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A direct comparison of the diagnostic efficacy of alternative scaffold-based radiopharmaceuticals [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 in patients with HER2-positive breast cancer

Bragina O.D.[1, 2], Deyev S.M.[2, 3], Garbukov E.Yu.[1], Goldberg V.E.[1], Chernov V.I.[1, 2], Tolmachev V.M.[2, 4]

[1] *Cancer Research Institute, Tomsk National Research Medical Center (NRMC) of the Russian Academy of Sciences 5, Kooperativny Str., Tomsk, 634009, Russian Federation*

[2] *National Research Tomsk Polytechnic University 30, Lenina Av., Tomsk, 634050, Russian Federation*

[3] *Shemyakin – Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences 16/10, Miklukho-Maklaya Str., Moscow, 117997, Russian Federation*

[4] *Uppsala University 7, Dag Hammarskjöldsväg, Segerstedthuset, Uppsala, Sweden*

ABSTRACT

Aim. To perform a direct comparison of the diagnostic efficacy of [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-$(HE)_3$-G3 in HER2-positive breast cancer patients before the systemic treatment.

Materials and methods. The study included 11 patients with HER2-positive breast cancer (T1–4N0–2M0–1) before the initiation of systemic treatment. All patients underwent a radionuclide examination with [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-$(HE)_3$-G3 with the interval of 3–4 days. Single-photon emission computed tomography (SPECT) /computed tomography (CT) was performed 2 and 4 hours after [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-$(HE)_3$-G3 administration, respectively.

Results. The analysis of [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-$(HE)_3$-G3 distribution showed their high uptake in the kidneys and liver. Breast tumors were visualized in all cases. The average tumor uptake of [99mTc]Tc-ADAPT6 was 4.7 ± 2.1, which was significantly higher than in the [99mTc]Tc-(HE)3-G3 injection (3.5 ± 1.7) ($p < 0.005$, paired *t*-test). The tumor-to-background ratio (15.2 ± 7.4 and 19.6 ± 12.4, respectively) had no statistical differences in both cases ($p > 0.05$, paired *t*-test). Liver metastases were visualized in patients 1 and 5 and corresponded to the projection of metastases according to contrast-enhanced abdominal CT. The accumulation of [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 in the projection of metastases in both cases was significantly higher compared to the primary tumor (1.3 and 1.7 times higher in patient 1; 2.2 and 3.5 times higher in patient 5, respectively).

Conclusion. Both [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 demonstrated the diagnostic efficacy in visualizing a primary HER2-positive tumor in breast cancer patients. However, [99mTc]Tc-ADEPT6 had higher accumulation values, which makes it a more promising diagnostic agent.

Keywords: breast cancer, SPECT / CT, ADAPT6, 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. The study was approved by the Bioethics Committee at Cancer Research Institute of Tomsk NRMC (Protocol No. 6 of 04.03.2022). All patients signed an informed consent to participate in the study.

✉ *Bragina Olga D.,* bragina_od@mail.ru

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Прямое сравнение диагностической эффективности радиофармацевтических препаратов на основе альтернативных каркасных протеинов [^{99m}Tc]Tc-ADAPT6 и [^{99m}Tc]Tc-(HE)$_3$-G3 у больных HER2-позитивным раком молочной железы

Брагина О.Д.[1, 2], Деев С.М.[2, 3], Гарбуков Е.Ю.[1], Гольдберг В.Е.[1], Чернов В.И.[1, 2], Толмачев В.М.[2, 4]

[1] Научно-исследовательский институт (НИИ) онкологии, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук
Россия, 634009, г. Томск, пер. Кооперативный, 5

[2] Научно-исследовательский центр (НИЦ) «Онкотераностика», Национальный исследовательский Томский политехнический университет (НИ ТПУ)
Россия, 634050, г. Томск, пр. Ленина, 30

