Journal of Nuclear Medicine, published on August 17, 2020 as doi:10.2967/jnumed.120.248799

Phase I study of ^{99m}Tc-ADAPT6, a scaffold protein-based probe for visualization of HER2 expression in breast cancer.

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Conflict of interest statement. This research was financially supported by the Ministry of Science and Higher Education of the Russian Federation (grant 075-15-2019-1925). The authors disclose no potential conflicts of interest.

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Word count: 4920

Running tittle: ^{99m}Tc-ADAPT6 imaging of HER2

ABSTRACT

Radionuclide molecular imaging of human epidermal growth factor (HER2) expression may help to stratify breast and gastroesophageal cancer patients for HER2-targeting therapies. ADAPTs (albumin-binding domain derived affinity proteins) are a new type of small (46-59 amino acids) proteins useful as probes for molecular imaging. The aim of this first-in-human study was to evaluate biodistribution, dosimetry, and safety of the HER2-specific ^{99m}Tc-ADAPT6.

METHODS: Twenty-nine patients with primary breast cancer were included. In 22 patients with HER2-positive (n=11) or HER2-negative (n=11) histopathology an intravenous injection with 385±125 MBq ^{99m}Tc-ADAPT6 was performed, randomized to an injected protein mass of either 500 μg (n=11) or 1000 μg (n=11). Planar scintigraphy followed by SPECT imaging was performed after 2, 4, 6 and 24 h. An additional cohort (n=7) was injected with 165±29 MBq (injected protein mass 250 μg) and imaging was performed after 2 h only. **RESULTS:** Injections of ^{99m}Tc-ADAPT6 at all injected mass levels were well tolerated and not associated with adverse effects. ^{99m}Tc-ADAPT6 cleared rapidly from blood and most other tissues. The normal organs with the highest accumulation were kidney, liver and lung. Effective doses were 0.009±0.002 and 0.010±0.003 mSv/MBq for injected protein masses of 500 and 1000 μg, respectively. Injection of 500 μg resulted in excellent discrimination between HER2-positive and HER2-negative tumors already 2 h after injection (tumor-to-contralateral breast ratio was 37±19 vs 5±2, $p<0.01$). The tumor-to-contralateral breast ratios for HER2-positive tumors were significantly ($p<0.05$) higher for injected mass of 500 μg than for both 250 and 1000 μg.

CONCLUSION: Injections of ^{99m}Tc-ADAPT6 are safe and associated with low absorbed and effective doses. Protein dose of 500 μg is preferable for discrimination between tumors with high and low expression of HER2. Further studies are justified to evaluate if ^{99m}Tc-ADAPT6 can be

used as an imaging probe for stratification of patients for HER2-targeting therapy in the areas where PET imaging is not readily available.

KEYWORDS: HER2; ADAPT6, ^{99m}Tc, SPECT, Phase I

INTRODUCTION

Human epidermal growth factor type 2 (HER2) is a molecular target for several therapeutics for treatment of breast and gastroesophageal cancers. Response to such therapeutics depends on the HER2 expression level, and assessment of HER2 status in tumors is required to avoid under- and overtreatments (*1,2*). The current standard of care includes collecting biopsy material followed by an assessment of HER2 status using immunohistochemistry (IHC) and in situ hybridization (ISH) analysis. Tumors with 3+ IHC score or 2+ IHC and ISH positive are considered as HER2-positive and eligible for HER2-targeting treatment. However, a major issue is HER2-expression heterogeneity, and breast cancer patients often have both HER2-positive and HER2-negative metastases *(3,4*). The invasiveness of biopsies prevents sampling of all metastases, which is associated with a risk of non-representative findings.

Radionuclide molecular imaging of HER2 expression is a non-invasive approach for patients' stratification, offering advantages of repetitive mapping of HER2 expression in multiple metastases (*5-7*). One of strategies is immunoPET, which utilizes specific recognition of HER2 by monoclonal antibodies and the superior spatial resolution, registration efficiency and quantification accuracy of positron emission tomography (PET). Therapeutic anti-HER2 antibodies trastuzumab (*3, 8-12*) and pertuzumab (*13*), labeled with the long-lived positron emitters ^{89}Zr or ^{64}Cu have been evaluated in patients. Clinical studies demonstrated a potential of radionuclide molecular imaging of HER2. For example, ^{89}Zr-trastuzumab PET imaging has resulted in altered therapeutic decisions for 40% of patients in cases when clinically important lesions could not be biopsied (*12*). However, the use of full-length antibodies is complicated by slow penetration into tumors and slow clearance. This prolongs the time between injection and

imaging, with the best results obtained 4-8 days after injection (*8,13*). Full-size antibodies also accumulate in tumors unspecifically, creating a risk of false-positive diagnostics (*14*).

A possible alternative to immunoPET is the use of much smaller targeting vectors, such as single domain antibodies or engineered scaffold proteins (*15*). Both ^{68}Ga-labeled HER2-specific nanobody (*16*) and affibody (*4*) enabled high-contrast imaging of HER2-expressing breast cancer tumors and their metastases within 1.5-4 h after injection. This shows that the use of scaffold proteins is a promising strategy for development of probes for radionuclide molecular imaging. ,

ADAPTs are affinity proteins, derived from the three-helical scaffold of the albumin-binding domain of streptococcal protein G (*17*). The small size of ADAPTs (46-59 amino acids, molecular weight of 5-7 kDa) and affinity in the low nanomolar range create preconditions for their successful use as imaging agents. A series of ADAPT was previously preclinically evaluated as HER2-imaging probes (*18*). To facilitate clearance of unbound agent from blood, an ADAPT variant, ADAPT6, that does not bind to albumin has been created (*18*). We have demonstrated that radiolabeled ADAPT6 is capable of specific high-contrast imaging of HER2-expressing human xenografts in mice a few hours after injection (*19*). Our studies suggested that labeling of hexahistidine-containing ADAPT6 using $^{99m}Tc(H_2O)_3(CO)_3$ provides an agent with affinity of 2.8 nM, which is suitable for imaging of HER2 using SPECT or planar gamma-scintigraphy (*20*).

In this first-in-human study (ClinicalTrials.gov Identifier: NCT03991260) we evaluated distribution of ^{99m}Tc-ADAPT6 in patients with primary HER2-positive and HER2-negative breast cancer.

The primary objectives of the study were:

To assess distribution of ^{99m}Tc-ADAPT6 in normal tissues and in tumors over time;

To evaluate dosimetry of ^{99m}Tc-ADAPT6;

To obtain initial information concerning safety and tolerability of ^{99m}Tc-ADAPT6 after single intravenous injection.

The secondary objective was to compare the tumor imaging data with HER2 expression data obtained by immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH) analysis of biopsy samples.

Since the data for another scaffold proteins, affibody, demonstrated that increase of injected protein mass could increase tumor-to-liver ratio and improve discrimination between tumors with high and low HER2-expression (*4*), planar scintigraphy and SPECT imaging 2, 4, 6 and 24 h after injection of 500 and 1000 μg of ^{99m}Tc-ADAPT6 were evaluated. In addition, imaging 2 h after injection of 250 μg of ^{99m}Tc-ADAPT6 was performed in a smaller cohort of patients.

