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Possibilities of predicting the HER2 / neu status in a primary tumor in breast cancer patients using ^{99m}Tc-DARPinG3

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ABSTRACT

Aim. To determine informative prognostic criteria for assessing the HER2 / neu status in primary breast cancer using ^{99m}Tc-DARPinG3.

Materials and methods. The study included 10 patients with breast cancer ($T_{1-4}N_{0-2}M_0$) before systemic therapy, who underwent a radionuclide study using ^{99m}Tc-DARPinG3 at a dose of 3,000 μg. Five patients were characterized by HER2 / neu overexpression in primary breast cancer, whereas 5 patients were HER2-negative. For all patients, morphological and immunohistochemical studies and fluorescence in situ hybridization (FISH) of the primary tumor nodule were carried out. Single-photon emission computed tomography (SPECT) of the chest was performed for all patients 4 hours after the injection of ^{99m}Tc-DARPinG3.

Results. The total activity of ^{99m}Tc-DARPinG3 was 522.4 ± 341.8 MBq. The comparative analysis showed that higher uptake of the labeled protein in HER2-positive breast cancer was significant ($p = 0.0159$, Mann – Whitney U test). The analysis of the ratios showed significant differences in the tumor-to-background ratios in patients with HER2-positive breast cancer ($p < 0.0159$, Mann – Whitney U test). Based on the logistic regression analysis, a mathematical model was developed to predict the status of HER2 / neu in primary breast cancer patients (specificity and sensitivity 100%; $p = 0.0004$) using ^{99m}Tc-DARPinG3 at a dose of 3,000 mcg 4 hours after the injection of the radiopharmaceutical.

Conclusion. The results of the study allow to consider the tumor-to-background ratio 4 hours after the injection of ^{99m}Tc-DARPinG3 as an additional prognostic parameter for determining the HER2 / neu status in primary breast cancer.

Keywords: breast cancer, radionuclide diagnosis, alternative scaffolds, DARPinG3, HER2 / neu

Conflict of interest. The authors declare the absence of obvious or potential conflict of interest related to the publication of this article.

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the Bioethics Committee at Cancer Research Institute, Tomsk NRMC.

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Возможности прогнозирования статуса рецептора HER2/neu в первичной опухоли у больных раком молочной железы с применением таргетного радионуклидного препарата «^{99m}Tc-DARPinG3»

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РЕЗЮМЕ

Цель. Определить информативные прогностические критерии для оценки статуса HER2/neu в первичной опухоли у больных раком молочной железы (РМЖ) с применением радиофармпрепарата «^{99m}Tc-DARPinG3».

Материалы и методы. В работу включены 10 больных РМЖ ($T_{1–4}N_{0–2}M_0$), которым до начала системного лечения выполнялось радионуклидное исследование с применением препарата «^{99m}Tc-DARPinG3» в дозировке основного вещества 3 000 мкг: у пяти пациентов была выявлена гиперэкспрессия HER2/neu в первичной опухоли молочной железы, у пяти – нет. Во всех случаях проводились морфологическое и иммуногистохимическое исследования и FISH-анализ ткани основного опухолевого узла. Через 4 ч после введения препарата всем больным выполнялась однофотонная компьютерная томография органов грудной клетки.

Результаты. Суммарная активность препарата «^{99m}Tc-DARPinG3» составила 522,4 ± 341,8 МБк. При сравнительном анализе статистически значимым являлось более высокое накопление меченного протеина в HER2-позитивных опухолях молочной железы (p = 0,0159, U-критерий Манна – Уитни). Анализ соотношений продемонстрировал значимые различия показателя опухоль/фон у больных в подгруппе с HER2-позитивными опухолями молочной железы ($p < 0,0159$, U-критерий Манна – Уитни). На основании проведенного исследования с применением метода логистической регрессии разработана математическая модель для прогнозирования статуса HER2/neu в первичной опухоли у больных РМЖ (специфичность и чувствительность 100%; $p = 0,0004$) при использовании препарата «^{99m}Tc-DARPinG3» в дозировке 3 000 мкг через 4 ч после введения.

Заключение. Результаты данного исследования позволяют рассматривать показатель опухоль/фон через 4 ч после введения препарата «^{99m}Tc-DARPinG3» в качестве дополнительного перспективного параметра для определения статуса HER2/neu в первичной опухоли у больных РМЖ.

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

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено биоэтическим комитетом НИИ онкологии Томского НИМЦ.

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INTRODUCTION

HER2-positive breast cancer (BC) is diagnosed in more than 20% of BC cases. This cancer subtype is characterized by an unfavourable prognosis and a high risk of distant metastasis. High expression of HER2 / neu is a predictor of tumor sensitivity to specific (targeted) therapy, which requires strict selection of BC patients [1, 2].

Immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH), currently used in clinical practice, do not have optimal characteristics for determining the HER2 / neu status. In particular, they do not allow for a simultaneous study of the state of a primary tumor and regional and distant metastasis, as well as for determination of molecular characteristics of detected tumor growth areas. This fact is of particular importance in terms of heterogeneity of HER2 / neu expression in primary tumors and metastatic sites, which can occur in 6–48% of cases. Harvesting material for a morphological examination in this case is not always technically possible or may result in serious complications [3, 4].

One of the modern directions in determining the HER2 / neu status is targeted radionuclide diagnosis using alternative scaffolds [5–7]. DARPinG3 molecules, which are ankyrin repeat proteins, belong to scaffolds [8, 9]. Results of phase I clinical trials of ^{99m}Tc-DARPinG3 in BC patients revealed the absence of complaints and toxic effects on the patient's body throughout the entire follow-up and higher uptake of the compound in HER2-positive BC. Besides, the optimal dose of the protein (3,000 μg) and the optimal time interval for the study after administration of the radiopharmaceutical (4 hours) were determined [10].

To continue the study in the subgroup of patients who received DARPinG3 at a dose of 3,000 μg, we conducted an additional analysis to identify prognostic criteria and the cut-off value for the tumor-to-background ratio for ^{99m}Tc-DARPinG3 to assess the HER2 / neu status in primary BC.

The aim of the study was to determine informative prognostic criteria for assessing the HER2 / neu status in primary BC patients using 99mTc-DARPinG3.

MATERIALS AND METHODS

The clinical trial was registered on ClinicalTrials.gov (Identifier: NCT04277338) and approved by the Bioethics Committee at Cancer Research Institute of Tomsk NRMC (Protocol No. of). The study included 10 BC patients (T1-3N0-1M0) who underwent a radionuclide study using ^{99m}Tc-DARPinG3 at a dose of 3,000 μg 4 hours after the injection of the radiopharmaceutical: 5 patients had HER2 overexpression, while 5 patients were HER2-negative.

All patients underwent a standard morphological examination and IHC of breast tumors; verification of axillary lymph node metastasis was carried out by cytology. IHC was performed according to a standard procedure; Dako oncoprotein c-erbB-2 antibodies were used. The expression of HER2 / neu was assessed according to the American Society of Clinical Oncology (ASCO) guidelines adopted in 2018 [1].

^{99m}Tc-DARPinG3 was prepared in sterile conditions at the Department of Radionuclide Diagnosis of Cancer Research Institute, Tomsk NRMC using the "CRS Isolink" kit (Center for Radiopharmaceutical Science, Paul Scherrer Institute, Villigen, Switzerland). Purification of the radiopharmaceutical was performed using NAP-5 columns (GE Healthcare, Sweden). After the purification, ^{99m}Tc-DARPinG3 was diluted to 10 ml with a sterile 0.9% sodium chloride solution, filtered, and slowly injected to the patient [9].

Single-photon emission computed tomography (SPECT) of the chest was conducted in the supine position 4 hours after the injection. SPECT acquisition included 32 projections. The data were evaluated using the E. Soft software package (Siemens, Germany) with determination of the radiopharmaceutical uptake in the primary breast tumor, the same area in the opposite breast, and the area projected at the latissimus dorsi and liver by outlining the region of interest (ROI) on axial slices with the best visualization (v = 3.53 cm^3). Tumor-to-background ratio (TBR), tumor-to-latissimus dorsi ratio, and tumor-to-liver ratio were calculated.

Statistical processing of the results was carried out using the STATISTICA 10.0 software package and Prism 9 (GraphPad, USA). The normality of distribution of variables was checked using the Shapiro – Wilk test. Taking into account non-normal distribution of the studied quantitative variables, the nonparametric Mann – Whitney test was used to assess the significance of differences for independent samples. The results were presented as the median and the interquartile range *Me* [Q_1–Q_3]. The prognostic value of the studied parameters was assessed using the ROC analysis. The logistic regression analysis was used to assess the risk. The differences were considered statistically significant at $p < 0.05$.

RESULTS

The activity of ^{99m}Tc-DARPinG3 before administration to the patient was 522.4 ± 341.8 MBq. The comparative analysis of ^{99m}Tc-DARPinG3 uptake revealed that higher uptake of the radiopharmaceutical in primary breast tumors with HER2 / neu overexpression ($p = 0.0159$, Mann – Whitney *U* test) was significant (Table 1, Fig. 1).

