Therapeutic Efficacy of Antiglioma Mesenchymal Extracellular Matrix $^{131}$I-Radiolabeled Murine Monoclonal Antibody in a Human Glioma Xenograft Model

Yi-Sheng Lee, Dennis E. Bullard, Michael R. Zalutsky, R. Edward Coleman, Carol J. Wikstrand, Henry S. Friedman, Edward V. Colapinto, and Darell D. Bigner

ABSTRACT

The development of Mabs, particularly those reactive with primary brain tumors but not with normal brain, provides a potential means of delivering therapeutic agents selectively to human malignant gliomas. Mab 81C6, an IgG2b immunoglobulin, which defines an epitope of the glioma-associated extracellular matrix protein tenascin, has been shown to bind to human glioma cell lines, glioma xenografts in nude mice, and primary human gliomas, but not to normal adult or fetal brain. To test the therapeutic potential of this Mab for targeted delivery of isotopes, nude mice bearing progressively growing s.c. xenografts of D-54 MG, a human glioma cell line, were given injections via the tail vein of either buffer, unlabelled 81C6, $^{131}$I-labeled 81C6, or $^{131}$I-labeled 45.6, a nonspecific control Mab of the same isotype. Specific activities of the Mab range from 6.0 to 15.5 mCi/mg with protein doses from 7.6 to 167 µg. The doses given by injection per animal for labeled 81C6 were 50, 250, 500, and 1000 µCi and 500 and 1000 µCi for 45.6. Tumor response was measured by growth delay in reaching 1000 or 5000 mm$^3$ tumor volumes using the Wilcoxon rank sum test, and by comparing the proportion of tumors that had regression in volume after treatment using the Fisher exact test. Statistically significant growth delays at 1000 mm$^3$ were noted in 1 of 3 experiments with 500 µCi 81C6 ($P < 0.001$) and 2 of 3 for 1000 µCi 81C6 ($P = 0.001$ and $<0.001$). At 5000 mm$^3$, statistically significant growth delays were seen with radiolabeled 81C6 in 2 of 2 experiments at 250 µCi ($P = 0.01$ and 0.02), 4 of 4 at 500 µCi ($P = 0.03$<0.001), and 2 of 2 at 1000 µCi ($P = 0.001$) and with radiolabeled 45.6 in 1 of 1 at 1000 µCi ($P = 0.01$). The percentage of animals with tumor regression progressively increased with increasing doses of isotopes. For radiolabeled 45.6, there were 0 of 10 regressors at 500 and 1 at 1000 µCi. For radiolabeled 81C6, there were 0 of 6 regressors at 50 µCi, 1 of 6 (16%) at 250 µCi, 7 of 38 (18%) at 500, and 15 of 28 (54%) at 1000 µCi. Statistically significant tumor regression was seen only at doses of 500 and 1000 µCi of 81C6. The initial tumor size for those regressing was significantly smaller than those not regressing ($P = 0.01$ for 500 µCi and $0.0009$ for 1000 µCi). The estimated dose to tumor was 9719 cGy for 1000 µCi 81C6 and 2346 cGy for 1000 µCi 45.6. Doses to other organs for 81C6 and 45.6 were equivalent ranging from 135 cGy for brain to 2415 cGy for lung. Whole body dose determined by total body radiation to tumor tissue with an associated therapeutic re
dose-limiting propensity to toxicity of the normal CNS and among the dominant factors (1-6).

With the development of hybridoma technology, Mabs reactive with a wide spectrum of human tumor-associated antigens have been reported (4, 11-22). $^{131}$I-labeled antibodies against tumor-associated antigens have shown selective radiation delivery to tumors in several animal models (4, 11-22). However, both among and within models, a wide variability in dose distribution and response have been seen. To date, the therapeutic efficacy of radioiodinated antiglioma Mabs has not been evaluated in a human xenograft model.

In previous work, we have demonstrated that 81C6, an IgG2b immunoglobulin, defines an epitope of the extracellular matrix protein tenascin present in human glioma cell lines, primary human gliomas, and glioma xenografts in nude mice and rats, but not in normal adult or fetal brain (12, 23, 24). When radiolabeled, 81C6 has allowed external imaging of s.c. and i.c. xenografts (12, 25). We present here data evaluating unlabeled and radiolabeled 81C6 and a control myeloma Mab of the same class as therapeutic agents against a progressively growing s.c. human glioma cell line xenograft in athymic mice.

