Lymphoscintigraphy of melanoma: Lymphatic channel activity guides localization of sentinel lymph nodes, and gamma camera imaging/counting confirms presence of radiotracer in excised nodes

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Lymphoscintigraphy has become a standard preoperative procedure to map the cutaneous lymphatic channel for progression of nodal metastasis of melanoma of the skin. Lymphoscintigraphy was employed to visualize lymphatic channels as a guide to identify sentinel lymph nodes (SLNs). Excised tissue was imaged with a gamma camera to verify the findings of presurgical lymphoscintigraphy. Percent counts of SLN(s) among the total counts of the excised melanoma tumor or scar tissue and SLN(s) were calculated.

Methods: Eleven patients with cutaneous melanoma received four to ten intradermal injections of Tc-99m sulfur colloid at elual distances around the melanoma site. Images were made immediately after injection: 1 minute per image for 15 min; and then 5 minutes or 1,000,000 counts per image for 30 min. After surgery, the excised melanoma tumor or scar and SLN(s) were imaged/counted with a gamma camera. Percent counts of SLNs among the total counts of the excised melanoma tumor or scar tissue and SLNs were calculated. To validate the specimen count accuracy, an experimental phantom study was done.

Results: Linear lymphatic channels were identified between the injected sites and the SLNs in each patient. Gamma camera images demonstrated radioactivity in the SLNs of all patients, verifying the lymphoscintigraphy findings. Uptake in the SLNs of ten of the eleven patients ranged from 0.4 to 7.2% (mean 2.2%) of the total counts in excised tissue. We noted that a node with lower uptake should not be ignored because a lower percent of SLN activity does not necessarily rule out existing metastasis. In two of eleven patients, histopathologic showed metastases. One patient's melanoma on the middle back had lymphatic channel activity directed to both axillae. The results of the phantom study validated accuracy of our specimen counts.

Conclusions: Because linear lymphatic channels existed between lymph nodes and the injected sites in all eleven patients, these lymphatic channels could be used as a guide for localizing SLNs. The SLNs indicated by presurgical lymphoscintigraphy were verified by postoperative gamma camera imaging, and radiotracer localization in the SLNs averaged 2.2%.

Key words: melanoma, lymphoscintigraphy, sentinel node, lymph node, Tc-99m sulfur colloid, phantom study

INTRODUCTION

MELANOMA is a cutaneous malignancy with straightforward lymphatic flow, which metastasizes first to the regional lymph nodes. The direction of lymphatic drainage often varies, especially in the torso.[1-5] Preoperative

lymphoscintigraphy in a patient with melanoma allows surgeons to identify all nodal basins at risk for metastasis, to estimate the number of sentinel lymph nodes (SLNs) in the regional basin,[6-11] and to detect drainage to the contralateral axillary or inguinal nodes on the torso.[6-9] SLN is defined as the first node to receive lymphatic drainage from a tumor site and it will develop into a tumor if there has been any lymphatic spread,[9] so that pathological sampling of the SLN is sufficient to assess the involvement of the lymphatic bed.[9]

There is great variation between patients in cutaneous lymphatic drainage. Lymph node drainage is especially unpredictable if the melanoma is located in the lower chest wall, the upper abdominal wall, or the back near the midline.[1] To confirm the SLNs detected by lymphoscintigraphy, we measured the removed SLN uptake of ten patients with gamma camera imaging/counting, and we also compared SLN uptake with the uptake of the surgically removed scar tissue or melanoma tumor tissue of the skin, which had been injected radiopharmaceutically.

MATERIALS AND METHODS

Eleven consecutive patients who were proven to have malignant melanoma histopathologically, by exisional, incisional, punch, or saucer biopsy, were enrolled. Seven to 21 days after the biopsy, these eleven patients were referred to the Nuclear Medicine Service for lymphoscintigraphy for lymph node dissection. Lymphoscintigraphy, surgery, and *ex vivo* counting were carried out on the same day. Typically a patient with malignant lymphoma underwent scintigraphy at 8:30 in the morning, and surgical procedures at 1:30 in the afternoon; and *ex vivo* counting for scar/tumor tissue dissected sentinel lymph node was performed at 4:00 in the afternoon. Surgical procedures performed included the excision of the scar/tumor tissue where the melanoma was located, and sentinel lymph node dissection where the lymphoscintigraphy was localized. Examination of the specimens included the presence or absence of malignant melanoma in the scar/tumor tissue, dissected sentinel lymph node(s), and excisional margins were whether free or not free of malignant melanoma cells.

1. Radiopharmaceutical preparation. Tc-99m sulfur colloid with a reduction of heating method and used in this study. The radiopharmaceutical was prepared by the reduced heating method filtration were (i.e., the boiling-water-bath incubation period was shorter than that for making conventional sulfur colloid).[12] With that modification, $\geq 36\%$ of Tc-99m sulfur colloid was less than 0.03 μm.[12] The prepared radiopharmaceutical was filtered and drawn into a 1-m*l* syringe with a 25- or 26-gauge needle. Each syringe contained about 3.7 MBq (100 μCi) in 0.1 m*l*; each patient received four to ten intradermal injections, and usually two to five syringes

were used. The injected radiopharmaceutical dose ranged from 4.4 MBq to 18.5 MBq (119 μCi to 500 μCi).

2. Imaging. With the scar or tumor site of the skin exposed, the patient was placed supine or in the prone position on an imaging table. After cleansing the skin over the scar or tumor lesion area with alcohol, four to eight skin wheals were created circumferentially by intradermal injections at equal distances from the melanoma or scar, and each injection was 1 cm away from the edge of the tumor lesion or scar. Injection sites were shielded with lead, and imaging was performed with a large field-of-view and a low-energy, all-purpose parallel hole collimator. The following sequences were obtained: 1 image per min for 15 min; then 5 minutes per images for 30 min. After obtaining the routine anterior-posterior views or posterior-anterior views of the chest/abdomen, back, upper extremity, lower extremity and axillary/inguinal regions were performed; and lateral, oblique, or appropriate views (of the axillary region, for example) were also obtained as required. Marks were made with indelible ink on the skin over SLN(s). The surgeon to do resection of the SLN was usually present to discuss the surgical approach and position.