MATERIALS AND METHODS

Patients

This was a prospective, open-label, non-randomized Phase I diagnostic study in patients with untreated primary breast cancer (ClinicalTrials.gov Identifier: NCT03991260). The initial protocol and further extension of patients' cohort have been approved by Scientific Council of Cancer Research Institute and Board of Medical Ethics, Tomsk National Research Medical Center of the Russian Academy of Sciences and all subjects signed a written informed

consent. Female patients (18-80 years) with a primary breast cancer were eligible. The inclusion criteria were:

Diagnosis of primary breast cancer with possible lymph node metastases;

Availability of HER2 status determined according to the guidelines of the American Society of Clinical Oncology (*1*);

Volumetrically quantifiable tumor lesions on CT, with at least one lesion > 1.0 cm in greatest diameter;

Hematological, liver and renal function test results within the normal limits;

Negative pregnancy test;

Capability to undergo the diagnostic investigations planned in the study

The exclusion criteria were

Second, non-breast malignancy;

Autoimmune disease;

Active infection or history of severe infection;

Administration of other investigational medicinal product.

Twenty-nine patients were enrolled (Table 1; Fig. 1).

As a local standard of care, biopsy sampling, mammography (Giotto Image), bone scan (Siemens E.Cam 180) using ^{99m}Tc-pyrophosphate, chest CT (Siemens Somatom Emotions 16 ECO) and ultrasound of breast, regional lymph nodes and liver (GE LOGIQ E9) imaging were performed for all patients. For patient 4, an additional MRI (Siemens Magnetom Essenza 1.5T) examination was performed.

The level of expression of HER2 in biopsy samples was determined by immunohistochemistry (IHC) using Herceptest (DAKO). In tumors with a score of 2+ or in a case of questionable results, HER2 amplification was assessed using fluorescent in situ hybridization (FISH). The tumors were classified as HER2-positive (HercepTest score 3+ or HercepTest score 2+ and FISH-positive) and HER2-negative (HercepTest score 0 or 1+, or score 2+ but FISH-negative).

Imaging Protocol

Labeling of ADAPT6 was performed in aseptic conditions according to the method described earlier (*19*). The yield was 77±9 %, and radiochemical purity was 99±1 %.

^{99m}Tc-ADAPT6 was injected as an intravenous bolus. Patients 1-11 were injected with 500 µg ADAPT6 (416±135 MBq), and patients 12-22 with 1000 µg (349±133 MBq). Patients 23-29 were injected with 250 µg (165±29 MBq) and planar whole-body and SPECT scans were performed at 2 h. Imaging was performed using (Siemens E.Cam 180) scanner equipped with a high-resolution low-energy collimator and interfaced with a computer system for scintigraphic data processing (E.Soft system). Anterior and posterior planar whole-body imaging (at a scan speed of 12 cm/min, 1024x256 pixel matrix) and SPECT scans (32 projections, 30 seconds each, 128x128 pixel matrix) were performed at 2, 4, 6 and 24 h. The SPECT data was reconstructed using iterative reconstruction with a Gaussian filter applying scatter correction.

Monitoring of vital signs and possible side effects was performed during imaging study (0-24 h after injection) and 3-7 days after injection. Blood and urine analyses were performed 5 and 14 days after injection.

Assessment of Distribution and Dosimetry

Regions of interest (ROI) were drawn over organs of interest and the whole body on the anterior and posterior whole-body images of patients injected with 500 and 1000 μg ^{99m}Tc-ADAPT6; a geometric mean at 2, 4, 6 and 24 h was calculated for each ROI. For quantification, known activity of ^{99m}Tc in a water-filled phantom in combination with Chang's correction was used. To assess kinetics in blood, a ROI was placed over the heart. Data were fitted by single exponential functions and residence time was calculated as areas under fitted curves using Prism 8 (GraphPad Software, LLC). Absorbed doses were calculated by OLINDA/EXM 1.1 using Adult Female phantom.

To calculate tumor-to-contralateral breast and tumor-to-liver ratios, a 3.5-cm^3 volume of interest (VOI) was drawn on tomograms centered on the highest tumor uptake, and counts were recorded. Thereafter, this VOI was copied to the contralateral breast and liver to obtain reference counts. Tumor-to-contralateral breast ratio for each primary tumor was calculated and matched with the biopsy-based data concerning HER2-expression in this tumor.

Statistics

Values are reported as mean ± standard deviation. The significance of differences between uptakes in organs at different time points was analyzed using 1-way ANOVA. The significance of differences between tumor-to-contralateral breast and tumor-to-liver ratio values for HER2-positive and HER2-negative tumors was analyzed using the nonparametric Mann–Whitney U-test. A two-sided P value of less than 0.05 was considered significant.

RESULTS

Safety and Tolerability

^{99m}Tc-ADAPT6 was administered in twenty-nine patients. The administration was well tolerated. No drug-related adverse reactions or changes in vital signs were observed during imaging or follow-up period. No changes in blood or urine analyses were detected.

Distribution and Dosimetry

The highest uptake in normal organs was observed in kidneys, liver and lungs (Fig. 2 and Table 2). Moderate activity was observed in the gastrointestinal tract. Uptake in salivary and lacrimal glands was also visualized. Distribution of activity was similar after injection of 500 µg and 1000 µg. The only significant difference ($p<0.05$) was in uptake in intestines content at 6 and 24 h after injection, which was lower for 500 µg. The decay-corrected uptake in kidneys, liver, lungs and intestines content plateaued by 2 h after injection.

The blood kinetics of ^{99m}Tc-ADAPT6 is shown in Fig. 3. The elimination rates for 500 µg (half-life 3.1 h, 95% CI from 2.4 to 4.0 h) and 1000 µg (half-life 3.0 h, 95% CI from 2.3 to 3.9 h) were similar.

Estimated absorbed doses are presented in Table 3. The highest absorbed organ dose was in kidney. Doses to adrenal, gall bladder wall, liver, spleen and pancreas were also noticeable, but they were several fold lower than the renal dose. Doses to adrenals, stomach wall, spleen, thyroid and uterus were significantly ($p<0.05$) higher for 1000 µg, but absolute difference was prominent only for adrenal and thyroid. Total effective dose was 0.009±0.002mSv/MBq for 500 µg and 0.010±0.003 mSv/MBq for 1000 µg. For a typical injected activity in this study, 380 MBq, this would result in an effective dose of 3.4 and 3.8 mSv.

Discrimination between Tumors with High and Low HER2 Expression

All tumors (including 8 multicentric) and involved lymph nodes (n=9) with both high and low HER2 expression were visualized using ^{99m}Tc-ADAPT6 as early as 2 h after injection, and remained visible throughout the study (Fig. 2 and 4). A number of visualized tumor lesion was in agreement with the data from other radiology investigations. Involvement of lymph nodes was confirmed by histology analysis after core biopsy (n =3) or cytology analysis after fine-needle biopsy (n=6) with subsequent histology analysis of operation materials.