Table 1

Comparative analysis of ^{99m}Tc-DARPinG3 uptake in breast cancer patients 4 hours after the injection, *Me* [Q_1–Q_3]

^{99m}Tc-DARPinG3 uptake	HER2-negative breast tumors (total number of impulses)	HER2- positive breast tumors (total number of impulses)
Tumor	835.0 (654.5–2,534.0)	8,184.0 (5,174.0–13,453.0)
	$p = 0.0159$	
Background	450.0 (81.0–1,206.0)	413.5 (391.5–566.0)
	$p = 0.9048$	
Latissimus dorsi muscle	183.0 (58.0–790.5)	390.5 (298.8–588.0)
	$p = 0.7302$	
Liver	1,060.0 (690.5–6,421.0)	4,481.0 (2,300.0–5,126.0)
	$p = 0.2857$	

Analysis of the ^{99m}Tc-DARPinG3 uptake ratio demonstrated significant differences in the tumor-to-background ratio in patients with HER2-positive BC ($p < 0.0159$, Mann – Whitney *U* test) (Table 2, Fig. 2).

Table 2

Tumor-to-background ratio, tumor-to-latissimus dorsi ratio, and tumor-to-liver ratio in breast cancer patients 4 hours after the ^{99m}Tc-DARPinG3 injection, *Me* [Q_1–Q_3]

Parameter	HER2-negative breast tumors	HER2- positive breast tumors
Tumor-to-background ratio	2.4 (1.8–8.0)	15.3 (12.6–32.0)
	$p = 0.0159$	
Tumor-to latissimus dorsi ratio	4.5 (3.2–12.8)	22.5 (9.4–45.1)
	$p = 0.0635$	
Tumor-to-liver ratio	0.8 (0.4–1.1)	2.3 (1.0–5.6)
	$p = 0.0635$	

Additionally, to determine the prognostic value of the tumor-to-background ratio in assessing the HER2 / neu status in the primary tumor, we constructed ROC curves following the ROC analysis. The area under the curve (AUC) was 1.000 (95% confidence interval (CI) 1.000–1.000), $p < 0.0143$. The cut-off value was 10.39, sensitivity was 100.0%, and specificity was 100.0% (Fig. 3).

The logistic regression analysis revealed that the tumor-to-background ratio of more than 10.39 4 hours after the injection of ^{99m}Tc-DARPinG3 at a dose of 3,000 μg was a prognostic factor for the positive HER2 / neu status in primary BC patients (Chi-square = 12.36, $p = 0.0004$). The sensitivity and specificity of the model were 100%.

CONCLUSION

Determining the HER2 / neu status is an essential component for the prescription of optimal systemic therapy for BC patients. Unfortunately, despite a big number of currently used diagnostic techniques, they cannot simultaneously analyze the spread of the tumor in the patient's body and assess the molecular characteristics of the detected metastatic sites. Rapid development of targeted radionuclide imaging methods and addressing the potential of this research method at international scientific and clinical sites have significantly expanded the understanding of the role of this technique in the diagnosis of patients with BC and confirmed its potential.

In particular, the first clinically tested scaffold (a novel group of synthetic proteins with optimal properties for delivering a radionuclide to a tumor cell) was the affibody molecule. Radiolabeled ^{111}In-ABY-025 and ^{68}Ga-ABY-025 demonstrated its

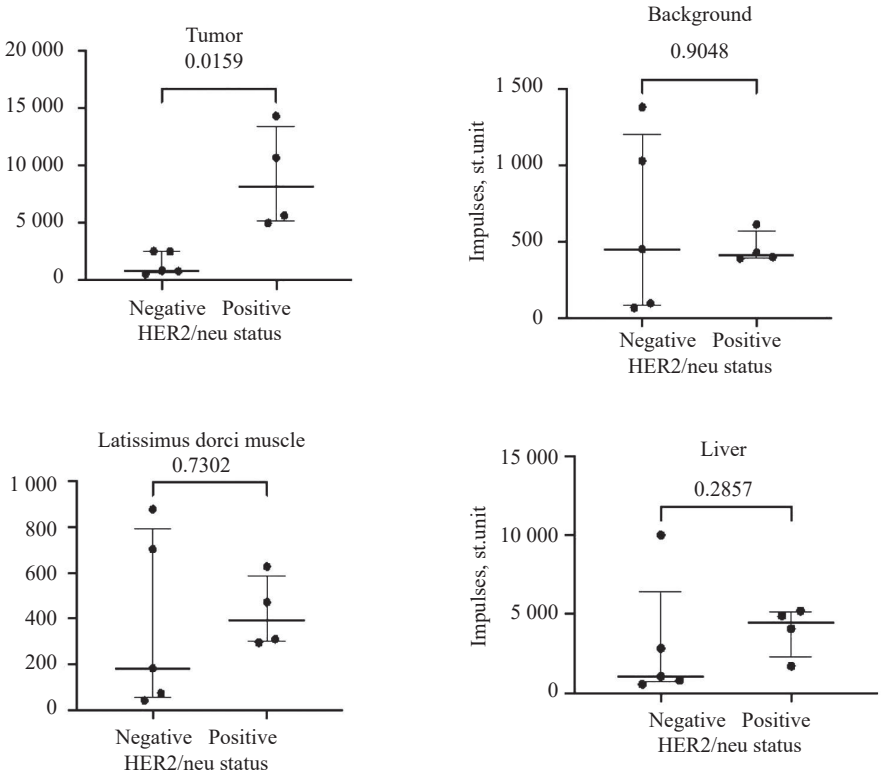

Fig. 1. Uptake of ^{99m}Tc-DARPinG3 in breast cancer patients 4 hours after the injection of the radiopharmaceutical

