Imaging of Non-Small Cell Lung Cancers with a Monoclonal Antibody, KC-4G3, Which Recognizes a Human Milk Fat Globule Antigen


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ABSTRACT

To determine the role of lung cancer tumor imaging with monoclonal antibodies directed against high molecular weight human milk fat globule antigens, we administered i.v. \(^{111}\text{In}-\text{KC-4G3} to 24 patients with advanced non-small cell lung cancer. One mg of \(^{111}\text{In}-\text{KC-4G3} was mixed with 0, 9, 49, 99, or 499 mg of unlabeled KC-4G3 and infused i.v. over 1 to 5 h. The mean \(^{111}\text{In}-\text{KC-4G3} radiochemical purity was \(>97\%\) and the result-
ant immunoreactivity averaged 62\%. Successful imaging of cancer sites was accomplished in 92\% of 24 patients, and 57\% of 91 total lesions were visualized. Successful localization of tumor sites related to size (\(P < 0.001\)), with 81\% of lesions >3.0 cm in diameter, 50\% of lesions 1.5 to 3 cm, and 6\% of lesions <1.0 cm successfully imaging, and to location (\(P < 0.05\)), with 69\% of pulmonary lesions, 80\% of soft tissue lesions, and only 32\% of bone metastases being visualized. Nonspecific reticulo-
endothelial uptake of radioactivity was a major problem. Approximately 35\% of \(^{111}\text{In}\) was chelated to serum transferrin by 24 and 48 h after infusion. The mean \(t_{1\alpha}\) for plasma radioisotope and immunoreactive KC-
4G3 was 29 and 27 h, respectively. There was no correlation between total infused antibody dose and imaging success or between total dose and effect on \(^{111}\text{In}\) and KC-4G3 kinetics. Circulating free KC-4 antigen was measurable in all but one patient before study. Tumor biopsy following infusion could demonstrate antibody presence but not saturable antigen binding. We conclude that (a) \(^{111}\text{In}-\text{KC-4G3}\) demonstrates suc-
cessful tumor localization in non-small cell lung cancers bearing generally high expression of its antigen and (b) further investigations to diminish nonspecific radioactivity for imaging and utilization of high dose radio-
labeled antibody for therapeutic intent are warranted.

INTRODUCTION

Lung cancer is the most common and lethal cancer in the United States, with 157,000 cases and 142,000 deaths estimated in 1990 (1). Non-small cell lung cancers (squamous, adenocar-
cinoma, and large cell undifferentiated carcinomas) represent 75\% of all cases. Surgical resection of early lesions is the only established method of cure, and this occurs in only about 10\% of cases (1, 2). Standard presurgical staging includes CT4 scans of the chest and upper abdomen, with radionuclide scans of bone and head CT scans in selected patients. Because of the suboptimal accuracy of these procedures and the importance of staging, surgical biopsy at mediastinoscopy or thoracotomy is often required for complete staging. Most patients die from disseminated disease, for which there is little effective treat-
ment. Thus, new methods of early detection and systemic therapeutic modalities are sorely needed.

Monoclonal antibodies which react with human cancer anti-
gens have been evaluated in the imaging and treatment of several tumors, but there are few studies in lung cancer. Several murine monoclonal antibodies which react with high molecular weight antigens present in HMFG have been reported to react with high percentages of human epithelial cancers, especially of breast, ovarian, and colon origin (3–6). Radiolabeled anti-
HMFG antibodies have been evaluated for the detection of breast, gastrointestinal, and ovarian cancers and for i.p. therapy (7–9). Indium-111-labeled anti-HMFG F(ab\(^{'\})\), fragments have recently been reported for targeting non-small cell lung cancer (10). Related antibodies and combinations of antibodies to similar epitopes have also been used to detect circulating tumor antigens in patients with epithelial malignancies (11).

We have shown that an \(^{111}\text{In}-\text{DTPA}\) chelate of KC-4G3 retains its immunological reactivity and can be used to specifically image human adenocarcinomas in athymic nude mice (12). Thus, this study was designed to determine whether \(^{111}\text{In}-\text{KC-4G3}\) can be used for detecting sites of spread in patients with non-small cell lung cancers.

