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Tumor Resection Cavity Administered Iodine-131-Labeled Anti-tenascin 81C6 Radioimmunotherapy in Patients with Malignant Glioma: Neuropathology Aspects

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Abstract

Introduction— The neurohistological findings in patients treated with targeted β emitters such as ^{131}I are poorly described. We report a histopathologic analysis from patients treated with combined external beam therapy and a brachytherapy consisting of a ^{131}I -labeled monoclonal antibody (mAb) injected into surgically created resection cavities during brain tumor resections.

Methods— Directed tissue samples of the cavity walls were obtained because of suspected tumor recurrence from 28 patients. Samples and clinical follow-up were evaluated on all patients (Group A) based on total radiation dose received and a subset of these ($n=18$; Group B, proximal therapy subset) who had received external beam therapy within ≤ 3 months of mAb therapy, and undergoing 26 biopsies over 37 months. Histologic outcomes were “proliferative glioma”, “quiescent glioma” and negative for neoplasm. Statistical analysis was used to assess the casual relation between total absorbed dose (^{131}I -mAb + external beam) and histologic diagnosis.

Results— The lesions observed after ^{131}I -mAb therapy were qualitatively similar to those reported for other types of radiation therapy; however, the high localized dose rate and absorbed doses produced by the short range of ^{131}I β particles seem to have resulted in an earlier necrotic reaction in the tumor bed. Among all 28 (Group A) patients, median survival from tissue analysis post mAb therapy depended on histopathology and total radiation absorbed dose. Median survival for patients with tissue classified as proliferative glioma, quiescent glioma, and negative for neoplasm were 3.5, 15, and 27.5 months, respectively. Without categorization, total dose was a significant predictor of survival ($p < 0.002$) where patients with higher doses had better prognoses. For example, median

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survival in patients receiving a total radiation dose greater than 86 Gy was 19 months compared with 7 months for those receiving less than 86 Gy.

Conclusions— Histopathologic analysis correlated with prognosis. Among all patients (Group A) there was a significant correlation between biopsy outcome, survival, and total radiation absorbed dose. Among the Group B proximal therapy patients, the neuropathologic changes were qualitatively similar to those described for external beam therapy and interstitial brachytherapy.

Keywords

Radioimmunotherapy; Neuropathology; ^{131}I ; Brain Tumor; Monoclonal antibody; Radiation therapy

1. Introduction

Targeted radiotherapy has great promise for increasing the selectivity of radiation treatment compared to conventional approaches such as external beam radiation. The histopathologic effects of external beam radiation are well established in cancer treatment and, as such, the consequences of its application are understood. On the other hand, the effects of targeted β emitters such as ^{131}I on treated tissues have largely been unexplored. Administration of targeted radiotherapeutics via loco-regional approaches is appealing because this tactic should act not only to increase tumor radiation dose but also to help confine normal tissue effects to regions close to the site of injection. For example, patients with malignant brain tumors have been treated with ^{131}I -labeled monoclonal antibodies (mAbs) administered directly into surgically created tumor resection cavities [1].

What is known to date concerning the safety and efficacy of treating human gliomas with radiation has come from animal models and clinical biopsy and autopsy studies [2,3]. Radiation sensitivity of benign central nervous system (CNS) tissue at 60 Gy absorbed dose is the major factor limiting external beam therapy with standard fractionation [4,5]. Interstitial brachytherapy and stereotactic radiosurgery represent alternative methods of radiation delivery which focus the dose of therapy locally to the tumor [6–8] and their histopathologic effects are becoming known. These reports indicate that radiation necrosis is not only delayed 3 to 6 months following treatment but also may be seen years after therapy and is associated with vascular hyalinization, intravascular thromboses, and coagulative necrosis.

Iodine-131 labeled anti-tenascin 81C6 mAb delivered directly into the surgically created resection cavity of malignant brain tumor patients has been shown to deliver absorbed doses of 2000 Gy to the cavity interface, 90 Gy to the 1-cm cavity margins and 34 Gy to the 2-cm cavity margins [9]. We have established an optimal absorbed dose of 44 Gy to the 2-cm cavity margin limiting the damage of normal brain tissue [10].

