
Radioimmunodetection of Hodgkin's Disease and Non-Hodgkin's Lymphomas with Monoclonal Antibody to Eosinophil Peroxidase

Michael K. Samoszuk, Anne-Line Anderson, Eiman Ramzi, Felix Wang, Philip Braunstein, Jose Lutsky, Haresh Majmundar and Lewis M. Slater

Departments of Pathology, Nuclear Medicine and Medicine, University of California, Irvine, California

The purpose of this study was to determine if a radiolabeled murine monoclonal antibody (EOS) directed against eosinophil peroxidase would localize specifically to tumor sites in patients with lymphomas infiltrated by eosinophils. Ten patients with Hodgkin's disease and eosinophilia, three patients with non-Hodgkin's lymphomas and eosinophilia and five control patients received an intravenous injection of 3–10 mg of EOS antibody radiolabeled with 74–155 MBq (2.0–4.2 mCi) of ^{111}In . At intervals of 24, 48 and 72 hr after injection, gamma camera images were obtained along with blood and urine specimens and the imaging results were correlated with the results of other staging modalities. As early as 24 hr after antibody injection, there was clear visualization of identifiable sites of lymphoma with eosinophilia greater than 1 cm in size, including the spleen, bone marrow and lymph nodes. Although EOS also localized nonspecifically to the liver and, in some patients, to the nasopharynx, there was no appreciable uptake in normal bone marrow, spleen, uninvolved lymph nodes, lymphomas without eosinophilia or various other pathologic conditions without eosinophilia. Except for transient pain at tumor sites in three patients, no adverse reactions were noted. We conclude that a radiolabeled monoclonal antibody directed against eosinophil peroxidase localizes to lymphoma sites infiltrated by eosinophils.

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Because of the presence of numerous, reactive inflammatory cells in Hodgkin's disease tissues, Hecht et al. recently proposed that Hodgkin's disease may be "more amenable to monoclonal antibody treatment than many other tumors" (1). Despite this somewhat optimistic assessment, there has been only limited success in attempts to image or to treat Hodgkin's disease with immunoconjugates that specifically bind to the Reed-Sternberg cells that are characteristically present in low numbers in this neoplasm (2,3).

Attempts to stage and treat Hodgkin's disease with immunoconjugates and with other novel approaches (4–6)

have been prompted by the need for a highly accurate, noninvasive method to stage Hodgkin's disease and to identify and treat recurrent or residual disease in patients who have failed initial therapy (7–11). Conventional imaging procedures (12–14) are useful in many situations for imaging Hodgkin's disease, but they each have certain limitations with regard to sensitivity or specificity.

We and others (15,16) have demonstrated extensive degranulation of eosinophils and deposition of eosinophil peroxidase, a major component of eosinophil granules, in tissues involved with Hodgkin's disease and some non-Hodgkin's lymphomas. The eosinophilia in these lymphomas appears to be the result of production of interleukin-5 by malignant cells (17,18). Degranulation of the eosinophils in Hodgkin's disease may also be due to the deposition of IgE within the tumor (19,20).

At this time, the number of eosinophils within tumors does not have any known impact on the therapeutic strategies or the differential diagnosis. Eosinophil peroxidase (EPO), however, has a number of properties which make it a promising *in vivo* target for imaging lymphomas with an immunoconjugate. First, it is abundant in most cases of Hodgkin's disease and in many types of nonHodgkin's lymphomas, where it can coat the vascular endothelium within the tumor, thereby improving exposure to a systemically administered immunoconjugate (15,18,21). Unlike many tumor-associated antigens, such as carcinoembryonic antigen and immunoglobulin idiotypes, free EPO does not circulate in the peripheral blood and therefore should not interfere with immunoconjugate binding to target tissues. Moreover, because of its avidity for tumor cell membranes (22), EPO remains localized to tumor sites and is not subject to antigenic modulation since it is not produced directly by tumor cells. In normal bone marrow and blood, EPO is sequestered exclusively within intracellular granules of intact eosinophils (23); consequently, we felt that binding of anti-EPO immunoconjugates to normal bone marrow and to other normal tissues would be minimal. Finally, our pre-clinical studies in mice have demonstrated that EPO is an excellent *in vivo* target for a radiolabeled monoclonal antibody called EOS (24).