Statistically greater tumor regression and growth delay were seen with radiolabeled 81C6 than with equivalently radiolabeled control Mab. This paralleled dosimetry data in which a 4-fold advantage in radiation dose delivered to tumor was seen with radiolabeled 81C6. It was noted that the size of the tumor at the time of treatment was important for tumor regression but not for growth delay. These data show an increased delivery of radiation to tumor tissue with an associated therapeutic response using a radiolabeled antiglioma extracellular matrix Mab. Retrospective comparison of the dosimetry estimates derived from these therapeutic studies to those derived from tracelabeled localization studies indicate that the latter may significantly underestimate the dose received by the tumor and that continued tumor growth within nontherapeutic studies plays a major role in this error.

The abbreviations used are: CNS, central nervous system; Mab, monoclonal antibody; T-C, growth delay; ECM, extracellular matrix.
THERAPEUTIC EFFICACY OF ANTIGL1OMA MONOCLONAL ANTIBODY

MATERIALS AND METHODS

Animal Model. Human glioma cell line D-54 MG has demonstrated the ability to progressively grow and be serially transplantable as both s.c. and i.c. tumors in athymic nude mice (BALB/c, nu/nu) (5, 26). The nude mice were obtained from a colony maintained at Animal Laboratory and Isolation Facility, Duke University. Equal numbers of male and female animals were used. Animal age ranged from 6 to 8 wk and weight ranged from 15 to 25 g. Briefly, D-54 MG s.c. tumors were passaged in nude mice by injections of 30–50 µl of a 50% w/v tumor homogenate s.c. in the right flank of recipient animals (5). The tumors grew as well-circumscribed masses that were histologically similar to the cell line from which they were derived. Estimated tumor volume was determined 3 times weekly according to the formula

\[(\text{short dimension})^2 \times (\text{long dimension}) / 2\]

(5, 26). Animals were then used for therapeutic trials when mean tumor volumes ranged between 100 and 500 mm³. At the time of therapy, animals were randomized.

Monoclonal Antibody. Monoclonal antibody 81C6 and a control Mab of the same isotype, IgG2b, 45.6, were purified from hybridoma culture supernatant using Protein A Sepharose 4B. Mab 45.6 is produced by myeloma cell line 45.6.TG1.7; the ligand(s) for this myeloma protein is unknown. Extensive analysis by immunohistology and in vivo localization studies demonstrates no specific antigen-antibody binding by 45.6 to either rodent or human tissues (23, 25). Immunoglobulin was labeled with 111I (New England Nuclear, Boston, MA) using the chloramine T method (27). Radiolabeled immunoglobulins were chromatographed on a 10-ml Sephadex G-25 fine column in order to separate labeled immunoglobulin from free iodine and were subsequently injected within 4 h of labeling. The binding characteristics of purified Mab 81C6 were analyzed by immunoreactivity assays. Briefly, 100 ng of 125I-labeled 81C6 or 45.6 Mab was incubated overnight with 300 µg of washed D-54 MG s.c. tumor homogenate in 1 ml of 0.15 m phosphate saline buffer. The amount of 45.6 bound to the tumor homogenate after washing was considered nonspecific binding and was subtracted from the percentage of 81C6 bound in order to calculate the specific binding percentage of 111I-labeled 81C6. No change of immunoreactivity (range, 35–60%) was found for 81C6 and 45.6 Mab labeled with 111I or 131I at specific activity ranging from 0.5 to 30 mCi/mg protein. In these preparations, 111I activity was 95–98% precipitable in trichloroacetic acid and a range of 40–60% immunoreactivity was obtained.

