

# Localization of $^{131}\text{I}$ -Labeled p97-specific Fab Fragments in Human Melanoma as a Basis for Radiotherapy

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**ABSTRACT** 33 patients with advanced malignant melanoma were studied after intravenous administration of  $^{131}\text{I}$ -labeled Fab fragments specific for p97, an oncofetal glycoprotein of human melanoma. In all, 47 gamma camera imaging studies were performed for the purpose of localization of metastatic deposits. In addition to tumor,  $^{131}\text{I}$ -Fab uptake was also seen in liver and kidney. 20 of these studies included simultaneous administration of both an  $^{131}\text{I}$ -labeled Fab specific for p97, and an  $^{125}\text{I}$ -labeled Fab not specific for p97. Blood clearance of p97-specific Fab was significantly more rapid than for nonspecific Fab. Eight of these patients had biopsies of subcutaneous nodules at 48 and 72 h postinjection in order to assess whether localization of radioactivity was antigen specific. Antigen-specific localization was observed with average ratios of specific/nonspecific uptake of 3.7 (48 h) and 3.4 (72 h); uptake was strongly correlated with tumor p97 concentration ( $r = 0.81$ ,  $P < 0.01$ ). Also, imaging studies of the biodistribution of  $^{131}\text{I}$ -labeled anti-p97 Fab in patients selected for high p97 tumor concentration showed avid tumor uptake and more prolonged retention of labeled Fab in tumor than in normal tissues.

Based on these studies, we estimated that total  $^{131}\text{I}$  doses of 500 mCi could be safely given to patients

before dose-limiting toxicity would be observed. Accordingly, in seven selected patients, phase I radiotherapeutic trials were begun. For improved radiation safety, we developed automated methods to label Fab fragments with up to 200 mCi of  $^{131}\text{I}$ . So far, a total of 12 individual therapeutic doses, ranging from 34 to 197 mCi of  $^{131}\text{I}$ -labeled to 5 to 10 mg of Fab, have been administered with excellent tumor localization and without major target organ toxicity. Cumulative doses ranged from 132 to 529 mCi  $^{131}\text{I}$ . Side effects attributable to the radiation were mild, with a transient drop slightly  $>50\%$  in platelet and absolute neutrophil counts being observed in the two patients who received cumulative doses  $>500$  mCi. In the combined series of 47 diagnostic and 12 therapeutic studies, four acute reactions were observed: one episode each of transient chills and fever; flushing and hypotension; and two skin rashes. All of these reactions responded promptly to symptomatic therapy. After multiple administrations of  $^{131}\text{I}$ -(anti-p97) Fab (IgG1), isotype-specific immunity was observed in three patients. In two of these patients it was possible to successfully reinfuse after immunity had developed with  $^{131}\text{I}$ -(anti-p97) Fab of a different isotype (IgG2a). Dosimetry estimates were performed based on the biodistribution of  $^{131}\text{I}$ -Fab in these patients, and for every 100 mCi of  $^{131}\text{I}$ -Fab given, tumor received 1,040 rads; liver, 325 rads; and bone marrow, 30 rads. Marrow would be expected to be the critical organ, if doses  $>500$  mCi  $^{131}\text{I}$ -Fab are given. These studies demonstrated that, with proper precautions, large doses (of an  $^{131}\text{I}$ -labeled murine Fab fragments immunologically

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specific for a human melanoma-associated antigen) could be safely given to humans by using repetitive intravenous injections.

## INTRODUCTION

Disseminated malignant melanoma is incurable with presently available therapeutic regimens, and median survival of patients with this disease ranges from a few months to one year. We are evaluating a new therapeutic strategy based on the availability of monoclonal antibodies for an oncofetal antigen, p97, which is strongly expressed in many melanomas and only weakly in normal adult tissues (1-3). The goal of this technique is to use monoclonal antibodies or antibody fragments as vehicles for selective delivery of high doses of radiation to metastatic melanoma tumor deposits, while sparing normal radiosensitive tissues.

In a previous study, we used  $^{131}\text{I}$ -labeled IgG antibodies to image metastatic deposits in human patients with malignant melanoma (4). We found that murine monoclonal antibodies to p97 could be given safely to human patients in milligram doses, and that immunologically specific uptake could be demonstrated. 88% of known metastatic sites >1.5 cm were detected by this technique, including lesions that were otherwise occult. However, the use of the radiolabeled whole IgG was associated with the undesirable feature of significant retention in blood and soft tissue, resulting in a relatively low concentration gradient between tumor and surrounding tissue, particularly at early imaging times. Also, antibodies to mouse IgG appeared regularly in the patients' sera within 2 wk after intravenous injection of 1 mg or more of murine IgG. Although no clinical effects were evident in subsequent injections, these patients cleared the radiolabeled murine antibody more rapidly into liver, and tumor uptake was reduced. Thus, the possibility of sequential administrations of radioactivity to the patient for either diagnosis or therapy was obviated by the immune response.

On the basis of differences in size, Fab fragments would be expected to be significantly less immunogenic than whole IgG (5), and are cleared from the blood and extracellular fluid much more rapidly than whole IgG (6). The purpose of the present study was to determine if high dose radiolabeled (anti-p97) Fab localized in human melanoma in vivo and whether the procedure was safe enough for more extensive trials as a treatment for disseminated disease.

## METHODS

**Radioiodination of Fab fragments.** Two antibodies specific for p97 (2) were used: antibody 8.2 (IgG1) and antibody 96.5 (IgG2a). For most of the studies, antibody 8.2 was used, and as a control, antibody 1.4 (IgG1 specific for murine leu-

kemia gp 70, J. P. Brown, unpublished work). Antibodies were purified by affinity chromatography on protein A-Sepharose from the ascites fluid of mice, transplanted intraperitoneally with hybridoma cells (1). Fab fragments were prepared as described in reference 2.

All monoclonal Fab fragments used were radioiodinated by the chloramine T (CT)<sup>1</sup> method. Both  $^{131}\text{I}$  and  $^{125}\text{I}$  were obtained at high specific concentrations as protein labeling grade in 0.1 M NaOH (New England Nuclear, No. Billerica, MA). The radioiodine and Fab fragments were reacted together in a phosphate buffer (pH 7.3) at a CT level of ~25  $\mu\text{g}$  per mg Fab. The resultant iodinated Fab was purified by passage over a Sephadex column and sterilized by 0.22  $\mu\text{m}$  filtration.

For preparations involving >10 mCi of activity, the iodination, separation, and sterilization were performed remotely in a hot cell containing a four-channel peristaltic pump and three remotely activated valves to sequentially load 0.5 ml buffer, 0.1 ml (5 mg) Fab, and 0.1 ml (125  $\mu\text{g}$ ) CT to a vial containing 100-200 mCi of  $^{131}\text{I}$ -iodide. The reaction was automatically quenched 5 min after addition of oxidant by addition of sodium metabisulfite and carrier sodium iodide, and the reaction mixture was remotely pumped onto a Sephadex G-10 column (8-ml bed volume). The same pump transferred the product to the top of the column and then continually supplied sterile isotonic eluent while another channel pulled effluent from the bottom of the gel column and pushed it past a beta-sensitive radiation monitor for peak detection.

The final preparation for both high and low level iodinations was in 0.05 M phosphate-buffered saline suitable for injection. The percentage of protein bound radioiodine was determined using cellulose acetate thin-layer electrophoresis (typically >99%). Before injection, the product was determined to be pyrogen-free by using the limulus amoebocyte lysate test (Associates of Cape Cod, Woods Hole, MA).

**Assay for binding of labeled antibody to melanoma cells.** SK-MEL 28 melanoma cells ( $2 \times 10^6$ ) were suspended in 100  $\mu\text{l}$  test sample (5 ng Fab, diluted with fetal calf serum) and incubated with mixing for 15 min. Phosphate-buffered saline (10 ml) was added, the mixture was centrifuged, and the cells were counted for  $^{131}\text{I}$ . Results were expressed as percentage total added radioactivity bound to cells.

**Immunohistological staining.** We used an immunocytochemical technique for antigen localization in tissue sections, which has been described as the unlabeled antibody technique of Sternberger (7) and has been modified and used by us in the past (8).