3. Specimen imaging/counting. Surgically resected SLN(s) and removed scar or tumor tissue with skin were imaged in the afternoon of the day of surgical resection. These specimens were placed directly on an inverted large field-of-view gamma camera and imaged simultaneously for 10 minutes in all cases but two (case 1: scar tissue not imaged, case 3: inadvertent counting time of 2.1 minutes). The collimator face was covered to prevent contamination. Because the scar or tumor specimen contained the majority of counts, the distance between this and the SLN(s) was kept to at least 10 cm. That prevented any photons originating in the scar or tumor tissue in being detected in the area of the SLN(s). SLN activity was expressed as a percent of the total background corrected counts from individual regions-of-interest around the SLN(s) and scar or tumor specimens.

4. Phantom imaging/counting. To validate specimen imaging/counting accuracy, a phantom study was performed. Technetium-99m samples (~0.1 m*l* each) were injected into 1.5 m*l* Eppendorf tubes with a 1 m*l* syringe. Activities in each tube were determined by dose calibrator assays of the syringe before and after sample dispensing. The samples were allowed to decay to typical activities found in our actual cases. Six high activity inverted tubes were equally spaced (at 1 cm) around a circular area (4.2 cm diameter) that represented the injection site. These six tubes were placed on 1.2 cm thick polystyrene material to represent typical specimen thickness. Three low activity tubes representing SLNs were placed about 13 cm away from the injection site phantom and 8 cm apart from each

Table 1 The eleven patients' age, sex, primary melanoma tumor site, method of biopsy, presence of lymphatic channels, site(s) of SLN(s), per cent of SLN, and final pathological diagnosis of the primary site and SLN(s)

Case	Age	Sex	Primary tumor site	Method of biopsy	Lymphatic channels	SLN site(s)	% SLN activity[#]	Pathological excise Primary
1	77	M	R anterior abdomen	Excisional	Present	1, R axilla 1, R sternal	Axillary SLN = x^2 sternal uptake	Negative
2	81	M	R anterior chest	Incisional	Present	2, R axilla	0.4%	Positive
3	56	M	R forearm	Excisional	Present	3, R axilla	1.0%	Negative
4	73	M	Middle back	Excisional	Present	3, R axilla	0.7%	Negative
5	66	M	R upper back	Punch	Present	1, R axilla	1.3%	Negative
6	70	M	R upper arm, near shoulder	Saucer	Present	2, R axilla	2.7%	Negative
7	61	M	R forearm	Incisional	Present	2, R axilla	2.3%	Positive
8	62	M	R upper abdomen	Punch	Present	[##]2, R axilla 2, L axilla	0.8%	Positive
9	76	M	Back, center	Excisional	Present	2, R axilla	2.8%	Negative
10	43	M	R planta, near heel	Excisional	Present	[†]1, R groin	7.2%	Negative
11	78	M	L upper back	Excisional	Present	[††]1, L axilla	3.3%	Negative

mean 2.2% (n = 10, s.d. 2.0)

Note: Rt, right; SLN(s). sentillel lymph node(s). % SLN activity = percent SLN counts/total counts of SLN and scar or tumor tissue.
Axillary SLN = x^2 sternal uptake, counts of the axillary SLN were equal to twice counts of sternal SLN.
*One of the left axillary SLN(s) (hot and faintly blue, containing 0.4% of total counts) was positive for metastatic melanoma.
[#]The mean percent of SLN activity is 1.6. [##]The first node contained 0.3% of total counts, while the second node contained 0.5%.
[†]Only one lymph node was dissected. [††]Only the left axillary lymph node was dissected.

Table 2 Injection site and SLNs phantom activity expressed as a percent of total background corrected counts from individual regions-of-interest around each phantom

	Total Activity at Time of Assay/ Imaging MBq (μCi)	% of Total Phantom Activity/Counts			
		Injection Site	SLN #1	SLN #2	SLN #3
Initial Phantom Dose Calibrator Assays (% based on syringe assays)	66.3 (1791)	96.1	2.4	1.1	0.4
Simultaneous Phantom Acquisition—High Counts	9.8 (266)	96.2	2.3	1.1	0.4
Simultaneous Phantom Acquisition—Low Counts	3.4 (91)	96.1	2.4	1.1	0.4
Individual Phantom Acquisition—High Counts	10.1 (273)	96.2	2.3	1.1	0.4
Individual Phantom Acquisition—Low Counts	3.4 (93)	96.0	2.4	1.2	0.4
Individual Phantom Acquisition—Low Counts & w/Acrylic Plastic	3.1 (85)	95.7	2.6	1.2	0.5

other. The same specimen simultaneous imaging and counting parameters were used for the phantom studies. Additional individual imaging of the SLNs and injection site phantoms was done. An additional injection site phantom image was taken with 0.9 cm thick of acrylic plastic in place of the polystyrene material.