MATERIALS AND METHODS

Patients. Twenty-four patients with histologically confirmed non-
small cell carcinoma of the lung were studied. Histological subtypes included adenocarcinoma (12), squamous carcinoma (6), adenosqua-
rous carcinoma (3), large cell undifferentiated carcinoma (1), and bronchoalveolar carcinoma (1). All but 1 patient had immunohisto-
chemical staining of tissue before study, and all were found to have KC-4 antigen present by those studies (see below). There were 16 males and 8 females; mean age was 59 years (range, 35–83 years); all were caucasian. The patients were clinically staged prior to study; 22 had Stage IV with one or more sites of metastases, 1 had Stage II, and 1 had Stage III disease. The 91 sites of disease are shown in Table 1. All but 4 patients had received some prior therapy, including surgery alone (3), chemotherapy alone (1), radiotherapy alone (4), and two or three modalities (12). All patients had adequate renal, pulmonary, hepatic, cardiac, and marrow function and gave informed consent on a human subjects approved protocol.

Antibody. KC-4G3 (Coulter Immunology, Hialeah, FL) is a murine IgG3 monoclonal antibody which recognizes a high molecular weight membrane \((M, 438,000)\) and cytoplasmic \((M, 490,000)\) glycoprotein antigen on fresh human tumors, milk fat globule membranes, and lactating human breast tissue (13). A KC-4G3-DTPA chelate was prepared by a mixed anhydride method similar to that of Krejcarek and Tucker (14). The latter 4 patients were imaged with a KC-4G3-DTPA chelate prepared according to the 1-(p-isothiocyanatobenzyl) method (15). By each chelation technique, approximately 0.8 DTPA molecules were conjugated/immunoglobulin molecule. The preparations were free of viral, bacterial, and endotoxin contamination. KC-4G3 and KC-4G3-
DTPA chelate was administered under approved Investigational New Drug, Bureau of Biologies 2251.
IMAGING NON-SMALL CELL LUNG CANCER WITH MONOCLONAL ANTIBODY KC-4G3

Table 1 ¹¹¹In-KC-4G3 image results by lesion location and size

* Areas of "cold" uptake compared to normal liver.

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Location</th>
<th>1-(p-Isothiocyanatobenzyl)-DTPA</th>
<th>Mixed anhydride-DTPA</th>
<th>Totals*</th>
</tr>
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<tbody>
<tr>
<td>23</td>
<td>Pulmonary</td>
<td>11/15 (73%)</td>
<td>20/30 (66%)</td>
<td>31/45 (69%)</td>
</tr>
<tr>
<td>4</td>
<td>Soft tissue</td>
<td>0/4 (0%)</td>
<td>4/5 (80%)</td>
<td>4/5 (80%)</td>
</tr>
<tr>
<td>8</td>
<td>Lymph node</td>
<td>1/1 (100%)</td>
<td>5/18 (28%)</td>
<td>6/19 (32%)</td>
</tr>
<tr>
<td>8</td>
<td>Bone</td>
<td>2/2 (100%)</td>
<td>2/2 (100%)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Liver*</td>
<td>1/1 (100%)</td>
<td>1/3 (33%)</td>
<td>1/3 (33%)</td>
</tr>
<tr>
<td>4</td>
<td>Adrenal</td>
<td>2/2 (100%)</td>
<td>2/4 (50%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Kidney</td>
<td>1/3 (33%)</td>
<td>1/3 (33%)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Brain</td>
<td>0/4 (0%)</td>
<td>1/12 (8%)</td>
<td>1/16 (6%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>13/21 (62%)</td>
<td>39/70 (56%)</td>
<td>52/91 (57%)</td>
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</table>

<table>
<thead>
<tr>
<th>Size (cm)</th>
<th>No. of patients</th>
<th>1-(p-Isothiocyanatobenzyl)-DTPA</th>
<th>Mixed anhydride-DTPA</th>
<th>Totals*</th>
</tr>
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<tbody>
<tr>
<td>&gt;3.0</td>
<td>23</td>
<td>6/8 (75%)</td>
<td>29/35 (83%)</td>
<td>35/43 (81%)</td>
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<tr>
<td>1.5-3.0</td>
<td>15</td>
<td>7/9 (78%)</td>
<td>9/23 (39%)</td>
<td>16/32 (50%)</td>
</tr>
<tr>
<td>&lt;1.5</td>
<td>10</td>
<td>0/4 (0%)</td>
<td>1/12 (8%)</td>
<td>1/16 (6%)</td>
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<tr>
<td></td>
<td>Total</td>
<td>13/21 (62%)</td>
<td>39/70 (56%)</td>
<td>52/91 (57%)</td>
</tr>
</tbody>
</table>