These observations prompted us to undertake a clinical

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For correspondence and reprints contact: Michael Samoszuk, MD, Pathology Department, Medical Sciences I, D-440, University of California, Irvine, CA 92717.

study, using Hodgkin's and nonHodgkin's lymphomas as a model system, to determine if degranulated eosinophils are a suitable *in vivo* target for a radiolabeled immunoconjugate. We report our study of thirteen lymphoma and five control patients, which demonstrates clear and specific visualization of tumor tissues with a radiolabeled antibody directed against EPO.

MATERIALS AND METHODS

Immunoconjugate Properties

EOS is an IgG2a murine monoclonal antibody that binds to human EPO (21). For the clinical studies described, the antibody was purified from *in vitro* cell cultures of the SF 25.5 hybridoma cell line by Techniclone Corporation (Tustin, CA) and then conjugated with diethylenetriamine-pentaacetic acid anhydride (DTPA) and radiolabeled with ^{111}In -chloride (MediPhysics, Arlington Heights, IL) (25).

In preclinical stability studies, we demonstrated that the radiolabeled antibody loses less than 10% of the ^{111}In label after incubation for 48 hr in human plasma at 37°C. The percentage of immunoreactivity of the immunoconjugate was calculated to be approximately 54% using a Lineweaver-Burke approach (26) that employed freeze-thawed human eosinophils as the target.

Prior to initiation of the human studies, the general safety, sterility and pyrogenicity of the immunoconjugate were verified, and an Investigational New Drug Application was filed with the United States Food And Drug Administration (FDA), which approved a Phase I imaging study of the immunoconjugate in a limited number of lymphoma patients and in certain, specified control patients. At the suggestion of the FDA, control patients with nontumor related eosinophilia were specifically excluded from this study, except for one control patient with idiopathic hyper eosinophilia. This exclusion was intended to minimize the possible occurrence of adverse reactions such as anaphylactic shock in control patients participating in the Phase I study.

Indium-111-immunoconjugates for each of the 18 patients were prepared separately. In a typical preparation, 10 mg of antibody were radiolabeled with 185 MBq (5 mCi) of ^{111}In . Before human administration, each radiolabeled immunoconjugate was tested for the presence of unbound radionuclide by thin-layer chromatography (24) and for binding to EPO in a solid-phase radioimmunoassay (RIA) (24). Less than 5% of the ^{111}In in each preparation was unconjugated to antibody, and the immunoconjugates had at least 10-fold greater binding to EPO than to negative control targets (ARH-77 cell line) in the RIA at an antibody concentration of 5 $\mu\text{g}/\text{ml}$. Thus, we confirmed the specific binding of the radiolabeled antibody to human EPO by two methods: RIA and immunoreactivity assay. The labeled antibody was injected within 0.5–3 hr after preparation.

An irrelevant, negative control immunoconjugate was not included in this study because three reports have documented that such antibodies do not localize to Hodgkin's disease or to non-Hodgkin's lymphomas (3,27,28). Moreover, in this pilot study, our Institutional Review Board believed that exposure to a radiolabeled negative control antibody would subject patients to additional risks without any direct benefits. Finally, we felt that the inclusion of subjects whose lymphomas contained little or no evidence of eosinophilia would provide indirect but still useful information regarding the specificity of the antibody localization *in vivo*.

Histology of Tumor Tissues

In this study, the degree of eosinophilia within a tumor was presumed to correlate directly with the degree of eosinophil degranulation and release of EPO. Patients were eligible for participation in this study if they had Hodgkin's or nonHodgkin's lymphomas with none to extensive eosinophilia, as assessed by microscopic examination of routinely processed, Giemsa-stained tissue sections.

For this study, we counted the number of eosinophils in five nonoverlapping 400 \times microscopic fields of the tumor stained with Giemsa to highlight eosinophils (Table 1). Those patients whose tumors contained two or fewer eosinophils per five 400 \times fields were classified as negative controls and are listed in Table 2. A retrospective review of our pathology files at UCI Medical Center indicated that 58 of 65 (89%) cases of mixed cellularity and nodular sclerosis Hodgkin's disease contained microscopic evidence of significant eosinophilia within the tumor (29). This relatively high figure was obtained by using a highly sensitive autofluorescence procedure in addition to Giemsa staining to detect intact and degranulated eosinophils in unfixed tissue sections (29).