In order to better understand the neuropathology of tissues biopsied following this treatment, we undertook a systematic histologic review of the biopsies obtained from the first 28 malignant glioma patients receiving ^{131}I -labeled 81C6 at Duke University Medical Center. Qualitatively, the lesions associated with ^{131}I irradiation were similar to those described for other forms of radiation therapy. Extensive radiation necrosis resulting from excessive permeation of mAb into brain tissue was not found. The high localized dose rate and high absorbed doses produced by the short range of ^{131}I β particles seem to have resulted in an earlier necrotic reaction in the tumor bed.

2. Material and Methods

2.1. Patients

Our study group consisted of the first 28 patients biopsied at Duke following ^{131}I -labeled 81C6 treatment. These 28 patients were selected because they developed one or more enhancing nodularities around the surgically resected cavities and developed some delayed clinical symptoms suggestive of recurrent glioma during the follow-up period. The FDG PET scans presented hypermetabolic activity in each of the cases, rendering a distinction between radiation necrosis and recurrent tumor impossible. The Karnofsky Performance Status for these patients at the time of treatment ranged from 70–100 and their ages ranged from 19 to 64 yr; 20 were male and 8 were female, 27 patients were Caucasian and one was African-American. Twenty-two patients were initially diagnosed with glioblastoma multiforme, two patients with anaplastic astrocytoma, three patients with anaplastic oligodendroglioma, and one patient with a well-differentiated oligodendroglioma that recurred as an anaplastic oligodendroglioma and was treated. This last patient had received radiation therapy more than 8 years prior to mAb therapy. The patients were treated with ^{131}I -labeled 81C6 at doses between 20 and 160 mCi. Table 1 presents a summary of ^{131}I -labeled 81C6 administered activities; 3 patients were treated twice.

2.1 Treatment

81C6 murine mAb reacts with tenascin-C, an extracellular matrix glycoprotein that is over expressed in human gliomas but not normal brain [11–13]. The mAb was labeled with ^{131}I using Iodogen and administered as described [14]. All patients received external beam radiation within one year of labeled mAb, except for one who refused after ^{131}I -labeled 81C6 mAb therapy. Patients were evaluated by FDG PET and MRI before and after treatment on a monthly basis for a minimum of one year or until death. Unless there was clear evidence of tumor progression, no additional radiotherapy was allowed in the first 3 months after mAb therapy and no additional chemotherapy could be administered for 6 wk. However, patients could have received either external beam radiation or chemotherapy before mAb therapy.

A total of 39 samples were obtained, 23 were by stereotactic biopsy and 16 by open craniotomy. Although stereotactic needle biopsy is limited with respect to volume sampling, it remains the state of the art for diagnosis of focal, small, brain lesions. All of the biopsies were performed by one of us (AHF). The patients were studied with regard to histopathologic effects related to total absorbed dose of irradiation and time interval between mAb therapy and biopsy.

2.3. Dosimetry

Absorbed dose calculations were carried out for the 2-cm cavity margins based on previously described methods [9,10]. Briefly, for every patient, contrast enhanced T_1 -weighted MR images were obtained to estimate the volume of the cavity, and whole-body scintigraphy images or head probe counts were used to estimate the effective residence time of ^{131}I -labeled 81C6 mAb in the cavity. From these two measurements, it was possible to estimate the average absorbed dose to the 2-cm cavity margins. The cavity sizes among these patients ranged between 2.4 cm^3 and 43.9 cm^3 . The average absorbed dose to the 2-cm cavity margins from ^{131}I -labeled 81C6 mAb was then added to that received during external beam radiotherapy (if any). A summary of total absorbed doses is given in Table 1; one patient refused external beam therapy.

2.4. Histology

Antibodies used in the histopathologic evaluation of the tissue samples included HAM-56 (1:1000 dilution; Enzo Diagnostics, New York, NY, USA; anti-macrophage and anti-

endothelial cell reactivity) [15]; Mac 387 (1:500 dilution; DAKO Corp., Santa Barbara, CA, USA; anti-macrophage reactivity) [16]; anti-GFAP antibody (1:1000 dilution, monoclonal cocktail; glioma cell reactivity) and anti-Ki-67 (1:50; DAKO Corp. anti-proliferating nuclear antigen). The technique for staining brain tissues has been previously reported in detail elsewhere [17].