5. *Histopathologic examinations.* The specimens were quarantined for 48 h before histopathological sectioning.[13] All histopathologic examinations included special stains for HMB45 (HMB45 is a mouse anti human mono-clonal antibody, and reactions with intracytoplasmic antigen have been defined by immunochemistry. This testing supports a diagnosis of malignant melanoma and S100 (S100 is rabbit anti cow polyclonal antibody for histochemistry testing to support a diagnosis of malignant melanoma; both reagents are commercially available from DAKO Corporation) to demonstrate metastatic melanoma. Surgeons employed both blue dye injection and a hand-held gamma probe. Excised specimens were labeled "blue" or 'not blue" and "hot" or "not hot" in accordance with intraoperative observations.
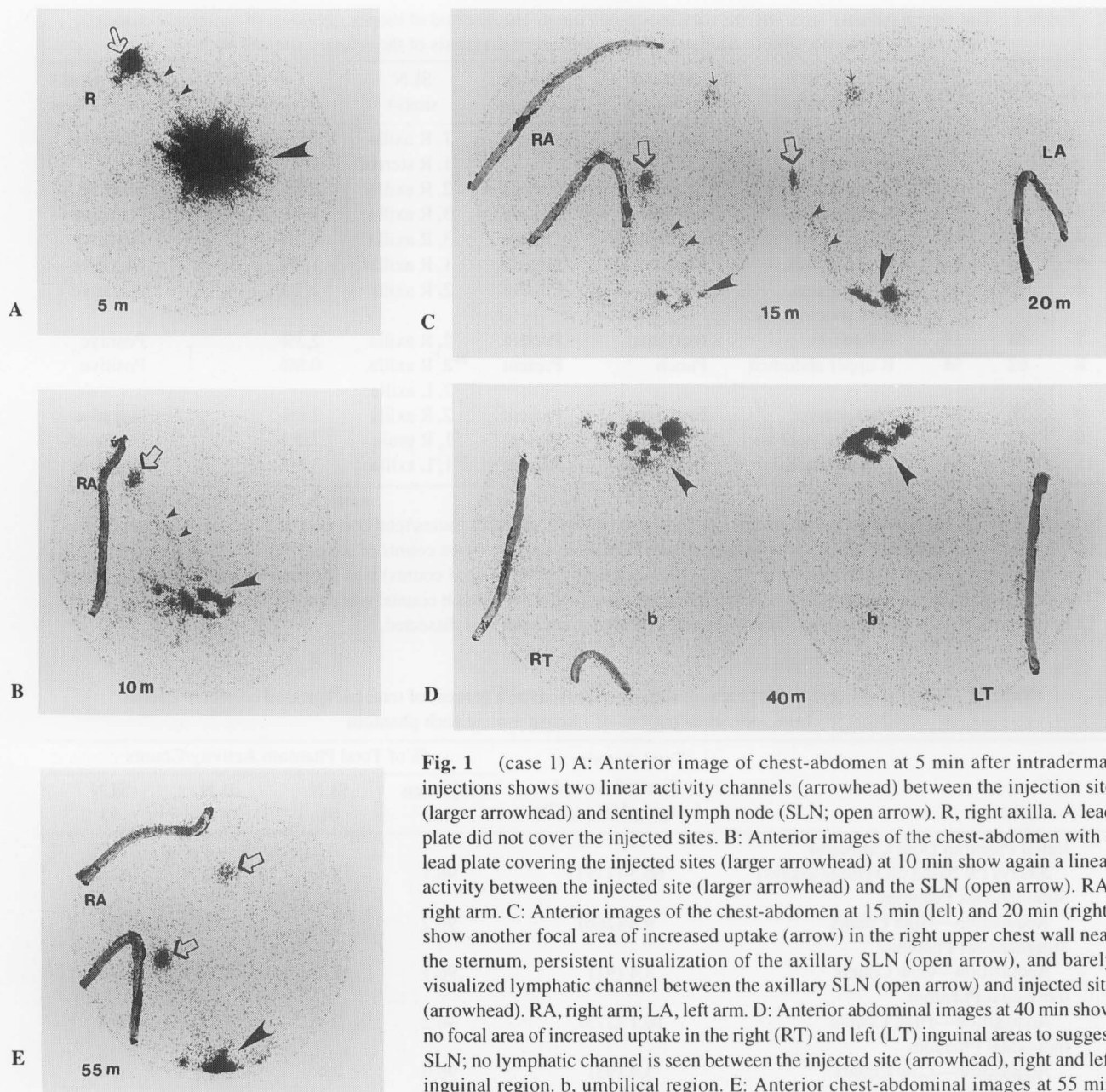
Fig. 1 (case 1) A: Anterior image of chest-abdomen at 5 min after intradermal injections shows two linear activity channels (arrowhead) between the injection site (larger arrowhead) and sentinel lymph node (SLN; open arrow). R, right axilla. A lead plate did not cover the injected sites. B: Anterior images of the chest-abdomen with a lead plate covering the injected sites (larger arrowhead) at 10 min show again a linear activity between the injected site (larger arrowhead) and the SLN (open arrow). RA, right arm. C: Anterior images of the chest-abdomen at 15 min (lelt) and 20 min (right) show another focal area of increased uptake (arrow) in the right upper chest wall near the sternum, persistent visualization of the axillary SLN (open arrow), and barely visualized lymphatic channel between the axillary SLN (open arrow) and injected site (arrowhead). RA, right arm; LA, left arm. D: Anterior abdominal images at 40 min show no focal area of increased uptake in the right (RT) and left (LT) inguinal areas to suggest SLN; no lymphatic channel is seen between the injected site (arrowhead), right and left inguinal region. b, umbilical region. E: Anterior chest-abdominal images at 55 min show activity of the axillary SLN (open arrow) to be higher than that of the upper chest SLN (open arrow 2); the lymphatic channel between the injected sites and axillary SLN is no longer seen.
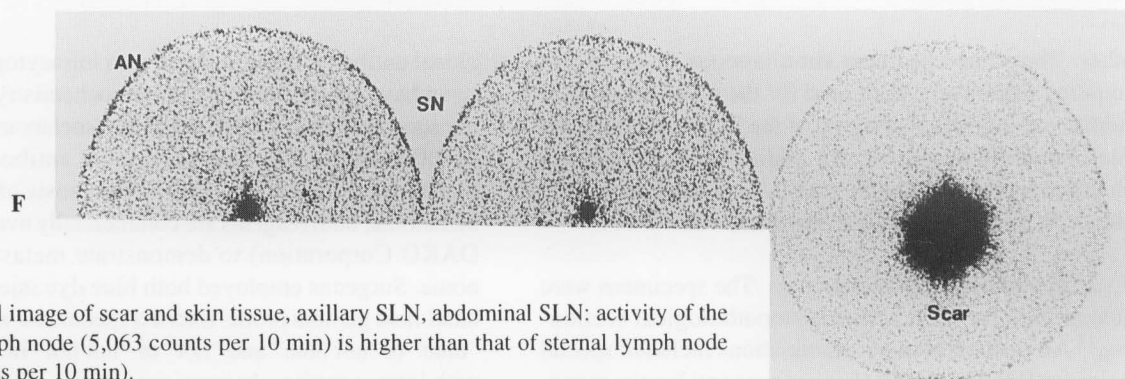
F: Individual image of scar and skin tissue, axillary SLN, abdominal SLN: activity of the axillary lymph node (5,063 counts per 10 min) is higher than that of sternal lymph node (2,613 counts per 10 min).