Patients

All studies were performed under a protocol approved by the Human Subjects Committee of the University of California, and all patients gave informed consent. Eleven males and seven females were enrolled in the study. There were 13 patients who had extensive or moderate eosinophilia in their lymphomas (Table 1). Five additional patients constituted various negative, positive and specificity controls (Table 2). Because two of the patients in Table 1 (Patients 1 and 7) also had inflammatory or neoplastic conditions other than lymphoma, they were included in Table 2 as specificity controls. Consequently, Table 2 consists of a total of seven patients.

Table 1 includes ten patients with Hodgkin's disease and three patients with nonHodgkin's lymphomas with eosinophilia in their tumors. Ten of the patients in Table 1 had lymphomas which had been previously treated by various means, and three patients had newly diagnosed, untreated Hodgkin's disease. Patients 3 and 7 received chemotherapy concurrently with their participation in this study.

The patients in Table 2 were selected to demonstrate the specificity of antibody binding to degranulated eosinophils. Two of the negative control patients had newly diagnosed Hodgkin's disease which contained minimal or no eosinophilia. In addition, Table 2 included a negative control patient with recurrent lymphocyte predominant Hodgkin's disease, a tumor without eosinophilia. The specificity controls included patients with resolving pneumonia, chronic sinusitis, uterine leiomyomata and pulmonary tuberculosis. Although these pathologic conditions were not biopsied in our subjects, they ordinarily do not contain degranulated eosinophils. The "positive control" was a patient with idiopathic hyper-eosinophilic syndrome and biopsy-proven, eosinophilic (allergic) sinusitis.

Within 2 wk of enrollment in this study, all subjects underwent staging to determine the extent of their tumors by means of x-ray studies, physical examinations and computed tomography (CT) scans. In addition, nine of the patients underwent gallium scanning within 2 wk of their participation in this study, and some of the patients also underwent nuclear magnetic resonance imaging, biopsies and staging laparotomy as appropriate. Serum, plasma and urine specimens were obtained for routine analysis from subjects at the start of their participation. Patient 2 died due to

TABLE 1
Results of Staging and Imaging Studies in Patients with Eosinophilia in Lymphomas

Patient no.	Age/Gender	Diagnosis/Stage	Prior treatment	Tumor eosinophilia per five 400× fields	Conventional staging studies	Imaging with EOS
1	28/M	HD-MC/IIIA	RT and chemo; "remission"	15	Right iliac wing mass on CT scan	Right iliac wing mass
2	39/M	HD-NS/IVB	Chemo; refractory	26	Cervical, axillary, supraclavicular, abdominal nodes; iliac bone marrow	Same sites
3	41/M	Large-cell lymphoma, T cell type	Chemo; refractory	38	Right and left retromandibular region and submandibular nodes. ? spleen by CT scan	Upper cervical and right mandibular node and mandible; spleen. No marrow uptake.
4	46/F	HD-NS/IVB	Chemo; relapsed	11	Right hemipelvis and soft tissues, retroperitoneal nodes	Same sites, except retroperitoneal nodes
5	44/M	HD-NS/IIIB	Chemo; relapsed	14	Posterior auricular, cervical, supraclavicular, mediastinal axillary nodes	Same sites
6	40/M	HD-NS/IIIB	RT and chemo; relapsed	21	Spleen by CT scan	Strong uptake in spleen
7	65/F	Large-cell lymphoma, B cell type	Chemo; surgery	5	Sigmoid colon, spleen, cervical and para-aortic lymph nodes	Moderate uptake in sigmoid colon and right upper quadrant; spleen
8	36/F	HD-NS/IVB	Chemo; RT; refractory	9	Cervical, supraclavicular, mediastinal, inguinal, para-aortic nodes; left axilla; right iliac crest bone marrow	Same sites. Faint left cervical nodes and uptake
9	28/F	HD-MC/IIIA	None; newly diagnosed	14	Left cervical and supraclavicular lymph nodes. Spleen	Faint left cervical and supraclavicular nodes; strong splenic uptake
10	62/F	HD-NS/IIIB	None; newly diagnosed	12	Supraclavicular, axillary, mediastinal nodes	Supraclavicular and mediastinal nodes
11	22/F	HD-NS/IIA	None; newly diagnosed	18	Right cervical lymph nodes	Right cervical lymph nodes
12	63/M	Large-cell lymphoma, B cell type	Radiation; chemo	29	Orbital tumor	Orbital tumor
13	39/M	HD-NS/IIIB	RT only; relapse	25	Para-aortic and iliac lymph nodes pelvic bones	Same sites

progressive tumor and pancytopenia 13 wk after his participation in this study. His autopsy results were correlated with the results of our EPO imaging studies and with other staging modalities.