**Human studies.** Studies in humans were performed under the auspices of Investigational New Drug Bureau of Biologics No. 1609 and after approval by all appropriate Institutional Review committees. Patients with widespread inoperable melanoma were selected for study after they had given their informed consent. In most cases these patients had failed all conventional forms of therapy. The concentration of p97 in tumors was measured for each patient in biopsy material before scintigraphic study using a two-site immunoradiometric assay (9). Before intravenous injection for the imaging study, 100  $\mu\text{l}$  (20-100  $\mu\text{g}$ ) of Fab was tested intradermally. Thyroid uptake was blocked with 10 drops of a saturated solution of potassium iodide (SSKI) 60 min before injection, and twice per day for 14 d postinjection. This dose of SSKI reduces thyroidal uptake of  $\text{Na}^{131}\text{I}$  to ~1% of the dose (10) and is administered because some free iodide is liberated

<sup>1</sup> *Abbreviations used in this paper:* CBA, cell binding assay; CT, chloramine T; SSKI, saturated solution potassium iodide.

from the  $^{131}\text{I}$ -(anti-p97) Fab during the course of its metabolism. Unless these precautions are taken, the free iodide will be concentrated in the thyroid. Base-line laboratory studies obtained before injection and repeated at 48 h included hemoglobin, leukocyte count, platelet count, serum creatinine, bilirubin, lactate dehydrogenase, aspartate amino transferase, alkaline phosphatase, complement, and urinalysis.

All patients received at least one diagnostic dose of 1 mg (anti-p97) Fab labeled with 5 mCi  $^{131}\text{I}$  and injected over 10 min. In most cases, 1 mg (control) Fab labeled with 2 mCi  $^{125}\text{I}$  was injected concomitantly. In seven patients with avid tumor concentration of diagnostic doses, therapeutic amounts of  $^{131}\text{I}$ -(anti-p97) Fab of 34–197 mCi  $^{131}\text{I}$  labeled to 5–10 mg protein were administered by constant infusion over 1 h. After terminating the infusion, serial blood samples ( $5\text{ cm}^3$ ) were drawn at 5, 15, 30, 45, 60, 90, and 120 min and at 24 and 48 hours. Clearance curves were obtained by plotting decay corrected counts per minute per cubic centimeter of plasma and plotting the results on semi-log paper. Several patients had biopsies of accessible skin nodules at 48 or 72 h postinjection.

The plasma samples and biopsies were assayed for radioactivity in a gamma counter (Autogamma, 5900 series, Packard Instruments Co., Inc., Downers Grove, IL). Patients were imaged at 2, 24, and 48 h with a 410 gamma camera (Ohio Nuclear Inc., Solon, OH) with high energy collimator. At least 500,000 counts were obtained per view. Gamma camera energy settings were 20% window-centered at 364 keV. In all patients and at most time points, a  $^{99\text{m}}\text{Tc}$ -human serum albumin (1 mCi) blood pool subtraction (11) method was employed. Data processing was performed using a VIP-450 computer (Technicare Corp., Solon, OH). Clearance curves were generated by collecting sequential counts over an organ. Clearance half-times were calculated as described above. Five of the seven patients who received therapeutic amounts of  $^{131}\text{I}$  Fab have died, and autopsies were obtained on three of five patients.

## RESULTS

*Effect of iodination on Fab binding to melanoma cells in a cell binding assay (CBA).* The  $^{131}\text{I}$ -(anti-p97) Fab fragments were tested for binding to melanoma cells as a measure of the radiolabeled Fab's reactivity with antigen. For the clinical studies, the CBA varied from 15 to 50% (average 38%). There was continual improvement in CBA throughout the course of these investigations, and the higher CBA's occurred in the more recent studies. Work in progress indicates that in future studies, CBA of 65% should be routinely achievable.

*Measurement of p97 antigen in patient tumors.* The presence of p97 antigen in tumor was demonstrated by the immunoperoxidase technique in several of the patients. An example is shown in Fig. 1, A and B, and was obtained from a subcutaneous nodule. Cell-associated intense antigen staining is seen within the clusters of tumor cells, but not in the adjacent stroma.

*In vivo studies (see Table I).* In all, a total of 47 studies have been performed on 33 patients with  $^{131}\text{I}$ -Fab, for the purpose of tumor localization and assessment of biodistribution.

20 studies were performed with <2 mg (0.4–1.7 mg,

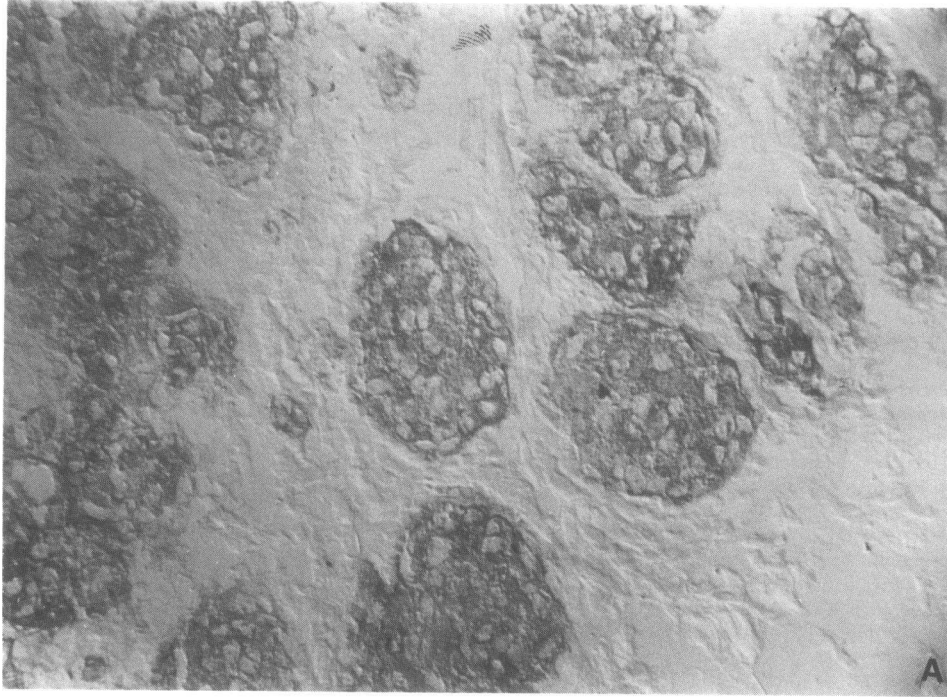
$x = 0.96\text{ mg}$ ) of specific  $^{131}\text{I}$ -Fab (see Table I, 3 a, b, f, and j) and an exactly equal amount of nonspecific  $^{125}\text{I}$ -(1.4) Fab. Blood clearance, whole body retention, urinary excretion, and whole body imaging studies were performed on all patients, and when subcutaneous nodules were accessible, patients had biopsies (six at 48 hours, 11 nodules; and four at 72 hours, seven nodules). (See Table I for details of antibody injection technique and blood clearance data.)

In patients with advanced melanoma, the biodistribution of anti-p97 Fab was significantly different from the nonspecific Fab. Within minutes after injection in five patients, an average of 37% (SD of 9%) more specific than nonspecific Fab was cleared from the circulation (See Table I, 3 a).

We compared whether there might be systematic differences between  $^{131}\text{I}$ - and  $^{125}\text{I}$ -labeled anti-p97 Fab as a further control in a single patient. The clearances of both preparations were identical and were characteristic of the more rapid clearance that we observed in other patients for anti-p97 Fab. Thus, the differences in the behavior of  $^{131}\text{I}$ - and  $^{125}\text{I}$ -labeled Fab in patient studies were presumably due to biologic differences between the anti-p97 and control Fab with respect to their in vivo metabolism. Four patients who received an infusion of antibody had their studies repeated a week later, within 15 min of receiving 1.0 mg of non-radioactive anti-p97 Fab. There was a significant reduction in the difference between specific and nonspecific blood concentration at 5 min postinjection (before: nonspecific-specific was 48.5%; after: nonspecific-specific was 30%;  $P < 0.01$ ).