Surgically resected SLN(s) and removed scar tissue or tumor tissue with skin were imaged in the afternoon of the day of surgical resection. Excised scar/tumor tissue and sentinel lymph node was counted with a gamma camera for 5 minutes simultaneously. The detector of a gamma camera was set upward direction which as covered a sheet of plastic draper for prevention of contamination purpose, then the specimens were put on the draper and counted. For individual SLN, the specimens was counted for 5 minutes; for the specimens of skin/tumor tissue and SLN simultaneously counted for 10 min. SLN activity was calculated as percent of the total counts of SLN and scar or tumor tissue: SLN counts were divided by total counts. The specimens of the excised scar/tumor tissue and dissected lymph nodes of case 1 were counted separately and not counted simultaneously. The specimens were quarantined for 48 h before histopathologic sectioning.[13] All histopathologic examinations included special stains for HMB45 (HMB45 is mouse anti human monoclonal antibody, reactions with intracytoplasmic antigen have been defined by immunochemistry. This testing is supporting a diagnosis of malignant melanoma and S100 (S100 is rabbit anti cow polyclonal antibody for histochemistry testing to support a diagnosis of malignant melanoma; both reagents are commercially available from DAKO Corporation) to demonstrate metastatic melanoma. Surgeons employed both blue dye injection and a hand-held gamma probe. Excised specimens were labeled "blue" or "not blue" and "hot" or "not hot" in accordance with intraoperative observations.

RESULTS

The locations of these 11 patients' cutaneous melanomas were as follows: the right anterior abdomen in 2 patients, middle back in 2 patients, right forearm in 2 patients, right anterior chest in 1 patient, right upper back in 1 patient, left upper back in 1 patient, right upper arm near the shoulder in 1 patient, and right planta near the heel in 1 patient. Among these 11 patients six patients underwent excisional biopsy before lymphoscintigraphy, and all the excised specimens of these patients were cited as resection margins free of tumor cells. The patient's specimen from the saucer biopsy was noted as the resection margin free from tumor cells. The excised specimens of the two patients undergoing incisional biopsy and of the 2 patients undergoing punch biopsy confirmed melanoma histopathologically, but their resection margins were of tumor cells. The interval between diagnostic (excisional, incisional, punch, and saucer) biopsies and the corresponding lymphoscintigraphy was from 2 to 5 weeks.

Table 1 summarizes the eleven patients age, sex, primary location of the cutaneous malignant melanoma, methods of initial biopsy, status of visualization of lymphatic channels, site of SLN localization, percent of SLN activity and final pathological findings in the tissue ex-

cised from the primary tumor site and axillary SLN. A gamma camera confirmed the presence of radiotracer in each SLN that had been identified through lymphatic channels. Immediate imaging after injections of Tc-99m sulfur colloid allowed us to visualize lymphatic channels and note their direction over time in all ten patients. "Hot or not hot" and "blue or not blue" nodal detection accorded with postoperative gamma camera imaging/counting. The patients' SLNs were confirmed as shown on the respective lymphoscintigraphies. The percent of radioactivity in the SLN(s) over total counts of SLN(s) and scar or tumor tissue was calculated in ten of the eleven patients (except for patient No. 1), showing uptakes of 0.4%, 1.0%, 0.7%, 1.3%, 2.7%, 2.3%, 0.8%, 2.8%, 7.2%, and 3.3% (mean, 2.2%).

Phantom imaging/counting. Phantom activity was expressed as a percent of total background corrected counts from individual regions-of-interest around each SLNs and injection site phantom. Percent activities in both the injection site and SLNs phantoms when imaged simultaneously or individually were similar to initial percent activities determined from actual dose calibrator assays (Table 2).

CASE REPORTS

Case 1
This 77-year-old white man with malignant melanoma in the right upper abdominal skin underwent lymphoscintigraphy. Immediately after injection of Tc-99m sulfur colloid, images were obtained of the chest and thoracoabdominal region. The first 5-mill image showed a focal area of increased uptake in the right axillary area, and two linear activity areas were noted between the injected site and the axillary lymph node (Fig. 1A). At 10 min, the area of the abnormally increased uptake in the right axial area and the linear activity between the injected sites and the axillary activity were persistently visualized (Fig. 1B). The 15-min and 20-min images (Fig. 1C) showed another focal area of increased uptake located in the upper chest wall near the sternum, though with less intense activity than that of the first node. In the 40-min images of the right lower abdomen and inguinal area and of the left lower abdomen and inguinal area, no tracer localization appeared (Fig. 1D). The right chest wall images at 55 min showed that an area of high radioactivity in the right axillary region and one of relatively lower activity still persisted, but the linear activity between the axillary area and the injected site no longer existed (Fig. 1E). Three excised specimens—an axillary lmph node, a lymph node near the sternum, and the skin tissue with scar—were imaged individually. The axillary lymph node count (5063 counts/10 min) was nearly twice that of the sternal lymph node count (613 counts/10 min) (Fig. 1F). The results of the pathological examinations of these SLNs and the scar tissue were negative for malignancy.
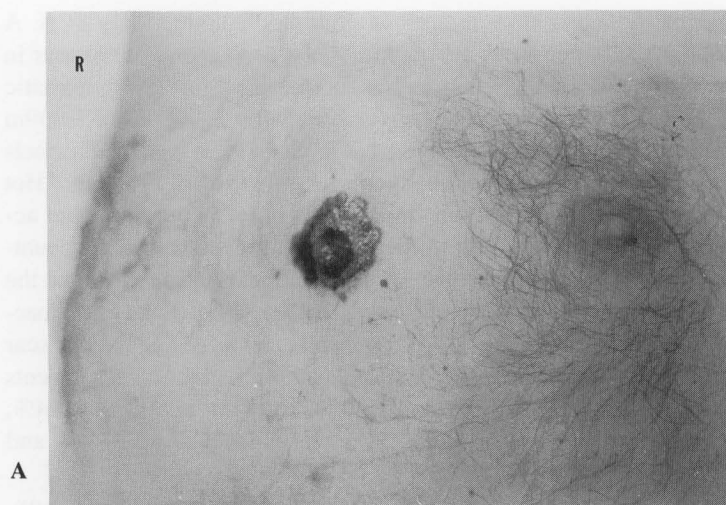
A