Relation between data concerning expression of HER2 in biopsy samples and tumor-to-contralateral breast ratios is presented in Fig. 5 and Supplemental Fig.1. The best discrimination between tumors with high and low HER2 expression was provided by injection of 500 μg protein, where the mean value of tumor-to-contralateral breast ratio for HER2-positive tumors was 37±19 already 2 h after injection, significantly higher ($p<0.001$, Mann-Whitney test) than the value for HER2-negative tumors (5±2) (Fig. 5). The ratio tended to increase with time. The tumor-to-contralateral breast ratio for 500 μg was significantly ($p<0.05$) higher than for 1000 μg. The difference in tumor-to-contralateral breast ratio value between HER2-positive and HER2-negative tumors in the case of injection of 1000 μg was not significant ($p>0.05$, Mann-Whitney test) at any time point (Fig. 5). The tumor-to-contralateral breast ratio for 250 μg at 2 h (6.5±4.9) was also significantly ($p<0.05$) lower than for 500 μg (Fig. 5).

Patient 17 was enrolled into this study because initial IHC evaluation of her biopsy suggested 3+ expression level. However, her image showed unusually low tumor-to-contralateral breast ratio (1.33 at 2 h). The biopsy samples were re-evaluated by pathologists and the HER2 status was reassigned as 2+ and FISH-negative. Based on the new pathology evaluation, her treatment was changed by the cancellation of HER2-targeting therapy.

Imaging of patient 5 revealed, besides the primary tumor and axillary metastases, suspicious bone metastases in rib 5 and vertebrae Th8 and Th9 (Fig. 6A). CT imaging and bone scan using ^{99m}Tc-pyrophosphate (Fig. 6B, Supplemental Fig. 2A,B) did not reveal any metastases. However, MRI confirmed metastases in Th8 and Th9 (Supplemental Fig. 3). The treatment strategy of Patient 5 was changed from surgical treatment to chemotherapy and HER2-targeting therapy. ^{99m}Tc-pyrophosphate bone and CT scans (Fig. 6C; Supplemental Fig. 2C,D) confirmed metastatic lesions in rib 5 and Th8 and Th9 vertebrae six months after ^{99m}Tc-ADAPT6 imaging.

Injections of 500 or 1000 μg ^{99m}Tc-ADAPT6 enabled higher uptake in tumors than in liver (Fig. 7). There was a tendency of higher tumor-to-liver ratios for the 500 μg protein dose, but the difference between ratios for doses of 500 and 1000 μg was not significant. Injections of 250 μg ^{99m}Tc-ADAPT6 resulted in significantly lower tumor-to-liver ratio than injection of 500 μg.

DISCUSSION

This study demonstrates that injections of ^{99m}Tc-ADAPT6 are safe and well tolerated. The mean effective dose of 0.010 mSv/MBq in this study corresponds to 3.8 mSv per patient. This is slightly lower compared to doses for imaging using ^{68}Ga-ABY25 affibody molecule (5.6 mSv) (*21*) or for ^{68}Ga-nanobody (4.6 mSv) (*16*), and appreciably lower than effective doses for ^{89}Zr-trastuzumab (18-38 mSv) (*8,10*) or ^{89}Zr-pertuzumab (39 mSv) (*13*). It has to be noted that clear discrimination between HER2-positive and negative tumors already 2 h after injection might permit further two-fold reduction of injection activity while preserving good imaging quality.

Discrimination between HER2-positive and HER2-negative lesions is a primary aim of molecular imaging since this helps to identify patients potentially responding to HER2-targeting therapy. However, the term "HER2-negative", i.e. unsuitable for treatment with HER2-targeting therapies, is deceptive. Breast tumors with IHC score of 2+ (and ISH negative) are considered as HER2-negative, but they may express up to 500,000 HER2 molecules per cell (*22*). Thus, some accumulation of imaging probes is expected even in HER2-negative lesions. Studies with anti-HER2 affibody molecules demonstrated that it is possible to improve discrimination between tumors with high and low expression by increasing injected protein mass (*4,23*). Furthermore, clinical data have shown that increase of injected mass of ^{68}Ga-labeled anti-HER2 affibody molecules from 100 to 500 μg reduces binding to hepatocytes, which express low levels of HER2, and improves imaging of hepatic metastases (*4*). Studies in mice demonstrated that an increase of injected dose of ^{68}Ga-labeled ADAPT6 from 1 to 15 μg improved discrimination between human xenografts with high and low HER2 expression (*19*). However, an upscaling from mice to human is quite uncertain. Therefore, we initially evaluated injection of ^{99m}Tc-ADAPT6 at two dose levels, 500 and 1000 μg. Injection of 500 μg provided excellent discrimination already 2 h after injection (Fig. 5), and the tumor-to-contralateral ratio had a tendency to increase with time. On the opposite, injection of 1000 μg did not enable discrimination between HER2 positive and negative tumors. To check if further reduction of injected protein mass would improve the discrimination, an additional smaller cohort of patients was injected with 250 μg ^{99m}Tc-ADAPT6. However, the contrast of such imaging was clearly inferior to contrast of imaging using 500 μg ^{99m}Tc-ADAPT6 (Fig. 5). Thus, a 500 μg dose appears optimal, and deviation from this dose would result in decrease of both sensitivity and specificity of imaging of HER2-expression (Supplemental Fig. 4). Most likely, such dose provides a delicate balance between saturation of HER2 in liver, which increases bioavailability

of radiolabeled ADAPT6, and saturation of HER2 in tumors, which decreases ^{99m}Tc-ADAPT6 uptake in HER2-positive lesions. Capacity of early imaging enables reduction of injected activity and, accordingly, effective dose to patients. Obviously, clinical imaging using ^{99m}Tc-ADAPT6 should be performed approximately 2 h after injection. Increasing the time interval between injection and imaging would require either increasing of injected activity (and consequently the effective dose) or decreasing counting statistics at the time of injection (and therefore decrease of reconstruction fidelity).

PET provides better resolution, sensitivity and quantification than SPECT, but is costlier and requires substantially more infrastructure. Modern PET/CT facilities are mainly installed in Europe and North America, while SPECT is the most common nuclear imaging modality in Asia, Africa and South America. SPECT imaging might be of interest also in other regions where PET is available but much more expensive or accessibility restricted. It is conceivable that ^{99m}Tc-labeled targeting proteins and peptides will be useful in these regions (*24*).

CONCLUSION

^{99m}Tc-ADAPT6 is safe and well tolerated. Injections of ^{99m}Tc-ADAPT6 are associated with low absorbed and effective doses. An injected protein dose of 500 μg provides the best discrimination between primary tumors with high and low expression of HER2 among tested doses. Further studies concerning the use of ^{99m}Tc-ADAPT6 for stratification of patients for HER2-targeted therapy are justified.

ACKNOWLEDGEMENTS

This research was financially supported by the Ministry of Science and Higher Education of the Russian Federation (grant 075-15-2019-1925).