In a group of four patients who received <2 mg of anti-p97 Fab (8.2), it was possible to compare plasma clearance of an equal amount of nonspecific antibody. The CBA on the patients' plasma was also determined. The results are shown in Fig. 2 as a function of time postinjection. Only the averages of the data are plotted. About 50% of specific antibody was rapidly cleared over the first 20 min postinjection. Thereafter, both specific and nonspecific Fab were cleared with about the same  $t_{1/2}$  (83 min), and the clearance was essentially monoexponential over the first 4 h postinjection. The CBA rapidly declines over the first 20 min also, and then leveled off to be cleared more slowly. Note that the CBA was 50% of total activity at the time of injection, and then declined to ~15% very rapidly. Taken together, these data suggest that the active Fab 8.2 was cleared more rapidly than the inactive Fab 8.2, which did not bind to SK MEL 28 cells.

In four patients, seven accessible subcutaneous nodules were biopsied at 48 h and the content of  $^{131}\text{I}$  and  $^{125}\text{I}$  radioactivity was assayed. In the same samples, the amount of p97 was measured by a double determinant radioimmunoassay (9). The results are expressed in Fig.



**FIGURE 1** Frozen sections from lymph node biopsy of patient with documented melanoma. Hematoxylin-stained sections (not shown) demonstrated tumor cells occurring in round clusters within a fibrous stroma. (A) Monoclonal antibody 96.5 treated; (B) control antibody treated. The clusters of tumor cells are stained by the immunoperoxidase technique (A), while the normal cells in the same section are unstained. Neither tumor nor normal cells are stained with control antibody (B).

TABLE I  
Localization Studies with Anti-p97 Fab\*

| 1. Patient injections 47 studies (33 patients)                  |     |                          |         |                      |             |
|---|-----|--------------------------|---------|----------------------|-------------|
| a. Fab 8.2 (IgG1)   |     | 25 studies (17 patients) |         |                      |             |
| b. Fab 96.5 (IgG2a)   |     | 17 studies (16 patients) |         |                      |             |
| c. Fab 96.5 + 8.2   |     | 5 studies (4 patients)†  |         |                      |             |
| 2. Results summary  |     |                          |         |                      |             |
| a. Negative   |     | 14 studies (13 patients) |         |                      |             |
| size $\leq 1\frac{1}{2}$ cm (10); p97 levels low (2); other (2) |     |                          |         |                      |             |
| b. Positive   |     | 33 studies (20 patients) |         |                      |             |
| 3. Blood clearance studies                                      |     |                          |         |                      |             |
| Injection   | No. | Antibody                 | Amount  | % "rapid component"‡ | $t_{1/2}$ ‡ |
| <i>min</i>  |     |                          |         |                      |             |
| a. Pulse  | 5   | 8.2/1.4                  | <2.0 mg | 37±9                 | 61±28       |
| b. Infusion   | 8   | 8.2/1.4                  | <2.0 mg | 48±9                 | 91±20       |
| c. Infusion   | 2   | 8.2                      | <2.0 mg | N/A                  | 67±19       |
| d. Infusion   | 6   | 8.2                      | 5 mg    | N/A                  | 116±38      |
| e. Infusion   | 1   | 8.2                      | 10 mg   | N/A                  | 73          |
| f. Infusion   | 2   | 96.5/1.4                 | <2 mg   | 38±5                 | 53±27       |
| g. Infusion   | 3   | 96.5                     | 5 mg    | N/A                  | 101±13      |
| h. Infusion   | 13  | 96.5                     | 10 mg   | N/A                  | 93±16       |
| i. Infusion   | 2   | 96.5                     | 20 mg   | N/A                  | 113±33      |
| j. Infusion   | 5   | 96.5 + 8.2/1.4           | <2 mg   | 45±10                | 62±34       |
| 4. Clearance studies (other)                                    |     |                          |         |                      |             |
| Tissue  | No. | Antibody                 | Amount  | $t_{1/2}$ (h)‡       |             |
| a. Whole body   | 15  | 8.2                      | 2-20 mg | 33 (30-48)           |             |
| b. Liver  | 5   | 8.2                      | 2-5 mg  | 20±6                 |             |
| c. Tumor  | 7   | 8.2                      | 2-5 mg  | 46±18                |             |
| (3 parts)   |     |                          |         |                      |             |

N/A = not available.

\* 0.4-20 mg anti-p97 Fab; 1-20, mCi  $^{131}\text{I}$ .

† All patients had previous injections with single agent.

‡ Immediate clearance determined by reference to control Fab (1.4).

‡  $t_{1/2}$  refers to biologic  $t_{1/2}$ .

3. There is a strong positive correlation ( $r = 0.81$  and  $P = 0.02$ ) between the concentration of p97 and the ratio of specific ( $^{131}\text{I}$ ) to nonspecific ( $^{125}\text{I}$ ) radioactivity content. Similar results were obtained on 11 samples from an additional four patients biopsied at 72 h ( $r = 0.79$ ,  $P = 0.01$ , data not shown). Thus, antigen-specific localization was observed with average ratios of specific to nonspecific uptake of 3.7 (48 h) and 3.4 (72 h).

A comparison of whole body clearance using probe counts and cumulative urinary excretion showed that virtually all of the radioactivity cleared was via the urine for both specific and nonspecific Fab. Significantly less specific than nonspecific Fab was excreted in the urine, however. Cumulative percent injected dose ex-

creted by 24 h was 36% (specific) vs. 46% (nonspecific),  $P < 0.01$ ; by 48 h, 61% (specific) vs. 82% (nonspecific),  $P < 0.01$ . Initially, over the first 2 h, >60% of the activity in the urine was in the form of Fab, but at 24 and 48 h, 90% was in the form of free iodide, indicating that some in vivo deiodination had occurred.

Whole body imaging studies after intravenous infusion of  $^{131}\text{I}$ -(anti-p97) Fab, over 3-10 min, demonstrated initial distribution of radiolabeled antibody in the blood pool and liver and kidney. The greatest concentration even at earliest imaging times was in the liver, the major repository for the 40% of radiolabeled anti-p97 antibody which disappeared rapidly from the circulation. All patients had some concentration of radioiodine in their thyroid gland, again suggesting that

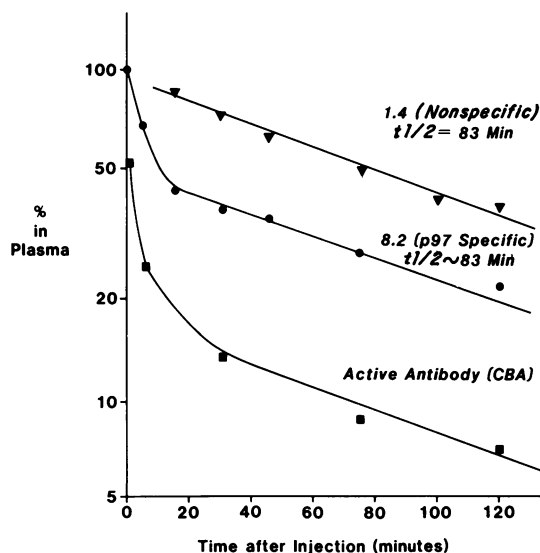


FIGURE 2  $^{125}\text{I}$ -Fab plasma clearance in four patients with widespread malignant melanoma. The average of the data points is plotted at each time interval, for clearance of  $^{125}\text{I}$  nonspecific Fab ( $\blacktriangledown$ ),  $^{125}\text{I}$ -(anti-p97) specific Fab ( $\bullet$ ), and the CBA for p97 reactivity ( $\blacksquare$ ). In preparing this graph, the plasma volume was computed for each patient from a nomogram (29) which takes into account height and weight. Based on the counts in plasma per cubic centimeter, the amount of radioactivity retained in the entire plasma space was computed as percentage of the administered dose in plasma as a function of time postinjection. Patients received  $<2$  mg Fab over a 20-min infusion interval and  $t = 0$  is taken as the time immediately postinfusion.

some in vivo deiodination occurred, and SSKI was required to block thyroidal uptake. Kidneys and bladder were prominently visualized because of continuous renal excretion of the Fab fragments and/or free  $\text{I}^-$  over the period of study. A typical scan image of these normal organs at 2, 24, and 48 h is shown in Fig. 4.