[3] Институт биоорганической химии (ИБХ) им. академиков М.М. Шемякина и Ю.А. Овчинникова Российской академии наук (РАН)
Россия, 117997, Москва, ГСП-7, ул. Миклухо-Маклая, 16/10

[4] Уппсальский университет
Швеция, Уппсала, Segerstedthuset, DagHammarskjöldsväg, 7

РЕЗЮМЕ

Цель: проведение прямого сравнения диагностической эффективности препаратов [^{99m}Tc]Tc-ADAPT6 и [^{99m}Tc]Tc-(HE)$_3$-G3 у больных HER2-позитивным раком молочной железы до начала системного лечения.

Материалы и методы. В исследование включены 11 больных HER2-позитивным раком молочной железы (T1–4N0–2M0–1) до начала системного лечения. Всем больным в интервале 3–4 дней выполнялись радионуклидные исследования с использованием препаратов [^{99m}Tc]Tc-ADAPT6 и [^{99m}Tc]Tc-(HE)$_3$-G3. Однофотонная эмиссионная компьютерная томография/компьютерная томография (КТ) проводилась через 2 ч для препарата [^{99m}Tc]Tc-ADAPT6 и через 4 ч для [^{99m}Tc]Tc-(HE)$_3$-G3.

Результаты. При анализе распределения обоих препаратов больший захват нормальными тканями был отмечен в почках и печени. Опухоли молочных желез визуализировались всех случаях. Средний захват опухолью при использовании препарата [^{99m}Tc]Tc-ADAPT6 составил 4,7 ± 2,1, что было значительно выше, чем при [^{99m}Tc]Tc-(HE)$_3$-G3 (3,5 ± 1,7) ($p < 0,005$, парный *t*-тест). Соотношение опухоль/фон (15,2 ± 7,4 и 19,6 ± 12,4 соответственно) в обоих случаях не имело статистических различий ($p > 0,05$, парный *t*-тест). Метастазы в печень визуализированы у пациенток № 1 и 5, что соответствовало проекции метастазов по данным КТ органов брюшной полости, выполненной с контрастированием. Аккумуляция [^{99m}Tc]Tc-ADAPT6 и [^{99m}Tc]Tc-(HE)$_3$-G3 в проекции метастазов у обеих больных была значительно выше по сравнению с первичной опухолью (в 1,3 и 1,7 раза у пациентки № 1; в 2,2 и 3,5 раза у пациентки № 5 соответственно).

Заключение. Препараты[^{99m}Tc]Tc-ADAPT6 и [^{99m}Tc]Tc-(HE)$_3$-G3 продемонстрировали свою эффективность в отношении визуализации первичныхHER2-позитивных опухолей молочных желез. При этом аккумуляция [^{99m}Tc]Tc-ADAPT6 имела более высокие показатели накопления, что делает это соединение более перспективным диагностическим агентом.

Ключевые слова: рак молочной железы, однофотонная эмиссионная компьютерная томография, компьютерная томография ОФЭКТ/КТ, DARPinG3, ADAPT6, HER2/neu

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

Источник финансирования. Работа выполнена в рамках гранта Министерства науки и высшего образования (соглашение № 075-15-2022-1103) по теме «Разработка таргетных молекул на основе каркасных белков для диагностики и терапии злокачественных новообразований: тераностический подход».

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено биоэтическим комитетом НИИ онкологии Томского НИМЦ (протокол № 6 от 04.03.2022).

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INTRODUCTION

Overexpression of the human epidermal growth factor receptor 2 (HER2 / neu) occurs in 20–25% of breast cancer patients and indicates a need for targeted therapy, which significantly improves overall and relapse-free survival rates [1, 2]. Despite the availability and prevalence of commonly used immunohistochemistry (IHC) and fluorescent *in situ* hybridization (FISH), there are still problems that significantly limit the assessment of the HER2 / neu status in patients with breast cancer. In particular, the impossibility of performing a simultaneous assessment of tumor spread and a molecular analysis of identified sites remains obvious and is due to difficulties or the impossibility of performing a core biopsy [3, 4].