2.5. Histologic analysis

All patient samples were initially studied using routine H&E stains. At the time of surgery, intraoperative frozen section evaluations were performed and all of the biopsies were evaluated for the presence of viable, abnormal tissue and the operative procedure was continued until such tissue was obtained. Permanent sections of these biopsies were evaluated for the presence of tumor and necrosis. Those exhibiting only necrosis (negative for tumor in the biopsy tissues) were diagnosed as "*gliosis, necrosis and macrophages*". Tumor recurrences, were characterized as either "proliferative" or "quiescent" accordingly: "quiescent glioma" was designated for those exhibiting mild hypercellularity with nuclear pleomorphism but no evidence of vascular proliferation, macrophage infiltrates, or mitotic activity (and had an anti-Ki67 labeling index of 2% or less.); "proliferative gliomas" were those tumors that were densely cellular, mitotically active or briskly labeled with anti-Ki67 (>3%) and lacked a component of HAM56 labeling (microglia/macrophage) cells.

A Cox's proportional hazards model was used to examine the relationship between the status of the glioma and survival from the time of the first biopsy. To allow for changes in status due to re-biopsies, status was incorporated into the statistical analysis as a time-dependent covariable.

2.6. Histopathologic examination

Histologically, the biopsies from all 28 patients (designated Group A) were evaluated for the following histologic findings: neoplasia and its status, necrosis, macrophage infiltrates, reactive gliosis, and vascular change including hyaline vascular wall change and intraluminal thrombi. In order to better understand the onset of histologic findings as related to the time interval between mAb treatment and biopsy, a subset of 18 patients (who had undergone 26 biopsies) from the Group A patients, was designated as the proximal therapy group, or Group B, with the defining characteristic being that they had either received chemotherapy alone or external beam therapy within three months or less of ^{131}I -labeled 81C6 mAb treatment. Sixteen of these eighteen (89%) patients were newly diagnosed with no prior chemotherapy or external beam radiation. Their age range was 19–57 yr, and 11 of 18 were male. Sixteen patients were diagnosed with glioblastoma and two with anaplastic astrocytoma. One patient had a recurrent brain tumor following external beam radiation and chemotherapy. The dose breakdown of these 18 Group B patients was: 20 mCi (n=2), 60 mCi (n=2), 80 mCi (n=3), 100 mCi (n=1), 116.5 mCi (n=1), 120 mCi (n=4), 140 mCi (n=3) and 160 mCi (n=2). Two patients (11%) were treated a second time, one was treated at 120 mCi following an initial treatment with 20 mCi and the other received 100 mCi following a 60-mCi treatment. Both patients had recurred prior to second treatment. Five patients treated at doses equal or higher than 120 mCi developed acute or delayed toxicities, including two 160 mCi and one at 140 mCi developing dose limiting neurological toxicities. Biopsy samples were obtained from all of these patients due to increased enhancement around their cavity, with increased or irregular nodularity, suggestive of recurrent tumor. In order to illustrate the appearance and persistence of these histologic findings, the results for each category from all 26 biopsies from these 18 proximally treated (Group B) patients were plotted according to their post treatment date on a time-line after ^{131}I -labeled 81C6 mAb treatment (Table 2).

3. Results

The biopsies on eight patients showed recurrences of their malignant gliomas mixed with necrosis. Four patients revealed only quiescent glioma mixed with reactive effects. Of these first 28 patients treated and subsequently biopsied at Duke, six (21.4%) patients underwent open craniotomies with debulking surgery due to apparent clinical progression and the tissue from all 6 patients revealed proliferative glioma. Sixteen patients presented with changes only of radiation necrosis effects characterized by chronic inflammation, gliosis and macrophage infiltration with no evidence of glioma in the biopsy. Eight (28.6%) patients developed chronic irreversible neurological toxicity from the mass effect caused either by their tumor or radiation necrosis.

