Abstract
Evaluation of $[^{99m}Tc]oxotechnetium(V)$ complexes of the amine-amide-dithiol (AADT) chelates containing tertiary amine substituents as small-molecule probes for the diagnostic imaging of metastatic melanoma has shown that technetium-99m–labeled AADT-(CH$_2$)$_2$-NEt$_2$ ($^{99m}$Tc-1) has the highest tumor uptake and other favorable biological properties. We have, therefore, assessed this agent in a more realistic metastatic melanoma model in which, after i.v. tail injection, a highly invasive melanoma cell line, B16F10, forms pulmonary tumor nodules in normal C57BL6 mice. Small melanotic lesions develop in the lungs and, on histologic examination, appear as small black melanoma colonies, increasing in size and number with time after tumor cell injection. Groups of mice received tumor cell inocula of 2 $\times$ 10$^5$, 4 $\times$ 10$^5$, or 8 $\times$ 10$^5$ B16F10 cells; 14 days later, 2 hours after $^{99m}$Tc-1 administration, lung uptake of 2.83 $\pm$ 0.21%, 3.63 $\pm$ 1.07%, and 4.92 $\pm$ 1.61% injected dose per gram of tissue (% ID/g), respectively, was observed, compared with normal lung uptake of 2.13 $\pm$ 0.2% ID/g ($P < 0.05$). Additionally, a higher level of $^{99m}$Tc-1 accumulation was seen 17 days after tumor cell inoculation as the lung lesions grew. These in vivo studies coupled with additional in vitro and ex vivo assessment show that $^{99m}$Tc-1 has high and specific uptake in melanoma metastases in lungs and can potentially follow the temporal growth of these tumors. (Cancer Res 2005; 65(12): 4979-86)

Introduction
Malignant melanoma is a public health challenge with a rising incidence rate and estimates of 47,700 new cases to be diagnosed annually (1–3). The significant mortality of this cancer is associated with its high cellular proliferation rate and the early occurrence of metastases. Because early detection of the melanoma and its associated metastases considerably improves management and prognosis of the disease, there has been intense interest in the search for melanoma-specific diagnostic imaging probes and therapeutic agents (4, 5).

$[^{18}F]$Fluoro-2-deoxy-D-glucose ($[^{18}F]$FDG) is widely used in positron emission tomographic (PET) imaging of various tumors including melanoma. In patients with stage III or in-transit melanoma, prospective whole-body $[^{18}F]$FDG PET studies display a sensitivity of 87.3% with a positive predictive value of 78.6%, which can be further improved with the help of other pertinent clinical information (6). Other prospective clinical studies have also shown the utility of whole-body $[^{18}F]$FDG PET imaging in stage II to IV melanoma with sensitivity and specificity of 94.2% and 83.3%, respectively, for lesions in the soft tissue, lymph nodes, and liver (7); false-positives are due to the accumulation of $[^{18}F]$FDG in surgical wounds, pneumonia, and other etiologies including infection/inflammation (6–9).

A number of radiolabeled, single-photon emission computed tomography (SPECT) metabolic tracers, peptides, and monoclonal antibodies have also been investigated for melanoma detection, staging, and follow-up (4, 5). Among these SPECT agents, the most promising are the radiiodinated benzamides (10–14). Although the nature of the affinity of iodobenzamides for melanoma is not clearly understood, it has been shown that radiolabeled iodobenzamides possess a considerable in vivo affinity for melanotic melanoma lesions compared with amelanotic lesions (10). Animal and human studies of several radioiodobenzamide derivatives indicate a promising profile for their use in melanoma diagnosis. A scintigraphic phase II clinical study in 110 patients for detecting malignant melanoma and its metastases with $N$-(2-diethylaminoethyl)-4-$[^{123}$I$]$iodobenzamide ($[^{123}$I$]$BZA; Fig. 1) found 81% diagnostic sensitivity, 87% accuracy, and 100% specificity (11) for the compound. Additional studies to optimize the pharmaco kinetic characteristics of $[^{123}$I$]$BZA derivatives have shown that $N$-(2-diethylaminoethyl)-3-$[^{123}$I$]$iodo-4-methoxybenzamide ($[^{123}$I$]$IMBA; Fig. 1) has an 8-fold higher melanoma/nontarget tissue ratio than $[^{123}$I$]$BZA at 1 hour postinjection and a 4-fold higher ratio at 4 hours in the C57BL6 mouse model (12, 13). Preliminary scintigraphic studies in patients with melanoma metastases have confirmed the efficacy of this compound (12). More recently, a single-center clinical trial in 25 patients using another radioiodinated iodobenzamide derivative, $N$-(2-diethylaminoethyl)-2-[$^{[123}I$]iodobenzamide ($[^{123}$I$]$BZA$_2$; Fig. 1), showed 100% sensitivity, 95% specificity, a positive predictive value of 86%, and a negative predictive value of 100% on a per patient basis (14).