One of the solutions to these problems is targeted radionuclide diagnosis [5], which is currently being actively studied and uses alternative scaffold proteins with optimal characteristics as a "targeting" module for delivering radioisotopes to tumor cells [6–8]. Thus, the results of phase I clinical trials of [^{99m}Tc]Tc-ADAPT6 (ClinicalTrials.gov Identifier: NCT03991260) and [^{99m}Tc]Tc-(HE)3-G3 (ClinicalTrials.gov ID: NCT04277338) in breast cancer patients demonstrated good tolerability and absence of adverse reactions both at the time of injection and for the entire follow-up periods. Also, in both cases, differences in the accumulation of radiopharmaceuticals in patients with HER2-positive and HER2-negative breast tumors were shown ($p < 0.05$, Mann – Whitney test), and optimal dosages and study periods after the injection of labeled proteins were revealed: 500 μ and 2 hours for [^{99m}Tc]Tc-ADAPT6 and 3,000 μ and 4 hours for [^{99m}Tc]Tc-(HE)3-G3 [9, 10].

The results of the preclinical comparative analysis of [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-$(HE)_3$-G3 showed high uptake of both radiopharmaceuticals by the HER2-positive SKOV-3 cell line compared to the HER2-negative MDA-MB-468 cell line [11]. The aim of the study was to perform a direct comparison of the diagnostic efficacy of [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 in HER2-positive breast cancer patients before the initiation of systemic treatment.

MATERIALS AND METHODS

The study was approved by the Bioethics Committee at Cancer Research Institute of Tomsk NRMC (Protocol No. 6 of 04.03.2022) and registered before recruitment of patients (ClinicalTrials.gov Identifier: NCT05376644). The study included 11 breast cancer patients (T1–4N0–3M0–1) with overexpression of HER2 / neu in the primary tumor before the initiation of chemo / targeted therapy (Table 1). All patients signed an informed consent to participate in the study.

In all patients, the HER2/neu expression status was established according to the immunohistochemistry (IHC) of the biopsy material obtained from the primary tumor according to the American Society of Clinical Oncology (ASCO) / College of American Pathologists (CAP) guidelines (2018). Tumors were classified as HER2-positive if an IHC score was 3+. The study was carried out according to the standard methodology. Lesions of the lymph nodes in all patients were confirmed by the results of the histologic examination.

Before treatment, all patients underwent mammography (Giotto Image), osteoscintigraphy with ^{99m}Tc pyrophosphate (Siemens Symbia Intevo Bold), computed tomography (CT) of the chest (Siemens Somatom Emotions 16 ECO), and ultrasound

Table 1

Characteristics of breast cancer patients before the administration of [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3

Patient number	Age, years	HER2 in primary breast tumor (IHC)	ER, PR, Ki67 expression in primary breast tumor	Clinical stage before tumor visualization
1	61	3+ (IHC)	ER +; PR +; Ki67 40%	IV (T4N3M1)
2	48	3+ (IHC)	ER +; PR +; Ki67 18%	IIB (T2N1M0)
3	26	3+ (IHC)	ER +; PR +; Ki67 45%	IIB (T2N1M0)
4	49	3+ (IHC)	ER +; PR +; Ki67 20%	I (T1N0M0)
5	41	3+ (IHC)	ER +; PR +; Ki67 45%	IV (T1N1M1)
6	65	3+ (IHC)	ER +; PR +; Ki67 60%	IIB (T2N1M0)
7	59	3+ (IHC)	ER –; PR –; Ki67 55%	IIA (T1N1M0)
8	55	3+ (IHC)	ER –; PR –; Ki67 18%	IIB (T2N1M0)
9	38	3+ (IHC)	ER +; PR + Ki67 25%	IIB (T2N1M0)
10	65	3+ (IHC)	ER –; PR –; Ki67 18%	I (T1N0M0)
11	63	3+ (IHC)	ER +; PR + Ki67 10%	I (T1N0M0)

Note: ER – estrogen receptor, PR – progesterone receptor, Ki67 – marker of cell proliferation.

examination of the mammary glands, regional lymph nodes, and liver (GE LOGIQ E9). Patients 1 and 5 additionally underwent contrast-enhanced abdominal CT. The size of the primary tumor and metastatic lymph nodes was determined according to the ultrasound findings.