B

C

20 m

D

E

Fig. 2 (case 2) A: A photo taken from the right anterior chest wall of case 2 shows an elevated and rounded dark-reddish to black skin lesion measuring 3.5 × 3 cm in the right upper chest wall lateral to the right nipple. B: Sequential anterior chest images made every 1 min show barely visualized linear lymphatic channel (arrows) radiating from the injected sites to the upper right. RS, marked on the right shoulder. C: Anterior chest image at 20 min shows two SLN in the right axilla with linear lymphatic channels (smaller arrows) between the injected sites (right) and SLN (arrows). D: Surgical specimens consist of the tumor lesion from the right chest wall with injection of blue dye and SLN 1, SLN 2, and adipose tissue marked X. E: The specimens shown on figure 2D imaged simultaneously show tracer accumulation in the tumor of the skin, SLN 1 and SLN 2; two specimens with adipose tissue marked X do not have any activity. Arrowhead indicates Co-57 marker activity.
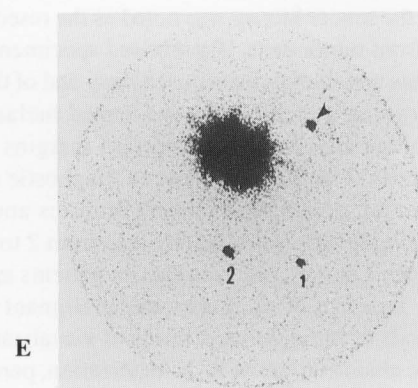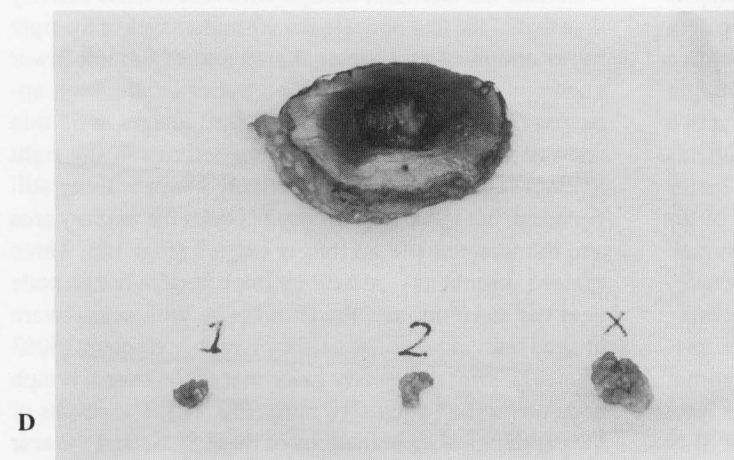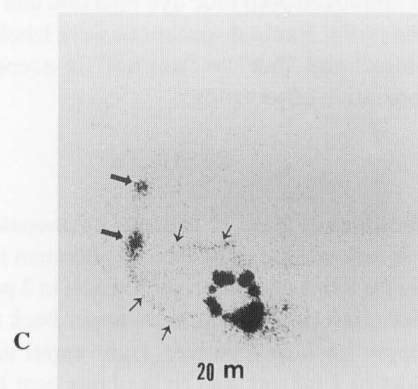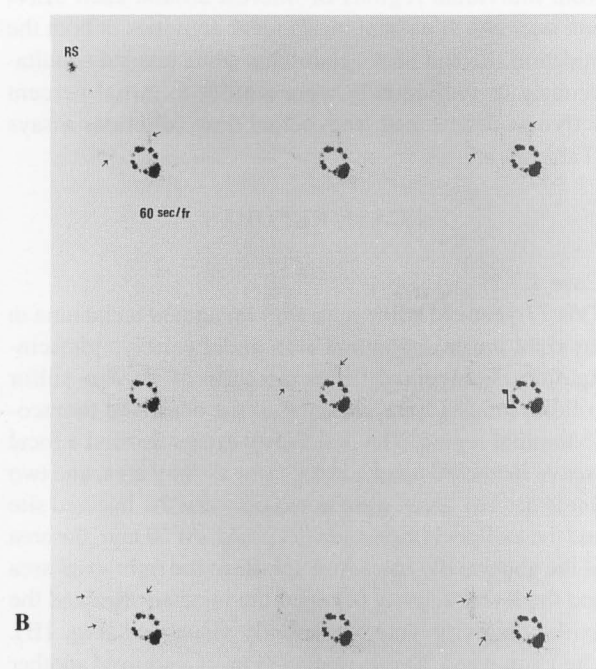
Case 2