KEY POINTS

- **QUESTION: Is imaging of HER2 using ^{99m}Tc-ADAPT6 safe and informative?**
- **PERTINENT FINGINGS: Imaging using ^{99m}Tc-ADAPT6 is safe and well tolerated. An injected protein dose of 500 μg provides discrimination between HER2-positive and HER2-negative primary breast tumors 2 h after injection.**
- **IMPLICATION FOR PATIENT CARE:** Data from this first-in-human study support further clinical evaluation of **^{99m}Tc-ADAPT6, which might result in development of a probe for imaging of HER2 expression in the regions, where PET is not readily available.**

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TABLES

Table 1. Patient Characteristics Before Injection with ^{99m}Tc-ADAPT6

Patient no	Age (y)	HER2 status of primary tumor	Primary tumor ER/PgR	Stage
500 µg; mean tumor size 23±8 mm				
1	36	3+ (IHC)	ER +/PgR -	IIB (T2N1M0)
2	63	3+ (IHC)	ER -/PgR -	IIIA (T3N1M0)
3	50	3+ (IHC)	ER +/PgR -	IIIA (T2N2M0)
4	61	3+ (IHC)	ER -/PgR -	IIB (T2N1M0)
5	64	2+ (IHC)/FISH+	ER-/PgR -	IIIB (T2N1M0) /IV (T2N3M1) [a]
6	34	0 (IHC)	ER +/PgR +	I (T1N0M0)
7	47	0 (IHC)	ER +;/PgR +	IIA (T2N0M0)
8	41	0 (IHC)	ER +/PgR +	IIA (T2N0M0)
9	63	1+ (IHC)	ER +/PgR +	IIB (T2N1M0)
10	59	1+ (IHC)	ER +/PgR -	IIA (T2N0M0)
11	40	0 (IHC)	ER -/PgR -	IIIA (T3N1M0)
1000 µg; mean tumor size 31±11 mm				
12	34	3+ (IHC)	ER +/PgR -	IIA (T2N0M0)
13	37	3+ (IHC)	ER +/PgR -	IIA (T1N1M0)
14	43	2+ (IHC)/FISH+	ER +/PgR +	IIA (T2N0M0)
15	36	3+ (IHC)	ER +/PgR +	IIA (T1N1M0)
16	33	3+ (IHC)	ER -/PgR -	IIA (T2N0M0)
17	58	3+ (IHC)/ 2+ (IHC)/FISH- [b]	ER +/PgR +	I (T1N0M0)
18	51	1+ (IHC)	ER+/PgR -	IIA (T2N0M0)
19	63	1+ (IHC)	ER +/PgR +	IIA (T2N0M0)
20	62	0 (IHC)	ER -/PgR -	IIA (T2N0M0)
21	71	1+ (IHC)	ER +/PgR -	IIA (T2N0M0)
22	42	0 (IHC)	ER+/PgR +	IIA (T2N0M0)
250 µg; mean tumor size 30±10 mm				
23	51	2+ (IHC)/FISH+	ER +/PgR -;	IIA (T2N0M0)
24	48	2+ (IHC)/FISH+	ER +/ PgR -	IIA (T2N0M0)
25	61	2+ (IHC)/FISH+	ER +/ PgR +	IIA (T4N1M0)
26	39	3+ (IHC)	ER+/PgR -	IIA (T2N0M0)
27	29	2+ (IHC)/FISH-	ER +/ PgR +	IIA (T2N0M0)
28	62	1+ (IHC)	ER +/ PgR +	I (T1N0M0)
29	48	1+ (IHC)	ER -; PgR -	I (T1N0M0)

[a] staging was changed as imaging revealed distant metastases;

[b] FISH analysis after imaging confirmed HER2-negative status.

Table 2. Uptake of ^{99m}Tc in Tumor-Free Areas of Organs with Highest Uptake on SPECT Images After Injection of ^{99m}Tc-ADAPT6 (decay corrected).

	Kidney			Lungs			Liver			Small intestines		
	250 µg	500 µg	1000 µg	250 µg	500 µg	1000 µg	250 µg	500 µg	1000 µg	250 µg	500 µg	1000 µg
2 h	26±10	27±10	35±9	2.7±0.9	3.3±0.8	2.7±0.6	3.1±0.3	3.2±1.1	2.4±0.8	1.8±0.4	0.8±0.3	1.0±0.3
4 h		31±12	36±10		2.5±0.8	2.2±0.4		2.8±1.1	2.4±1.0		0.9±0.3	1.3±0.5
6 h		32±9	45±11		2.0±0.6[c]	2.0±0.4[c]		2.6±0.8	2.0±0.7		0.8±0.3[a]	1.3±0.5
24 h		29±10	38±8		1.4±0.5[b]	1.2±0.4[b]		2.4±1.0	1.8±0.8		0.6±0.2[a]	1.0±0.3

Data are presented as percent injected radioactivity per organ (mean values and SD from all patients).

[a] Significantly ($p<0.05$) lower uptake in intestines content after injection of 500 µg compared to 1000 µg;

[b] Significantly ($p<0.05$) lower uptake in lungs at 24 h after injection compared to 2 and 4 h;

[c] Significantly ($p<0.05$) lower uptake in lungs at 6 h after injection compared to 2 h;

Table 3. Absorbed Doses after Injection of 500 and 1000 µg ^{99m}Tc-ADAPT6

	500 µg	1000 µg
Adrenals	0.023±0.005*	0.032±0.009
Brain	0.001±0.000	0.001±0.000
Breasts	0.007±0.002	0.009±0.005
Gallbladder wall	0.013±0.008	0.012±0.003
Lower large intestine wall	0.005±0.001	0.005±0.001
Small intestine	0.006±0.001	0.008±0.002
Stomach wall	0.006±0.001*	0.008±0.002
Upper large intestine wall	0.007±0.001	0.008±0.002
Heart wall	0.004±0.001	0.004±0.001
Kidney	0.135±0.042	0.191±0.047
Liver	0.011±0.008	0.008±0.002
Lungs	0.005±0.001	0.006±0.001
Muscle	0.003±0.000	0.003±0.001
Ovaries	0.008±0.002	0.010±0.003
Pancreas	0.011±0.002	0.014±0.004
Red Marrow	0.004±0.001	0.005±0.001
Osteogenic Cells	0.006±0.001	0.008±0.002
Skin	0.001±0.000	0.002±0.000
Spleen	0.011±0.003*	0.015±0.004
Thymus	0.005±0.002	0.006±0.002
Thyroid	0.009±0.004*	0.014±0.005
Urinary bladder wall	0.012±0.007	0.012±0.006
Uterus	0.005±0.001*	0.007±0.002
Total body	0.004±0.001	0.005±0.001
Effective Dose equivalent (mSv/MBq)	0.017±0.004	0.022±0.005
Effective Dose (mSv/MBq)	0.009±0.002	0.010±0.003

Data presented as mean mGy/MBq ± SD (n = 11).

* Significant ($p<0.05$) difference between doses after injection of 500 and 1000 µg.