Antibody Administration and Tumor Imaging

Patients received a total dose of between 3–10 mg of antibody labeled with 74–155 MBq (2.0 to 4.2 mCi) of ¹¹¹In in 5 ml of sterile, phosphate-buffered saline. In order to minimize any possible adverse reactions, the immunoconjugate was administered intravenously in three divided doses. Vital signs were monitored every 15 min. The first (test) dose consisted of 1% of the total immunoconjugate and was followed by a 15-min observation period to detect adverse reactions. The second and third doses each consisted of half of the remaining immunoconjugate and were administered every 15 min, followed by a 15-min observation period. After the infusion and observation periods were completed, patients were allowed to resume their normal activities.

Scintillation camera images were acquired at intervals of 24, 48, and in a few cases, 72 hr after injection of the immunoconjugate. Anterior and posterior spot views (500,000–700,000 counts; 3–5 min per image) were recorded with a GE Starcam gamma camera. In a limited number of patients, single-photon emission computed tomography (SPECT) was also performed to localize and verify areas of abnormal uptake seen in the planar images. Blood-pool or organ subtraction was not performed, and we did not utilize any computerized image enhancement techniques. The images were stored in a digital format for subsequent reanalysis and also recorded on film.

The imaging studies were interpreted by two nuclear medicine physicians without prior knowledge of the results of the other staging studies performed on the patients. A scan was considered positive if both physicians identified the same abnormal-appearing localization of antibody in an anatomic site. The criteria for ab-

TABLE 2
Results of EOS Imaging Studies in Control Patients

Patient no.	Age/Gender	Diagnosis	Tissue eosinophilia per five 400× fields	Description	Imaging with EOS
1	28/M	Bronchopneumonia, resolving	Not evaluated	Specificity control	No imaging of pneumonia
7	65/F	Uterine leiomyoma; tuberculosis	Not evaluated	Specificity control	No imaging of uterus or lungs
14	21/M	HD-NS (IIB); newly diagnosed, in cervical, supraclavicular, mediastinal nodes and spleen	0	Negative control	No uptake in cervical, supraclavicular, mediastinal nodes, spleen
15	26/M	HD-NS (IIB); newly diagnosed, in cervical, supraclavicular, and mediastinal nodes	0	Negative control	No imaging of tumor in supraclavicular, cervical, mediastinal nodes
16	31/M	Recurrent lymphocyte predominance, Hodgkin's disease in inguinal lymph nodes	0	Negative control	No imaging of tumor in inguinal, femoral lymph nodes
17	34/F	HD-NS (IIB) in complete remission. Sinusitis; migraine headaches	Not applicable	Specificity control	No specific localization to any site
18	43/M	Eosinophilic (allergic) sinusitis; "idiopathic" hypereosinophilia	39	Positive control	Strong imaging of sinuses, spleen

normal uptake included assymmetric or nodular patterns of uptake in anatomic sites where such patterns would not normally be expected to occur.

Because there was no definitive "gold standard" in this study for identifying all tumor sites in all subjects, the interpretations of the EOS scans were subsequently compared to the results of the other staging modalities, with special emphasis given to the results of planar gallium and CT scans correlated with clinical findings. Gallium scans were performed with 5 mCi of ⁶⁷Ga given intravenously within 2 wk of the EOS imaging studies. Planar images of the head, chest, abdomen and pelvis were obtained 48 and 72 hr after injection (and, rarely, even later). Bowel cleansing and additional planar and SPECT images were obtained as needed.

CT scans were performed on a GE 9800 scanner, generally using 10-mm thick contiguous slices. Intravenous contrast was administered and noncontrast images through the liver were also obtained. The resulting images were reviewed with soft-tissue, bone or lung windows as appropriate.

Pharmacokinetic Studies

To determine the urinary clearance of the radiolabeled antibody, patients were instructed to collect their urine daily for up to 72 hr. Serial whole blood samples were also obtained from each patient for up to 72 hr. The blood and urine samples were counted in a well scintillation counter along with diluted aliquots of the injected material. Total urine volume was measured, and total blood volume was estimated using a nomogram. The percent of injected dose in urine and blood at the various time intervals was then calculated.