Tumor recurrence was typically proliferative (Fig. 1) and the tumors manifested typical findings of high grade glioma including mitotic activity, vascular proliferation, and necrosis pseudopalised by tumor cells. Regions in these tumors in which few macrophages were identified by the anti-macrophage mAbs HAM-56 and/or Mac-387 were helpful in characterizing these proliferative gliomas. Necrosis lacking pseudopalising was commonly found, even within obvious tumors. Such instances of necrosis suggested an obfuscation of the World Health Organization (2000) guidelines for grading these gliomas induced by the therapeutic intervention and in such instances, we opted for the diagnosis of “proliferative glioma with therapeutic effects”.

Quiescent tumor recurrence was manifested by the presence of a modestly cellular collection of glial cells with mild nuclear pleomorphism, lacking in mitotic activity, vascular proliferation or tumor associated necrosis. Cellular regions in these tumors, which exhibited GFAP reactive neoplastic glial cells not associated with a cellular mAb HAM-56 and mAb Mac-387 reactive macrophage population, were used to identify quiescent gliomas. Patients with quiescent gliomas were not retreated.

In order to determine the prognostic significance of distinguishing between proliferative and quiescent gliomas, a Cox's proportional hazards model was used to describe the survival as a function of time-dependent measure of grade. By a factor of 2.0 (1.3–3.0 95% confidence interval), the risk of dying among patients with proliferative glioma was significantly higher than the risk among patients with “necrosis, gliosis and macrophages” ($p < 0.001$). No difference in the risk of survival was noted between the quiescent glioma and the “necrosis, gliosis and macrophages” diagnostic categories. These findings support the use of the needle biopsy method to characterize status in recurrent gliomas in concurrence with the findings of Forsyth [18].

3.1.1 Proximal therapy histopathologic study (Group B patients)

A subset of 18 patients were chosen for further study (Group B patients) as these patients had undergone external beam therapy within 3 months of mAb therapy (2 prior to mAb therapy and 16 after mAb therapy). These 18 patients underwent 26 biopsies over a period of 37 months following therapy. Necrotic tissue was identified in 23/26 biopsies and was found in all post-treatment intervals. In all 23 of these biopsies, coagulative type necrosis was found and small areas in 15 biopsies also lacked cell structural outlines suggesting liquifactive type necrosis. These patterns of necrosis were not different from those typically found after surgery and external beam therapy. In particular, the small foci of devitalized brain tissue surrounding a small vessel and associated with minimal reactive gliosis ascribed to radiation effects following external beam therapy were also found in these tissues (Fig. 2A & B).

Intramural vascular hyalinization of small intraparenchymal vessels (Fig. 3) was found in 15 biopsies, becoming evident in those biopsies taken 6 months or more following ^{131}I -labeled

mAb treatment. The hyalinization of the vessel wall was circumferential and eosinophilic and expanded the vascular wall, typical in every way to that described previously in patients undergoing external beam therapy. Blood vessel thrombosis was identified in small parenchymal vessels in 12 biopsies.

No evidence of glioma was noted in 15 biopsies; typically, reactive type gliosis manifest by cells with prominent eosinophilic processes that were densely knitted together formed by cells with abundant cytoplasm and enlarged nuclei. Bizarre nuclear change could be found in occasional reactive astrocytes as identified by their sparse distribution among otherwise benign astrocytes and macrophages (Fig. 4). A dense collection of gemistocytic cells with significant nuclear pleomorphism was a useful clue for tumor recurrence. Mitotic figures in these cells were considered to be a sign of proliferative glioma. Concurrent use of the immunohistochemically based proliferation marker, Ki-67 [17], clearly delineated malignant, proliferating glioma populations from benign, non-proliferating, reactive populations of glial cells (although macrophage populations were also noted occasionally to be proliferative). So-called “granular mitoses”, characterized by glial cells with nuclei exhibiting chromatin in membrane bound, irregularly sized packets, could be found in tissues diagnosed as “gliosis, necrosis, and macrophages.”