Whereas iodine-123 is commercially available and the radioiodobenzamide derivatives, such as $[^{123}$I$]$IMBA and $[^{123}$I$]$BZA$_2$, exhibit promise as potential SPECT agents for imaging metastatic melanoma, a technetium-99m–based radiopharmaceutical would have considerable advantages. Technetium-99m decays with a 6-hour half-life and is widely available on demand in most nuclear medicine facilities as a $^{99}$Mo–$^{99m}$Tc generator. It has a high photon flux and emits 140 keV $\gamma$-photons with ideal characteristics for SPECT imaging, making it very cost-effective and the radiolabel of choice for routine clinical imaging. Technetium complexes that contain structural elements of the benzamides have been explored, including technetium-99m–labeled nitridotechnetium-bis(aminethiol)benzamide (TcN-BAT-BZA) and oxotechnetium-bis(aminethiol)benzamide (TcO-CF; Fig. 1); the
latter has limited tumor uptake (<1.6% injected dose/gram (% ID/g) at 60 minutes postinjection) in the B16 mouse melanoma model (15–17).

Our attempts to design technetium-based, small molecules that possess structural elements of the benzamides are based on the strategy of replacing the aromatic moiety in the benzamide structure with a chelated metal-oxo core linked to the tertiary amine substituent. Applying this strategy via a “3 + 1” tridentate/monodentate chelating approach has produced [99mTcO(SNS)(S-C2-NEt2)] (Fig. 1) and [99mTcO(SNS)(S-C2-NBu2)] complexes with significantly higher tumor accumulation (3.1% and 5% ID/g, respectively, at 60 minutes postinjection; ref. 18) compared with the benzamide-containing technetium complex TcO-Cf. Because the use of these “3 + 1” complexes as imaging agents could be hampered by their known tendency to form transchelation products with glutathione in vivo (19), we have modified the strategy and have synthesized a focused library of complexes based on the tetradentate chelate, amine-amide-dithiol (AADT), containing only tertiary amine substituents [AADT-(CH2)n-NEt2 (n = 1, 2; R = Et, n-Bu, piperidino, morpholino); refs. 20–22]; most of these complexes display considerable affinity for melanoma both in vitro and in vivo, with the [99mTcOAADT-(CH2)2-NEt2] derivative ([99mTc-1]) having a high in vivo melanoma uptake of 7.6% ID/g (melanoma/nontarget tissue = 9.5) at 1 hour postinjection in the s.c. C57Bl/6/B16F0 mouse model (20). Described herein is a study of this small, neutral complex [99mTcO-S]-1 (Fig. 1) in a more realistic lung metastatic melanoma model with a temporal tumor growth pattern that illustrates the potential use of [99mTcO-S]-1 as a small-molecule SPECT imaging probe for the in vivo diagnosis of metastatic sites in malignant melanoma.

Materials and Methods

General. All chemicals and reagents were purchased from commercial sources (Sigma-Aldrich, St. Louis, MO; Gibco Life Technologies, Gaithersburg, MD). Technetium-99m pertechnetate was obtained via a 99Mo-99mTc generator (DuPont NEN, Bilirica, MA). High-performance liquid chromatography (HPLC) purification was done on a Waters Millennium Chromatography System equipped with a 996 UV-Vis diode-array detector attached in series to a γ-detector consisting of a shielded photomultiplier powered by a Canberra voltage amplifier and connected to a ratemeter. For the analytic and preparative HPLC purification of the radiolabeled complex, a reverse-phase C8 column equipped with a C18 guard was eluted with methanol (solvent A) and 5 mmol/L PBS, pH 7.4 (solvent B, Gibco Life Technologies) using a 30-minute linear gradient from 15:85 (A/B) to 90:10 (A/B) at a 1.0 mL/min flow rate.