Assay for Human Anti-Mouse Antibody (HAMA) Response and Toxicity

Each patient was tested 2–4 wk after antibody administration for the development of HAMA response. During this follow-up visit, patients were also questioned about any adverse reactions associated with the immunoconjugate and routine blood and urine tests were again performed.

The HAMA response in each patient was determined using a two-stage ELISA which detects and quantifies human humoral antibodies to mouse IgG (ImmuSTRIP, Immunomedics, Warren, NJ). Duplicate assays of diluted patient serum samples were analyzed along with serial dilutions of a reference standard provided by the manufacturer. The results were expressed as ng/ml of human anti-mouse IgG.

RESULTS

Antibody Administration and Tumor Imaging

The results of the imaging studies are summarized in Tables 1 and 2, and representative images are illustrated in Figures 1–5. In the 13 patients whose lymphomas contained eosinophilia, 41 of 41 known and suspected tumor sites greater than approximately 1 cm in diameter were visualized at 24 hr, but the best image quality was obtained at or after 48 hr. In the positive control patient with eosinophilic sinusitis, there was intense antibody uptake in the frontal sinuses.

Figure 1 illustrates the specific localization of the radiolabeled antibody to the orbit of the patient with an orbital large-cell lymphoma containing extensive eosinophilia. In the Hodgkin's disease patients, there was obvious uptake

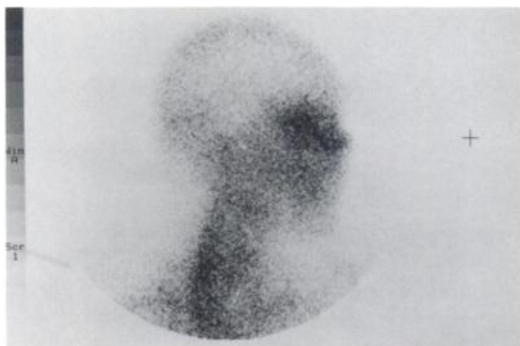


FIGURE 1. Gamma camera image of right head and neck of Patient 12 demonstrates antibody localization to orbital lymphoma.

of antibody by tumor in cervical lymph nodes (Fig. 2), axillary lymph nodes, mediastinal lymph nodes and para-aortic abdominal lymph nodes (Fig. 3). Hodgkin's disease involving the iliac bone marrow also had strong uptake of radiolabeled EOS antibody (Fig. 4).

In the patients without splenic involvement by Hodgkin's disease, hepatic uptake was considerably greater than splenic uptake (Fig. 3). By contrast, the four patients with known splenic involvement by lymphoma had splenic uptake that was significantly more intense than hepatic uptake (Fig. 5).

With regard to the specificity of imaging, all patients had significant hepatic uptake unrelated to tumor involvement. It should be noted, however, that hepatic uptake is a general property of all indium-labeled whole monoclonal antibodies (26). Three patients had unexplained moderate uptake in the nasopharynx. There was no substantial localization of antibody, however, to uninvolved spleen, bone marrow, lymph nodes, breast or previous sites of tumor that had regressed after treatment, including Patient 17 whose tumor originally contained extensive eosinophilia.

As summarized in Table 2, there was also no localization of antibody to sites of resolving pneumonia, chronic sinusitis, uterine leiomyomata, pulmonary tuberculosis, lymphocyte predominant Hodgkin's disease or nodular sclero-



FIGURE 2. Gamma camera image of head and neck of Patient 5. There is obvious uptake of radiolabeled antibody by Hodgkin's disease in the neck, left supraclavicular area, sternum and near the sternoclavicular joints bilaterally.

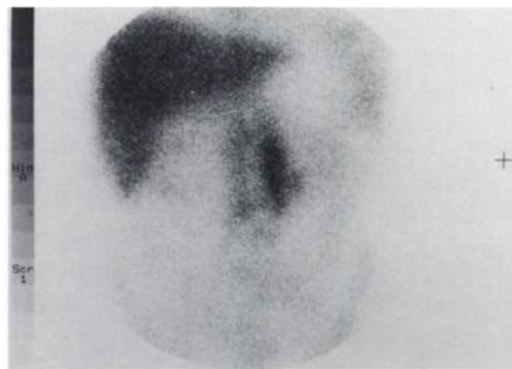


FIGURE 3. Anterior abdominal view of Patient 2. A chain of intensely positive, para-aortic lymph nodes is visible. The spleen is also visible but is much less intense than the liver. At autopsy, this patient's spleen contained no evidence of Hodgkin's disease, but there was extensive Hodgkin's disease in the para-aortic lymph nodes.

sis Hodgkin's disease without eosinophilia. The results from these specificity control patients therefore provide further evidence of the specificity of antibody localization to tumor sites containing EPO.