Radionuclide studies. Radiopharmaceuticals [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 were prepared using the aseptic technique at the Department of Radionuclide Therapy and Diagnosis of Cancer Research Institute of Tomsk NRMC immediately before the study. Labeling was carried out according to the methods described earlier [12, 13]. The radiochemical yield was 97 ± 1% for [^{99m}Tc]Tc-ADAPT6 and 97 ± 2% for [^{99m}Tc]Tc-(HE)3-G3. The protein dose was 500 μ for [^{99m}Tc]Tc-ADAPT6 and 3,000 μ for [^{99m}Tc]Tc-(HE)3-G3. Consecutive intravenous injections of ready-made compounds [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 were performed in all patients with an interval of 3–4 days.

All patients underwent single-photon emission computed tomography (SPECT) / computed tomography (CT) of the chest using a Siemens Symbia Intevo Bold scanner equipped with a high-resolution low-energy collimator. SPECT / CT scans were performed 2 hours after the injection of [^{99m}Tc]Tc-ADAPT6 and 4 hours after the injection of [^{99m}Tc]Tc-(HE)3-G3. The images were reconstructed using the xSPECT Reconstruction Protocol (Siemens) based on the Ordered Subset Conjugate Gradient (OSCG) method. A 3D Gaussian filter with a 10-mm FWHM (soft tissue) was used. The images were processed using the Syngo.via software package (Siemens).

Maximum standardized uptake values (SUVmax) normalized for the body surface were calculated for the primary tumors and the liver. SUVmax were also determined in the contralateral symmetrical areas of the breast to calculate the tumor-to-background ratio, as well as in areas of the liver free from metastases to calculate the liver / metastasis ratio. To assess the uptake of the radiopharmaceuticals in healthy organs, which may contribute to the occurrence of background in typical metastatic sites, SUVmax were also measured in unaffected lymph nodes, lungs (S3 of the right lung in the projection of the aortic arch), and bones (five thoracic vertebrae).

The data were presented as $M \pm m$, where M is the mean and m is a standard deviation. A paired t-test was used to compare the uptake values and derived parameters. The differences were statistically significant at $p < 0.05$.

RESULTS

According to the results of IHC, all 11 patients were diagnosed with HER2-positive breast cancer (3+). Liver metastases were detected in two patients (patients 1 and 5). When analyzing the distribution of both radiopharmaceuticals, their greater uptake by healthy tissues was noted in the kidneys and the liver, while a greater uptake in the liver was detected using [^{99m}Tc]Tc-(HE)3-G3 (the distribution is shown in Fig. 1). The uptake of [^{99m}Tc]Tc-ADAPT6 (SUVmax 0.3 ± 0.1) in the contralateral breast area was significantly higher than that of [^{99m}Tc]Tc-(HE)3-G3 (SUVmax 0.2 ± 0.1) ($p < 0.01$, paired t-test). At the same time, the uptake of both radiopharmaceuticals

did not differ in the unaffected lymph nodes ($p > 0.05$, paired *t*-test). The accumulation of the radiopharmaceuticals in unaltered lung tissue was 0.4 ± 0.2 for [^{99m}Tc]Tc-ADAPT6 and 0.4 ± 0.1 for [^{99m}Tc]Tc-(HE)3-G3; in bones – 0.6 ± 0.2 and 0.9 ± 0.5, which had no significant difference according to the statistical analysis ($p > 0.05$, paired *t*-test) (Fig. 2). Accumulation of the radiopharmaceutical was also visualized in the salivary and thyroid glands.