Synthesis of [99mTcOAADT-(CH2)n-NEt2]. The AADT-(CH2)n-NEt2 ligand and its technetium-99m complex were synthesized following procedures described previously (20). Briefly, 5.0 mg trityl-protected ligand was dissolved in 10 mL trifluoroacetic acid (TFA), and the resulting yellow-colored solution was titrated with triethylsilylhydride until it turned colorless. TFA was completely evaporated from the colorless solution on a...
rotary evaporator followed by additional drying via high vacuum. The dry residue was later redissolved in argon-saturated methanol and distributed into 10 vials that were dried and kept under vacuum for later preparation of the technetium-99m–labeled compound. 99mTc-1 was synthesized using a vial containing 0.5 mg of the above thiol-deprotected ligand which was dissolved in 0.25 mL PBS (5 mmol/L, pH 7.4). To this was added the required 99mTc activity in the form of a 99mTc-glucoheptonate solution (0.3 mL), and the reaction was heated at 75°C for 25 minutes in a water bath. HPLC analysis of the reaction mixture typically indicated >90% radiochemical yield of the 99mTc-1 complex (Fig. 2). In addition, coinjection of the structurally characterized rhenium complex (20) with the analogous 99mTc-1 complex resulted in coelution of the radioactive species with the corresponding UV-active rhenium complex. All in vitro and in vivo experiments were conducted with the 99mTc-1 complex isolated via preparative HPLC. The solution was subsequently evaporated to complete dryness and the complex reconstituted in sterile distilled water to the required radioactivity concentration.

In vitro studies with murine B16F10 melanoma cells. B16F10 cells (2 × 10^6) from the American Type Culture Collection (Manassas, VA) were plated and grown in three T-175 flasks in 14 mL DMEM (pH 7.4; Gibco Life Technologies) containing 4,500 mg/L D-glucose, L-glutamine, and pyridoxine hydrochloride, 110 mg/L sodium pyruvate, 10% fetal bovine serum, 0.2% gentamicin, and 0.5% penicillin-streptomycin solution. At the time of the cell uptake assay, all cells were harvested from the culture flasks by trypsinization with 1 mL trypsin-EDTA solution (0.25% trypsin, 1 mmol/L EDTA/4Na; Gibco Life Technologies). After being washed twice with 12 mL cold PBS (Gibco Life Technologies), the cells were counted and resuspended in Ca2+-free MEM with reduced Mg2+ content (S-MEM, Gibco Life Technologies) to a final concentration of 5.0 × 10^6 cells/mL. The effect of inhibition of melanin synthesis on the uptake of 99mTc-1 was studied by substituting DMEM containing 500 μmol/L N-butyldeoxynojirimycin (NBdNJ; ref. 23) as the growth medium 48 hours before the assay, and replenishing the NBdNJ medium every 24 hours. Similarly, the effect of increased melanin synthesis/content on the uptake of 99mTc-1 was assessed by stimulating melanin synthesis in

**Figure 3.** In vitro cell uptake of 99mTc-1 by intact B16F10 cells at 37°C (A) and 4°C (B).

**Figure 4.** Comparison of in vivo tumor accumulation of 99mTc-1 (Tc-1), [18F]FDG (FDG), [18F]DOPA (F-DOPA), and [123I]IMBA (IMBA) in s.c. B16-melanoma tumor model. Tumor uptake expressed as differential absorption ratio (DAR) as reported by Ishiwata et al. (28) for [18F]FDG and [18F]DOPA. Data for IMBA calculated from Eisenhut et al. (13).

