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Detection of melanoma lesions using ^{131}I–IMBA obtained by electrophilic substitution of ^{131}I for metal organic substituent — a preliminary communication

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Abstract

BACKGROUND: Compounds of N-alkylated benzamide derivatives have been the subject of investigations in the last few decades from the standpoint of their possible application for scintigraphic detection of melanoma. Positive results have been observed in studies on biodistribution when using animal models and the compound IMBA (N-(2-diethylaminoethyl)--3-iodo-4-metoxybenzamide).

The present study presents preliminary results of scintigraphic studies in patients with documented melanoma metastases, who were administered ^{131}I-IMBA synthesized by modified labelling procedure (electrophilic substitution of radioactive ^{131}I to metal organic substituent).

MATERIAL AND METHODS: The study was made in three patients with diagnosed melanoma metastases to tissues and organs. To each patient 111 MBq of ^{131}I-IMBA was intravenously administered and whole body scintigraphy was performed 4 and 24 hours post injection of the radiopharmaceutical. Additionally, after 24 hours, SPECT/CT of selected regions of the body was performed.

RESULTS: In 3 patients a total of 20 lesions of increased activity were found (15 were detected previously by other methods, 5 in the head, 4 in thorax, 2 in liver and spleen, 3 in abdomen and 6 in extremities). In the scintigrams performed 4 hours after ^{131}I-IMBA administration, there were found 12 lesions of enhanced accumulation of the radiopharmaceutical. After 24 hours, due to reduction of background activity, there were 8 additional hot lesions detected. The mean activity tumour/background ratio for 20 lesions 4 hours post injection amounted to 1.51 ± 0.64, and the ratio increased to 2.94 ± 2.32 24 hours after administration of a radiopharmaceutical.

CONCLUSIONS: ^{131}I-IMBA preparation, obtained by a modified labelling procedure, enabled detection of metastatic lesions in the patients. This may indicate that there is a possibility of using radioiodinated IMBA (with ^{123}I or ^{131}I) for diagnosis of melanoma in humans. From our results it follows that scintigraphy should be performed 24 hours post injection. Further studies on diagnostic efficacy (sensitivity and specificity) of the method are necessary.

Key words: melanoma, aminoalkyl-iodobenzamides, radioiodinated IMBA

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Introduction

Melanoma is a neoplasm with the highest dynamics of morbidity increase among all malignant carcinomas [1]. A credible assessment of the stage of the disease is of high importance for prognosis and therapy planning [2].

Investigation of possible use of benzamide derivatives in nuclear medicine diagnostics has been under way since the 1990s. The most studied compound of this group has been IBZA (*N*-(2-diethylaminoethyl)-4-iodobenzamide) and its isomers, differing in the position of the iodine atom in the benzene ring [3–5]. In spite of long studies the only compound available on the market is ^{123}I-IBZM (N-[(1-ethyl-2-pyrrolidinyl)methyl]-2-hydroxy-3-iodo-6-methoxybenzamide; GE Health Care, Amersham), which is used due to its affinity to dopamine receptors (D2) in the diagnosis of neuropsychiatric diseases, mostly Parkinson's disease [6, 7].

IMBA (N-(2-diethylaminoethyl)-3-iodo-4-metoxybenzamide), as a derivative representing this group of compounds, was described by Nicholl et al. in 1997 [8]. The iodine labelled preparation of IMBA was obtained by thallation reaction, from the derivative cleared from the halogen atom. The same method has been used by Edreira and Pozzi in their exploration of IMBA [9]. These authors have demonstrated that their compound was more convenient in imaging the melanoma lesions when compared with the precursor of the group — IBZA.

In our previous study [10] we were able to obtain encouraging results when studying the biodistribution of ^{131}I-IMBA on animal models, using the compounds obtained by applying two methods for radiolabelling. The highest tumour/background ratios where obtained when using ^{131}I-IMBA labelled by means of electrophilic substitution of iodine isotope for metal organic substituent.

The reported new study presents preliminary results obtained when imaging metastases of melanoma tumours by whole body scintigraphy, using ^{131}I-IMBA labelled by the more convenient method.

Material and methods

Preparation of the ^{131}I-IMBA

The labelling was based on substitution of ^{131}I molecules for metal organic substituent ($SnBu_3$) in ($SnBu_3$)MBA derivative. The procedure was first described by John et al. when utilizing other benzamide derivatives (IPAB, PIMBA) for labelling [11, 12].