Breast tumors were visualized using both [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3. The average tumor uptake of [^{99m}Tc]Tc-ADAPT6 was 4.7 ± 2.1, which was significantly higher than for [^{99m}Tc]Tc-(HE)3-G3 (3.5 ± 1.7) ($p < 0.005$, paired *t*-test). At the same time, the tumor-to-background ratio (15.2 ± 7.4 and 19.6 ± 12.4, respectively) when using both compounds showed no significant differences ($p > 0.05$, paired *t*-test) (Fig. 3).

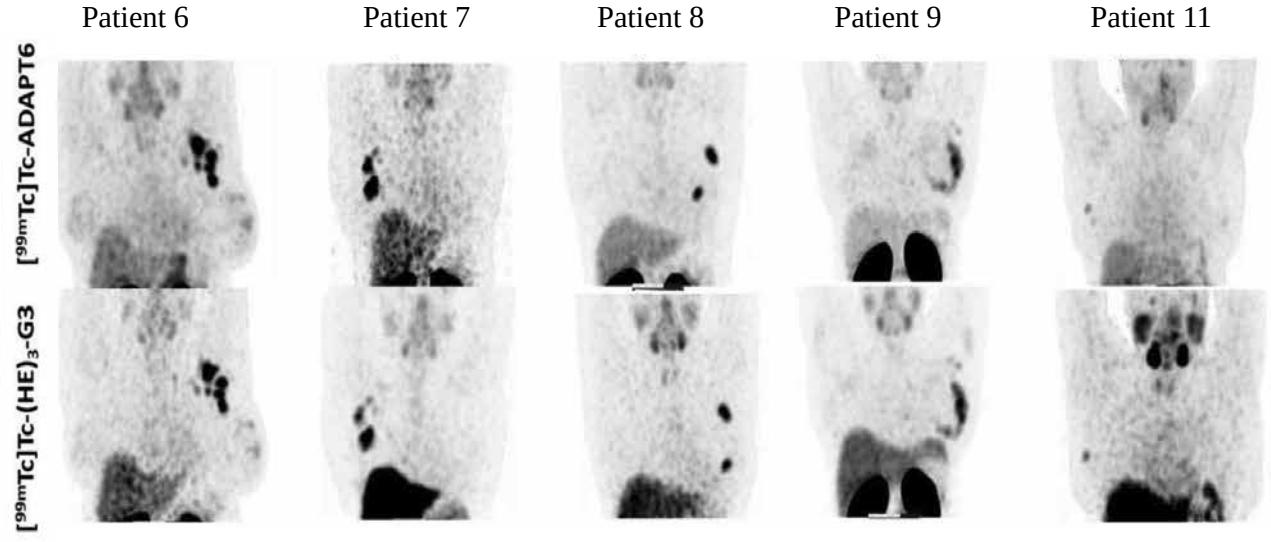

Fig. 1. Comparison of SPECT / CT using [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 in patients with HER2-positive breast cancer (SUV is 6.8 in all images)