13 of the 33 patients studied had initial diagnostic scans that showed no tumor localization. These negative scans were expected in that these patients either had lesions that were too small ( $<1.5$  cm) to be readily imaged (10 patients) or negative scans associated with low p97 antigen levels (two patients). In an additional patient the Fab iodination was a technical failure, with CBA of only 3%.

20 patients had positive scans; an example is shown of antigen-specific tumor localization in Fig. 5. A detailed study of rates of clearance from tumor, liver, blood, and whole body was performed at 2, 24, and 48 h and 5 d with the aid of a dedicated computer. On the basis of the biodistribution data obtained (Table I and Fig. 2), dosimetry estimates were prepared (see below).

In seven patients with advanced disease whose preliminary studies showed a particularly favorable ratio between tumor and potential target organs, liver and marrow, we began a phase I assessment of the localization and safety of higher doses of  $^{131}\text{I}$ -(anti-p97) Fab. Radiolabeled Fab (5–10 mg protein) was administered by slow infusion over 1 h. A summary of the patient data is shown in Fig. 6 A. The total millicuries ad-

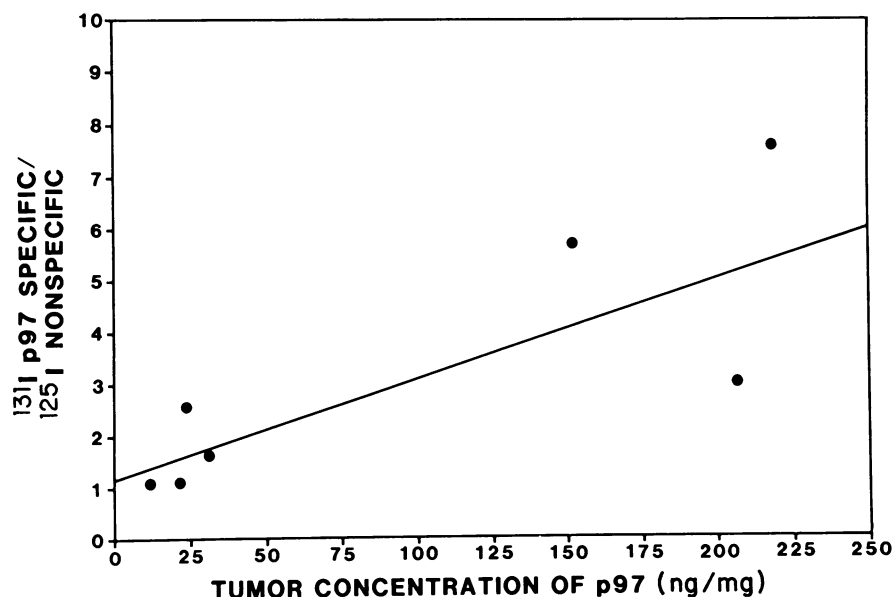


FIGURE 3 Specific localization of  $^{131}\text{I}$ -(anti-p97) Fab at 48 h vs. p97 antigen concentration in a series of seven subcutaneous metastatic melanoma nodules from four patients.  $r = 0.81$ ;  $P = 0.028$ . See text for details.

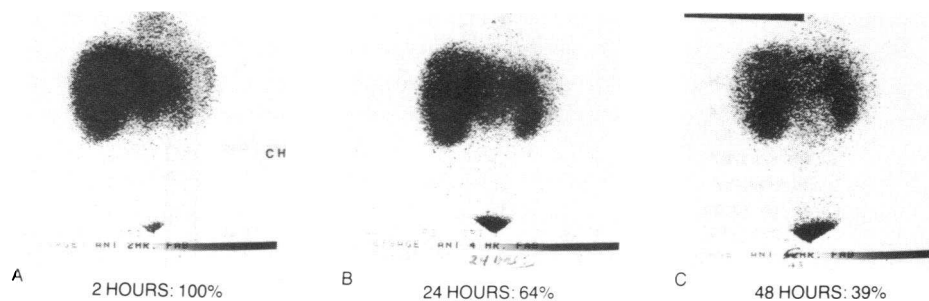


FIGURE 4 Typical  $^{131}\text{I}$ -(anti-p97) Fab image in a patient (CH) with metastatic melanoma (anterior abdomen). 5 mCi of  $^{131}\text{I}$ -(anti-p97) Fab (0.8 mg, CBA = 66%) was administered over 3 min, and 2, 24, and 48 h images are shown along with percent whole body retention at each time interval. The patient had recently had a brain metastasis removed. The only known residual disease was a small left hilar mass. Liver, but not spleen and bone marrow, was prominent, suggesting that a hepatic mechanism was responsible for the blood clearance of Fab fragments. No retention in lungs and mediastinum was seen and little gut activity was noted. The main route of excretion was the kidneys.

ministered to these patients and the number of doses are as follows: 132 mCi (4); 148 mCi (4); 134 mCi (4); 185 mCi (2); 207 mCi (2); 510 mCi (4); and 529 mCi (4). No abnormalities of liver and kidney function were seen in any of the patients. Abnormalities of marrow function were not observed except in the two patients who received a total of 500 mCi (see Fig. 6 B). In these two patients, a transient drop in total neutrophil and platelet counts were observed, which reached a nadir at 2–3 wk after the last injection (Fig. 6 B). Clinical sequelae were not noted from this mild marrow suppression. A total of 24 infusions were given to these seven patients, and on four occasions there were acute reactions. One patient experienced fever and tachycardia of uncertain cause but possibly due to pyrogens,

another had flushing and transient hypotension, and the other two had allergic skin reactions that responded rapidly to benadryl and epinephrine. One of the patients receiving 500 or more millicuries (P.L.) developed a human anti-mouse IgG that promoted rapid clearance of the Fab from the circulation, and blocked tumor uptake on the last therapeutic dose. This patient's disease subsequently progressed to death. It was of interest that P.L. developed cross-reactivity with an IgG1 (48.7) which he had not received before, but reactivity with an IgG2a was weaker. The other two patients who developed anti-mouse Fab antibodies (M.V. and T.C.) also had type-specific immunity (see Table II). In the assay system used to measure anti-mouse antibody titers, a percent precipitable activity of >2–3% is considered significant. In the case of T.C. and M.V., the 96.5 Fab was used for therapy after anti-mouse antibody to 8.2 Fab was documented. Good localization in tumor with 96.5 Fab was documented.

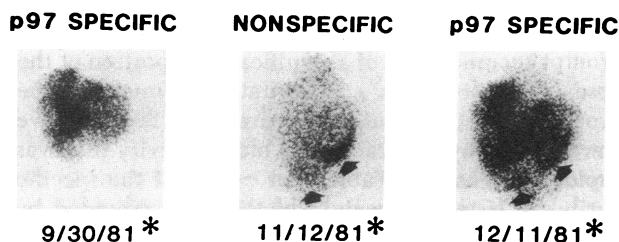


FIGURE 5 Gamma camera images from M.V., a patient with metastatic melanoma to liver. 4 mCi of  $^{131}\text{I}$ -(anti-p97) Fab (monoclonal antibody) (8.2; 0.8 mg, CBA = 53%) was injected intravenously on 9/30/81. Uptake in the periphery of a large intrahepatic tumor mass was observed. A control  $^{131}\text{I}$ -Fab preparation (nonspecific, 1.4; 1.32 mg, CBA = 1%) was injected 2 wk later (11/12/81). No uptake was observed in tumor. A repeat  $^{131}\text{I}$ -(anti-p97) Fab injection (8.2; 0.76 mg, CBA = 69%) was given 1 mo after the nonspecific study (12/11/81). Tumor localization was again seen. Uptake in stomach and intestine (arrow) was seen on 11/12/81 and 12/11/81, and probably represents free  $\text{I}^-$  concentrating in these organs. \*, 48 h postinjection.