**Table 1.** Biodistribution of 99mTc-1 at 2, 3, and 6 hours postadministration in C57BL6 mice bearing s.c. B16F10 melanoma

<table>
<thead>
<tr>
<th>Organ</th>
<th>2 h</th>
<th>3 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0.71 ± 0.13^a</td>
<td>0.56 ± 0.02</td>
<td>0.23 ± 0.09</td>
</tr>
<tr>
<td>Heart</td>
<td>0.67 ± 0.13</td>
<td>0.45 ± 0.03</td>
<td>0.16 ± 0.05</td>
</tr>
<tr>
<td>Liver</td>
<td>10.03 ± 0.62</td>
<td>7.57 ± 0.22</td>
<td>4.32 ± 0.59</td>
</tr>
<tr>
<td>Lungs</td>
<td>3.19 ± 0.51</td>
<td>1.94 ± 0.20</td>
<td>0.70 ± 0.23</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.54 ± 0.12</td>
<td>0.22 ± 0.02</td>
<td>0.09 ± 0.05</td>
</tr>
<tr>
<td>Kidneys</td>
<td>3.06 ± 0.54</td>
<td>2.40 ± 0.34</td>
<td>1.39 ± 0.85</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.35 ± 0.25</td>
<td>0.69 ± 0.05</td>
<td>0.53 ± 0.53</td>
</tr>
<tr>
<td>Brain</td>
<td>0.19 ± 0.03</td>
<td>0.11 ± 0.00</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td>Intestines</td>
<td>6.51 ± 2.18</td>
<td>6.21 ± 3.57</td>
<td>2.39 ± 0.62</td>
</tr>
<tr>
<td>Tumor</td>
<td>7.60 ± 1.09</td>
<td>6.60 ± 2.16</td>
<td>4.72 ± 1.93</td>
</tr>
<tr>
<td>Skin/Fur</td>
<td>0.67 ± 0.11</td>
<td>0.57 ± 0.31</td>
<td>0.19 ± 0.09</td>
</tr>
<tr>
<td>Tumor/Blood</td>
<td>10.53 ± 3.01</td>
<td>11.83 ± 3.76</td>
<td>24.01 ± 15.90</td>
</tr>
<tr>
<td>Tumor/Muscle</td>
<td>16.34 ± 2.30</td>
<td>30.58 ± 11.52</td>
<td>67.54 ± 58.30</td>
</tr>
<tr>
<td>Tumor/Liver</td>
<td>0.77 ± 0.07</td>
<td>0.88 ± 0.30</td>
<td>1.15 ± 0.65</td>
</tr>
<tr>
<td>Tumor/Lung</td>
<td>2.21 ± 0.31</td>
<td>3.41 ± 1.04</td>
<td>8.21 ± 6.55</td>
</tr>
<tr>
<td>Tumor/Spleen</td>
<td>5.88 ± 0.90</td>
<td>9.53 ± 2.79</td>
<td>15.01 ± 9.63</td>
</tr>
</tbody>
</table>

^a% ID/g ± SD; n = 4–6.
similar biodistribution studies were done 14 days after i.v. injection in organs were weighed. The excised lung tissues were then prepared for with a known standard of technetium-99m, and subsequently the tissues/organs were processed for histopathologic examination. In vivo biodistribution studies with 99mTc-1 were also done in normal (control) mice that did not receive an i.v. tumor cell inoculation.

Subcutaneous tumors were created using 2.5 × 10^6 B16F10 cells (0.1 mL) transplanted s.c. on the left hind flank of C57BL/6 male mice. After 10 to 14 days, the animals developed palpable tumor nodules 0.5 to 1.0 cm in diameter. The biodistribution studies were carried out in these mice at 2, 3, and 6 hours after tail vein injection of 25 to 40 μCi (0.1 mL) 99mTc-1. The organs and tumors were harvested and counted in a γ-counter along with technetium-99m standards of the injected dose. The results are expressed as % ID/g.

All animal experiments were done in compliance with the Principles of Laboratory Animal Care (NIH publication no. 85-23, revised 1985) and under approved institutional animal protocols.