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FIGURES CAPTIONS

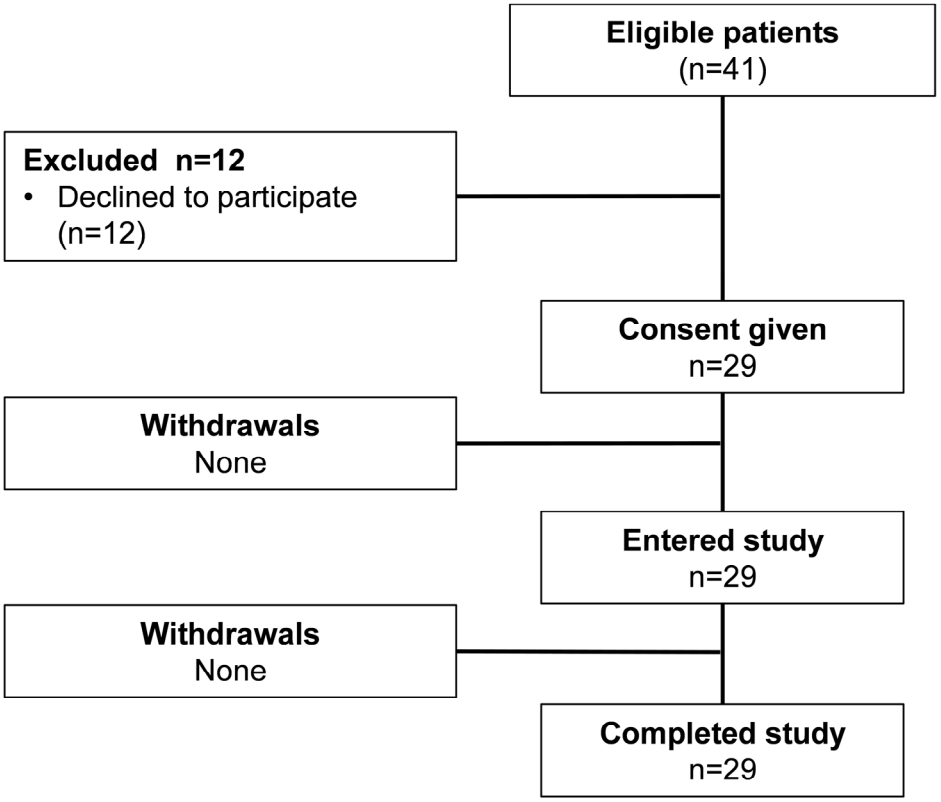

FIGURE 1. Standards for Reporting of Diagnostic Accuracy Studies (STARD) flow diagram.

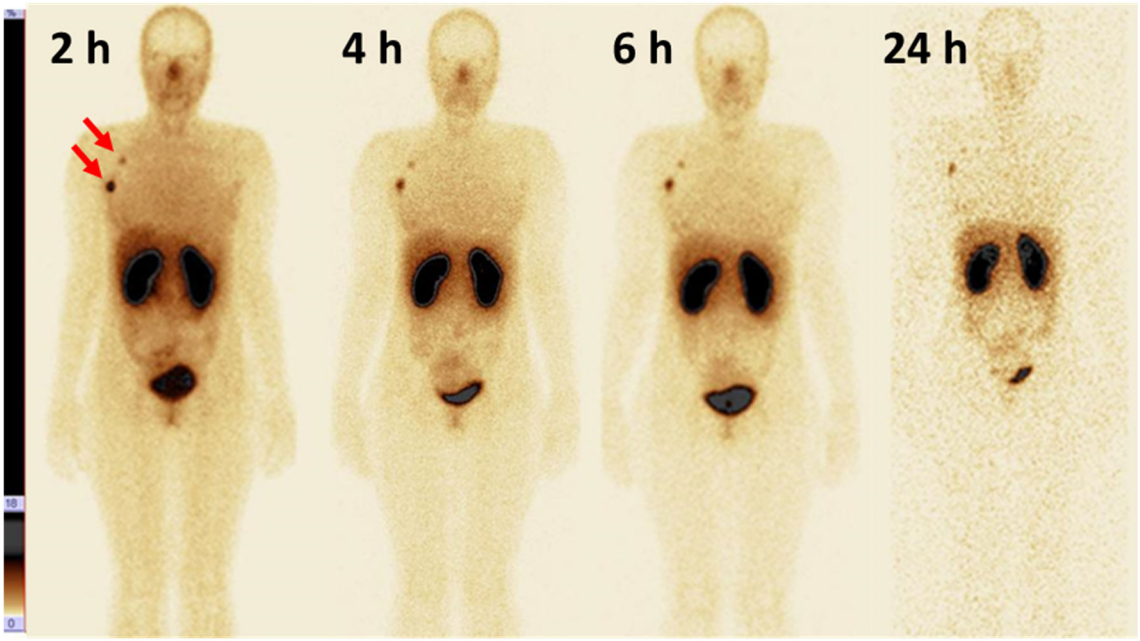

FIGURE 2. Whole-body images at 2, 4, 6 and 24 h after injection of 500 µg ^{99m}Tc-ADAPT6 (Patient 1). The upper setting of the scale window is 18% of maximum counts.

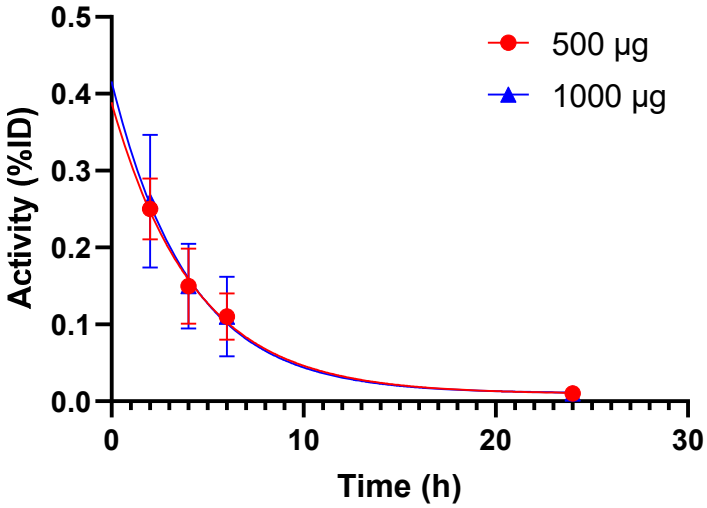

FIGURE 3. Kinetics of elimination of ^{99m}Tc-ADAPT6 from blood. Data were calculated based on count rates in ROIs placed over hearts.

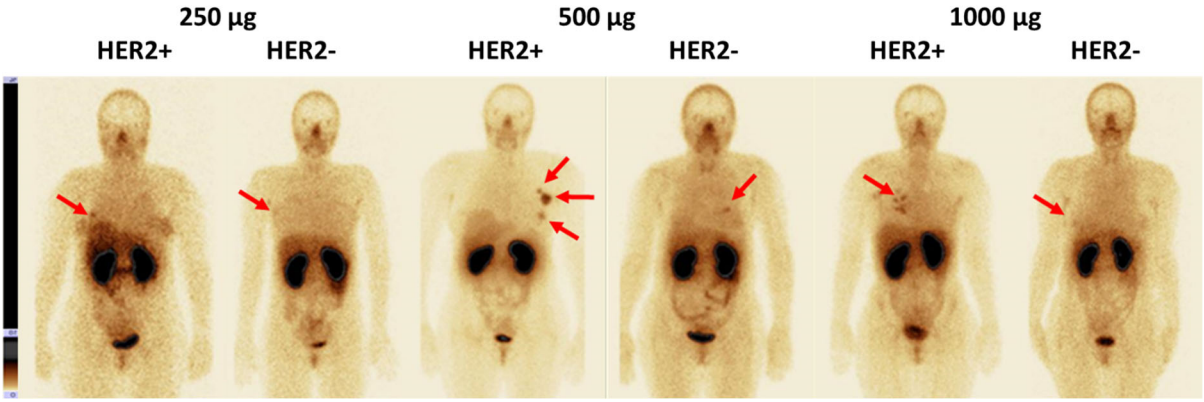

FIGURE 4. Representative anterior images of patients with HER2 negative and HER2 positive tumors after injection of 250, 500 or 1000 µg ^{99m}Tc-ADAPT6. The upper setting of the scale window is the same for all images, 18% of maximum count rate.