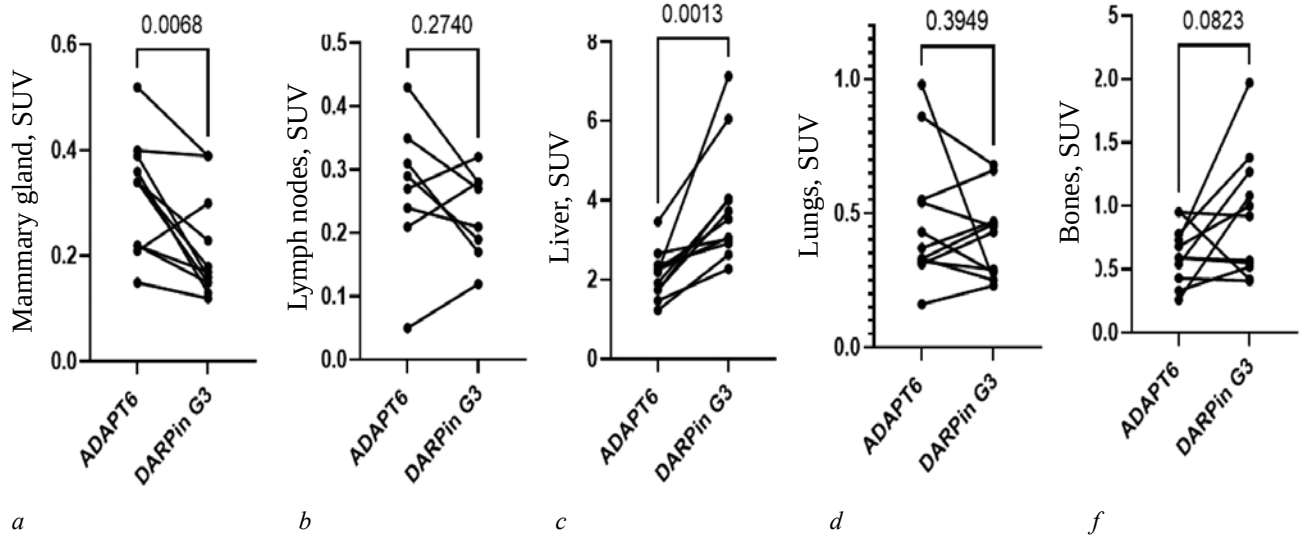

Fig. 2. Accumulation (SUV) of [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 in healthy tissues: *a* – mammary gland; *b* – unaffected lymph nodes; *c* – liver; *d* – lungs (S3 of the right lung in the projection of the aortic arch); *e* – bones (fifth thoracic vertebra)

Also, when using both [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3, metastatic lymph nodes were detected in all patients (Fig. 4).

Liver metastases were visualized in patients 1 and 5 using both [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 and corresponded to the projection of metastases according to contrast-enhanced abdominal CT (Fig. 5). In both cases, morphological verification of the foci was not performed due to refusal of the patients to undergo a biopsy. Interestingly, the accumulation of [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 in the projection of metastases in both patients was significantly higher compared to the primary tumor (1.3 and 1.7 times in patient 1; 2.2 and 3.5 times in patient 5, respectively).

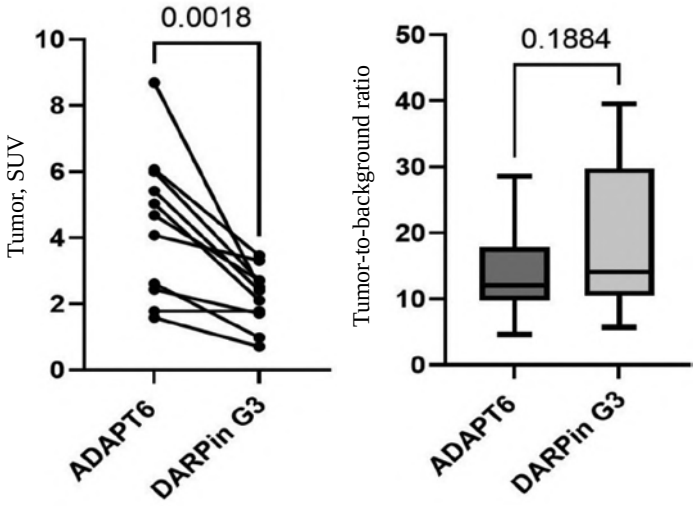

Fig. 3. Accumulation (SUV) of [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 in primary tumors and contralateral symmetrical areas of the breast in patients with HER2-positive breast cancer