Patient examples of tumor specific localization are shown in Figs. 7 and 8. Patient M.V. initially received 34 mCi intravenously and 90 mCi 4 wk later. Excellent localization of the therapy dose was noted on both occasions. Fig. 7 shows the precise localization of  $^{131}\text{I}$ -Fab to a large tumor area in the left lobe of liver on 20 April 1982. Patient M.M. received 125 mCi of  $^{131}\text{I}$ -(anti-p97) Fab. Fig. 8 shows localization of radioactivity in pleural metastases over diaphragm and in retrocardiac mass (arrow). There was also generalized avid concentration in tumor involving the omentum and pelvis (not shown). Both of these patients have now died from the progressive effects of their disease, and autopsies were obtained at 10 d (M.M.) and 24 d (M.V.) after the last therapeutic dose. The areas of radioactivity localized on scan corresponded to large metastatic tumor deposits. Marrow, liver, and kidney samples showed

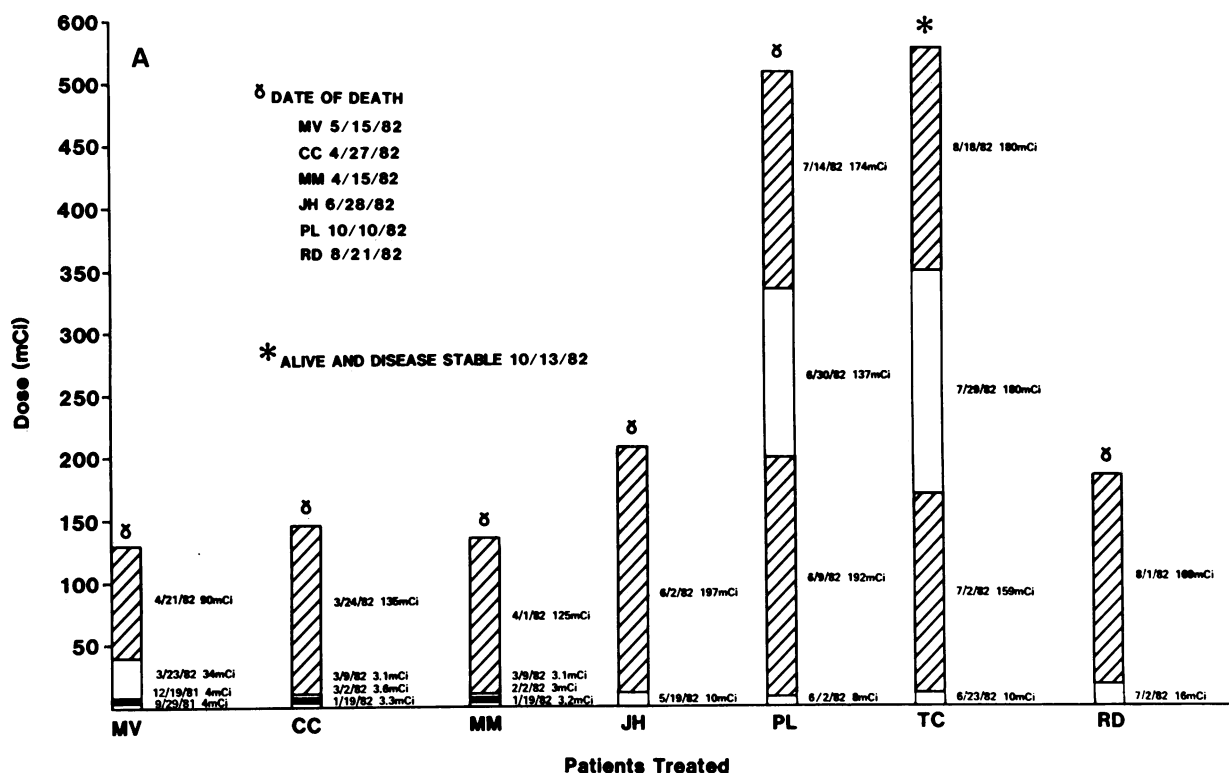


FIGURE 6 Phase I studies with  $^{131}\text{I}$ -anti-p97 Fab monoclonal antibodies. Patient data from phase I radiotherapeutic trial with  $^{131}\text{I}$ -labeled anti-p97 Fab. (A) Dosage scheme including dates of administration. (B) Effect of high dose  $^{131}\text{I}$ -anti-p97 Fab on neutrophil and platelet count, in two patients who received cumulative doses of  $^{131}\text{I} > 500$  mCi.

no abnormalities. There was good correlation of gamma camera images and autopsy findings, with demonstrable uptake in all known tumor sites of appreciable size ( $>1.5$  cm). However, the uptake was greater in visceral lesions than in subcutaneous lesions. Uptake in brain tumor metastases, although present, was less avid than in systemic sites.

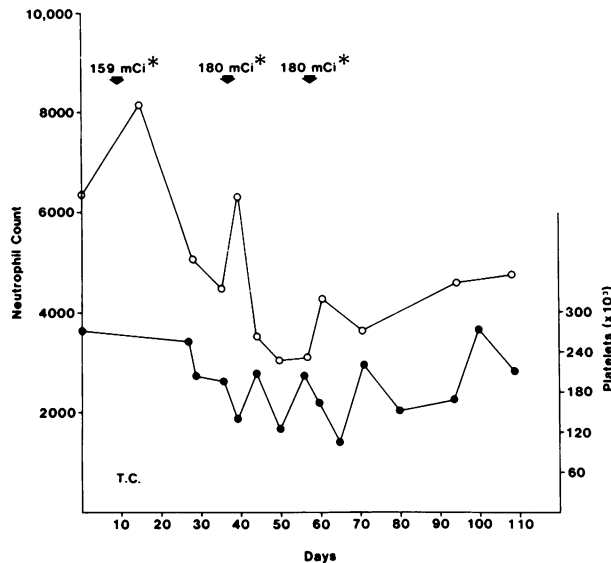
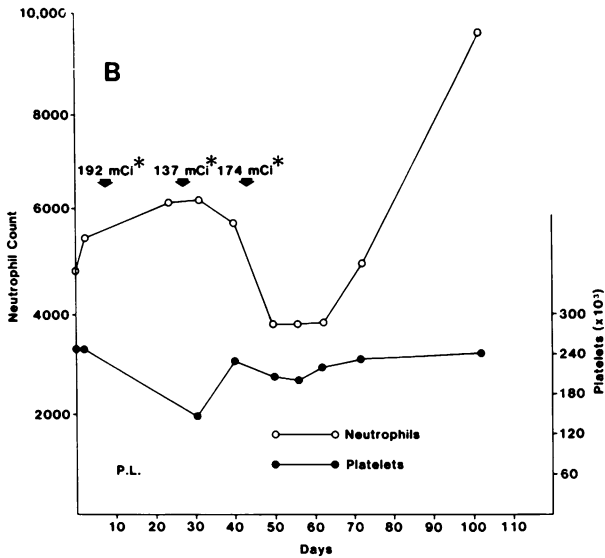
#### Calculation of dosimetry

Dose rate and cumulative dose were calculated using the Medical Internal Radiation Dose Committee (MIRD) schema and the biologic data obtained from the localization studies. Based on the biologic clearance rates and the physical half-life of the isotopic label, effective half-lives were computed for  $^{131}\text{I}$  anti-p97 Fab from the total body, liver, tumor, kidney, and bladder. These five tissues are essentially the sites of accumulation of the injected Fab preparation. For each iodine isotope an effective half-life ( $t_{1/2}$  eff) was computed from the formula  $1/(t_{1/2})_{\text{eff}} = 1/(t_{1/2})_b + 1/(t_{1/2})_p$ , where  $(t_{1/2})_b$  and  $(t_{1/2})_p$  are the biological and physical half-lives, respectively. On the basis of these considerations, the time course of  $^{131}\text{I}$  in each of these source tissues was calculated, assuming an initial in-

jected dose of 100 mCi of  $^{131}\text{I}$ -anti-p97 Fab. The data are shown in Fig. 9 A. More detailed description of the assumptions underlying estimates of dosimetry for the target tissues are described below.