The reaction was carried out in a glass vial, to which 500 μl of ethanol solution of ($SnBu_3$)MBA (1 mg/ml), 50 μl of Na^{131}I solution (185 MBq RI-10, POLATOM, Swierk), 250 μl of 0.05 M HCl, and 250 μl of freshly prepared chloramine T (1 mg/ml), were added. After mixing, the reaction mixture was incubated for 10 minutes at room temperature in a shaker. The iodination reaction was discontinued by adding to the mixture 1000 μl of sodium metabisulphite in a saturated solution of sodium bicarbonate (200 mg/ml) and 1000 μl of saturated solution of sodium bicarbonate. Next, 1 ml of chloroform was added and the mixture was shaken. The chloroform layer was collected in a fresh vial and dried in a stream of argon. The dried remnant, after removal of chloroform was dissolved in physiological saline and sterilized by 0.20 μm syringe filters.

The radiochemical purity was tested by ascending thin-layer chromatography using silica gel plates with fluorescent indicator (Silica Gel 60 F_{254} — Merck). A mixture of chloroform:methanol in a ratio of 1:7 was used as the developing solution. Retention factor (Rf) for ^{131}I-IMBA amounted to 0.2–0.25, and Rf for ^{131}I$^-$ was 0.90–0.95.

Scintigraphy

Scintigraphic examination of 3 patients with melanotic metastases was made using a hybrid SPECT/CT GE Infinia Hawkeye installation. Before injecting the patients with ^{131}I-IMBA, permission was obtained from the Bioethics Commission of the Medical University of Lodz. Patients were given Lugol fluid — 20 drops × 3 times daily from one week before the planned investigation up to 1 week after scintigraphy.

The patients subjected to scintigraphy with use of ^{131}I-IMBA had an earlier diagnosis and surgically removed primary skin lesions of melanoma. They were referred for radionuclide study after diagnosis of metastases found by other imaging modalities (RTG, CT, and ultrasonography).

On the day of scintigraphic scanning 111 MBq of ^{131}I-IMBA in 5 ml of physiological saline was administered intravenously to each patient. Scintigraphy of whole body was performed in anterior and posterior projections, 4 and 24 hours post injection. High-energy collimators were used (energy window for ^{131}I of 364 keV $\pm$ 10%) and acquisition lasted 10 minutes per image. Processing of images was made using an Xeleris station with appropriate programs from the manufacturer.

Whole body scintigrams were evaluated visually by two nuclear medicine specialists, and the final result was arrived at by consensus.

Additionally, 24 hours after administration of ^{131}I-IMBA SPECT-CT, examination of selected regions of the patient's body was performed with the aim of more precise localization of the activity lesions (60 images in the matrix of 64 × 64 at an acquisition time of 50 seconds per image). Evaluation of the uptake of activity 4 and 24 hours post administration on whole body images was made using the tumour/background ratios.

Results

The method of electrophilic substitution of a metal organic fragment by ^{131}I atom had an efficacy of 80-85%. The radiochemical purity of the final product exceeded 95%.

Examination of whole body scans, made four hours after ^{131}I-IMBA administration, disclosed enhanced uptake of a radiopharmaceutical in the liver, kidneys, and the urinary bladder (Figure 1A). This reveals two probable routes of tracer elimination from the organism.

Twenty-four hours after ^{131}I-IMBA injection the activity in the liver was substantially reduced, but higher activity was found in the intestines, which seems to indicate that elimination of the tracer — and its metabolites — took place with bile (Figure 1B). A reduction of activity over 24 hours was also observed in the lungs, where a relatively high uptake of ^{131}I-IMBA, compared with the neighbouring tissues, could be seen earlier — after 4 hours (Figure 1). In 2 patients — in spite of Lugol fluid administration to prevent activity uptake by the thyroid — there was low but visible activity in the gland.