An 81-year-old man with a malignant melanoma of his right anterior chest wall, confirmed by an incisional biopsy three weeks earlier, was reterred for SLN mapping for surgical resection of the tumor. On examination, an elevated and rounded dark-reddish to black lesion measuring 3.5 × 3 cm was identified in the right upper chest wall, lateral to the right nipple (Fig. 2A). Tc-99m sulfur colloid (18.5 MBq) was intradermally injected around the
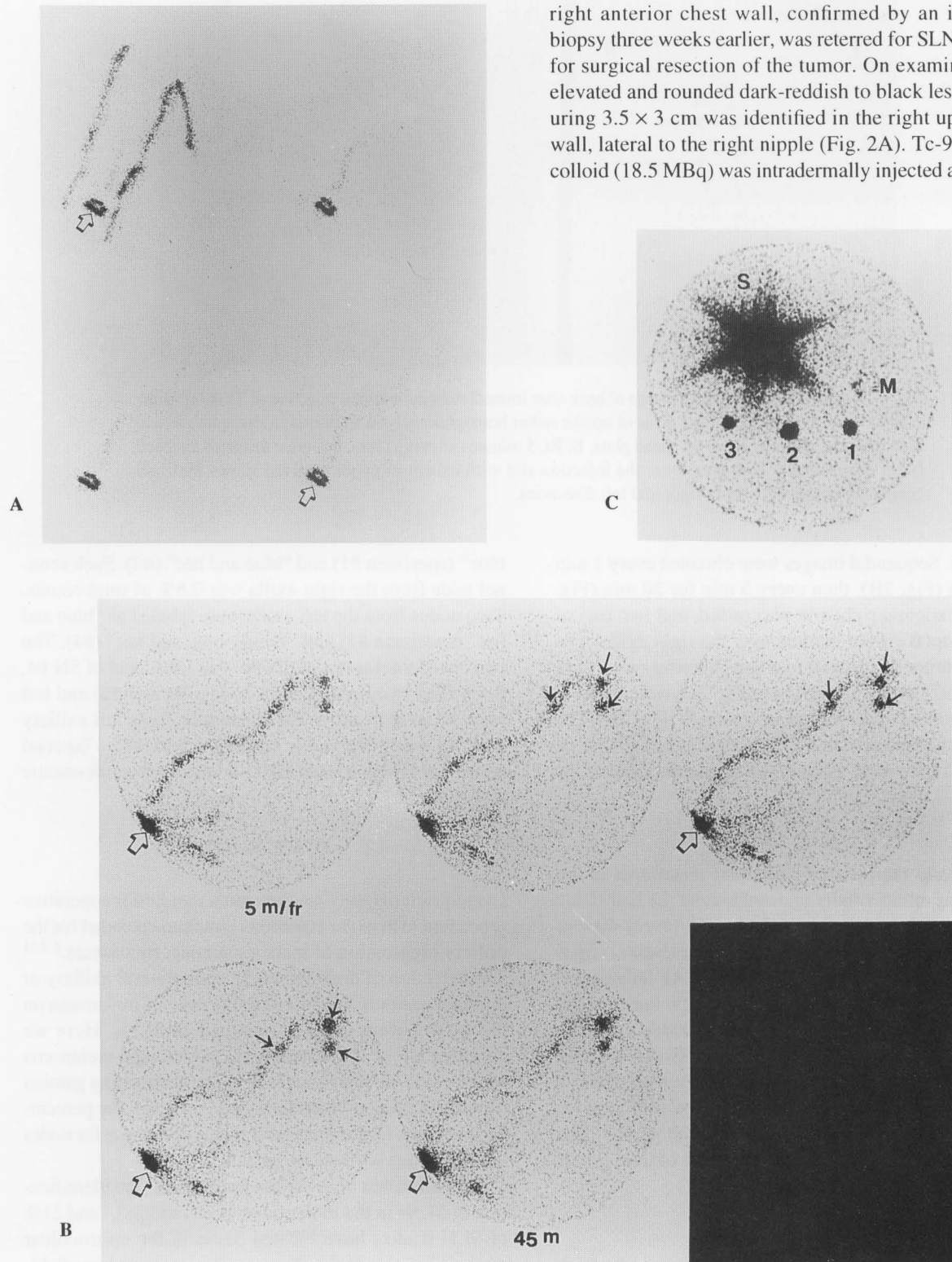
Fig. 3 (case 3) A: Sequential images made every 60 seconds show a barely visualized linear lymphatic channel (arrows) from the injected sites (opell arrow) in an axillary direction. B: Subsequent images taken every 5 min show two linear lymphatic channels from injected sites (open arrow) in an axillary direction connecting the SLNs (arrows). C: Scar tissue and skin with injected blue dye, three SLNs marked 1, 2, and 3, and adipose tissue from axillary region.
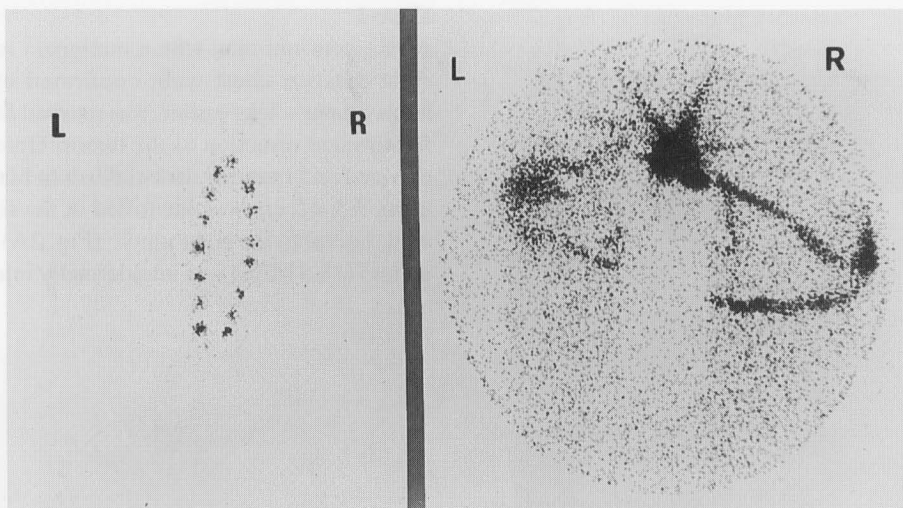
Fig. 4 (case 9) A: Lt, posterior image ol back after immediately intradermal injection of Tc-99m sulfur colloid show multiple small dot areas of uptake rather homogeneously in an ellipse in shape on vertical direction; the skin was cover by a lead plate. B: Rt, 5 minutes after injection, posterior image of the back (with covered by a lead plate over the injection site with except in upper portion) shows multiple lymphatic channels running right and left directions.