Therapeutic Trials. Following establishment of progressively growing 100–500 mm³ s.c. tumors, animals were given injections via the tail vein or Mab in equivalent volumes. The choice of using immunoreactive Mab rather than protein mass as the treatment unit was based on two facts: (a) no change in binding activity as determined by immunoreactive percentage was noted between preparations with different specific activities; and (b) the range of protein dose (7.6–76 µg) used was considerably lower than that (~500 µg) required for antigenic saturation (25) in the D-54 MG nude mouse tumor systems. The use of subcutaneous Mab doses, therefore, should result in equivalent binding percentages of injected Mab, allowing comparison of varying radiation doses. Thus, for therapeutic evaluation of radiation, it is most reasonable to use radiation as the dose unit. Ten animals/treatment group were routinely used. In Experiment 1, five treatment groups were studied: buffer (2% bovine serum albumin in 0.15 m phosphate-buffered saline), unlabeled 81C6 (76 µg), and 131I-labeled 81C6 at 50, 250, and 500 µCi per animal. The specific activity of the preparation was 6.9 mCi/mg. Increased isotope dose was given by increased delivery of protein over the range, 7.6 to 76 µg, respectively. In Experiment 2, the same format was utilized with 131I doses of 250, 500, and 1000 µCi and a specific activity of 6.0 mCi/mg. The protein doses were 41 to 167 µg; unlabeled 81C6 dose was 167 µg. Since statistical evaluation of the two initial studies demonstrated no difference between buffer and unlabeled 81C6, in Experiment 3, three groups were studied: control and radiolabeled 81C6 at 500 and 1000 µCi. The specific activity was 15.5 mCi/mg and the protein doses were 64.5 µg for both groups with unlabeled Mab added to make the protein dose constant. For Experiment 4, radiolabeled nonspecific Mab, 45.6, was also evaluated. The treatment groups were: buffer, 500 µCi of 131I-labeled 45.6 or 81C6, and 1000 µCi of 45.6 or 81C6. The specific activities were 9.9 mCi/mg for 45.6 and 11 mCi/mg for 81C6 with constant protein doses of 64.4 µg per animal.

Antibody Localization and Radiation Dosimetry. In Experiment 4, an extra 20 athymic mice with 100–500 mm³ progressively growing D-54 MG s.c. tumors were randomized and received i.v. injections of either 1000 µCi of 131I-labeled 45.6 or 81C6 Mabs. Two animals from each group were then killed on Days 1, 4, 6, 8, and 11 following injection. The bodies were frozen until 35 days after Mab injection when the 131I activity in the tumors and other organs had decayed enough to be counted in a gamma counter (Packard Instruments, Downers Grove, IL). Before the tumor and organs were dissected and weighed, total body activity was measured by a Mediac Dose Calibrator (Nuclear Chicago Co., Chicago, IL). The levels of 131I in tissue samples were corrected for isotope decay and compared to dose standards. For antibody biodistribution analysis, data were corrected to Day 0 of Mab injection. All results were expressed as the percentage of the original antibody injection dose per g (% dose/g). For radiation dosimetry calculation, the data were corrected for radiation decay of the 131I isotope to Days 1, 4, 6, 8, and 11 postinjection when the individual animal was sacrificed and expressed as radiation dose per g tissue (µCi/g).

Absorbed cumulative radiation doses to tumor, various organs, and whole body were calculated from the mean specific activities (µCi/g) of each tissue at Days 1, 4, 6, 8, and 11 postinjection using the trapezoid integration method for the area under the curve (11). The initial concentrations of radiolabeled Mabs in each organ were assumed to be 0 µCi/g.

cGy were further calculated by multiplying the integrated µCi·h/g by the g·cGy/µCi·h factor published by the Medical Internal Radiation Dose committee (28) for 131I of 0.4313. This includes the dose from all β particles, low-energy X-rays, and Auger electrons, all of which were considered to be absorbed completely in the tissues where 131I localized. For 131I, γ-radiation dose was not included because of the low absorbed fractions for small tumor and organs such as in a nude mouse.

In a separate experiment, 20 athymic mice with 100–500 mm³ progressively growing D-54 MG s.c. tumors were randomized and given i.v. injections of 5 µCi/5 µg each of 125I-labeled 45.6 or 81C6. Three animals from each group were then killed on 16 h and Days 1, 2, 3, 5, 7, and 9 following injection and their organs collected and weighed for subsequent counting in a gamma counter. The percentage of injected dose per g and localization index of tissue were determined for each organ (19). Similar dosimetry calculations were performed as in the therapy experiments.