**Liver.** Gamma camera images obtained as early as 2 h after intravenous injection of radiolabeled Fab show prompt accumulation of a significant proportion of the dose in the liver. As a quantitative estimate of the proportion of dose that was cleared into the liver, we were guided by the fraction of blood activity that was rapidly cleared (see Table I) or  $\sim 40\%$  of the specific antibody. Using the weight of the liver provided by MIRD for their "standard man," of 1,800 g (13), the liver concentration is 0.022% of the total injected dose per gram of tissue. For purposes of dosimetry, liver uptake of this amount of antibody was assumed to be instantaneous. Measurements of clearance of radioactivity from liver were made as described above and a biologic half-time of 20 h was obtained (see Table I). In many patients who do not have extensive hepatic involvement, the standard man liver assumption is accurate. However, in patients with extensive hepatic involvement, the "S" values for normal liver are only approximate. Still, the majority of dose to liver ( $>90\%$ )





\* Dose of I-131-anti p97 Fab administered

FIGURE 6 (Continued)

TABLE II  
Human Anti-Mouse Antibodies in Patients Receiving  
Murine, Anti-p97 Fab

| Patient | Ab received | Fab 8.2 (IgG1)* | Fab 96.5 (IgG2a)* | Fab 48.7 (IgG1)* |
|---------|-------------|-----------------|-------------------|------------------|
| P.L.    | 8.2         | 26              | 6.8               | 23%              |
| T.C.    | 8.2         | 12              | 3                 | —                |
| M.V.    | 8.2         | 8.5             | 2                 | —                |

\* Percent of total radiolabeled anti-p97 Fab radioactivity precipitated by human anti-mouse antibody (Ab).

comes from nonpenetrating radiation and even if the normal liver is distorted by compression of invading tumor, no major error in estimating dose will be introduced.

**Total body.** The total body receives the remaining radiolabeled antibody that is not rapidly taken up into the liver and tumor. Some antibody, however, is rapidly cleared in first pass (estimated ~5%), and, therefore, the total body receives the total amount injected - (liver dose) - (rapidly excreted component), or ~43% of the total dose. For dosimetry purposes, distribution is assumed to be instantaneous. Whole body clearance studies using a gamma probe gave a biologic  $t_{1/2}$  of 33 h (see Table I).

**Bladder.** The amount of retained activity in the bladder was determined by considering the fractional clearance per 24 h of 0.3 or 0.0125/h ( $\lambda_b$ ). The rate of excretion into urine ( $A_u$ ) was related to the amount of activity in the body ( $A_b$ ) and the physical decay rate for the appropriate iodine isotope ( $\lambda_p$ ). It can be shown that

$$\frac{dA_u(t)}{dt} = A_b(t) \cdot \lambda_b - A_u(t) \cdot \lambda_p$$

$$A_b(t) = A_0 e^{-(\lambda_b + \lambda_p)t} \quad (1)$$

$$\frac{dA_u(t+1)}{dt} = A_b(t+1) \cdot \lambda_b - A_u(t+1) \cdot \lambda_p$$

$$A_b(t+1) = A_0 e^{-(\lambda_b + \lambda_p)(t+1)} \quad (2)$$

$$A_u(2 \text{ or } 4 \text{ h}) = 0; \quad \sum_0^{720 \text{ h}} A_u = \tilde{A}_u, \quad (3)$$

where  $A_b(t)$  is the initial activity in the total body. Note that  $\lambda_b$  is both a fractional body clearance and a fractional urinary excretion, since urinary excretion is essentially the only mode of clearance from the body. With a computer, the derived expression for urinary excretion was integrated numerically for both 2 and 4 h, and then reset to zero bladder activity. The integration was then determined again for the next 4-h period, and the cumulative activity in the bladder was computed, taking care to take into account the appropriate reduction into total body activity. The process was then repeated until all the radioactivity was excreted via the urine.

**Kidney.** We considered the input to the kidney to be the fraction of total body activity ( $A_b$ ) that is excreted in the urine as  $\lambda_b A_b$  (see discussion of bladder activity above). Clearance of contained material from the kidney is rapid, with a transit time ( $T$ ) of  $1/6$  h. The amount of activity in the kidney  $A_k$  at any time can be shown to be  $A_k = A_b \cdot \lambda_b \cdot T$ . This equation was used to calculate retained renal activity.

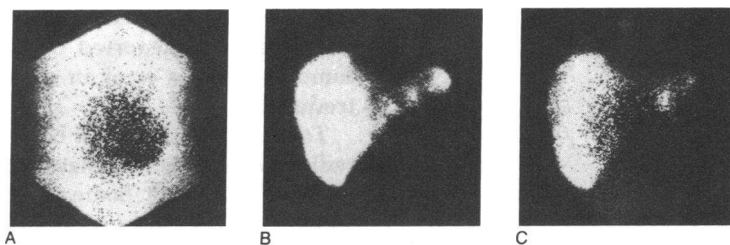


FIGURE 7  $^{131}\text{I}$  Fab images at 24 h posttherapy dose (90 mCi) in comparison to Technetium Sulfur Colloid (TcS) liver scan in anterior abdomen (patient M.V.; see Fig. 5). (A)  $^{131}\text{I}$ -(anti-p97) image of anterior abdomen with most intense localization of radioactivity photographed as black area. Image of 4/20/82. (B) TcS liver scan shows replacement of left lobe of liver by tumor. (C) Superimposition of (A) and (B) showing localization of  $^{131}\text{I}$ -(anti-p97) Fab in mass in the left lobe of the liver.

**Tumor.** The amount of radioactivity in tumor as a function of time was estimated by using a dedicated computer based on gamma camera images from patient studies in order to determine clearance rates from tumor and to estimate amount retained in tumor. For example, in patient M.V. (Fig. 5), the patient's lesion was assumed to be approximately spherical, and a volume was calculated based on the maximum diameter in the anteroposterior projection. The entire lesion, including a central cold area, was  $\sim 600$  g. Assuming a spherical geometry, the volume of the most radioactive portion of the tumor was  $\sim 450$  g. From the blood clearance curve, the percentage of the injected radiolabeled antibody that was rapidly removed from the blood was determined as described above. Gamma camera images obtained at 2 h after injection (not shown) revealed that the uptake of this portion of radioactivity was in tumor and liver. Counts were obtained over liver and tumor. On the basis of the percentage of injected activity cleared into liver, and the proportion of counts in tumor as compared with liver, the percentage taken up into tumor was determined. This was calculated at 17.2%

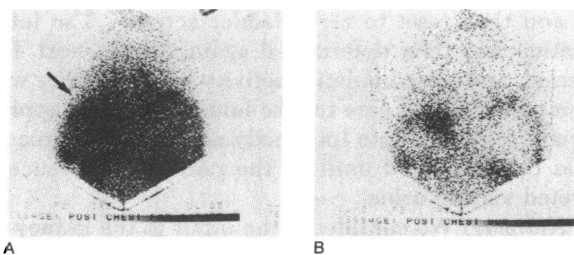


FIGURE 8 Posterior chest gamma camera image at 48 h after intravenous administration of 125 mCi  $^{131}\text{I}$ -(anti-p97) Fab. Patient M.M., a 38 year old man with widely metastatic melanoma. (A) Localization is seen in autopsy documented retrocardiac mass (arrow) and pleural implants. (B) A blood pool subtraction image shows the extent of pleural uptake particularly well.