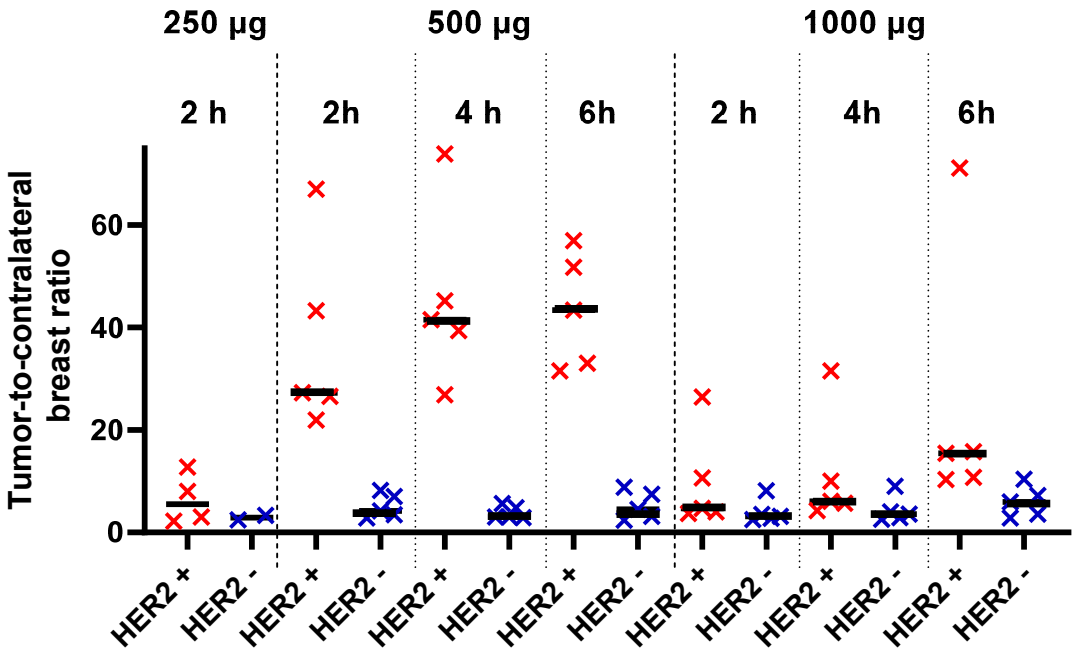

FIGURE 5. Primary tumor-to-contralateral site ratio at 2 h after injection of 250 µg, and 2, 4 and 6 h after injection of 500 and 1000 µg ^{99m}Tc-ADAPT6. Red symbols represent data for HER2 positive tumors, blue from HER2-negative.

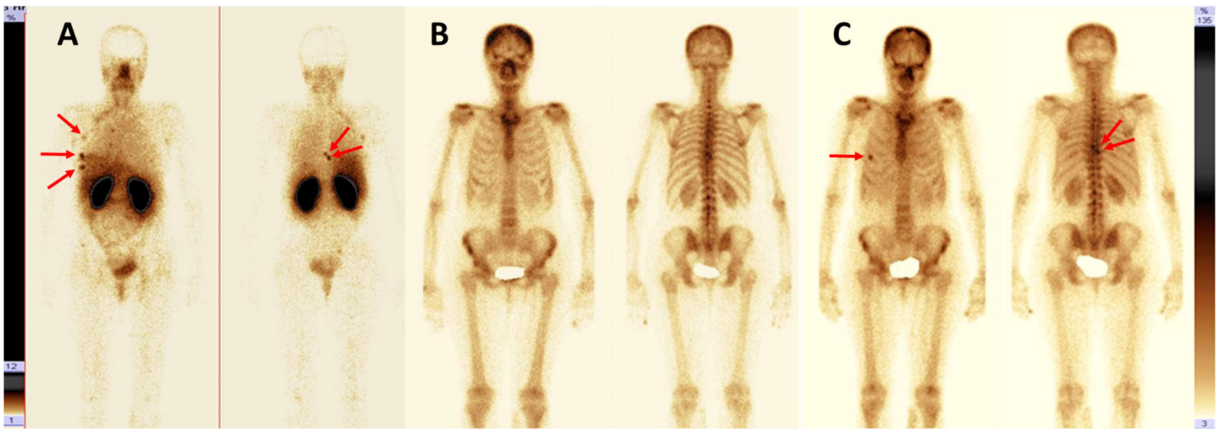

FIGURE 6. Tumor sites visualization with planar scintigraphy in patient 5: (A) ^{99m}Tc-ADAPT6; 2 h after injection (the upper setting of the scale window is 12% of maximum counts); (B) ^{99m}Tc-pyrophosphate at the time of imaging with ^{99m}Tc-ADAPT6; (C) ^{99m}Tc-pyrophosphate 6 months after ADAPT6 injection.