It is notable that peripheral blood eosinophilia ($1600/\mu\text{l}$) or bone marrow eosinophilia (35% eosinophils) in otherwise normal bone marrow of patients did not interfere with the imaging of lymphoma sites. Furthermore, neutropenia ($1200\text{--}2000/\mu\text{l}$) by itself did not diminish uptake of antibody by those lymphomas that were extensively infiltrated by eosinophils (Patients 2 and 4). The uptake of EOS antibody was, however, somewhat less intense in the nonHodgkin's lymphoma patients who were concurrently receiving chemotherapy.

In general, the most intense antibody uptake occurred in patients with relatively large masses of relapsed or refractory Hodgkin's disease that contained extensive eosinophilia. Newly diagnosed patients with Hodgkin's disease in lymph nodes between 1–1.5 cm in size tended to have less intense, but still obvious, nodal imaging.

No acute or late toxicity was attributable to administration of the immunoconjugate. None of our patients developed fevers, chills, urticaria or other allergic responses to

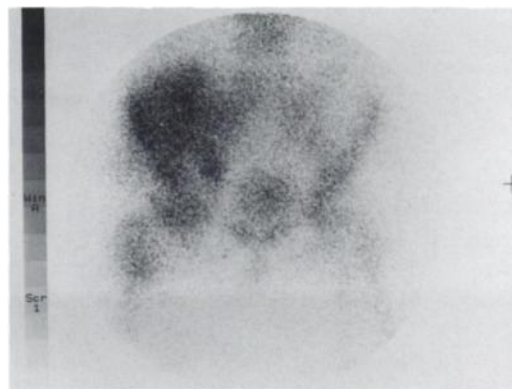


FIGURE 4. Anterior pelvic image of Patient 4. There is striking imaging of Hodgkin's disease tumor in the right hemipelvis and right proximal femur.

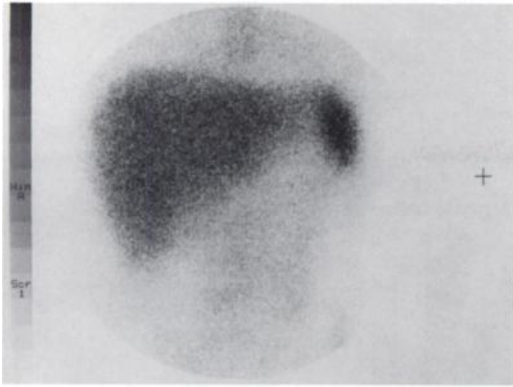


FIGURE 5. Anterior abdominal image of Patient 9. In the gallium scan (not shown), there was equivocal imaging in the region of the spleen. By contrast, in EOS imaging shown here, the spleen was more intensely and sharply visualized than the liver. At laparotomy, the spleen was found to be focally infiltrated by Hodgkin's disease.

the antibody. Three patients complained of transient, painful lymphadenopathy at tumor sites commencing approximately 24 hr after administration of the immunconjugate and continuing for up to 3 days afterwards.

Comparison with Gallium and CT scans

The results of the EOS imaging studies in patients from Table 1 are compared with the results of gallium and CT scans in Tables 3 and 4, respectively. In Table 3, there was concurrence between EOS imaging and gallium scan results for 10 separate sites of lymphoma. Three additional lymphoma sites were detected only by EOS imaging and were thought to represent false-negatives of the gallium scan (biopsy-proven and CT-proven Hodgkin's disease in the iliac bone marrow of Patient 1; splenic Hodgkin's disease in Patients 6 and 9, proven by CT scan and/or laparotomy).

Also in Table 3, there were five sites in which the gallium scan was positive and EOS imaging was negative. Three of these five sites were in the parotid and submandibular glands of Patient 1 and were considered to be false-positives of the gallium scan because there was no other clinical or radiological evidence of recurrence in those sites. Two small lymph nodes were visualized on the gallium scan before treatment in Patient 7 but were not visualized by EOS imaging performed 2 wk later after chemotherapy had begun.

