuclide study with ^{99m}Tc-ADAPT6 (arrows indicate tumor in the right breast); *C* – FISH with negative amplification of the *ERBB2* gene in the biopsy material

DARPins (Designed Ankyrin Repeat Proteins) are representatives of scaffold proteins which were designed on the basis of ankyrin proteins. Ankyrins are involved in the attachment of membrane proteins to the cytoskeleton. The framework of DARPins can include 4–6 ankyrin domains, each of which contains 33 amino acids; the domains are organized as two antiparallel alpha helices with a beta turn between them [25]. Since the molecular weight of one module is slightly more than 3.5 kDa, and DARPins consist of 4-6 modules, their molecular weight ranges from 14 to 21 kDa and is about one tenth the size of a conventional antibody (IgG) or one third the size of Fab [26].

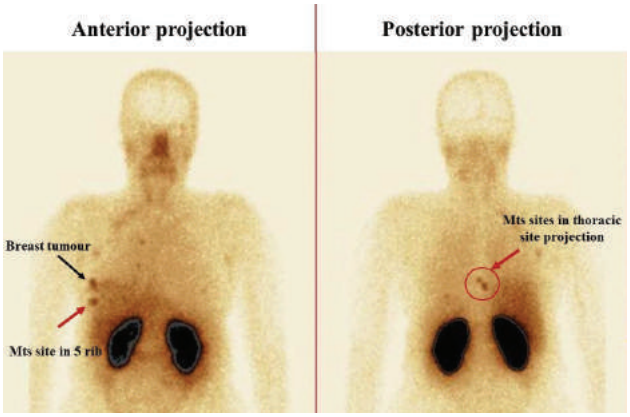

Fig. 4. Planar scintigraphy of the HER2-positive breast cancer patient 2 hours after the ^{99m}Tc-ADAPT6 injection (anterior and posterior projection): visualization of pathologic accumulation in the primary breast tumor projection (indicated by the black arrow); metastatic sites projected at the 5th rib (right) and Th 8–9 (indicated by the red arrow)

The phase I clinical trial of ^{99m}Tc-DARPinG3 was performed on 28 breast cancer patients with different HER2 / neu expression using three doses of the protein: 1,000, 2,000, and 3,000 μg. Whole-body planar scintigraphy and SPECT of the chest were performed in all patients 2, 4, 6, and 24 hours after the injection of the protein. The results of the study showed the absence of toxic effects of ^{99m}Tc-DARPinG3 over the entire follow-up period, its rapid excretion with the blood flow, as well as a relatively low effective dose on the patient when injecting the protein at a dose of 1,000, 2,000, and 3,000 μg (0.011 ± 0.001, 0.012 ± 0.006, and 0.012 ± 0.003 mSv / MBq, respectively) (Fig.5). The best tumor-to-background ratio was observed in patients with HER2 / neu overexpression 2 and 4 hours after the injection of the protein at a dose of 1,000 and 2,000 μg; and 2, 4, and 6 hours after the injection of the protein at a dose of 3,000 μg ($p < 0.05$, Mann – Whitney test). At the same time, the most effective dose of ^{99m}Tc-DARPinG3, allowing to visualize liver metastasis, was 3,000 μg [27].

One of the patients with HER2-negative breast cancer (HER2 /neu 1+ according to IHC of the biopsy material) included in the study showed a high tumor-to-background ratio of 12.5 (4 hours after the administration of the protein at a dose of 2,000 μg). FISH of the biopsy material revealed amplification of the *ERBB2* gene in 35% of tumor cells, and IHC data determined overexpression of HER2 / neu. As a result, the patient's tumor status was changed to "positive", and targeted therapy was added to the planned systemic treatment (Fig. 6).

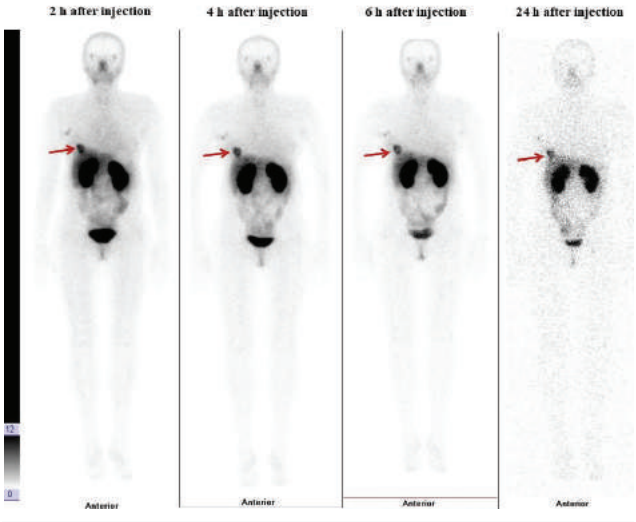

Fig. 5. Anterior projection of planar scintigraphy of the HER2-positive breast cancer patient 2, 4, 6, and 24 hours after the injection of ^{99m}Tc-DARPinG3 at a dose of 3,000 mμ (arrows indicate tumor in the right breast)