Radiolabeling was performed, in the radiopharmacy at the University of Colorado, by mixing 5 mCi ¹¹¹In (New England Nuclear, Billerica, MA) with approximately 1 mg KC-4G3 for 20 min at room temperature. The ¹¹¹In incorporation for ¹¹¹In-DTPA-KC-4G3 prepared by the mixed anhydride-DTPA method was 88.0 ± 12.1% (mean ± SD); incorporation for ¹¹¹In-DTPA-KC-4G3 prepared by the 1-(p-isothiocyanatobenzyl)-DTPA method was 84.8 ± 10.1%. After labeling, the reactants were loaded onto a Superose 12HR 10-x 300-mm column and eluted with a 0.9% saline solution pumped by a P-500 fast protein liquid chromatography system (Pharmacia Fine Chemicals, Piscataway, NJ) at a flow rate of 30 ml/h, to separate unbound radioisotope. The elution profile was monitored using a Geiger-Mueller meter. Radiochemical purity was determined by instant thin layer chromatography on SG paper in methanol:water (1:1) with 5% ammonium acetate for the mixed anhydride-DTPA-¹¹¹In preparation and by instant thin layer chromatography on SG paper in 0.9% saline containing 5 mM EDTA at pH 7.0 for the 1-(p-isothiocyanatobenzyl)-DTPA-¹¹¹In preparation. The mean radiochemical purity for the mixed anhydride-DTPA-¹¹¹In final product was 97.7 ± 4.7% and for the 1-(p-isothiocyanatobenzyl)-DTPA-¹¹¹In final product was 99.1 ± 0.6%, whereas the mean specific activity for the final product was 3.6 ± 1.0 mCi/mg and 3.7 ± 0.3 mCi/mg, respectively.

The immunoreactivity of ¹¹¹In-KC-4G3 was determined for each patient using a modification of the linear extrapolation method at infinite antigen excess which we previously described (16). We used 3-µm beads coated with KC-4 antigen, BSA, or goat anti-mouse IgG (Coulter Immunology) as the antigen sources for antigen binding, nonspecific binding, and mouse IgG bound counts. Triplicate borosilicate tubes (Fisher Scientific, Pittsburgh, PA) were prepared with concentrations of 3 x 10⁴, 1.5 x 10⁵, 0.75 x 10⁵, and 0.375 x 10⁵ beads/tube for each type of bead and ¹¹¹In-KC-4G3 was added to each tube in a concentration of 25 ng/ml diluent (1% BSA-phosphate-buffered saline). Assay tubes were incubated overnight on a shaker (Precision Scientific Co., Chicago, IL) at 25°C. The assay tubes were then diluted with 2.5 ml diluent, centrifuged, decanted, and counted on a Beckman 5500 gamma-counter (Beckman Instruments, Irvine, CA). Specifically bound ¹¹¹In-KC-4G3 was calculated as the mean determination of KC-4 antigen bead counts minus the nonspecific BSA bead counts. The immunoreactivity averaged 61% following labeling of KC-4G3-DTPA chelate prepared by the mixed anhydride method and averaged 83% for the KC-4G3-DTPA chelate prepared by the 1-(p-isothiocyanatobenzyl) method.

Procedure. Intravenous infusions of radiolabeled antibody were given over 1 to 5 h. Total KC-4G3 administered was 1 mg (3 patients), 10 mg (4 patients), 50 mg (6 patients), 100 mg (5 patients), or 500 mg (6 patients). Unlabeled KC-4G3 (9, 49, 99, or 499 mg) was added to 1 mg of ¹¹¹In-KC-4G3 immediately prior to infusion. Analogue images of regions of interest (skull, anterior and posterior chest, abdomen, and extremities) were obtained on a GE 400at gamma-camera (General Electric, Milwaukee, WI) and counted, and data were stored and processed in a DEC Scintigraphic data analyzer (Digital Electronics, Maynard, MA). Images were collected for 7 min. There was no blood pool or organ subtraction. Initially, patients were imaged at 2, 24, 48, 72, and 96 h after antibody administration. After 3 patients were serially imaged, it became apparent that early scans (2 and 24 h) had considerable blood pool activity, which decreased over time, and that tumor sites retained their activity for up to 96 h. These findings were consistent with our nude mouse imaging studies and human data with other ¹¹¹In-labeled antibodies (10, 12, 17, 18). Thus, the remaining patients were imaged only at 72 h. For the latter 4 patients studied with the KC-4G3 chelate prepared by the 1-(p-isothiocyanatobenzyl) method, 24-, 48-, and 72-h images were obtained. These demonstrated a similar decrease in blood pool over time and retention of tumor localization to the 72-h image (Fig. 1).