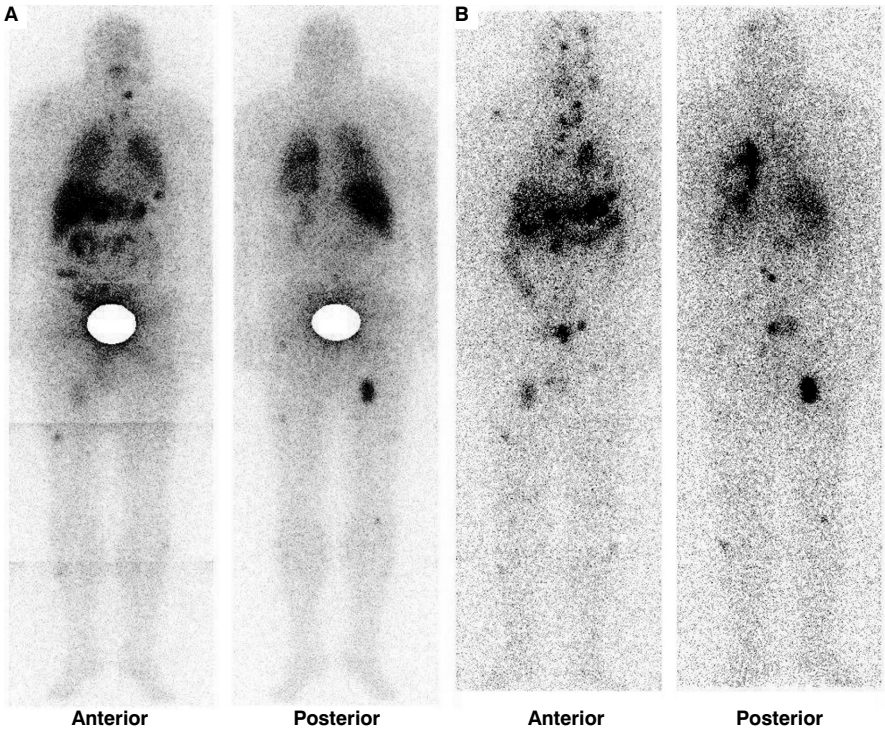

Figure 1. Whole body scintigram of patient with spread of metastatic melanoma. **A.** 4 hours after radiopharmaceutical injection; **B.** 24 hours after radiopharmaceutical injection; radioactivity in urinary bladder on the 4 h image has been masked; there are seen numerous melanoma metastases to soft tissue of the extremities and to many internal organs, including brain, lungs, and liver, particularly clearly visible on the image recorded 24 hours after radiopharmaceutical injection.

Table 1. Results of visual analysis of scintigrams recorded 4 and 24 hours after 131I IMBA injection

Localization (n = 20)	Number of detected lesions of of the radiopharmaceutical			
	4 hours		24 hours	
	Certain	Uncertain	Certain	Uncertain
Head	1	2	5	0
Thorax	1	1	4	0
Liver and spleen	2	0	2	0
Abdominal cavity/pelvis/groins	1	0	3	0
Extremities	3	1	6	0
Total	8	4	20	0

Summarizing, apart from liver, lungs and intestines, the remaining tissues and organs (muscles, skin, subdermal tissue, bones, and brain) showed a similar but low level of ^{131}I activity.

Altogether, in 3 patients reviewed for visual analysis of scintigraphic images, 20 lesions of elevated uptake of the radiopharmaceutical were revealed. Fifteen of these lesions were identical to those verified with classical diagnostic procedures. The scintigraphic examination detected five additional lesions not found previously.

Visual evaluation of whole body scintigrams revealed 12 melanoma lesions — 4 of these were classified as uncertain. Twenty-four hours after ^{131}I-IMBA injection, the observations indicated 8 additional lesions of higher accumulation of the tracer. This was a result of faster elimination of the radiopharmaceutical from non-affected tissues, and therefore lower activity concentration in the background was observed (Table 1).

At the same time, the lesions visible 4 hours after injection became more intensive in 24-hour images compared with the background. Respective scintigrams recorded 4 and 24 hours after radiopharmaceutical administration are presented in Figures 1 and 2.

The list of all revealed lesions, together with their localization and with calculated tumour/background ratios at the two time points (4 and 24 hours), are presented in Table 2.