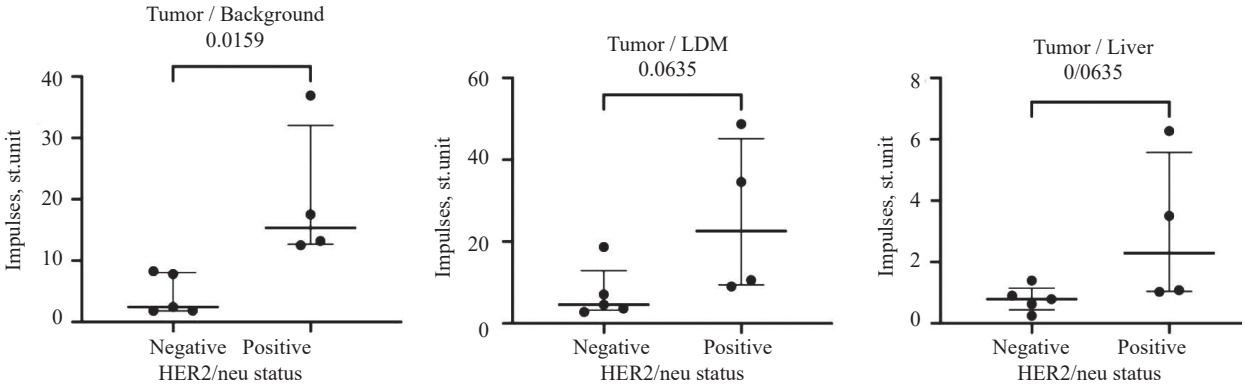

Fig. 2. Tumor-to-background ratio, tumor-to-latissimus dorsi ratio, and tumor-to-liver ratio in breast cancer patients 4 hours after the ^{99m}Tc-DARPinG3 injection

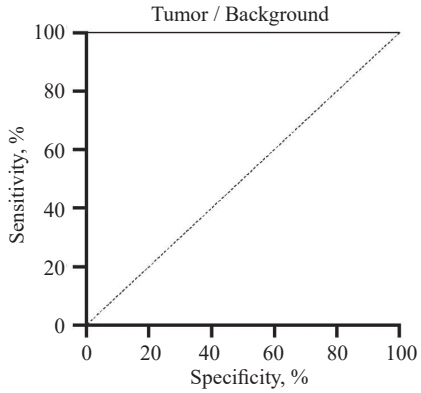

Fig. 3. ROC curve for the tumor-to-background ratio for assessing the HER2 / neu status in the primary tumor in breast cancer patients 4 hours after the ^{99m}Tc-DARPinG3 injection

effectiveness in SPECT / CT and PET for diagnosing metastatic BC with HER2/neu overexpression [11, 12]. A phase I clinical radionuclide study on alternative scaffolds in the diagnosis of HER2-positive BC was performed at Tomsk Polytechnic University and Tomsk NRMC using the synthetic ADAPT6 molecule labeled with technetium-99m (^{99m}Tc-ADAPT6) [13, 14]. The results demonstrated good tolerability of ^{99m}Tc-ADAPT6 and its high diagnostic efficiency in determining the HER2 / neu status in BC [15].

The present work is a continuation of the study using another alternative scaffold – the DARPinG3 molecule labeled with technetium-99m (^{99m}Tc-DARPinG3) with tropism to HER2 / neu. The results obtained during the recently completed phase I clinical trials also demonstrated good tolerability of ^{99m}Tc-DARPinG and its potential use for visualization of tumor sites in the breast, axillary lymph nodes, and visceral organs. This fragment of the study allowed to identify the most informative parameters for determining the HER2 / neu status in BC.

In particular, the tumor-to-background ratio in the mathematical model allows to predict the status of HER2 / neu in primary BC patients with high sensitivity and specificity (100 and 100%, respectively, $p = 0.0004$). Besides, it allows to consider the tumor-to-background ratio 4 hours after the injection of the radiopharmaceutical as an additional prognostic criterion for determining the HER2 / neu status in this group of patients.

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Authors contribution

Bragina O.D., Chernov V.I., Deyev S.M., Tolmachev V.M. – conception and design or analysis and interpretation of the data; justification of the manuscript, critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication. Tashireva L.A. – statistical processing of the material.

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