**Radiophosphor imaging.** Excised lung tissue collected from tumor-bearing mice was placed in preweighed tubes containing 15% formalin buffer solution and subjected to ex vivo radiophosphor imaging. The formalin-fixed lung tissues were blotted dry on tissue paper and placed on a clean microscope slide and photographed with a digital camera. The slides were then placed in a phosphor imaging cassette (Molecular Dynamics, Inc., Sunnyvale, CA). In the closed imaging cassette, the lung tissues were compressed to a thickness of 1 to 2 mm. The imaging plates were exposed overnight to the lung tissues to accumulate counts. These counts were then processed on a Molecular Dynamics Phosphor Imager (Storm 860 Scanner, Scanner Control Version 4.1, Molecular Dynamics) to obtain the radiophosphor images. Image analysis was carried out with the software program ImageQuant 1.2 (Molecular Dynamics).

**Histology.** The excised lung, liver, spleen, and brain tissues collected in 15% formalin buffer were processed for histopathologic studies after the complete decay of radioactivity. The tissue was embedded in paraffin and 5-μm-thin sections were cut on a microtome and stained with H&E. Slides of all the sample tissues were examined under light microscopy to determine the presence, extent, and size of melanoma metastases.

### Table 2. In vivo uptake of 99mTc-1 at 2 and 3 hours postadministration in C57BL/6 mice bearing 11-, 14-, and 17-day-old B16F10 melanoma lung metastases

<table>
<thead>
<tr>
<th>Organ</th>
<th>Normal</th>
<th>11-day-old*</th>
<th>14-day-old*</th>
<th>17-day-old*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 h</td>
<td>3 h</td>
<td>2 h</td>
<td>3 h</td>
</tr>
<tr>
<td>Blood</td>
<td>0.3 ± 0.03</td>
<td>0.31 ± 0.01</td>
<td>0.75 ± 0.15</td>
<td>0.54 ± 0.06</td>
</tr>
<tr>
<td>Heart</td>
<td>0.34 ± 0.03</td>
<td>0.28 ± 0.07</td>
<td>0.50 ± 0.07</td>
<td>0.32 ± 0.04</td>
</tr>
<tr>
<td>Liver</td>
<td>5.52 ± 0.35</td>
<td>2.42 ± 0.38</td>
<td>7.25 ± 0.97</td>
<td>6.48 ± 0.93</td>
</tr>
<tr>
<td>Lungs</td>
<td>2.13 ± 0.20</td>
<td>0.89 ± 0.26</td>
<td>4.55 ± 0.83</td>
<td>3.44 ± 0.94</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.21 ± 0.02</td>
<td>0.10 ± 0.06</td>
<td>0.31 ± 0.05</td>
<td>0.33 ± 0.29</td>
</tr>
<tr>
<td>Kidneys</td>
<td>1.59 ± 0.14</td>
<td>1.35 ± 0.13</td>
<td>2.35 ± 0.39</td>
<td>1.72 ± 0.34</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.72 ± 0.04</td>
<td>0.30 ± 0.12</td>
<td>0.80 ± 0.08</td>
<td>0.56 ± 0.10</td>
</tr>
<tr>
<td>Brain</td>
<td>0.12 ± 0.01</td>
<td>0.07 ± 0.02</td>
<td>0.16 ± 0.01</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>Upper intestine</td>
<td>6.62 ± 1.88</td>
<td>4.66 ± 0.97</td>
<td>8.43 ± 4.49</td>
<td>4.76 ± 4.15</td>
</tr>
<tr>
<td>Skin</td>
<td>0.69 ± 0.57</td>
<td>0.40 ± 0.20</td>
<td>0.73 ± 0.24</td>
<td>0.60 ± 0.30</td>
</tr>
<tr>
<td>Lung/Blood</td>
<td>7.05 ± 0.52</td>
<td>2.98 ± 0.78</td>
<td>6.29 ± 1.63</td>
<td>6.03 ± 1.17</td>
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<tr>
<td>Lung/Muscle</td>
<td>9.74 ± 0.97</td>
<td>11.02 ± 3.57</td>
<td>15.01 ± 3.12</td>
<td>15.68 ± 4.03</td>
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<tr>
<td>Lung/Liver</td>
<td>0.39 ± 0.03</td>
<td>0.36 ± 0.06</td>
<td>0.64 ± 0.20</td>
<td>0.50 ± 0.15</td>
</tr>
<tr>
<td>Lung/Spleen</td>
<td>2.98 ± 0.14</td>
<td>3.62 ± 2.18</td>
<td>5.73 ± 1.2</td>
<td>5.78 ± 1.54</td>
</tr>
</tbody>
</table>

*4 × 10^5 B16F10 cells inoculated i.v.