The tumour/background ratios calculated on the basis of the 4-hour image were, in each case, lower than those obtained from the second examination (24 hours post injection). The ratios calculated for 20 foci at 4 and 24 hours varied from 1.05 to 3.95 (mean

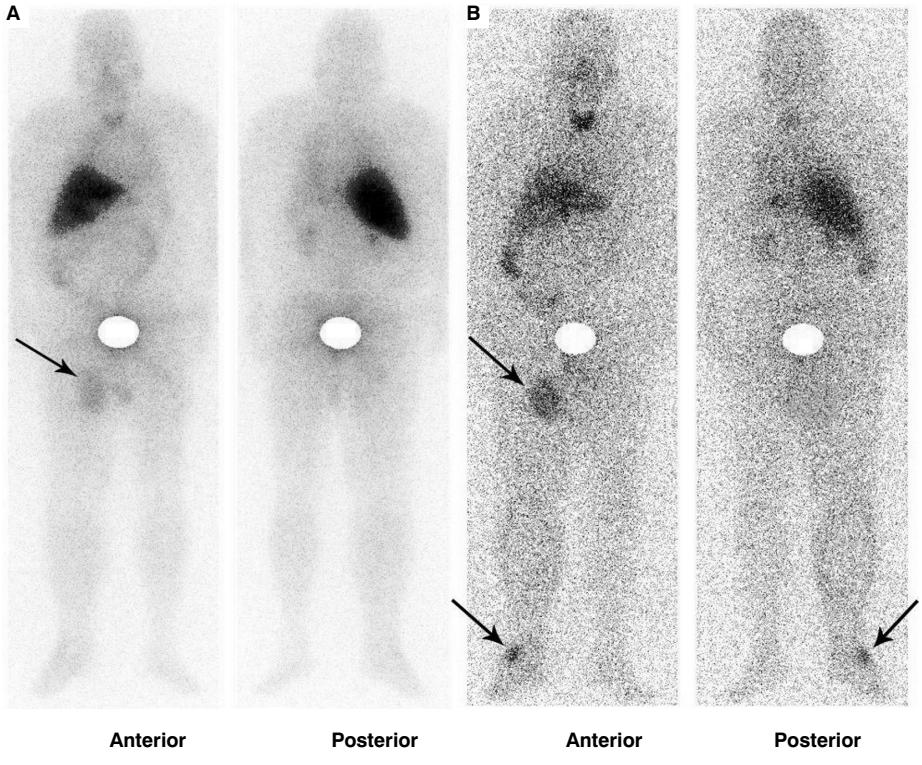

Figure 2. Whole body scintigram of a patient with melanoma metastases to soft tissue of the right leg (black arrows). **A.** 4 hours after radiopharmaceutical injection; **B.** 24 hours after radiopharmaceutical injection; radioactivity in urinary bladder on images has been masked; focus of increased radiopharmaceutical uptake in right foot visible only on image recorded 24 hours after tracer injection.

Table 2. Localization, calculated tumour/background ratios and methods of verification of all registered lesions of [131]I-IMBA uptake on scintigrams recorded 4 and 24 h after [131]I IMBA injection

	Localization	Tumor/background ratio		Verification
		4 hours	24 hours	
1	Brain	1.77	2.76	CT
2		1.42	2.37	
3		1.28	1.56	
4		1.27	1.55	
5	Mandible region (lymph nodes)	1.49	2.56	—*
6	Shoulder region (skin and subdermal tissue)	1.37	1.95	—*
7	Lungs	1.77	4.87	RTG, CT
8		1.24	2.21	RTG, CT
9		1.05	1.64	CT
10		1.16	1.35	CT
11	Spleen	1.70	2.92	—*
12	Liver	1.33	2.87	USG, CT
13	Abdominal cavity	1.06	2.70	—*
14	Pelvis	1.15	4.46	—*
15	Groin (lymph nodes)	2.11	2.67	Biopsy
16	Thigh (skin and subdermal tissue)	1.33	2.08	Visual and/or biopsy
17		3.95	12.04	
18	Shank (skin and subdermal tissue)	1.35	2.11	
19		1.40	2.18	
20	Foot (skin and subdermal tissue)	1.05	1.98	

*without verification

Table 3. Comparison of the mean value of tumor/background ratios calculated on the basis of the 4 and 24 h image

	Tumor/background ratios		Proportion of tumor/background ratio
	4 hours	24 hours	24 h *vs*. 4 h
Range	1.05–3.95	1.35–12.04	–
Mean	1.51	2.94	1.86
Standard deviation	0.64	2.32	0.70