Statistical Analysis. Tumors were measured 3 times weekly until the individual tumor volume exceeded 5000 mm³. Tumor response was measured in two ways. T-C was the difference in days between the medians of the treatment and control groups to reach a tumor volume of 1000 or 5000 mm³. These results were compared for statistical significance by the Wilcoxon rank sum test while the number of tumors regressing in each treatment group was determined and compared to control by the Fisher exact test (29). Tumor regression was defined as any measured volume less than the tumor volume on the day of treatment. The Wilcoxon-Mann-Whitney rank test was used to compare tumor size at time of treatment for tumor regressors and nonregressors.

RESULTS

Growth Response. In Experiment 1, mean initial tumor volumes at the time of treatment were not statistically different among the treatment groups and ranged from 291 to 317 mm³ with standard deviations of 134–167 mm³. The number of days required for the control and treated tumors to reach 1000–5000

5 V. S. Lee, C. J. Wikstrand, C. N. Pegram, R. E. Coleman, and P. Humphrey, unpublished data.
mm$^3$ is given in Table 1. T-C comparing this buffer treatment group to each of the other groups was statistically insignificant at 1000 mm$^3$ (Table 2). Progressively, however, the difference in mean number of days required to reach 2000, 3000, 4000, and 5000 mm$^3$ widened between the buffer animals and those receiving 250 or 500 $\mu$Ci $^{131}$I-81C6. Comparison of the growth curves is shown in Fig. 1. Statistical evaluation at 5000 mm$^3$ then demonstrated both the 250 and 500 $\mu$Ci groups to significantly differ from buffer ($P = 0.02$ and 0.03) (Table 2). Only one animal in the 250 $\mu$Ci group, however, demonstrated any tumor regression.

In Experiment 2, the mean tumor volumes at the time of treatment were larger and ranged from 421 to 475 mm$^3$ with standard deviations of 140–206 mm$^3$ but with no statistical difference among the groups. The time required for tumors to reach 1000 mm$^3$ ranged from 3.8 to 6.1 days and a statistically significant difference was not noted between buffer and any of the treatment groups (Table 2). The time required to reach 2000 and 3000 mm$^3$ again showed progressive delays for the radiolabeled groups. Beyond 3000 mm$^3$, the 1000 $\mu$Ci group could not be evaluated because of animal death due to a watering system failure. For the remaining groups, the time required to reach 5000 mm$^3$ ranged from 8.7 to 16.6 days. Statistical comparison of buffer to both the 250 and 500 $\mu$Ci groups demonstrated a significant difference at 5000 mm$^3$ ($P = 0.01$ and 0.01) (Table 2). None of the animals in any of the groups demonstrated tumor regression.

In Experiment 3, the initial tumor volumes ranged from 215 to 227 mm$^3$ with standard deviations of 53–64 mm$^3$ and no statistically significant variation. The times required to reach 1000 and 5000 mm$^3$, respectively, were 3.2–19.3 and 11.2–33.2 days (Table 1). Statistical evaluation comparing buffer to the radiolabeled treatment groups for growth delay at 1000 mm$^3$ was statistically insignificant for 500 $\mu$Ci ($P = 0.057$). However, the difference of 3.2 days for buffer versus 19.3 days for 1000 $\mu$Ci was statistically significant ($P = 0.001$) (Table 2). At 5000 mm$^3$, both groups were statistically different from buffer ($P = 0.002$ and 0.001).