*1% ID/g ± SD; n = 6-6.

*2 P < 0.05 compared with normal.
In vitro cell uptake studies. B16F10 cell uptake studies over a 1-hour incubation period at 37°C (Fig. 3A) show rapid uptake of 99mTc-1, with a 30% maximum being reached within 20 minutes. Because L-tyrosine is the main substrate in the synthesis of melanin via the oxidative enzyme tyrosinase, melanin synthesis/content is enhanced by growing B16F10 cells in DMEM medium supplemented with additional L-tyrosine (2.0 mmol/L). There is a significant darkening of the cells/medium compared with control cells cultured in DMEM alone. These tyrosine-stimulated cells have a rapid and significantly enhanced cellular accumulation of the 99mTc complex at 37°C, such that within 5 minutes of incubation they display a 70% uptake of 99mTc-1, which reaches a maximum of 78% in 60 minutes. To distinguish an active accumulation component from passive diffusion, simultaneous experiments were done at 4°C (Fig. 3B). Under these conditions a significant reduction in cellular uptake is observed in the cells cultured in DMEM alone, with a maximum of only 8% observed over a 60-minute period, indicating that a significant fraction of the cellular uptake of 99mTc-1 by B16F10 melanoma cells is due to active, temperature-dependent processes. At 4°C, the cellular accumulation in tyrosine-stimulated cells is reduced from a maximum of 78% to 50%; moreover, the uptake is higher than that observed for control cells at 37°C, suggesting that the uptake and accumulation of the 99mTc-1 complex is related to the melanin synthesis/content of the cells, a phenomenon also observed with the radioiodinated benzamides (10, 22, 27).

NBdNJ added to the growth medium of melanoma cells produces functionally inactive tyrosinase, a result of inhibition of correct N-glycan processing, and thereby reduces melanin synthesis/content within intact melanoma cells. Despite this incomplete glycan processing, the enzyme is transported to the melanosome; however, it lacks catalytic activity, in effect, generating amelanotic B16F10 cells that appear pale in color and contain little or no melanin compared with normal control cells.
Cellular uptake studies in these NbNdNJ-treated cells show a dramatic decrease in accumulation of $^{99m}$Tc-1, with a maximum uptake of 6% at 37°C throughout the 1-hour incubation period (Fig. 3A). Taken together, these in vitro cell uptake data indicate the effect of modulating the melanin synthesis/content on the accumulation of $^{99m}$Tc-1 by intact melanoma cells and suggest that mechanistically the accumulation of $^{99m}$Tc-1 is related to their melanin synthesis/content.

In vivo biodistribution of $[^{99m}$TcOAADT]-($\text{CH}_2$)$_2$-NEt$_2$ in B16F10 metastatic melanoma models. In vivo distribution of $^{99m}$Tc-1 was examined in the s.c. mouse model bearing metastatic B16F10 tumors. The biodistribution values in Table 1 show high in vivo tumor uptake of $^{99m}$Tc-1 at 2 hours after its administration. This tumor uptake is similar to that observed previously (20) and is also comparable with that obtained with the iodobenzenes in the B16F0 tumor model (13). The biodistribution data over an extended 6-hour period also indicate that tumor uptake of $^{99m}$Tc-1 remains high whereas a relatively faster washout from normal organs results in increasing tumor/nontumor ratios (Table 1).