With regard to the visualization of small tumor sites, the gallium scan and EOS imaging produced equally intense

TABLE 3
Comparison of EOS Imaging and Gallium Scanning in Patients with Eosinophilia in Lymphomas

EOS imaging	Gallium scanning		Total
	Positive	Negative	
	Number of sites		
Positive	10	3	13
Negative	5	N/A	5
Total	15	3	18

TABLE 4
Comparison of EOS Imaging and CT Scanning in Patients with Eosinophilia in Lymphomas

EOS imaging	CT scanning		Total
	Positive	Negative	
	Number of sites		
Positive	33	2	35
Negative	4	N/A	4
Total	37	2	39

images of small (1–1.5 cm) cervical lymph nodes in two of our patients with newly diagnosed Hodgkin's disease (Patients 9 and 11). Consequently, our data suggest that gallium scans and EOS imaging have approximately equal capabilities to detect small tumor masses.

In summary, the major discordances between the gallium scan results and the EOS imaging results in this limited Phase I study were primarily attributable to three false-negatives and three false-positives in the gallium scan results. EOS imaging therefore appears to compare favorably with gallium scanning for specifically identifying sites of lymphoma and for detecting tumor masses below the diaphragm, particularly in the spleen and bone marrow.

Not listed in Table 3 are the three negative control patients (Patients 14–16) whose Hodgkin's disease contained minimal or no eosinophilia. In those patients, the gallium scans were positive at the sites of tumor except in the spleen of Subject 14 and the inguinal lymph nodes of Subject 16, where equivocal imaging was obtained. The EOS imaging studies, as expected, produced very faint or no visualization of tumor in the three negative control subjects. The results from these negative control patients therefore provide further evidence of the correlation between antibody localization in tumor sites and the presence or absence of degranulated eosinophils.

In Table 4, there was concurrence between EOS imaging and CT scan results for 33 separate tumor sites. CT scans, however, were able to detect four small (approximately 1 cm) lymph nodes not visualized by EOS imaging. Two of these lymph nodes (from Patients 4 and 10) probably represented small sites of Hodgkin's disease in the retroperitoneum and axilla. The third lymph node was visualized by CT scan in the para-aortic region of Patient 7 before the start of chemotherapy; EOS imaging was performed 2 wk after the start of treatment. In the fourth case, the CT scan detected an enlarged pulmonary lymph node in Patient 7 who had a history and radiologic evidence of pulmonary tuberculosis. Since the node was not biopsied, it was not possible to ascertain if the enlargement was neoplastic or inflammatory in origin.

In two sites listed in Table 4, EOS imaging correctly identified Hodgkin's disease in bone marrow incorrectly interpreted as normal on the CT scan (iliac bone marrow of Patients 2 and 8). EOS imaging also suggested the presence of Hodgkin's disease in the spleens of two patients in

TABLE 5
Pharmacokinetics of EOS Immunoconjugate

Patient no.	Amount injected (mCi)	%ID in Blood/Urine			T _{1/2} (hr)
		24 hr	48 hr	72 hr	
Table 1					
1	3.9	59.7/7.3	52.2/3.4	28/4.0	49
2	3.1	50.3/5.1	42.6/3.6	28/n.a.	48
3	2.5	36.0/3.5	20.8/3.4	12.7/2.5	16
4	4.2	75.2/8.5	n.a./3.4	31.7/3.5	41
5	3.0	59.9/5.1	31.9/4.6	n.a.	31
6	4.2	49.6/5.2	31.2/2.9	n.a.	24
7	3.3	60.6/4.0	34.8/n.a.	n.a.	32
8	3.2	30.3/4.1	18.1/n.a.	n.a.	14
9	3.1	46.8/5.8	32.5/2.5	n.a.	21
10	2.0	54.9 (19 hr)/6.6	33.7 (43 hr)/2.7	n.a.	22
11	2.2	46.8 (19 hr)/4.7	29.1 (43 hr)/3.4	n.a.	16
12	3.1	no data	no data	no data	n/a
Table 2					
13	3.6	44.8/7.2	22.6/4.2	n.a.	21
14	3.3	34.8/8.7	19.5/4.4	n.a.	16
15	4.2	56.0/4.7	30.6/1.8	n.a.	28
16	3.7	n.a./6.6	42.9/1.8	30.3/2.4	39
17	3.4	54.6/8.5	31.0/3.7	n.a.	27
Mean ± 1 s.d. (n)	3.3 ± 0.6 (17)	50.7 ± 11.2 (15)	31.6 ± 9.0 (15)	25.7 ± 7.6 (5)	28 ± 11 (16)
	Urine	6.0 ± 1.6 (16)	3.3 ± 0.8 (14)	3.1 ± 0.7 (4)	

whom the CT scans were reported as "suspicious" for splenic involvement.