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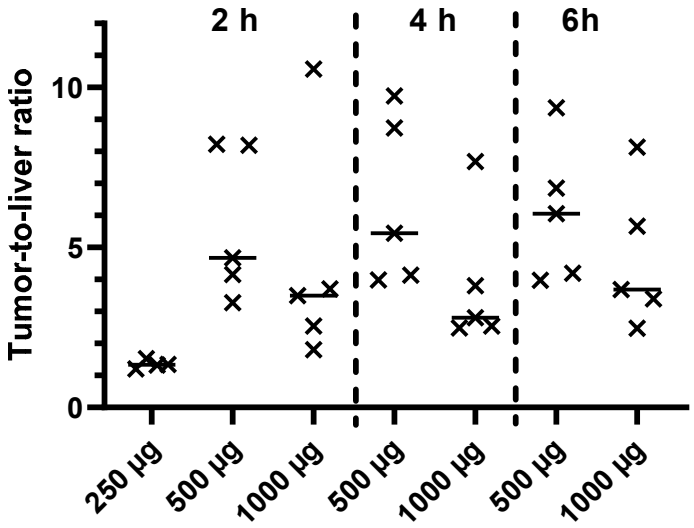
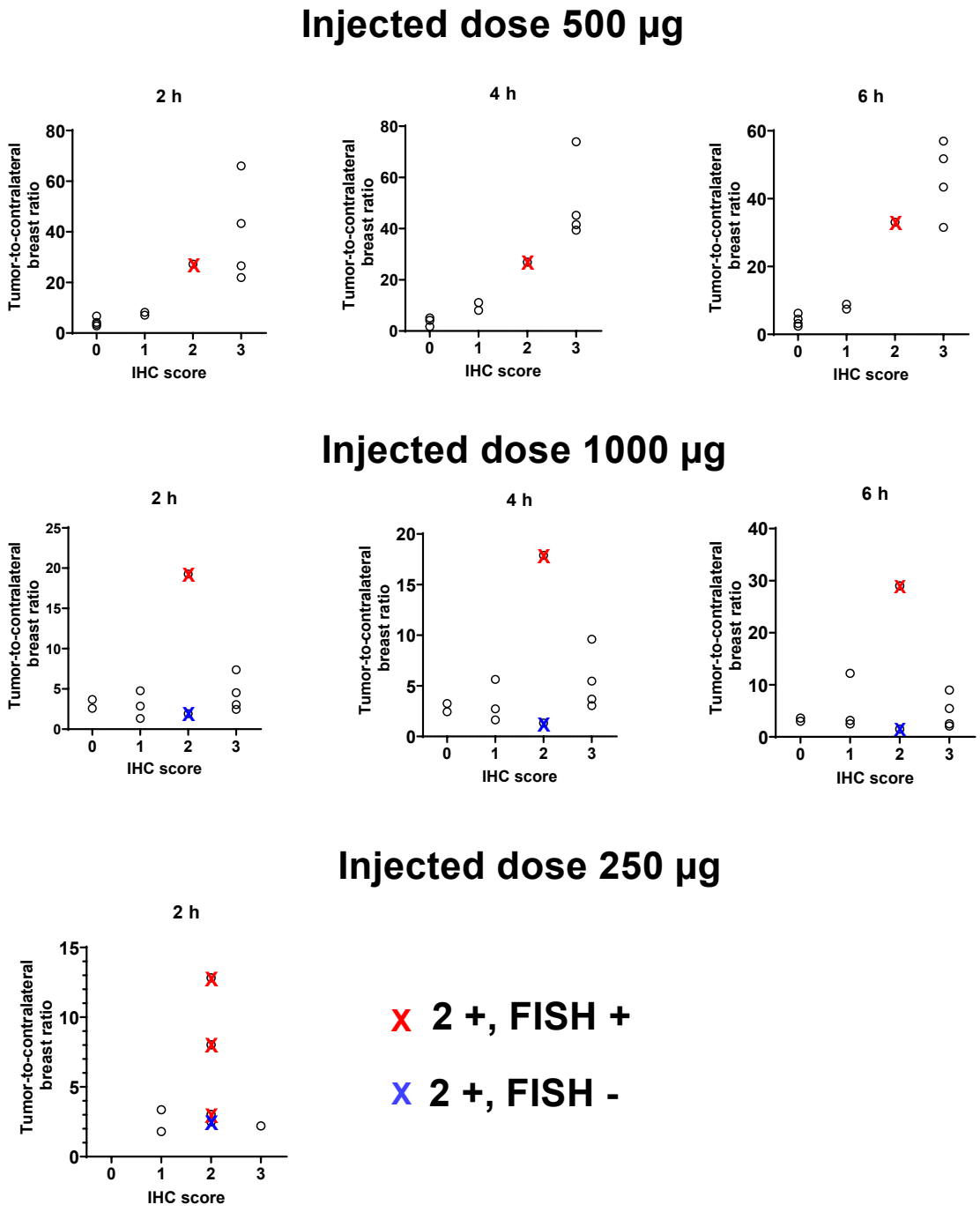
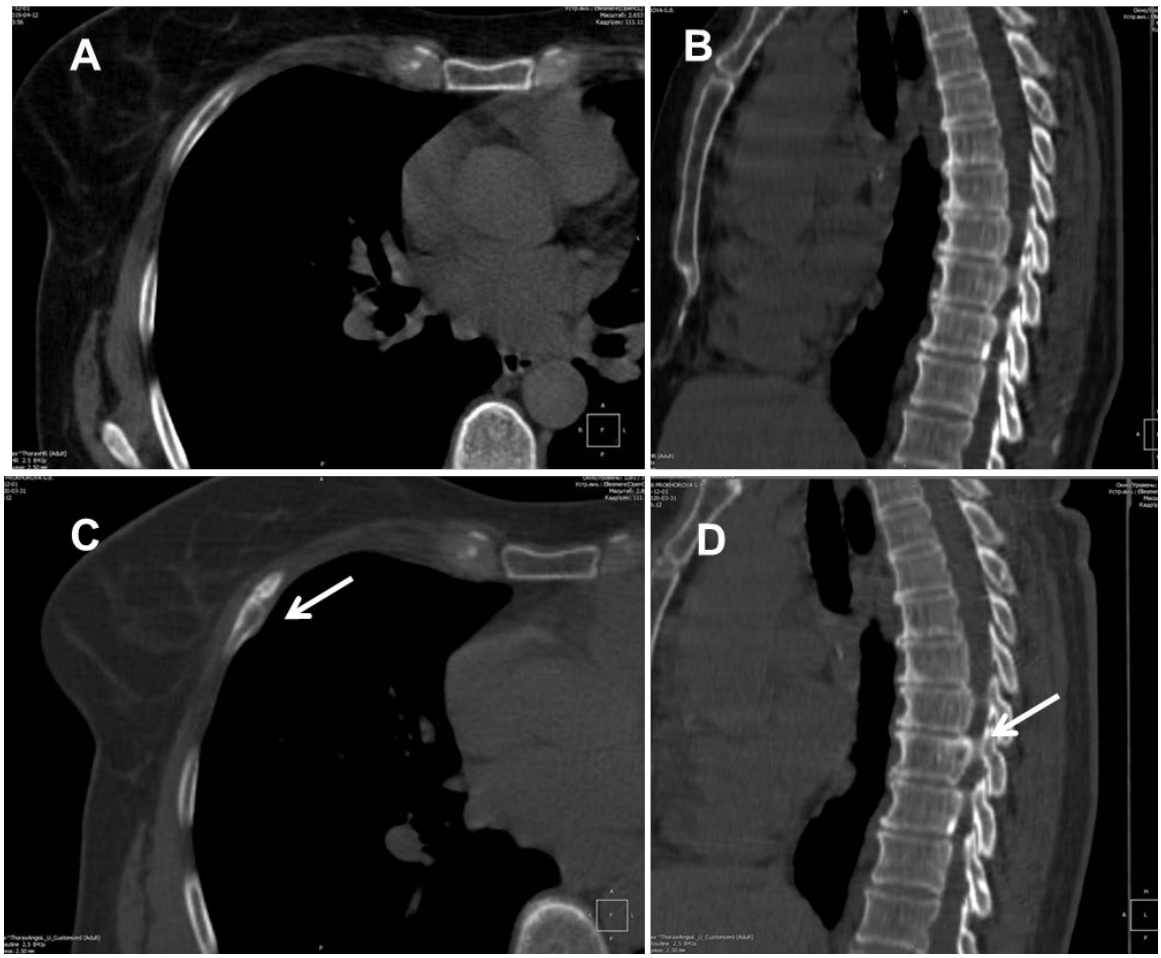