Pharmacokinetics. Serial blood and plasma collections were obtained before infusion and at 1, 2, 4, 8, 12, 24, 48, 72, and 96 h after infusion for determination of plasma ¹¹¹In radioactivity and KC-4G3 antibody levels. Indium-111 radioactivity was measured by gamma-counting of duplicate 1-ml aliquots, which were corrected for ¹¹¹In decay; immunoreactive KC-4G3 antibody levels were measured by radioimmunoassay, as described below. Gamma-counting of whole blood samples for the 4 patients given the 1-(p-isothiocyanatobenzyl)-DTPA preparation was corrected to plasma counts by the formula: counts in plasma = counts in blood/1 - hematocrit. The volume of distribution, clearance, elimination rate constant, and t½ were determined by computer modeling with the computer program ESTRIP (19), which provides estimates of slopes and intercepts for each exponential component. Statistical determination of the number of exponentials to describe the data (20) and further refinement of the slopes and intercepts were performed with the computer program PCNONLIN (Statistical Consultants, Lexington, KY). Urine clearance of ¹¹¹In was determined using data from gamma-counting of aliquots collected over 0–2, 2–12, 12–24, 24–48, 48–72, and 72–96 h with correction for ¹¹¹In decay.

Serum KC-4G3 Levels. Serum levels of total serum KC-4G3 antibody were determined by competition, using iodinated KC-4G3 tracer and unlabeled KC-4G3 antibody. Immunoreactive KC-4G3 was then measured by addition of KC-4 antigen-coated beads to infinite excess/sample and incubation with patient serum and ¹²⁵I-labeled KC-4G3 antibody.
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RESULTS

Gamma-Camera Imaging. Gamma-camera images obtained over the first 2–24 h contained considerable blood pool activity (Fig. 1), which gradually cleared over 48–72 h. Sites of specific tumor uptake remained during the 24–72-h period. The 24 patients imaged had 91 sites of disease detected by standard methods, including physical examination, chest roentgenogram, radionuclide scans, and CT scans, for an average of 3.8 cancer sites/patient (range, 1–7). Table 1 shows the success of gamma-camera imaging by the location and size of the lesion and by type of KC-4G3-DTPA chelate utilized [1-(p-isothiocyanatobenzyl)-DTPA versus mixed anhydride-DTPA]. Successful imaging occurred most often in pulmonary (31 of 45) and soft tissue (4 of 5) lesions. Successful images were also obtained for 4 of 11 lymph node metastases, 6 of 19 bone metastases, 2 of 4 adrenal metastases, 2 of 2 kidney metastases, and 2 of 2 liver metastases (p < 0.05). Overall, 22 of 24 (92%) patients had successful "In-KC-4G3 imaging of at least 1 cancer site, and 57% of 91 total lesions were detected. Examples of successful pulmonary, bone, and nodal tumor localization are shown in Fig. 2.

The size of the lesion had a great effect on imaging success. Large lesions (>3 cm) were detected in 35 of 43 (81%) occasions and lesions from 1.5 to 3 cm were detected in 16 of 32 (50%), whereas small lesions (<1.5 cm) were detected in only 1 of 16 (6%) (P < 0.001). Large pulmonary (24 of 29) and soft tissue lesions (3 of 3) were detected with high frequency because of their size and location apart from areas of nonspecific uptake. Although the small number of patients prevents definitive assessment, success of imaging intermediate lesions, from 1.5 to 3.0 cm, appeared to be superior for the 1-(p-isothiocyanatobenzyl)-DTPA chelate (7 of 9; 78%), compared to the mixed anhydride-DTPA preparation (9 of 23; 39%) (P < 0.05).