For comparison of the in vivo melanoma uptake of $^{99m}$Tc-1 with that of other small-molecule PET imaging probes such as $^2$-[${}^{18}$F]fluoro-3,4-dihydroxy-L-phenylalanine ($^1$F]DOPA) and $^1$F]FDG in a similar s.c. B16F10-melanoma tumor model, the tumor accumulation has also been calculated as a differential absorption ratio [$= ($counts of tissue/g tissue $\times$ g body weight) / total injected counts or (% ID/g $\times$ g body weight) / 100] as reported by Ishiwata et al. (28) for $^{[18F]$FDG and $^{[18F]$DOPA. Figure 4 contains a comparison of the differential absorption ratio of $^{99m}$Tc-1 with those previously observed for $^{[18F]$FDG, $^{[18F]$DOPA (28), and $^{[123I]$IMBA (13). Because the degree of $^{[18F]$FDG accumulation in an organ or lesion is also semiquantitatively determined by PET in terms of standardized uptake values [$= ($decay-corrected activity in tumor/g tumor) / (decay-corrected injected dose/g body weight of animal)] that serve as a normalized measure of target to background ratios (9, 29), these have also been calculated for the uptake of $^{99m}$Tc-1 in the tumor. Values of 2.36 ± 0.42, 2.37 ± 0.87, and 2.73 ± 1.09 are obtained for $^{99m}$Tc-1 tumor standardized uptake values at 2, 3, and 6 hours, respectively. Corresponding $^{99m}$Tc-1 standardized uptake values for blood are 0.22 ± 0.03, 0.26 ± 0.06, and 0.13 ± 0.05, and those for muscle are 0.17 ± 0.05, 0.11 ± 0.06, and 0.05 ± 0.03 at 2, 3, and 6 hours, respectively.

As the foremost concern in the evaluation of patients diagnosed with metastatic melanoma is the detection of the presence and extent of metastasis at distant sites, $^{99m}$Tc-1 was also assessed in...
mice that received an i.v. inoculation of B16F10 melanoma cells, resulting in the formation of metastatic melanoma lesions in the lungs. When mice are evaluated 11 days after receiving $4 \times 10^5$ B16F10 cells i.v., they have a significantly higher lung uptake of $^{99m}$Tc-1 2 hours postadministration of the radiopharmaceutical than normal mice ($4.55 \pm 0.83\% \text{ ID/g}$ versus $2.13 \pm 0.20\% \text{ ID/g}$, respectively, $P < 0.05$; Table 2). This difference in lung uptake increases further at 3 hours postinjection of the compound, with the uptake of 11-day tumor-bearing mice of $3.44 \pm 0.94\% \text{ ID/g}$ and that of normal mice of $0.89 \pm 0.26\% \text{ ID/g}$, indicating a relatively faster washout of $^{99m}$Tc-1 from normal lung tissue compared with lungs bearing metastatic melanoma lesions. When the time interval following tumor cell inoculation is prolonged to 14 days to allow further growth of the metastatic lesions, the accumulation of $^{99m}$Tc-1 in the lungs increases to $6.12 \pm 0.81\% \text{ ID/g}$ and $7.74 \pm 1.34\% \text{ ID/g}$ at 2 and 3 hours postadministration, respectively. Moreover, at 17 days post tumor cell injection, the uptake of $^{99m}$Tc-1 in the lungs reaches a value of $10.84 \pm 2.03\% \text{ ID/g}$ at 2 hours postadministration. This accumulation of $^{99m}$Tc-1 as a function of the growth of the metastatic pulmonary melanoma lesions over time is illustrated in Fig. 5A.

The uptake of $^{99m}$Tc-1 was also examined in a group of mice that received varying doses of B16F10 tumor cells 14 days before the assessment. The uptake of $^{99m}$Tc-1 in the lungs of these tumor-bearing mice has a positive correlation with the number of tumor cells administered, such that mice receiving $2 \times 10^5$, $4 \times 10^5$, and $8 \times 10^5$ B16F10 cells have lung uptake of $2.83 \pm 0.21\% \text{ ID/g}$, $3.63 \pm 1.07\% \text{ ID/g}$, and $4.92 \pm 1.61\% \text{ ID/g}$, respectively, compared with $2.13 \pm 0.20\% \text{ ID/g}$ observed in the lungs of normal mice (Table 3; Fig. 5B). Taken together, these in vivo experiments illustrate the potential use of $^{99m}$Tc-1 as an in vivo probe to assess both temporal growth and extent of metastatic tumor burden in the lungs.