Although highly variable in number and density, macrophage infiltrates were found in every biopsy and were most commonly associated with obviously necrotic foci (Fig. 5). Occasionally, the macrophage infiltrates, when compared against the background edematous neuropil, exhibited an appearance such that a distinction from infiltrating tumor could not be made by routine H&E staining (Fig. 6A). Therefore, immunohistochemical stains using the macrophage related markers HAM-56 and Mac-387, and glial marker GFAP, were evaluated for histologic phenotyping. Infiltrating microglia (Fig. 6B) often mimicked infiltrating gliomas as they lacked the typical foamy, net-like cytoplasm of the activated macrophage and exhibited round, regular nuclei in contrast to the typical elongated nuclei of microglia in resting brain. Macrophages occasionally accounted for a brisk (>3%) Ki-67 labeling index.

Five patients were biopsied within four months of mAb therapy (n= 5 biopsies). Tumor was identified in two biopsies. Four of the five biopsies revealed necrosis with macrophage infiltrates in all four and associated vascular thrombosis in two. Five patients coming to biopsy were long-term survivors (26 to 37 months post mAb treatment). Biopsies in two patients manifested proliferative glioma recurrence. Obvious necrosis was found in three long term survivors associated with macrophage infiltrates which indicated that necrosis was ongoing in the treated fields in these samples.

3.2. Total absorbed dose versus histologic findings (Group A studies)

A Kaplan-Meier plot (Fig. 7A) graphically displays survival of all 28 patients (Group A) stratified by last histologic diagnosis (negative for neoplasm, quiescent glioma, and proliferative glioma). Median survival for patients with tissue classified as proliferative glioma, quiescent glioma, and negative for neoplasm were 3.5, 15, and 27.5 months, respectively. A two-degree of freedom log-rank test showed significant differences in the pattern of survival among the three diagnoses ($p < 0.0001$). Patients with proliferative gliomas had the poorer survival. The relationship between total absorbed radiation dose and survival was examined using Cox's proportional hazards model. Without categorization, total dose was a significant predictor of survival ($p < 0.002$); patients with higher doses had better prognoses. The average total absorbed dose to the 2-cm cavity margins was categorized at higher and lower than 86 Gy, and the relationship between total dose and survival was examined. Again, categorized total dose was a significant predictor of survival ($p < 0.002$). A Kaplan-Meier survival curve (Fig. 7B) again graphically displays survival stratified by absorbed dose. Median survival in patients receiving a total radiation dose greater than 86 Gy was 19 months compared with 7

months for those receiving less than 86 Gy. The relationship between total dose and the diagnosis of radiation necrosis was examined using logistic regression. This analysis, which considered uncategorized total dose as a predictor of a “negative for neoplasm” histologic finding (i.e. “gliosis, necrosis and macrophages”), showed a statistically significant relationship ($p < 0.003$). Higher absorbed doses were more commonly associated with a “negative for neoplasm” histologic finding. If one were to dichotomize dose by its median, the relationship between dose and a “negative for neoplasm” biopsy again revealed that higher doses were associated with radiation necrosis, as expected.

4. Discussion

Loco-regional administration of a targeted radiotherapeutic in the CNS such as into a surgically created resection cavity is an attractive alternative to intravenous injection because of the potential for minimizing radiation dose to normal organs and for overcoming the blood brain barrier. On the other hand, radiation dose deposition to normal tissues in contact with a cavity will be increased. The nature of the effect will depend on several factors including the range-energy deposition profile of the radiation, cross reactivity of the targeting vehicle with normal cell populations and the diffusion of the labeled drug beyond the cavity interface. With regard to ^{131}I -labeled 81C6, ^{131}I is a relatively low energy β -emitter, tenascin is not present in normal brain, and because of their large size, intact mAbs diffuse slowly in tissue [11,12,19].

The present study incorporates the ten patients previously reported by Marriott [20] who were found by ^{18}F FDG-PET to be hypermetabolic along the surgically created rim after treatment with ^{131}I -labeled 81C6. That study noted that ^{18}F FDG accumulation along the rim correlated with macrophage infiltrates while nodular accumulations beyond the rim correlated with recurrent glioma. The present study undertook to evaluate these tissues and those from biopsies of additional patients more thoroughly from a histopathological standpoint.