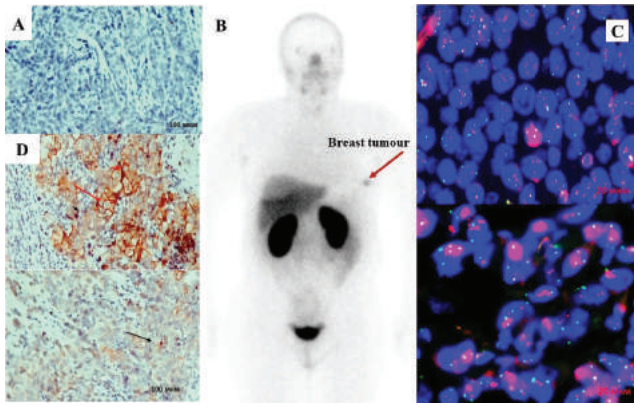

Fig. 6. Results of IHC, FISH, and radionuclide study with ^{99m}Tc-DARPinG3 injected at a dose of 2,000 μg in the breast cancer patient: *A* – IHC of the biopsy material showing negative HER2 / neu expression; *B* – radionuclide study with ^{99m}Tc-DARPinG3 (left breast tumor is indicated by the arrow); *C* – FISH results with *ERBB2* amplification in 35 % of tumor cells (operative material); *D* – IHC of the biopsy material with HER2 / neu overexpression

A similar example was observed in the patient with a HER2-negative breast tumor according to IHC, who also had a high tumor-to-background ratio after the ^{99m}Tc-DARPinG3 injection (the ratio of 14.4 4 hours after the injection of 2,000 μg of the protein). According to the results of FISH, amplification of the *ERBB2* gene was revealed, and the tumor status was changed to "positive" (Fig. 7) [28].

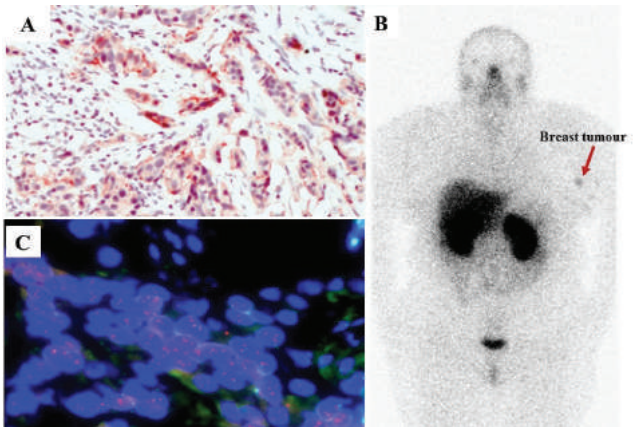

Fig. 7. Results of IHC, FISH, and radionuclide study with ^{99m}Tc-DARPinG3 injected at a dose of 2,000 μg in the breast cancer patient: *A* – IHC of the biopsy material showing negative HER2 / neu expression; *B* – radionuclide study with ^{99m}Tc-DARPinG3 (left breast tumor is indicated by the arrow); C– FISH results with *ERBB2* amplification in the biopsy material

DISCUSSION

HER2-positive breast cancer belongs to cancer subtypes with the most unfavorable disease prognosis, requiring highly specific targeted treatment. Unfortunately, the currently used IHC and FISH are not optimal and cannot solve all the problems. One of the options for optimizing the diagnostic algorithm for HER2-positive breast cancer detection is radionuclide imaging methods using alternative scaffold proteins labeled with various radionuclides.

CONCLUSION

The results of clinical studies on radiopharmaceuticals based on labeled affibody molecules, ADAPTs, and DARPins for SPECT and PET demonstrated in this review allow to consider various aspects of their application in clinical practice which are not available in conventional diagnostic methods. In particular, the possibility of simultaneous assessment of tumor spread and detection of molecular characteristics for identified tumor sites is the most relevant. The data obtained during the performed clinical studies undoubtedly indicate the importance of this research method and the need for its further study.

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