**Histology.** Evaluation of numerous histologic sections of the lung, liver, spleen, and brain of animals bearing s.c. B16F10 tumors does not indicate any metastatic lesions in these organs. However, as previously shown (19–21, 24), excised lungs from mice that received i.v. inoculations of B16F10 melanoma cells have distinct small black melanoma colonies, which increase both in number and in size with increasing dose of the tumor inoculum. Paraffin-embedded sections from metastatic tumor-bearing lungs of mice receiving i.v. inoculation of $2 \times 10^5$, $4 \times 10^5$, and $8 \times 10^5$ B16F10 melanoma cells 14 days before evaluation are shown in Fig. 6. The administration of $2 \times 10^5$ tumor cells results in the formation of a significant number of small distinct metastatic lesions with most of these extravasated near blood vessels (Fig. 6A and B). Increasing the tumor dose to $4 \times 10^5$ and $8 \times 10^5$ cells causes a significant increase in both the number and size of the metastatic lesions (Fig. 6C and D). As previously observed (24–26, 30), the metastatic lesions not only extravasate and grow near blood vessels but also grow near and at the lung surface; this is quite pronounced in the 14-day lung tissue when $8 \times 10^5$ cells have been administered (Fig. 6D).

An increase in both the number and size of metastatic melanoma lesions also occurs in the group where the time post tumor cell injection ($4 \times 10^5$ cells) is prolonged from 11 to 17 days. This was visually apparent at the time of dissection (Fig. 7). After biodistribution studies with this group of mice at 2 hours postadministration of $^{99m}$Tc-1 (Table 2), the excised lung tissue was photographed and subjected to ex vivo radiophosphor imaging. The distinct and specific localization of $^{99m}$Tc-1 by the melanotic lesions as observed in the radiophosphor images (Fig. 7) is underscored by the intensity and specificity of the accumulated activity in the individual tumor lesions. Also apparent in the correlative examination of the radiophosphor images with the digital images of the lung tissue displayed in Fig. 7 is the relationship of the level of accumulation of $^{99m}$Tc-1 to the degree of melanin in the...
individual melanotic lesions. Histologic examination of lung sections does not reveal any infiltrating inflammatory cells at or near the metastatic lesions. In conjunction with the specific uptake of $^{99m}$Tc-1 observed in the radiophosphor images, this observation emphasizes that the accumulation is specifically localized in the tumor cells.

Whereas examination of this group of mice (i.e. tumor inoculation) does not show any distant metastasis in the liver and spleen, the sections of the brain display small but noticeable infiltrated areas. A closer and more detailed examination of the brain sections adjoining the infiltrated regions reveals blockade in the blood vessels by small but distinct tumor metastases (Fig. 5E and F). Further detailed examination of the brain sections shows no metastatic tumor growth within the brain tissue, and small tumor metastases confined to the blood vessels supplying the neighboring infiltrated areas. The corresponding in vivo brain uptake of $^{99m}$Tc-1 in the biodistribution studies of these mice indicates a very small increase in uptake compared with normal mice (Table 3). This increase may be relatively small due to a number of factors: (a) the whole brain is excised and measured and the number and size of such metastases in the brain vasculature are very small; (b) a reduction in blood flow due to blockade of the vessels by the metastases produces decreased delivery and extraction of $^{99m}$Tc-1 in the relatively small tumor metastases; and (c) the presence of small infiltrated areas caused by the blockade may also bring about decreased brain uptake of $^{99m}$Tc-1. A more detailed study using this model will be required to derive any conclusions with respect to brain uptake of $^{99m}$Tc-1 and melanoma metastases within the brain vasculature.

$^{99m}$Tc-1, a small, neutral technetium complex, displays high melanoma uptake in both s.c. and pulmonary metastatic melanomas. The use of a more realistic model for evaluating metastatic melanoma-targeting agents shows that $^{99m}$Tc-1 not only has high and specific uptake by melanoma metastases in the lungs but also can potentially provide a means to assess noninvasively both temporal growth and extent of metastatic tumor burden in vivo.

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[\textsuperscript{99m}TcOADDT\textsubscript{(CH\textsubscript{2})\textsubscript{2}NEt\textsubscript{2}}: A Potential Small-Molecule Single-Photon Emission Computed Tomography Probe for Imaging Metastatic Melanoma

Zhen Cheng, Ashfaq Mahmood, Huazhi Li, et al.


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