To our knowledge the only other studies evaluating brain tissue from a patient treated with ^{131}I -labeled mAbs to glioma-associated antigens were by Papanastassiou and colleagues [21,22] and by Riva and colleagues [23,24]. The former mentions a biopsy that was performed 7 wk after ^{131}I -ERIC-1 therapy on one patient who required further surgery; the tissue was reported as necrotic without further description [21,22]. In a report on 17 patients treated with intra-cavitary ^{131}I -labeled anti-tenascin mAb, all patients exhibited necrosis, with neoplastic cells found in 14 patients [23,24].

Iodine-131 is a useful radionuclide for labeling mAbs because of its low cost, simple labeling chemistry and short-range β radiation. Iodine-125, the most common radionuclide used in brachytherapy and one of the more thoroughly studied radionuclides from the standpoint of CNS sensitivity, differs from ^{131}I in both its radiotoxicity and in its biological effects [10]. External beam radiation differs substantially from the β emissions of ^{131}I . The 90th percentile range of ^{131}I β particles in tissue is approximately 1 mm resulting in a steep dose gradient within this range. Based on cavity size and the limited diffusion of mAb in tissues, we would expect the radiotoxic effects of ^{131}I confined to be between a 2 to 5-mm shell of tissue adjacent to the cavity interface. Thus, for a 10-cm³ cavity, ^{131}I would deliver a localized absorbed dose to the cavity interface in the range of 4000 Gy and at 1-cm depth, an absorbed dose of 39 Gy. The resulting average absorbed dose over the 2-cm cavity margins will be approximately 48 Gy. Such high-absorbed doses and dose rate at the cavity interface will lead to immediate DNA damage and have cytotoxic effects that can be observed within three months post mAb therapy, in contrast to external beam irradiation in which early toxicity is considered controversial [25].

Coagulative necrosis and liquefactive necrosis were found in the biopsies of ^{131}I -labeled 81C6 treated patients in the first month following therapy (Table 2). This evidence of early necrosis must be interpreted with caution, recognizing that surgically induced changes may also be active at this time. Early-delayed and late-delayed effects of radiation from any source are largely attributed to vascular damage, specifically, breakdown of the blood brain barrier in early-delayed effects, and hyaline thickening of vessels and fibrin emboli in late delayed effects. Although all of the regions sampled in these biopsies demonstrated gadolinium enhancement on magnetic resonance imaging, the present study could not evaluate the functional effectiveness of the blood brain barrier. However, both hyaline thickening and fibrin emboli were present in tissues obtained from our patients undergoing biopsies beyond 3 months post mAb therapy. In contrast to the findings of ^{125}I brachytherapy experimental animals [26] or children exposed to external beam irradiation [27], no vascular calcifications were noted in our specimens.

Reactive benign gliosis has been consistently noted in experimental models and in human biopsy and autopsy studies of post-radiation CNS damage. The reactive gliosis has been noted to be proliferative in one BUdR study following gamma radiation in rats [28]; however, the proliferative index, as determined with the anti-Ki-67 mAb, indicates the index is less than 1% in benign post-radiation reactive gliosis exclusive of proliferative endothelial cells and macrophages.

Selection of the present specimens was keyed into radiographic images demonstrating regions of enhancement and increased metabolic activity by PET. By selecting regions that demonstrated uptake of ^{18}F FDG, our tissues would, by inference, be hypercellular. The present study, therefore, supports the earlier contentions of Marriott and colleagues [20] who suggested that bright PET regions related to radiation necrosis could be related to macrophage infiltrates and/or recurrent/residual glioma.