skin lesion. Sequential images were obtained every 1 min for 15 min (Fig. 2B), then every 5 min for 20 min (Fig. 2C). The patient's right arm was raised, and two foci of increased uptake were marked over the right axilla. The removed tumor lesion and two lymph nodes (each "not blue but hot") with adipose tissue (Fig. 2D) were simultaneously counted under the gamma camera (Fig. 2E). The counts of the two resected SLNs contributed 0.43% of total counts (one node's count was higher than that of the other).

Case 3
A 56-year-old man with a malignant melanoma of the forearm was intradermally injected around the scar tissue on the right forearm. Sequential imaging (every 60 sec) showed barely visualized linear lymphatic channels from the injection sites to the right axilla (Fig. 3A). Subsequent imaging every 5 min identified three lymph nodes in the right axilla (Fig. 3B). A skin marker was made while the patient's arm was raised. The three SLNs and the scar tissue (Fig. 3C) were simultaneously counted under a gamma camera. The three nodes were radioactive, confirming SLNs as shown on lymphoscintigraphy. The counts of the three SLNs contributed 1.0% of total counts of SLN and scar/tumor tissue.

Case 9
A 76-year-old man with melanoma in the middle back was referred for lymphoscintigraphy. Five minutes after intradermal injection of Tc-99m sulfur colloid, the images from the back showed lymphatic activity channels directing to the right and left axillae (Fig. 4). Two sentinel lymph nodes from the right axilla were labeled as "hot not

blue" (specimen #1) and "blue and hot" (#2). Each sentinel node from the right axilla was 0.6% of total counts. Two nodes from the left axilla were labeled as "blue and hot" (specimen #3) and "faintly blue and hot" (#4). The percent of total count of SN #3 was 1.2%, and of SN #4, 0.4%. The two right axillary nodes (#1 and #2) and left node #3 were negative for melanoma. Only left axillary node #4 was positive for metastatic melanoma (special stains for HMB 45 and S100 demonstrated metastatic melanoma).

DISCUSSION

Lymphoscintigraphy has become a standard preoperative procedure to map the cutaneous lymphatic channel for the orderly progression of melanoma nodal metastases.[8,9,11] The detection of drainage to the contralateral axillary or inguinal nodes is invaluable in the case of melanoma on the torso before surgical removal ot SLNs. Here we compare ten of the eleven patients' excised melanoma tumors, skin, or scar tissue with the corresponding gamma camera imaging of their SLNs, and compare the percentage of counts for the metastatic nodes with those for nodes and the nodes without metastasis.

For melanoma of the upper extremities, the identification of SLNs in the midarm has been reported,[2] and 21% of SLN studies have located SLNs in the epitrochlear region.[3] Imaging in the elbow region employ a large field-of-view gamma camera. In this way it is practically impossible to should therefore miss epitrochlear lymph nodes. Two patients (3 and 7) had a melanoma of the right forearm, from which potential lymphatic drainage might go through the right midarm or to the right axilla. Imaging

did not show SLNs in the mid-elbow region. Similarly, case 6's melanoma lesion was located in the right upper arm near the shoulder; the potential lymphatic drainage was to the right neck or to the right axilla. Since the imaging indicated that SLNs were limited to the right axilla, and were not present in the right cervical region, cervical surgical intervention was avoided. The results of the pathological section of SLN were all negative for melanoma.

Similarly for the melanoma lesion in the lower extremity, such as that of case 10, which was located in the right foot, planta near the heel, lymphatic drainage was potentially in the region of the posterior aspect of the knee. But our patient's sentinel lymph node(s) were located in the right groin, and linear lymphatic activity channels were directed cephalically toward the right groin area. This patient's % SN uptake was under calculated.

Presurgical lymphoscintigraphy was essential for surgeons to avoid unnecessary surgical intervention for lymph node dissection in malignant melanoma patients. Theoretically searching for sentinel lymph node localization may be in the following categories: between ipsilateral cervical versus axillary, between ipsilateral axillary versus inguinal, one side cervical versus contralateral cervical, one side axillary versus contralateral axillary, and one side inguinal versus contralateral inguinal region. For example, our cases 1, 2, 4, 5, and 11 belonged to the category of one side axillary versus contralateral axilla. These cases could also be belonged to the situations ipisilateral between cervical and axilla and ipsilateral between axillary and inguinal. In any case, sentinel lymph nodes were localized at only the axilla on one side in these cases, and surgical intervention in the contralateral axilla, ipsilateral cervical, and/or ipsilateral inguinal area had been avoided.

To determine the necessary surgical intervention for sentinel lymph node, presurgical lymphoscintigraphy was also important. Case 9 was a good example: because the melanoma was located in the central back, this patient's lymphatic channels ran to both left and right axillae as seen on lymphoscintigraphy. This patient therefore underwent lymph node dissection from both axillae, and from the left axillary node there was metastasis.