There was evidence of nonspecific uptake in all patients, which was most prominent in liver and bone. The mechanisms of this nonspecific uptake is uncertain, is probably multifactorial, and contributed to a lower specificity, especially for small lesions and lesions overlying or near liver and bone. One patient...
Fig. 2. Gamma-camera images following 10 mg $^{111}$In-KC-4G3, utilizing the mixed anhydride-DTPA chelate. A, $^{111}$In-KC-4G3, chest; B, anterior chest X-ray; C, $^{111}$In-KC-4G3, skull; D, bone scan, skull; E, $^{111}$In-KC-4G3, tibias; F, left tibia X-ray; G, $^{111}$In-KC-4G3, femoral nodes. All $^{111}$In-KC-4G3 images at 72 h. The arrows indicate the detected clinical lesions by $^{111}$In-KC-4G3 images compared to standard radiographic studies.
who received a 1-mg dose had 2 liver metastases detected by CT scan. These lesions appeared as "cold" abnormalities on the KC-4G3 image, since there was greater uptake in the normal liver than in the metastases. The nonspecific uptake in bone and blood pool obscured many smaller lesions.

To determine the amount of nonspecific uptake due to separation of $^{111}$In from KC-4G3, we utilized specific immunoprecipitation of radioactive transferrin to quantitate the percentage of free $^{111}$In or $^{111}$In-DTPA dissociated from the antibody molecule and bound to transferrin. The data shown in Table 2 indicate that, at 24 and 48 h after injection of the radioactive antibody dose, about one third of the radioactive indium is bound to transferrin. However, less than 50% of the injected dose of $^{111}$In was found in the plasma volume at these time points, so that less than one sixth of the total administered $^{111}$In was bound to transferrin within the plasma volume. Thus, for patients administered $^{111}$In-KC-4G3 prepared by the mixed anhydride-DTPA method, it seems likely that $^{111}$In bound to transferrin accounts for some of the radioactivity distributed in bone marrow and liver, tissues with relatively high densities of transferrin receptors.

There was no significant association between the type of histology of the tumor and the detection of clinical lesions or between the degree of tumor differentiation and success of lesion detection ($P > 0.05$) (Table 3). Both metastases in a patient with bronchoalveolar carcinoma were detected, but this was the only patient with this histology. There was no correlation between pre-study immunohistochemical staining intensity and the percentage of tumor cells staining and $^{111}$In-KC-4G3 image results ($P > 0.05$). Patients except 5 had at least 25% positive cells and all but 2 patients had 3+ to 4+ staining intensity. However, further subset analysis of the 2 patients with 1+ staining intensity and <25% of cells staining had 9 of 9 lesions identified ($P < 0.05$). Similarly, there was no association between the $^{111}$In-KC-4G3 immunoreactivity and image results.

The influence of antibody dose on tumor localization is shown in Table 4. The detection rate was lowest at the 50-mg and 100-mg doses and highest at the 500-mg dose but the data do not show a significant correlation between dose and successful imaging ($P > 0.05$). Large lesions (>3 cm) at the 500-mg dose of KC-4G3 were all detected (8 of 8) and accounted for the higher detection rate for that dose. The imaging results were also analyzed for correlation with the presence of free antigen prior to antibody administration. All but 1 patient had detectable circulating KC-4 antigen prior to $^{111}$In-KC-4G3 administration, with a mean level of 261 ng/ml and a range of 0 to 1702 ng/ml. There was no correlation between free antigen levels and image results ($P > 0.05$). Furthermore, the level of antibody in serum ($\mu$g/ml) far exceeded antigen levels (ng/ml) except at the 1-mg dose level.

In a few patients, there were sites of uptake which could not be correlated with known clinical lesions. For example, one patient without known liver metastases had evidence of hepatic metastases on the $^{111}$In-KC-4G3 image. He died within 1 month of progressive disease but prior to confirmatory tests, and a post-mortem examination was denied. Another patient was found to have probable left humerus metastasis on $^{111}$In-KC-4G3 scan and had a suspicious cortex lesion on plain film. However, an initially negative left humerus on bone scan became positive on repeat bone scan 7 months after the $^{111}$In-KC-4G3 scan.