In Experiment 4, the initial tumor volumes were equivalent for all groups, ranging from 152 to 165 mm$^3$ with standard deviations of 52–101 mm$^3$. The growth curves are shown in Fig. 2. Statistical evaluation at 5000 mm$^3$ was significantly different for 500 and 1000 $\mu$Ci $^{131}$I-81C6. At 5000 mm$^3$, 1000 $\mu$Ci $^{131}$I-45.6 ($P = 0.01$), 500 $\mu$Ci $^{131}$I-81C6 ($P < 0.001$) and 1000 $\mu$Ci $^{131}$I-81C6 ($P < 0.001$) were significantly different from buffer. Comparison of 500 to 1000 $\mu$Ci $^{131}$I-45.6 was not significant ($P = 0.053$), while significant statistical differences were noted between 500 and 1000 $\mu$Ci $^{131}$I-81C6 ($P = 0.006$), 500 $\mu$Ci $^{131}$I-45.6 and -81C6 ($P < 0.001$) and 1000 $\mu$Ci $^{131}$I-45.6 and -81C6 ($P < 0.008$). Tumor regression was noted in 1 of 10 animals that received 1000 $\mu$Ci $^{131}$I-45.6, 7 of 10 that received 500 $\mu$Ci $^{131}$I-81C6, and 9 of 9 that received 1000 $\mu$Ci $^{131}$I-81C6. Statistically, both 500 and 1000 $\mu$Ci $^{131}$I-81C6 were more effective than either buffer ($P = 0.03$ and 0.01) or the equivalent dose of $^{131}$I-45.6 ($P = 0.022$ and 0.037).

Table 1: Days required to reach specific tumor volumes

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<th>Mean tumor volume (mm$^3$)</th>
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<tr>
<td>1</td>
<td>Buffer</td>
<td>5.8 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>81C6</td>
<td>7.3 ± 3.8</td>
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<td>6.6 ± 2.7</td>
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<td></td>
<td>250 $\mu$Ci $^{131}$I-81C6</td>
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</tr>
<tr>
<td></td>
<td>81C6</td>
<td>4.4 ± 1.8</td>
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<td></td>
<td>50 $\mu$Ci $^{131}$I-81C6</td>
<td>4.7 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>250 $\mu$Ci $^{131}$I-81C6</td>
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</tr>
<tr>
<td>3</td>
<td>Buffer</td>
<td>3.2 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>50 $\mu$Ci $^{131}$I-81C6</td>
<td>5.3 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>1000 $\mu$Ci $^{131}$I-81C6</td>
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</tr>
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<td>6.1 ± 2.2</td>
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<td>25.5 ± 6.1</td>
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<td>1000 $\mu$Ci $^{131}$I-81C6</td>
<td>40.6 ± 10.3</td>
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* Mean ± SD.
Table 2 Statistical analysis of treatment response

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<th>5000 mm³</th>
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<th>P*</th>
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<td>50 μCi 131I-81C6</td>
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<td>NS</td>
<td>0/6</td>
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<td>500 μCi 131I-45.6</td>
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<tr>
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<td>&lt;0.001</td>
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<td>&lt;0.03</td>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>9/9</td>
<td>0.01</td>
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</table>

* Statistical P value of buffer control compared to treatment group using the Wilcoxon rank sum test.
* Statistical value compared to buffer using the Fisher exact test.
NS, not significant.

Fig. 1. Comparison of mean growth curves for the 4 experiments. The pooled controls and the mean growth curves for the radiolabeled Mab.

Individual tissues, including tail and carcass with tissues removed. The results were equivalent.

The localization of 131I-labeled 81C6 and 45.6 in various tissues and tumors is shown in Fig. 4. The concentration of 131I in the tumors reached a plateau (of 15.35% dose/g) in the first 24 h and remained elevated throughout the experimental period of 11 days. After injection of 45.6, the 131I level in the tumors, on the other hand, declined exponentially after Day 1 and decreased to 0.496% dose/g on Day 11.

In thyroid (neck tissue), heart, lung, liver, kidney, and spleen, both antibodies localized and cleared with similar kinetics (Table 3). Except for the Day 1 point in the lung, 45.6 levels were always slightly higher than 81C6 in all tissues at all time points. This may be a result of increased tumor uptake of 81C6 Mab; activity in the tumors ranged from 2.39% (Day 11) to 9.87% (Day 4) of total injected dose. There was a very small amount of Mab localized in both brain and muscle. When compared with data from trace-labeled localization experiments, similar biodistributions were noted.

Toxicity and Dosimetry. No animal died within the experimental period of 11 days. However, when individual tissue weights were examined (data not shown), spleen weights from both groups, either 81C6 or 45.6, decreased progressively from Day 1 (mean weight, 71.8 mg) to Day 11 (20.7 mg). No difference was noted in the weight of other organs except tumors in animals receiving specific Mab 81C6.