It is understandable that the percentage of SLN uptake was not necessarily correlated with nodal metastasis; for example, in case 9 the percentage of three metastatic nodes was the lowest (0.4%) among the four SLN uptakes. Likewise, it has been reported that one lymph node was full of metastatic cancer cells, but its counts were very low.[14] The low counts in the SLN(s) with melanoma depositions might be explained by the fact that the metastatic cells loaded in the SN might have resulted in complete or partial lymphatic obstruction and/or the SN has been occupied fully by metastatic cells that did not allow radiopharmaceutical drainage to the SLN. Therefore a SN with a low counts should not be disqualified as

an SLN and a node with lower uptake should not be ignored because a lower percent of SLN activity does not necessarily rule out existing metastasis[14]; in contrast, lower activity SLNs should be suspected of having malignant deposition.

The harvesting of *in vivo* SLNs in the axilla and *ex vivo* (counted with gamma probe) from breast cancer has been reported.[15] In a gamma camera study, delivery of about 1% of injected to the regional basin in a single case has also been reported.[16] Our report included a gamma camera study of ten patients, which showed % counts in sentinel nodes ranging from 0.4% to 7.2% with a mean of 2.2%. Their accuracies have been validated by our phantom study.

In this study, in such as cases 2 and 3 we simultaneously visualized more than one lymph node. In case 2, the only node proximal to the tumor injected site contained a lymphatic channel and the lymphatic channel of the node in the cranial aspect was from the node proximal to the injected site, not from the injected site. Theoretically the only proximal node should be considered as SLN; however, its radiotracer was instantly transported to the one distal node in case 2 and two distal nodes in case 3. Therefore, these nodes might be in case 3, a linear lymphatic channel and three consecutive lymph nodes were noted, as indicated by arrows; the proximal node in relation to the injection site had gradually radioactivity whereas the two distal nodes had gradually increased radioactivity. Therefore, these nodes might be in case 3, a linear lymphatic channel and three consecutive lymph nodes were noted, as indicated by arrows; the proximal node in relation to the injection site had gradually radioactivity whereas the two distal nodes had gradually increased radioactivity. Theoretical only the proximal node should be considered as a 'SLNs' but its radiotracer was instantly transported to the one distal node in case 2 and two distal nodes in case 3. Therefore, these nodes might be considered as 'SLN,' and our surgeons had dissected these three nodes which were counted as "SLNs." In such cases, if the distal nodes were not considered as sentinel nodes, the % uptake of the SLN appeared to have been overestimated. Nevertheless, in a recent report mammary lymphoscintigraphy illustrated 3 lymph in the internal mammary chain 4 hr after intratumor administration, and these 3 nodes were considered to be setinael nodes.[17]

A node is "in-transit" when there is demonstrable lymphatic channel activity from the injection sites to the SLN(s), as shown in images from our patients. All our patients had such in-transit nodes because of our imaging immediately after injections of Tc-99m sulfur colloid as shown on Figures 1–4.

Delay in obtaining an image runs the risk of missing the SLN.[11] In addition, dynamic imaging allows visualization of lymphatic vessels over time, which can improve tracking of the drainage pattern.[11] Our patients' lymphatic channels appeared to be more prominent than indicated by

Tc-99m human serum albumin.[18] We suggest that the presence of lymphatic channel activity may serve as a guide for SLN(s) localization. All ten patients had a few linear radioactivity channels, and in each an in-transit node between the injected site and the axillary SLNs was identified (Figs. 1–4). These channels of activity may be used as a guide to locate the SLNs.

Not all patientse' SLNs had metastatic malignant melanoma. In three reports,[1-3] 70 of 383 patients who underwent complete lymphadenectomy had nodal metastases (18%). In our report, two (cases 8 and 9) of eleven patients were positive for metastases in SLNs (18.2%).

During the skin marking procedure, it is preferable that the surgeon responsible for the biopsy be present. Just as in open rib biopsy guided by the radionuclide technique,[19] skin marking for the biopsy for SLNs should be done with the patient in a position that is comfortable and yet provides access for the operation. To make a skin marker in the axilla, the arm should be held above the head or the arm outstretched to image the SLN area in two different views. The raised arm position is preferable for axillary lymphadenectomy.[2]

A surgeons' intraoperative mapping techniques for SLN(s) include blue dye injection and a hand-held gamma probe. Blue dye provides the surgeon with a visual guide in identifying the SLN but several complications arise with blue dye in SLN mapping.[20] The radiocolloid used in the cases presented here more reliably migrates to the regional basin; thus the gamma probe method provides a sensitive method to identify SLNs. A study showed that the blue dye mapping technique identified only 69.5% of SLNs as compared with 83.5% identified by the gamma probe; the combined use of both techniques localized the SLN with a 96% success rate.[8] A gamma probe alone identified only two SLNs in case 2. Our patients' intraoperative "hot or not hot" and "blue or not blue" findings agreed with *ex vivo* gamma camera imaging/counting.

In case 4 there was a discrepancy between the *in vivo* images indicating one SLN in the right axilla and surgical findings in three nodes in this location. The discrepancy may be explained by the arrangement of the three nodes, either overlapping or in a cluster that could not be resolved by imaging from an anterior projection. Alternatively, the single image could be explained if these three nodes overlapped. In the latter situation, radioactivity of these three nodes would accumulate in one area (as imaged from one direction). This is why in the *in vivo* images; the activity in the right axillary region was greater than usual.

CONCLUSIONS

In conclusion, lymphoscintigraphy-mapped tracer localization allowed the SLN(s) to be detected prior to surgery, thus (a) guiding appropriate surgical exploration in the site(s) having SN(s) and (b) avoiding unnecessary surgical intervention in the site(s) with no tracer localization area(s). In addition, in this report we especially emphasize the following points: (1) the resected lymph nodes in our patients were counted with a gamma camera to verify that they were the SLNs shown on the respective lymphoscintigraphies; (2) these patients' SLNs ranged from 0.4% to 7.2% (mean, 2.2%) of total counts of SLN and scar or tumor tissue; (3) all 11 patients' lymphoscintigraphies demonstrated lymphatic channel activity (in-transit nodes); and its presence may serve as a visual road map in the identification of SLNs; and (4) two of the 11 patients were positive for SLN metastasis.

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