Pharmacokinetics. The mean plasma disappearance of the $^{111}$In by dose is shown in Fig. 3A. The $^{111}$In data fit either one-, two-, or three-compartment models. The clearance did not significantly vary as a function of total KC-4G3 or pre-study circulating antigen level dose, as shown by linear regression analysis, although the $t_{1/2}$ appeared slightly prolonged at higher doses. Table 5 shows the volume of distribution, clearance, elimination constant, and serum half-life as a function of dose. There were no significant associations with dose for any parameter. These parameters were also calculated by analysis of KC-4G3 antibody disappearance, and the data are shown in Fig. 3B and Table 5. In each instance, the kinetics determined by radioisotope or immunoreactive KC-4G3 were quite similar. The volume of distribution was similar to the total plasma volume and did not vary with dose. The $t_{1/2}$ and clearance did not vary with dose. The clearance kinetics for the 1-(p-isothiocyanatobenzyl)-DTPA chelate appeared similar to those for the mixed anhydride-DTPA chelate preparation and were not significantly different.

The cumulative urinary excretion of $^{111}$In label is shown in Table 4. The influence of antibody dose on tumor localization is shown in Table 4. The detection rate was lowest at the 50-mg and 100-mg doses and highest at the 500-mg dose but the data do not show a significant correlation between dose and successful imaging ($P > 0.05$). Large lesions (>3 cm) at the 500-mg dose of KC-4G3 were all detected (8 of 8) and accounted for the higher detection rate for that dose. The imaging results were also analyzed for correlation with the presence of free antigen prior to antibody administration. All but 1 patient had detectable circulating KC-4 antigen prior to $^{111}$In-KC-4G3 administration, with a mean level of 261 ng/ml and a range of 0 to 1702 ng/ml. There was no correlation between free antigen levels and image results ($P > 0.05$). Furthermore, the level of antibody in serum ($\mu$g/ml) far exceeded antigen levels (ng/ml) except at the 1-mg dose level.
Fig. 4. There was no association between urinary clearance and dose. The mean clearance over 96 h was 11.2%, with a range of 2.2 to 24.4%. There was 2.2% excreted over the first 24 h, with gradually increasing amounts over the next 72 h. A greater variability in urine radiolabel excretion was observed for the 1-(p-isothiocyanatobenzyl)-DTPA chelate than the mixed anhydride-DTPA chelate preparation, as shown by regression analysis and the F test ($P < 0.05$), characterized by an overall greater disappearance and more rapid rate of urine $^{111}$In excretion for the former chelate derivative. This may reflect improved antigen recognition and renal immune complex clearance with the 1-(p-isothiocyanatobenzyl)-DTPA derivative. Although we did not perform chromatography studies in these patients, our nude mouse studies have shown most of the urine $^{111}$In as free radioisotope or radioisotope bound to DTPA chelate or small molecular weight antibody fragments (12).

Toxicity. No clinical toxicity was observed in the patients in this imaging study. Specifically, there were no allergic reactions and no change in marrow, liver, or renal function. Many of the patients subsequently received twice-weekly injections of unlabeled antibody as part of a second therapy phase of study. The majority of patients developed human anti-mouse antibodies of the IgG class, detectable usually after the first or second antibody infusion. Low titers of IgG human anti-mouse antibodies did not prevent subsequent antibody administration or the achievement of anticipated high circulating serum KC-4G3 levels and were not predictive of potential allergic reactions.

Immunohistochemical Localization of KC-4G3. One patient underwent biopsy of a metastatic s.c. lesion 24 h after administration of 500 mg of antibody. Immunohistochemical localization of KC-4G3 using biotinolated goat anti-mouse immunoglobulin revealed the presence of the antibody in a few tumor cells and was scored at 1+ intensity for 25 to 50% reactive cells. However, the percentage of positive cells and intensity of staining was less than that obtained before the study, when paraffin sections were stained, which revealed 4+ staining intensity and 50 to 75% reactive cells (Fig. 5).

DISCUSSION

This study shows that the murine monoclonal antibody KC-4G3, which reacts with an ubiquitous high molecular weight mucin glycoprotein expressed on greater than 96% of non-small cell lung cancers, can be effectively radiolabeled with a $^{111}$In-DTPA chelate and safely administered to lung cancer patients. The radiolabeled antibody localizes in tumor sites. Although lesions were imaged in 22 of 24 (92%) patients, the sensitivity and specificity may not be sufficiently high to warrant general application of this i.v. administration, regional body-imaging approach. Nonspecific uptake is a major impediment to improved sensitivity/specificity and may lead to dose-limiting toxicities when therapeutic doses of radioisotope-antibody conjugates are utilized. Further studies to reduce nonspecific uptake and to evaluate other methods of administration and scanning, such as intrabronchial injections with single-photon emission computed tomography scanning of the mediastinum, may lead to important clinical applications.