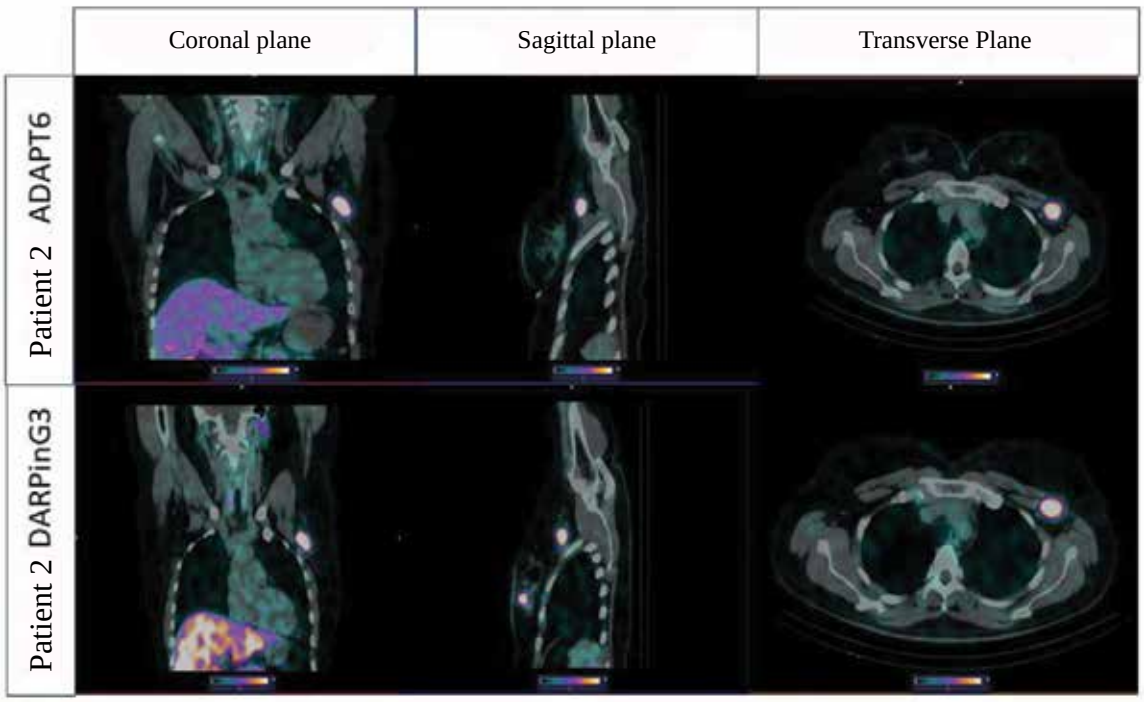

Fig. 4. Accumulation of [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 in metastatic lymph nodes in HER2-positive breast cancer patient. 2