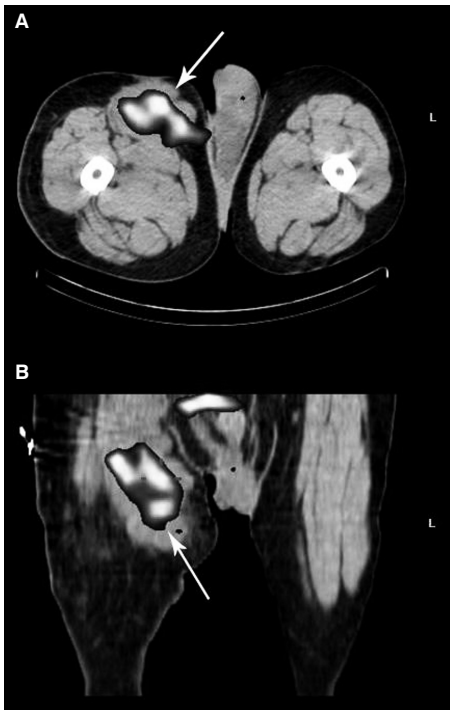

Figure 3. Fusion SPECT/CT image of melanoma metastases in right inguinal lymph nodes (arrows); **A.** Transversal slice; **B.** Sagittal slice.

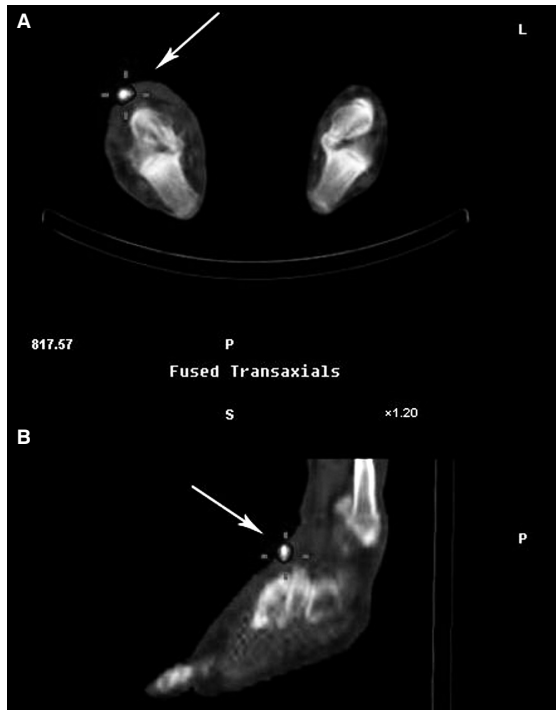

Figure 4. Fusion SPECT/CT image of melanoma lesions in skin and subdermal tissue of the right foot (arrows); **A.** Transversal slice; **B.** Sagittal slice.

1.51 ± 0.64) and from 1.35 to 12.04 (mean 2.94 ± 2.32), respectively (Table 3). The Wilcoxon test for pairs revealed statistically higher tumour/background ratios 24 hours than 4 hours post administration ($p < 0.0001$). The mean difference amounted to 1.86.

Additionally, on the second day of investigation, for better localization of the lesions in anatomical structures, SPECT/CT scans of the selected body regions were made. The example of additional fusion images (SPECT/CT) of the foci presented in Figure 2 (whole-body image) can also be observed in Figures 3 and 4.

Discussion

Preliminary studies made in patients with melanoma metastases revealed several tens of neoplastic lesions located in various organs. The mean tumour/background ratio in scintigrams acquired 24 hours post administration of the radiopharmaceutical amounted to 2.94. This value is of the same order as those found in the studies where investigations were made with other benzamide derivative (IBZA, IBZM) [3, 6, 7].

In our studies the highest value of the tumour/background ratio was found in the femur of the patient (in soft tissue) and amounted to 12.04 in the scintigraphic image recorded 24 hours after ^{131}I-IMBA injection. In one patient 4 lesions were located in the brain. One may anticipate that a study made using PET and ^{18}F-fluorodeoxyglucose as the radiopharmaceutical would not reveal these metastases because of the physiologically high uptake of the PET tracer by brain tissue.