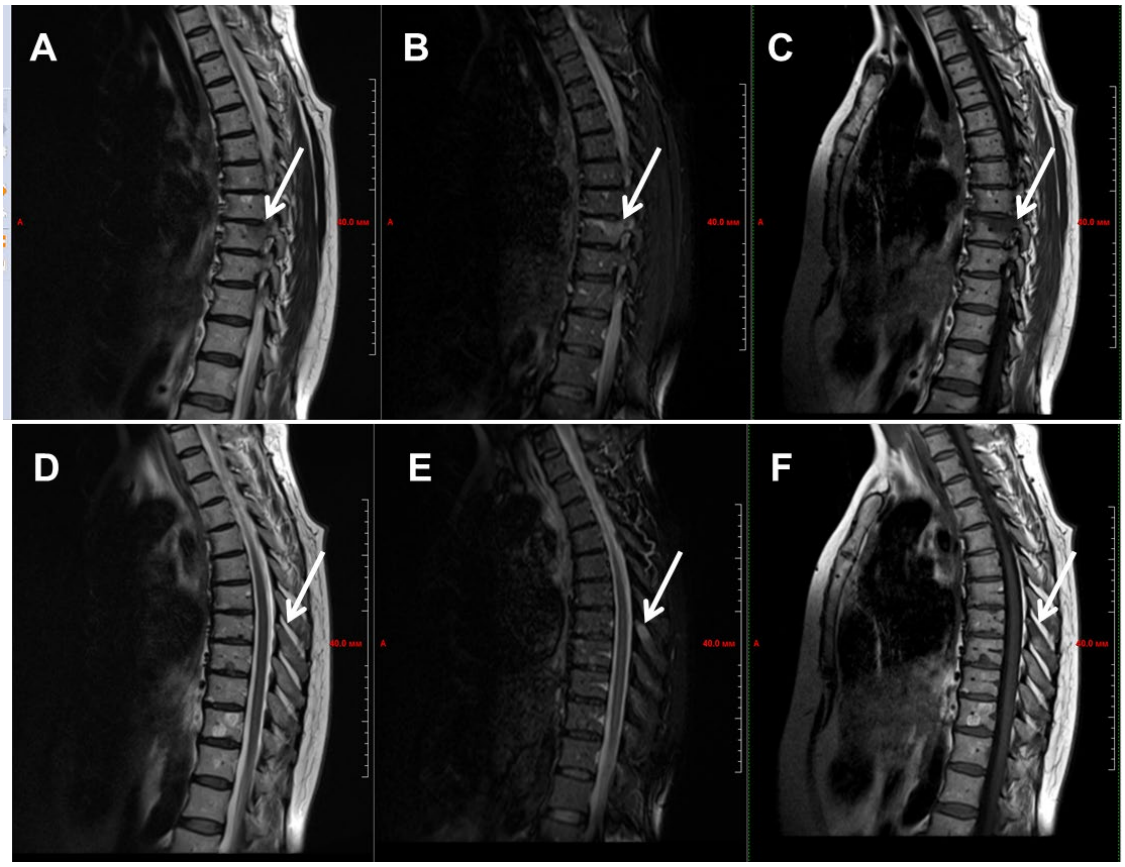
FIGURE 7. Tumor-to-liver ratio for HER2-positive tumors at 2 h after injection of 250 µg, and 2, 4 and 6 h after injection of 500 and 1000 µg ^{99m}Tc-ADAPT6.



Supplemental Fig. 1. Relation between data concerning expression of HER2 in biopsy samples and tumor-to-contralateral breast ratios at 2 h after injection of 250 µg, and 2, 4 and 6 h after injection of 500 and 1000 µg ^{99m}Tc-ADAPT6. Red crosses mark data for FISH-positive tumors with IHC score 2+, blue crosses mark data for FISH-negative tumors with IHC score 2+, .



Supplemental Fig. 2. CT scans of patient 5 at admission (A and B) and during follow-up (C and D). The follow-up scan show in front section of right rib 5 (C) an area of lytic destruction with expansion and sclerotic contours (6 x 13 mm). In the right posterior sector of the body Th9 (D) there is a lesion of 9x11 mm with destruction of the cortical plate and a sclerosis zone



Supplemental Fig. 3. Patient 5 magnetic resonance imaging at admission: T2WI (A, D); T2WI with fat suppression (B, E); T1WI (C, F). (A, B, C). Lesion in Th9 body. (D, E, F) Lesion in in the spinous process of Th8.

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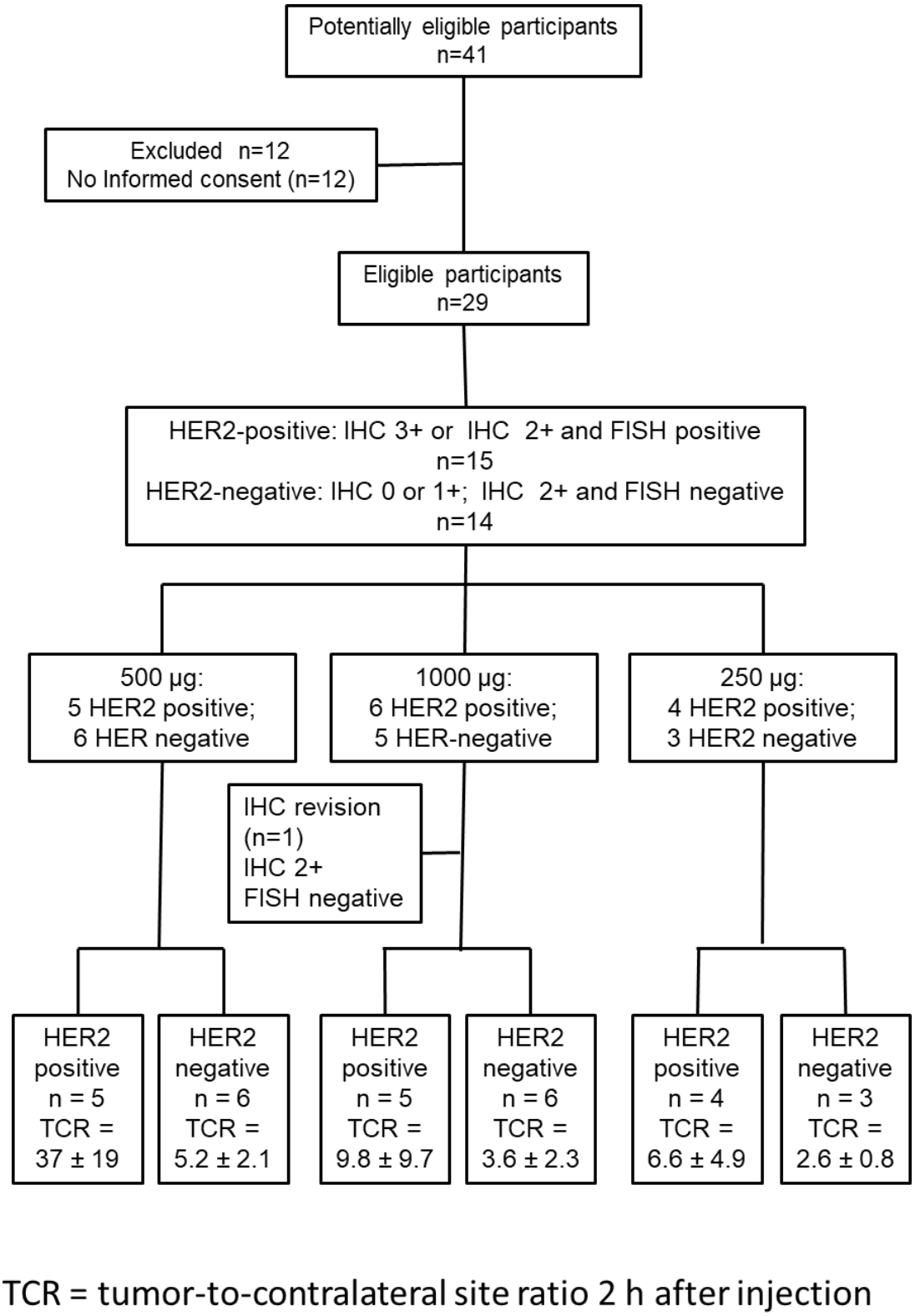

Supplemental Fig. 4. Study overview.