The high molecular weight glycoprotein mucin antigens recognized by KC-4G3 have been reported to be widely expressed on adenocarcinomas of the breast, colon, ovary, and other sites (4-6, 8, 23-25). Related antigen(s) have been termed HMFG and MAM-6 by various investigators (3-6). The antigen is complex, with a core protein and more than 50% carbohydrates including O-linked sugars, sialic acid, and uronic acid. Antibodies to a number of epitopes have been described including HMFG1, HMFG2, DF3, F36/22, W1, 115D8, Mc1, Mc3, Mc5, Mc8, Mc10, and others (3-6, 11, 23-26). These antibodies have demonstrated excellent tumor localization and some antitumor effects when radiolabeled or administered as unlabeled antibody cocktails in the nude rodent model (12, 27, 28).

Because of the frequent distribution of this antigen on epithelial malignancies, clinical application of these antibodies has wide applicability. Most clinical studies with related antibodies have been performed in breast and ovarian cancer patients, especially with iodine-131 as a radioisotope. Our study utilized an $^{111}$In conjugate and was confined to lung cancer patients. We found that tumor localization was significantly related to tumor size and location. Antibody dose and antigen density were less important. Tumor size has been identified as an important variable in other imaging trials (17, 18). For example, with $^{111}$In-labeled antimelanoma antibody 96.5, Murray et al. (17) found little localization with lesions less than 1 cm, while lesions greater than 1 cm imaged with greater frequency, to yield a total imaging detection rate of 50%. They also found that skin and lymph node metastases imaged better than bone, brain, or liver metastases. This suggests accessibility of antigen to antibody, blood supply, antigen expression, proximity of lesions to the gamma-camera and nonspecific uptake in certain organs play a role in discriminating metastases from background. The administration of up to 40 mg unlabeled antibody has been reported to improve detection of clinical lesions (17, 18, 29). In contrast, and of major importance, we found no
significant improvement in imaging detection with large infusion doses of up to 500 mg antibody and no significant correlation with preinfusion circulating serum KC-4 antigen levels. However, similar to other trials, the t_{1/2li} was prolonged slightly at higher antibody doses.

The nonspecific uptake appears to be the major factor in limiting sensitivity. This is especially true for the liver, where metastases in this and other studies (7, 17, 18, 29) may demonstrate less activity than surrounding hepatic tissues. The origin of the background activity appears to be multifactorial, including Fc receptors, \(^{111}\)In-transferrin transchelation, and uptake of foreign protein by the normal reticuloendothelial system (galactose, mannose, and other receptors). In athymic nude mouse xenograft models, both our own studies and those of others indicate that F(ab')\(_2\) fragments show faster plasma clearance and greater tumor: liver ratios than whole antibody, indicating that Fc receptor binding plays a role (30). Furthermore, utilizing the F(ab')\(_2\) fragment of the HMFG1 antibody labeled with \(^{111}\)In, investigators found lower hepatic uptake in humans than that previously reported for intact immunoglobulin (10). Even with these fragments, the liver remains a major site of radioisotope uptake. As shown in this study, a fraction of \(^{111}\)In label dissociates from the radioconjugate and binds to plasma transferrin and may account for radioactivity subsequently distributed to liver and marrow. Some variability exists regarding the degree of transchelation to transferrin, with other investigators estimating 9% serum \(^{111}\)In binding to transferrin/day (31). Use of better chelates and perhaps presaturation of transferrin by coadministered iron or gallium are potential ways to reduce this problem (32). There may, additionally, be binding of mouse immunoglobulin by receptors in the liver. It is uncertain whether human antibodies or chimeric antibodies would diminish hepatic uptake. This uptake may potentially be blocked by preadministration of large doses of nonspecific immunoglobulin or proteins such as asialofetuin to block specific galactose receptors (33). The removal of radiolabeled antibody from the blood pool following adequate tumor antigen exposure may be accomplished with administration of a secondary anti-mouse antibody 48 h after primary antibody infusion (34). Decreasing nonspecific uptake to bone marrow is also critical for therapeutic trials with high dose radiolabeled antibodies, where myelosuppression has been the dose-limiting toxicity (35).