Dosimetry calculations were carried out by direct integration of uptake data in tissues dissected from animals injected with 1000 μCi 131I-labeled 81C6 and 45.6. We assumed a uniform distribution of 131I in tumors and tissues and also a complete absorption of β particles as all tissues examined had dimensions larger than the 2-mm maximal range of the most abundant 131I-labeled β particles (particle energy, 0.606 MeV). Tumors in animals given 1000 μCi 131I-81C6 received 9719 cGy during the experimental period of 11 days (Table 4). For animals given 45.6, tumors received 2346 cGy. Both doses resulted in therapeutic effects as reflected by tumor growth delay (Figs. 1 and 2). Normal organs received radiation doses from 135 cGy (brains) to 2415 cGy (lungs). Mab 45.6 usually delivered only slightly higher radiation doses to normal tissues (Table 4). When the radiation doses delivered by 131I-81C6 to heart, lung, and liver were examined, doses equivalent to 131I-45.6 doses in the tumors were noted. This corresponds to similar distribution patterns seen in tumor-bearing mice and rats in prior studies (4, 25). Normal tissue uptake was assumed to have resulted from circulating Mabs residing in the tissue vessels. Such nonspecific toxicity is no doubt a real phenomenon in animal experiments where animal: tumor weight ratio is small (1-1.5 g:0.1-0.5 g) and the volume of distribution for normal tissues is relatively small. In human trials, such nonspecific toxicity may be less due to a much larger normal tissue:tumor volume ratio, e.g., liver in human adult:1500 g versus tumors:30 g. However, this assumes that high % of dose/g localization in human tumors will be as readily achievable as in the animal model. The theoretical calculation for liver dose in a human trial with proportional injected Mab dose will result in a much lower dosage: 2130 x (1.5 g/1500 g) x (30 g/0.5 g) = 127.8 cGy.

Dosimetry Comparison. The % of dose/g for tumor was compared to previous localization experiments which used 5 μg Mab/animal. For the localization studies, specific Mab 81C6 localization in the tumors reached peak levels in 48 to 72 h and declined progressively afterward. The equivalent growth curves

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Fig. 2. Results of Experiment 4 showing the mean growth curve for buffer-treated animals and the individual growth curves for the animals in each of the four treatment arms.

Table 3 Mean percentage of injected dose/g Days after Mab injection

<table>
<thead>
<tr>
<th>Days after Mab injection</th>
<th>1</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>45.6 Tumor</td>
<td>8.06</td>
<td>2.30</td>
<td>1.58</td>
<td>1.26</td>
<td>0.50</td>
</tr>
<tr>
<td>Brain</td>
<td>0.48</td>
<td>0.19</td>
<td>0.13</td>
<td>0.11</td>
<td>0.05</td>
</tr>
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<td>Thyroid</td>
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<td>1.17</td>
<td>0.50</td>
<td>0.42</td>
<td>0.18</td>
</tr>
<tr>
<td>Heart</td>
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<td>3.80</td>
<td>1.58</td>
<td>1.62</td>
<td>0.37</td>
</tr>
<tr>
<td>Lung</td>
<td>6.11</td>
<td>2.75</td>
<td>1.57</td>
<td>1.96</td>
<td>0.59</td>
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<td>1.01</td>
<td>0.89</td>
<td>0.23</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.85</td>
<td>0.24</td>
<td>0.29</td>
<td>0.18</td>
<td>0.10</td>
</tr>
<tr>
<td>Tail</td>
<td>1.16</td>
<td>0.55</td>
<td>0.57</td>
<td>0.42</td>
<td>0.20</td>
</tr>
<tr>
<td>Spleen</td>
<td>7.67</td>
<td>2.19</td>
<td>1.19</td>
<td>0.50</td>
<td>0.57</td>
</tr>
<tr>
<td>81C6 Tumor</td>
<td>15.29</td>
<td>13.97</td>
<td>14.10</td>
<td>13.20</td>
<td>18.44</td>
</tr>
<tr>
<td>Brain</td>
<td>0.50</td>
<td>0.10</td>
<td>0.05</td>
<td>0.08</td>
<td>0.05</td>
</tr>
<tr>
<td>Thyroid</td>
<td>1.51</td>
<td>0.45</td>
<td>0.25</td>
<td>0.16</td>
<td>0.24</td>
</tr>
<tr>
<td>Heart</td>
<td>5.23</td>
<td>0.53</td>
<td>0.51</td>
<td>0.26</td>
<td>0.43</td>
</tr>
<tr>
<td>Lung</td>
<td>10.74</td>
<td>1.20</td>
<td>0.65</td>
<td>0.27</td>
<td>0.41</td>
</tr>
<tr>
<td>Liver</td>
<td>5.57</td>
<td>0.98</td>
<td>0.59</td>
<td>0.26</td>
<td>0.25</td>
</tr>
<tr>
<td>Kidney</td>
<td>4.02</td>
<td>0.60</td>
<td>0.30</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.03</td>
<td>0.18</td>
<td>0.07</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>Tail</td>
<td>2.58</td>
<td>0.51</td>
<td>0.51</td>
<td>0.25</td>
<td>0.31</td>
</tr>
<tr>
<td>Spleen</td>
<td>5.19</td>
<td>0.82</td>
<td>0.82</td>
<td>1.34</td>
<td>1.72</td>
</tr>
</tbody>
</table>