Nicholl et al. in preliminary studies of a few patients 4 hours after ^{123}I-IMBA injection observed a satisfactory tumour/background ratio. The author stated, however, that visualization of some melanoma lesions was more satisfactory in images acquired later. The index of 24-hour/4-hour tumour/background ratio reported by the authors amounted to 3.6. In our study the mean value of tumour/background ratios increased by a factor of almost 2 between 4 and 24 hours after injection of the tracer.

Remembering that elimination of the radiopharmaceutical takes place with urine, the scintigraphic examination should be made when the bladder is empty.

Physiological uptake of ^{131}I-IMBA by liver and kidneys may cause difficulties in detection of melanoma lesions in these organs. However, radioactivity seen in these organs is substantially lowered

24 hours after injection. Difficulties resulting from the presence of activity at this time in intestines are most likely due to elimination of ^{131}I-IMBA with the bile. It should be possible to minimize this obstacle by giving cholagogues or laxatives to the patient.

As well as in excretory organs there is also an enhanced background in lungs (in early hours after ^{131}I-IMBA injection). Some activity is also present in the thyroid despite the fact that a Lugol liquid was given to patients. The same observation was made by Maffioli et al. when IBZM was used (in patients to whom potassium perchlorate was administered to block the thyroid uptake of activity) [7].

The optimal time for acquisition of scintigraphic images after administration of the radiopharmaceutical is a subject of discussion in the literature. Our results indicate that optimal time for scintigraphy appears to be 24 hours after injection of the tracer. None of the lesions detected in the first study (after 4 hours) in 3 examined patients showed a reduced tumour/background ratio in the second study (after 24 hours). Therefore, it seems that data acquisition performed 4 hours after injection may not supply complete information on advancement of the neoplastic process in the patient. This conclusion applies particularly to imaging of the liver and lungs.

The preliminary character of the investigation and small number of patients participating preclude assessing the diagnostic efficacy of the study. Such a conclusion would require a much larger group of patients and verification of all suspected neoplastic lesions with other diagnostic methods. In the literature the reported sensitivity and specificity of scintigraphy using benzamide derivatives (IBZA, IBZM) applied to melanoma patients reaches 80–100% and 91–100%, respectively [5, 6]. There are good reasons to use IMBA labelled with ^{123}I; image quality would be better and the effective radiation dose to the patients would be substantially lower. However, the high cost of the radiopharmaceutical and logistical difficulties form a substantial obstacle.

N-alkylated benzamide derivatives are also attractive from the standpoint of PET-diagnostics. Ren et al. [13] synthesized an analogue labelled with fluorine-18 [^{18}F]-FBZA. This compound was avidly taken up by melanoma cells (B16F10) and respective experimental tumours in C57BL/6 mice [13]. Thus benzamide derivatives may form an interesting alternative to fluorodeoxyglucose in PET diagnostics of melanoma.

The IMBA compound labelled with ^{131}I could perhaps also play the role of a therapeutic radiopharmaceutical, due to emission of β particles by this radionuclide. In recent studies a concept has been put forward that N-alkylated benzamides could play a role as specific agents in targeted radionuclide therapy due to their strong affinity for melanoma cells and their high retention in tumours [14, 15]. Joyal et al. tested a new benzamide derivative labelled with ^{131}I-N-(2-diethylaminoethyl)-4-(4-fluoro-benzamido)-5-iodo-2-methoxy-benzamide (MIP-1145). This compound was vividly accumulated — with prolonged retention — in experimental melanoma tumours in mice (SK-MEL3). This compound significantly retarded the tumour growth in the animals and after a series of administrations led to regression of the neoplasm [14]. A radiotoxic effect of administered benzamide derivative — [^{125}I]-N-(4-dipropylaminobutyl)-4-iodobenzamide ([^{125}I]BZ18) — was also observed by Labarre et al. in in vitro cultures of B16F0 melanoma [15].

Conclusions

Preliminary scintigraphic studies performed in patients with documented melanoma metastases led to visualization of the neoplastic lesions. Scintigraphy should be performed 24 hours after injection of the radiopharmaceutical (^{131}I-IMBA). Earlier scintigraphy is not recommended due to substantial accumulation of the compound in the lungs and liver.

These observations indicate the potential use of radioiodinated IMBA radiopharmaceutical in the diagnostics of melanoma in humans. To obtain more reliable data on the diagnostic efficacy of the procedure a study on a larger group of patients is required.

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