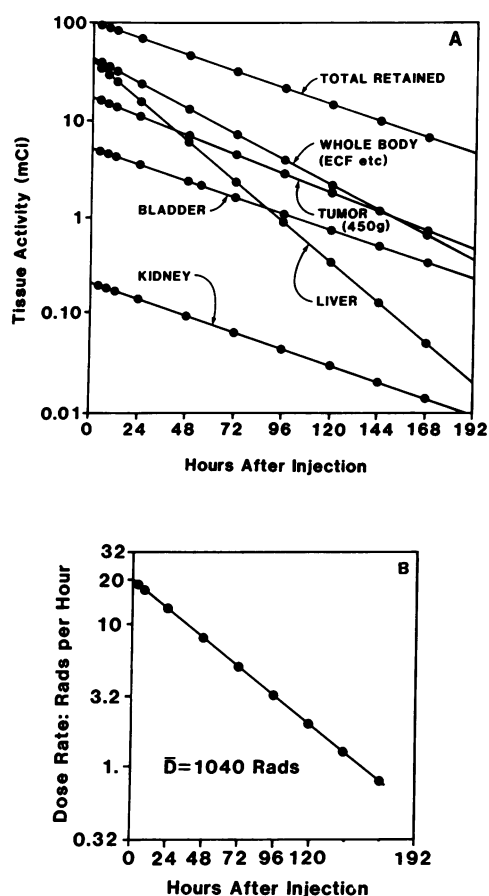


FIGURE 9 (A) Biodistribution: source tissue activity for 100 mCi  $^{131}\text{I}$ -(anti-p97) Fab as a function of time postinjection. Data are calculated based on measurements of Table I and Fig. 2. (B) Dose rate to tumor for  $^{131}\text{I}$ -(anti-p97) Fab. Given an initial dose of 100 mCi  $^{131}\text{I}$ -(anti-p97) Fab, a total of  $\sim 1,040$  rads will be delivered to tumor at a dose rate that is maximal at the early time points and declines rapidly over a period of 1 wk postinjection. Calculations were performed based on the MIRD schema (11) and the source organ activity in A.

of the injected dose. Assuming a mass of 450 g, the percentage injected dose per gram was  $\sim 0.04\%$ . The ratio of tumor to liver activity per unit area was slightly less than two on the anterior projection. Similar ratios were observed for posttherapy images of M.M. (Figure 8) and C.C. (image not shown). These ratios are in the same range as those observed by Leichner et al. (12) for radioimmunoglobulin concentrating in hepatic tumors using related but more elaborate techniques.

Uptake of  $^{131}\text{I}$ -Fab by tumor was rapid, with the maximum uptake being observed on images obtained at 2 h postinjection. In seven tumors from three patients (M.V., M.M., and C.C. in Fig. 6 A, determined from localization doses) clearance from tumor was determined to an average of 46 h (see Table I). It was of interest that a comparable tumor clearance (49 h) was calculated from the average concentration of subcutaneous tumors biopsied at 48 and 72 h, and assuming an exponential decline in radioactivity. 46 h was used as the biologic half-time for dosimetry purposes.

#### Target tissues for dosimetry

The following tissues were considered using  $S$  values and the methodology described by MIRDOSE (13): liver, kidney, bladder, red marrow, ovary, testes, whole body. For the tumor, the absorbed dose from irradiation due to radioactivity contained in liver, bladder, and whole body was calculated using  $S$  values for kidney, since kidneys are centrally located within the body, and have a mass (350 g) that is comparable to the tumor of our example (450 g). For tumor irradiation from radioactivity contained in the kidney,  $S$  values for spleen were used. These approximations probably result in a small underestimate, since both spleen and kidney are smaller than the tumor of our example. It is interesting that the contribution to tumor dose of these corrections is small, and not very dependent on shape or size. The majority of the dose to tumor ( $>90\%$ ) was due to tumor-contained radioactivity and calculations of this component of dose were computed using the equilibrium dose constants ( $\Delta_i$ ), and the specific absorbed fraction ( $\phi$ ) obtained from Table 10 of reference 14. For the contribution of tumor contained radioactivity to the radiation dose to other organs, the  $S$  values for kidney were again used, since this organ is adjacent to liver and is centrally located, comparable with the tumor of our examples. Similar approximations were employed by Leichner et al. (12) in their estimates of dosimetry. For all the tissues, both penetrating and nonpenetrating radiation was considered. Results are shown in Table III.

#### DISCUSSION

Building on previous work in which we reported early human imaging studies with murine monoclonal an-

TABLE III  
Dosimetry  $^{131}\text{I}$ -(Anti-p97) Fab (100 mCi IV)

| Target organ (tissue) | Dose (rads)  |
|-----------------------|--------------|
| 1. Tumor              | 1. 1,040     |
| 2. Liver              | 2. 325       |
| 3. Bladder            | 3. 254* 160† |
| 4. Kidney             | 4. 71        |
| 5. Whole body         | 5. 39        |
| 6. Marrow             | 6. 30        |
| 7. Ovary              | 7. 26        |
| 8. Testes             | 8. 20        |

\* Every 4 h emptying.

† Every 2 h emptying.

titumor antibodies (4, 15), we introduced a new concept in this paper: that (anti-p97) Fab, a mouse monoclonal immune fragment, can be safely used as a carrier for selectively and repeatedly depositing large doses of radiation in metastatic malignant melanoma in humans.

Experience with radiolabeled antibodies for therapy is quite limited at present. Ettinger et al. (16) used  $^{131}\text{I}$ -(antiferritin) IgG and  $^{131}\text{I}$ -(anti-carcinoembryonic antigen) IgG as part of an integrated treatment program for primary hepatic malignancies (cholangiocarcinoma and hepatoma). Data have been published on 18 patients who received 37–157 mCi of radiolabeled whole immunoglobulin antibody. An objective anti-tumor effect was noted in six of nine patients evaluable for disease. The treatment scheme involved the use of external radiation, chemotherapy, and radiolabeled antibody in the same patients. In our opinion, this makes it difficult to assess the toxicity and therapeutic benefits of the radiolabeled antibody as distinct from the other modalities. We have preferred to use radiolabeled Fab as the sole therapeutic modality in patients with disseminated melanoma, in order to more definitely determine the toxicity and therapeutic efficacy of the radiolabeled immune fragment itself.

In the present study, we estimated dosimetry to tumor and target organs and we assessed toxicity by gradually increasing the millicurie amount of radiolabeled antibody administered. Since up to 194 mCi acutely ( $\sim 2,018$  rads to tumor,  $\sim 57$  rads to marrow) and 529 mCi total ( $\sim 5,500$  rads to tumor, 159 rads to marrow) of  $^{131}\text{I}$ -(anti-p97) Fab have been well tolerated with minimal toxicity, our plan is to escalate the millicurie dose until antitumor effect or dose-limiting toxicity is seen. Our preliminary experience indicates that bone marrow will probably be the dose-limiting organ.

The problem of antitumor dosimetry with radiolabeled murine fragments has been studied by Leichner et al. (12) for the case of  $^{131}\text{I}$ -labeled anti-ferritin antibody in hepatoma. The kinetics of uptake and clear-

ance of whole IgG are very much slower than what we have observed for  $^{131}\text{I}$ -(anti-p97) Fab fragments, and so the cumulative radiation dose is delivered over a longer time period. Another way of looking at this is that the maximum dose rate to tumor is  $\sim 6$  rads/h for whole anti-ferritin IgG and for a comparable mCi amount of anti-p97 Fab ( $\sim 100$  mCi), the maximum dose rate is 20 rads/h (Fig. 9 B). For the anti-p97 Fab, tumor dose has been virtually totally delivered by 1 wk after treatment, whereas for whole IgG a full month is required. The concentration ratio for tumor to liver is about two for both the whole anti-ferritin IgG and the anti-p97 Fab, but there is a dramatic difference in the relative clearance rates as measured by effective half-life from liver, whole body, and tumor for these preparations. For the anti-ferritin whole IgG clearance rates are 7.4 d for liver and tumor and  $\sim 4$  d for total body. For the  $^{131}\text{I}$ -(anti-p97) Fab, tumor has an effective half-time of 37 h; liver, 17 h; and whole body, 28 h. These relative clearance rates are important factors in determining the dosimetry for tumor relative to other organs. From the data presented by Leichner et al. (12), a ratio of tumor to marrow exposure is  $\sim 10$  for the anti-ferritin IgG in hepatoma. In the present study, we have shown that for the anti-p97 Fab, the tumor-to-marrow ratio is  $\sim 30$ . Since the marrow is the critical organ, it would appear that the use of (anti-p97) Fab in melanoma results in more favorable dosimetry than the use of whole IgG (anti-ferritin) in hepatoma. Nonetheless, tumor response is a complex phenomenon that depends on the radiosensitivity of the tumors treated. Additional study is required before the efficacy of anti-p97 Fab for treating melanoma is established.