Table 4 Dosimetry of Mabs in D-54 MG s.c. tumor-bearing nude mice

<table>
<thead>
<tr>
<th>Organ</th>
<th>131I-81C6</th>
<th>131I-45.6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu$Ci/h/g</td>
<td>cGy</td>
</tr>
<tr>
<td></td>
<td>$\mu$Ci/h/g</td>
<td>cGy</td>
</tr>
<tr>
<td>Tumor</td>
<td>22,534</td>
<td>54,400</td>
</tr>
<tr>
<td>Brain</td>
<td>311</td>
<td>364</td>
</tr>
<tr>
<td>Heart (neck tissue)</td>
<td>1,015</td>
<td>1,764</td>
</tr>
<tr>
<td>Heart (whole body)</td>
<td>2,991</td>
<td>4,886</td>
</tr>
<tr>
<td>Lung</td>
<td>5,598</td>
<td>4,994</td>
</tr>
<tr>
<td>Liver</td>
<td>3,162</td>
<td>4,938</td>
</tr>
<tr>
<td>Kidney</td>
<td>2,196</td>
<td>2,575</td>
</tr>
<tr>
<td>Spleen</td>
<td>3,526</td>
<td>4,891</td>
</tr>
<tr>
<td>Muscle</td>
<td>570</td>
<td>627</td>
</tr>
</tbody>
</table>

Fig. 3. Total body clearance of 131I-81C6 and -45.6 calculated by Mediae dose calibrator.

Fig. 4. Biodistribution of 131I-81C6 and -45.6 in D-54 MG-bearing animals. Curves are shown using radiation doses obtained from whole body measurements with a dose calibrator. Equivalent results were obtained using individual tissue radioisotope levels.

are shown (Fig. 5). For the localization studies, tumors showed no growth delay. In therapeutic experiments, tumor size either stabilized or regressed. The % of dose/g for 81C6 therapy studies demonstrated rapid elevation by 24 h followed by a plateau over the next 7 days at which time there was a further increase between Days 8 and 11 postinjection. For 45.6, there
geneity in terms of antigen expression and vascular permeability.

tractive for CNS tumors which have a high degree of hetero.

0% for 50 itCi, 6% for 250 ¿tCi, 18% for 500 /¿Ci, and 54% for the dose of radioisotope was clearly seen for Mab 81C6 and, to a lesser extent, for 45.6. For 13II-81C6, both growth delay and tumor regression progressively increased with higher levels of the dose of radioisotope was clearly seen for Mab 81C6 and, to a lesser extent, for 45.6. For 13II-81C6, both growth delay and tumor regression progressively increased with higher levels of...
Therapeutic efficacy of antiglioma monoclonal antibody