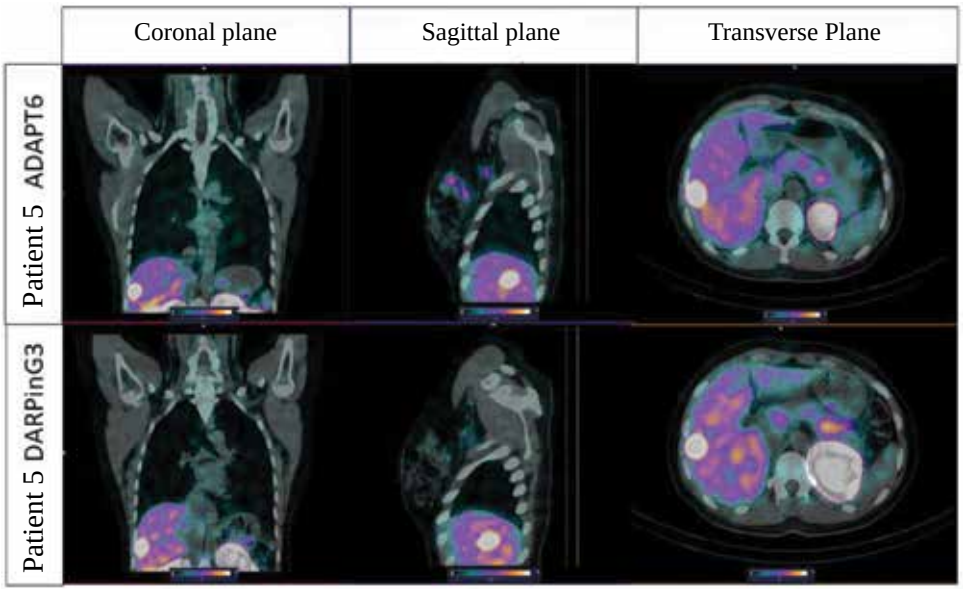

Fig. 5. Accumulation of [^{99m}Tc] Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 in primary tumors and liver metastases in patients with HER2-positive breast cancer

DISCUSSION

Performed phase I clinical trials of [^{99m}Tc] Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 clearly demonstrated the possibility of their use not only as primary tumor imaging agents, but also as markers that can effectively assess the HER2 / neu status in breast cancer patients [9, 10]. Taking into account the results of both analyses, a direct comparison of both [^{99m}Tc] Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 was naturally required by a complex of preclinical and clinical studies. Summarizing the data obtained, [^{99m}Tc]Tc-ADAPT6 showed its greatest efficacy in assessing the HER2 / neu status in breast cancer patients and, therefore, in selecting patients for targeted therapy. At the same time, [^{99m}Tc]Tc-(HE)3-G3 showed its sensitivity to a change (decrease) in the HER2 / neu expression in response to targeted therapy with trastuzumab, and, therefore, it can be used for monitoring an early response to systemic treatment [11].

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Authors' contribution

Bragina O.D., Chernov V.I., Deyev S.M., Tolmachev V.M., Garbukov E.Yu., Goldberg V.E. – conception and design, 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.

Authors' information

Bragina Olga D. – Dr. Sci. (Med.), Oncologist, Principal Researcher, Department of Nuclear therapy and Diagnosis, Cancer Research Institute, Tomsk NRMC; Senior Researcher, Oncotheranostics Research Center, National Research Tomsk Polytechnic University, Tomsk, bragina_od@mail.ru, http://orcid.org/0000-0001-5281-7758

Deyev Sergei M. – Dr. Sci. (Biology), Professor, Academician of RAS, Head of Molecular Immunology Department, Shemyakin – Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, deev_sm@tpu.ru, http://orcid.org/0000-0002-3952-0631

Garbukov Eugenii Yu. – Cand. Sci. (Med.), Senior Researcher, General Oncology Department, Cancer Research Institute, Tomsk NRMC, Tomsk, jrmaximum9@gmail.com, http://orcid.org/0000-0002-6016-7078

Goldberg Victor E. – Dr. Sci. (Med.), Professor, Head of the Chemotherapy Department, Cancer Research Institute, Tomsk NRMC, Tomsk, goldbergve@mail.ru, http://orcid.org/0000-0003-4753-5283

Chernov Vladimir I. – Dr. Sci. (Med.), Professor, Corresponding Member of RAS, Head of the Department of Nuclear Therapy and Diagnosis, Cancer Research Institute, Tomsk NRMC, Tomsk, chernov@tnimc.ru, http://orcid.org/0000-0002-5524-9546

Tolmachev Vladimir.M. – Dr. Sci. (Med.), Professor., Head of the Laboratory for Immunology, Genetics, and Pathology, Uppsala University, Uppsala, Sweden; Head of the Oncotheranostics Research Center, National Research Tomsk Polytechnic University, Tomsk, Vladimir.tolmachev@igp.uu.se, http://orcid.org/0000-0002-6122-1734

(✉) **Bragina Olga D.,** bragina_od@mail.ru

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