Another approach to decrease nonspecific background is to alter the route of administration and infuse the antibody by the i.p. or interstitial routes. This is most applicable in patients with intraabdominal disease or regional lymph node metastases. Others have shown decreased background and increased sensitivity with this approach utilizing related antibodies (36–38). We are evaluating the ability of interstitial administration of \(^{111}\)In-KC-4G3 to detect axillary metastases in breast cancer patients after injection into the breast tissue and/or the webs of the hands.

The kinetics and distribution of \(^{111}\)In-KC-4G3 appear similar to those of other \(^{111}\)In-labeled murine monoclonal antibodies in the literature (29, 39, 40). Other investigators have demonstrated improved tumor localization and retention of tumor radioactivity for the 1-(\(p\)-isothiocyanatobenzyl)-DTPA chelate, when compared with the mixed anhydride-DTPA chelate in the nude mouse xenograft model (15). We found only modest improvement in the imaging sensitivity and no significant

### Table 5 \(^{111}\)In-111 and KC-4G3 kinetics by total antibody dose

<table>
<thead>
<tr>
<th>Dose</th>
<th>Volume of distribution (liters)</th>
<th>Clearance (ml/min)</th>
<th>Elimination constant (h(^{-1}))</th>
<th>t(_{1/2li}) (h)</th>
<th>Volume of distribution (liters)</th>
<th>Clearance (ml/min)</th>
<th>Elimination constant (h(^{-1}))</th>
<th>t(_{1/2li}) (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.2 ± 0.9*</td>
<td>2.2 ± 0.9</td>
<td>0.033 ± 0.020</td>
<td>25 ± 15</td>
<td>3.8 ± 0.7</td>
<td>1.9 ± 0.3</td>
<td>0.030 ± 0.001</td>
<td>23 ± 1</td>
</tr>
<tr>
<td>10</td>
<td>9.1 ± 2.4</td>
<td>5.5 ± 2.3</td>
<td>0.035 ± 0.008</td>
<td>21 ± 5</td>
<td>4.7 ± 2.1</td>
<td>3.6 ± 4.3</td>
<td>0.040 ± 0.029</td>
<td>23 ± 12</td>
</tr>
<tr>
<td>50</td>
<td>6.0 ± 0.9</td>
<td>2.3 ± 1.0</td>
<td>0.022 ± 0.008</td>
<td>39 ± 23</td>
<td>4.5 ± 1.0</td>
<td>3.0 ± 1.3</td>
<td>0.039 ± 0.014</td>
<td>19 ± 6</td>
</tr>
<tr>
<td>100</td>
<td>5.2 ± 1.6</td>
<td>2.9 ± 1.0</td>
<td>0.033 ± 0.007</td>
<td>22 ± 5</td>
<td>5.2 ± 2.0</td>
<td>1.7 ± 0.5</td>
<td>0.020 ± 0.005</td>
<td>37 ± 10</td>
</tr>
<tr>
<td>500</td>
<td>7.0 ± 2.3</td>
<td>2.2 ± 0.8</td>
<td>0.019 ± 0.016</td>
<td>37 ± 4</td>
<td>4.7 ± 1.6</td>
<td>2.6 ± 2.4</td>
<td>0.032 ± 0.018</td>
<td>27 ± 12</td>
</tr>
<tr>
<td>All</td>
<td>6.5 ± 2.3</td>
<td>3.0 ± 1.7</td>
<td>0.027 ± 0.010</td>
<td>29 ± 14</td>
<td>4.7 ± 1.6</td>
<td>2.6 ± 2.4</td>
<td>0.032 ± 0.018</td>
<td>27 ± 12</td>
</tr>
</tbody>
</table>

* Mean ± SD.
plasma kinetic differences for the 1-(p-isothiocyanatobenzyl)-DTPA chelate preparation, compared to the mixed anhydride-DTPA chelate, although only four patients were studied in the former group. Improved sensitivity for the detection of intermediate-sized lesions of 1.5 to 3 cm in our study and the quality of tumor


Hansson, D. J., Grifflin, T. W., Kosciyczek, C., Ruskowski, M., Childs, R. L., Mattis, J. A., Shealy, D., and Doherty, P. W. Pharmacokinetics of an
imaging non-small cell lung cancer with monoclonal antibody KC-4G3


Imaging of Non-Small Cell Lung Cancers with a Monoclonal Antibody, KC-4G3, Which Recognizes a Human Milk Fat Globule Antigen

David G. Dienhart, Raymond F. Schmelter, James L. Lear, et al.


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