delivering 3300 cGy to a s.c. AKR T-cell lymphoma using 1000 
\( \mu Ci \) 131I-labeled anti-Thy 1.1. With a dose of 500 \( \mu Ci \) of 131I-
anti-Thy 1.1 they were able to deliver 1600 cGy to AKR tumor
cells as compared to 380 cGy with a similar dose of irrelevant
antibody. Zalckberg et al. (38) calculated a delivery of 700 cGy
to a human colon xenograft in athymic mice using 1000 \( \mu Ci \)
131I-labeled 250–30.6, a Mab against a cell surface antigen.
While Goldenberg et al. (45) calculated approximately 1500
cGy to a similar xenograft using 1000 \( \mu Ci \) of 131I-labeled anti-
CEA, Rostock et al. (21) estimated a 450 cGy tumor dose to a
rat hepatoma following infusion of 500 \( \mu Ci \) of 131I-labeled anti-
ferritin. The method of calculating radiation exposure in these
prior studies, however, was based upon extrapolation from low
specific activity localization experiments rather than directly
from therapeutic trials. Our data indicate that estimated dose
errors of 35–52% can result due to difference in tumor growth
rate. Badger et al. (11) acknowledged this potential source of
error based upon continued tumor growth in data obtained
from low specific activity studies. Our data suggest that tumor
growth was the major cause of the underestimation of dose
delivered. The plasma clearances were equivalent between the
two types of studies as were the general distribution patterns to
normal organs while the variation in % of dose/g paralleled the
differences in growth curves.

From our data, response to radiolabeled Mab generally corre-
related with the size of the tumor at initial treatment, although
this relationship was complex. If all experiments were com-
bined, tumor regression consistently correlated with initial tu-
more burden, but there was no direct relationship between regres-
sion and growth delay. Significant regression occurred only at
the smallest initial tumor size for 500 \( \mu Ci \) of 81C6 and at the
two smallest treatment sizes for 1000 \( \mu Ci \). If regressors were
compared to nonregressors on the basis of initial tumor size,
regressors had a significantly smaller initial tumor size. Several
possible explanations exist. Moshakis et al. (19) in a similar
athymic mouse model found that specific localization occurred
only in tumors smaller than 200–250 mg. This phenomenon
was seen in animals with multiple tumors and was therefore
tumor rather than animal dependent. However, many other studies,
including our previous experience with the localization of
81C6 within U-251 MG s.c. glioma xenografts, have shown higher
concentrations of both specific and nonspecific antibod-
ies in larger tumors (7, 8, 13, 17, 22, 25). It is unlikely that the
greater responsiveness of smaller tumors was based solely upon
greater radiation exposure, rather smaller tumors may have
been more susceptible to the 131I Mab for either geometric or
biological reasons. Assuming a total absorption of \( \beta \) particles
from the 131I and average and maximum ranges of 0.6 and 2
mm for these \( \beta \) particles, a smaller tumor would be more likely
to have a therapeutic response if any nonuniformity in antigen-
Mab complex distribution occurred or if distribution beyond
the tumor vasculature was limited. An alternative hypothesis is
the Goldie-Coldman quantitative model for multiple levels of
resistance (46). In this mathematical model of tumor growth
and therapeutic resistance, the probability of heterogeneity and
thus the potential resistance are highest in larger, more ad-
vanced tumors. This postulates that such a tumor may be
susceptible to "cure" early in its development while larger more
heterogeneous tumors are more likely to harbor spontaneously
resistant stem cells. Such a concept would explain why tumor
regression and growth curve were size dependent while growth
delay was not.

In summary, we have shown therapeutic response in the D-
54 MG s.c. human glioma xenograft model with a single dose
of radiolabeled anti-ECM monoclonal antibody. Response was
most noted in smaller tumors and was significantly greater than
that seen with control Mab. In the context of prior results
obtained with other radiolabeled Mabs and our prior localiza-
tion data, these data suggest that Mabs against ECM antigens
may prove to have a therapeutic advantage. While many factors
including size of tumor, antigen density, and species specificity
make it unlikely that equivalent localization indices and per-
centages of injected dose can be achieved in humans, the mouse
may be a reasonable screening model for Mabs prior to human
trials. Moreover, permeability or access of a macromolecule
such as IgG is much greater in a s.c. xenograft than in most
human or experimental i.c. tumor models. For successful
application of monoclonal antibodies to i. c. tumors, either highly
permeable tumors must be selected or strategies designed to
increase permeability to tumors in areas of frank tumors and
in invaded areas of normal brain.

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Yi-Sheng Lee, Dennis E. Bullard, Michael R. Zalutsky, et al.


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