In Table III, the radiation dose for 100 mCi of  $^{131}\text{I}$  Fab is shown for tumor and target organs. The accuracy of dose calculations made using the MIRD schema is thought to be  $\sim 30\%$  (percent standard deviation), although a precise number is difficult to obtain (11). The estimates of mean dose for normal tissues quoted in Table III are thought to be in this range. Biologic variability further confounds the errors; however, and on the basis of variation in clearance values and uptake estimates in individual patients, the relative error for liver could be as high as 50%, and for marrow 40%. Similarly, the tumor estimates contain even greater sources of potential variability, since some tumors concentrate the  $^{131}\text{I}$ -Fab avidly and other tumors do not. It should be noted that the tumor dosimetry estimates are for those areas of tumor that avidly concentrate the  $^{131}\text{I}$ . The dose estimates given for mean dose are based on data from three patients, and contain numerous assumptions, that relate the uptake in tumor to the absolute liver uptake. Thus, the error in tumor estimates are probably in the same range as liver, namely  $\sim 50\%$ . Thus, values in Table III should be

thought of as guidelines for planning the amounts of  $^{131}\text{I}$ -Fab to be used in therapy rather than precise measurements of dose to tissues. For example, using  $^{131}\text{I}$  therapy for hyperthyroidism, it has been the general experience that estimated marrow doses of 300 rad have been well tolerated (17). Thus, a dose of 500 mCi of  $^{131}\text{I}$  Fab, which gives  $\sim 150$  rad to marrow would be reasonably well tolerated, with a safety factor of about two. With 500 mCi of  $^{131}\text{I}$ -Fab, tumor doses of 5,200 rad may be expected from Table III, and these levels may potentially be sufficient to cause antitumor effect. It would seem, then, that there may be a sufficient ratio of tumor radiation exposure to normal tissue radiation exposure to warrant further more extensive trials of radiation therapy in melanoma with  $^{131}\text{I}$ -(anti-p97) Fab.

Additional important background for the present study was provided by several "radioimmunodetection" reports. A variety of radiolabeled antibodies (whole IgG) specific for tumor-associated antigens have been used to image cancers by external photoscanning techniques (radioimmunodetection) using antibodies specific for carcinoembryonic antigen (18), alpha-feto-protein (19), human chorionic gonadotropin (20), a kidney carcinoma antigen (21), and ferritin (22). Initial reports cited a diagnostic accuracy of 85% (17, 18). Image enhancement techniques (11) and absorption of xenoantisera (23) increase sensitivity. Nonetheless, the amount of radiolabeled antibody localized in tumor has been low compared with blood and other organs, particularly the liver, limiting the clinical utility of present methods (24). Furthermore, much of the published work lacks controls in which nonspecific immunoglobulin was used, control preparations often consisting of labeled serum albumin. In studies which lack appropriate controls, it is not clear that the observed tumor localization was antigen-specific and not simply the result of passive diffusion of radiolabeled IgG into the expanded extracellular space of such tumors. As far as we are aware, our data on specific localization of  $^{131}\text{I}$ -(anti-p97) Fab is the first in vivo study in humans to report that there is a strong positive correlation between tumor uptake and antigen concentration (see Fig. 3). Another way to look at the importance of antigen specific binding is illustrated in Fig. 5, in which anti-p97 Fab reproducibly localized in tumor, whereas nonspecific Fab did not.

The quantitative relationship between p97 antigen and tumor uptake deserves emphasis. The population reported in this paper was biased toward tumors containing larger amounts of p97, by using a double-determinant immunoassay (9) on tumor extracts to preselect patients before study. When sufficient antigen is present in tumor, excellent localization is observed. Imaging processing techniques are not required to de-

tect the lesions in such patients. On the other hand, we estimate that in the population at large, only ~50% of melanoma patients have sufficient p97 antigen for diagnostic imaging studies. Although diagnostic staging of such patients is possible, we believe that the major utility of imaging in such patients will be to select that subset of patients who have sufficient antigen specific localization to warrant therapeutic trials with radiolabeled (anti-p97) Fab.

Ultimately, monoclonal antibody technology should make it possible to rigorously compare the *in vivo* biodistribution of antibody and immune fragments which have identical immune specificity. No studies have been done in man, but such comparisons have been done in animals. In a study of antibodies specific for mouse T cell antigens, F(ab')<sub>2</sub> gave greater localization than either IgG or Fab (25). In athymic (nude) mice bearing human colon xenografts, IgG gave high tumor uptake although maximal concentration occurred at 5 d postinjection (26). In a study of the localization of cardiac myosin-specific antibody in infarcted dog myocardium, F(ab')<sub>2</sub> fragments were more effectively concentrated than whole IgG (27); others have expressed different views (28). Nonetheless, the bulk of evidence supports clear differences between antibody and antibody fragments, and demonstrates that nonimmunologic factors are also important in determining *in vivo* localization. More rapid diffusion of smaller molecules into the antigen-containing tissues and differences in the blood clearance of the various immune species have been invoked to explain the findings observed.

Some authorities maintain that the longer retention of IgG and F(ab')<sub>2</sub> in the blood is advantageous to tumor localization because the tumor uptake is thought to be a process that requires considerable time before actual tumor cell binding occurs (26). According to this view, Fab fragments are not useable for tumor localization because of their rapid clearance from the blood and excretion into the urine. Although this pessimistic view of Fab fragments for tumor localization appears warranted in these animal studies, our findings in humans lead to the conclusion that indeed <sup>131</sup>I-(anti-p97) Fab is useful in imaging of human melanoma.

There are no good animal models for the common human tumors, especially where biodistribution of immunologic proteins is concerned. Thus, to determine whether anti-p97 Fab, F(ab')<sub>2</sub>, or IgG is the best carrier for radioactivity to human melanoma, we plan to make more detailed comparisons in humans. At present, (anti-p97) Fab is the practical choice because this formulation is easier to make than F(ab')<sub>2</sub> and is less immunogenic than IgG.

In addition to antigen-specific localization of (anti-p97) Fab in tumors, there was also prompt clearance

from the blood and a significant accumulation of radiolabeled (anti-p97) Fab in normal tissues, particularly liver. The fact that this nonspecific localization could be reduced by increasing the amount of Fab suggests that the nonspecific component of (anti-p97) Fab clearance may be saturated by increasing the total amount of Fab infused. The basis for this rapid clearance is unclear at present. One possible explanation is that p97 antigen is held in liver in a form that is accessible to circulating radiolabeled antibody in blood. Radiolabeled antigen-antibody complexes are then formed *in situ* in the liver. On the other hand, the possibility exists that subdetectable amounts of antigen are circulating in blood, and when radiolabeled antibody is injected, enough antigen-antibody complexes are formed so that 30–40% of the radiolabeled antibody is cleared into the liver.

We recently examined the possibility that sufficient amounts of antibody are complexed to antigen in blood to account for the liver uptake. A sensitive radiometric assay has been developed (Reynolds, J., personal communication) which can detect levels of p97 circulating in serum. Patients with tumor have 5–15 ng of p97 per ml of serum. When 2 mg of (anti-p97) Fab is rapidly injected intravenously (see Table I), ~37% of the radiolabeled antibody fragment is cleared very rapidly into liver. The molecular weight of Fab fragments is ~50,000 or ~½ the mol wt of p97. For Fab and antigen, there is one binding site per molecule. The molarity of p97 is ~0.05 nM and for Fab, assuming uniform distribution in the extracellular fluid, ~2.6 nM or 52 times the p97 concentration. Therefore, the clearance of 30% of radiolabeled Fab is not attributable to complex formation with circulating antigen and subsequent hepatic uptake.

In summary, as far as we are aware, we are the first group to successfully use radiolabeled Fab fragments derived from mouse monoclonal antibodies for diagnostic and therapeutic studies in humans with tumors. Murine monoclonal Fab (anti-p97) localizes rapidly in tumor and can be used as a nontoxic carrier for repeated delivery of high dose radiation to human melanoma *in vivo*. These findings have important implications for both diagnosis and therapy of malignant melanoma.

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