Pharmacokinetic Studies

The pharmacokinetic properties of the immunoconjugate in each patient are listed in Table 5. Because of technical problems and compliance failures in some patients, some data points were not available for inclusion in the table.

In general, the limited pharmacokinetic data that were available suggest that there was no consistent relationship between the whole blood half-life or urinary clearance of the immunoconjugate and the extent of tumor or tumor-related eosinophilia, except for Patient 8 who had the most extensive tumor and also the shortest whole blood half-life. Patient 14 had the highest urinary clearance of radiolabel (8.7% of the injected dose at 24 hr). Interestingly, his tumor contained no detectable eosinophils. The third lowest urinary clearance (4.1% of the injected dose at 24 hr) was in Patient 8, the same patient with the shortest whole blood half-life and also the most extensive tumor.

Assay for HAMA Response

The HAMA titers in all patients were less than 40 ng/ml, which is the lowest detection limit with ELISA. The reference sample produced results within the expected range of the assay.

DISCUSSION

In this controlled study, we have demonstrated specific uptake of radiolabeled EOS antibody within tumor sites in 10 patients with Hodgkin's disease and in 3 patients with nonHodgkin's lymphomas whose tumors were infiltrated

by degranulating eosinophils. In addition, we have shown that EPO deposits were present in relapsed and widely disseminated lymphomas as well as in newly diagnosed lymphomas. We attribute the intensity and sensitivity of imaging of these tumors to the abundance and durability of the EPO target antigen within the tumor and the specificity of the immunoconjugate for EPO. Consequently, we conclude that eosinophilic infiltration in lymphomas is accompanied by degranulation of eosinophils and the release of EPO, which is an excellent *in vivo* target for a systemically administered immunoconjugate in humans.

Perhaps the most intriguing finding in this study was the presence of widespread and extensive deposits of EPO specifically in lymphoma sites infiltrated by eosinophils. This finding confirms and significantly extends previous *in vitro* studies of pathologic tissue specimens (15,16) and raises the possibility that EPO and other eosinophil granule proteins could have some biological significance within lymphomas. Curiously, other malignancies, such as lung, colon, cervical and thyroid carcinoma, sometimes also have extensive infiltration by eosinophils (30). Moreover, Gleich and co-workers have recently demonstrated extensive degranulation of eosinophils in pathologic tissue specimens obtained from a variety of non-neoplastic diseases, including retroperitoneal fibrosis, sclerosing mediastinitis, sclerosing cholangitis and a majority of cases of pulmonary fibrosis (31).

At this time, biologically specific, *in vivo* diagnostic and therapeutic studies of these neoplastic and inflammatory diseases containing degranulated eosinophils are still in an early stage of development. Although we confined this pilot imaging study to patients with Hodgkin's disease and

nonHodgkin's lymphomas, we believe that future studies should be performed to determine if it is possible to use EOS-derived immunoconjugates to deliver cytotoxic drugs or therapeutic radionuclides selectively to tumors or diseased tissues containing degranulated eosinophils. Such a therapeutic approach appears to be promising in view of the minimal uptake of the EOS immunoconjugate by normal bone marrow and the undetectable HAMA response in patients who have received a single, small dose of the immunoconjugate.

Our findings also suggest the need to study the possibility of using radioimmunodetection of EPO deposits to supplement conventional staging techniques for those lymphomas containing degranulating eosinophils. For example, patients with newly diagnosed Hodgkin's disease should be studied to determine if noninvasive imaging with radiolabeled EOS immunoconjugate could spare a subset of such patients from undergoing staging laparotomy and splenectomy in order to identify subdiaphragmatic disease when CT and lymphangiography are negative (10,11). Furthermore, imaging with EOS antibody should be formally evaluated in treated Hodgkin's disease patients who have an unconfirmed/uncertain complete remission (CR[u]) or persistent radiological abnormalities of uncertain significance (7).

In conclusion, we have demonstrated that human EPO is an excellent target for a systemically administered radiolabeled immunoconjugate in certain patients with Hodgkin's disease and nonHodgkin's lymphomas. Although the current study was not designed to be a definitive comparison between EOS antibody imaging and imaging with other modalities, our data have clearly demonstrated the potential utility of this approach as well as the need for further clinical studies.

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