5. Conclusion

The neuropathologic features of intracavitary ^{131}I -labeled mAb irradiation are qualitatively similar to those described for other radiation therapy modalities; however, the high dose rates and high absorbed doses produced by the short range of ^{131}I β particles seem to have resulted in an earlier necrotic reaction in the tumor bed than that reported for external beam irradiation [29]. Similar to ^{125}I interstitial brachytherapy, ^{131}I -labeled mAb treatment induced localized radionecrosis; however, toxic effects were less widespread, consistent with the shorter tissue range of ^{131}I β particles. This study demonstrated that there was a significant correlation between total absorbed dose and biopsy outcome, and total absorbed dose and survival, pointing out that patients whose biopsy revealed quiescent glioma or radionecrosis have statistically a higher median survival than those biopsies suggestive of proliferative glioma. Also, those patients that received total absorbed dose doses higher than 86 Gy had a median survival that was significantly higher than those with total absorbed doses less than 86 Gy.

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Figure 1. Tumor recurrence in these active malignant neoplasms was characterized by dense hypercellularity associated with nuclear pleomorphism and vascular proliferation. Although areas of necrosis were easily found, the influence of therapeutic effect was not often readily discernible; thus, these recurrences were characterized as “active malignant gliomas” (H&E; 250×).

Figure 2.

(A) Punctate areas of rarefied cerebral white matter, often associated with a central vessel. Similar lesions are reported to represent an early finding of external beam radiation necrosis. (B) These regions of rarefaction often revealed little if any reaction in the surrounding brain, further lending support to an early manifestation of radionecrosis. (A: H&E 100 $\times$; B: H&E 170 $\times$).

Figure 3. Hyalinization of small vessel wall was a common finding in biopsies taken more than 5 months after ^{131}I -labeled mAb therapy (H&E 320 $\times$).

Figure 4. Bizarre nuclear change could be found in occasional reactive astrocytes as identified by their sparse distribution among otherwise benign astrocytes and macrophages (H&E 325 $\times$).

Figure 5. Within areas of necrosis, macrophages could be easily identified by their foamy cytoplasm, round cytoplasmic outlines, and bland nuclei (H&E 400×).

Figure 6.

(A) Macrophages occasionally exhibit an appearance against the background edematous neuropil and made a distinction from infiltrating tumor difficult on H&E stain. (B) Here, the vast majority of cells present are infiltrating macrophages as confirmed by staining with the HAM 56 (A:H&E 325 $\times$; B:mAb HAM56 325 $\times$).

Figure 7.

(A) Kaplan-Meier plot displaying median survival stratified by last tissue diagnosis as radiation necrosis (“negative for neoplasm”), quiescent glioma, and active glioma. (B) Kaplan-Meier plot demonstrating median survival stratified by average total absorbed dose. Both plots display median survival since last tissue histopathological diagnosis post mAb therapy.

Histological diagnosis, radiation absorbed dose and survival data for the 28 patients in this study

Original Diagnosis*	¹³¹ I- labeled mAb Dose (mCi)	Absorbed Dose (Gy)		Results of Last Biopsy Post ¹³¹ I- mAb treatment	Survival (months)			Alive	
		¹³¹ I- mAb	XRT		Total	From Biopsy	From ¹³¹ I- mAb Treatment		From Original Diagnosis
GBM	100	73	60	133	12	21	25	N	
AO	80	68	62	130	43	74	74	N	
GBM	120	32	59	91	16	27	27	N	
GBM	100	49	60	109	98	117	127	Y	
GBM	160	46	60	106	41	51	53	N	
GBM	140	43	60	103	3	16	19	N	
GBM	80	17	60	77	4	11	13	N	
GBM	120	45	61	106	26	29	31	N	
GBM	120	52	59	111	56	71	72	N	
AA	80	19	66	85	3	5	47	N	
GBM	80	34	59	93	19	28	29	N	
GBM	120	33	61	94	4	18	24	N	
GBM	160	17	60	77	3	12	14	N	
AO	20	4	60	64	12	6	15	N	
GBM	80	50	60	110	2	42	43	N	
GBM	140	47	60	107	29	119	119	N	
AO	100	36	--	36	92	44	45	Y	
GBM	60,101	43	46	89	9	4	14	N	
AA	21	6	60	66	1	4	14	N	
GBM	140	5	60	65	10	10	14	N	
GBM	100	25	60	85	14	30	40	N	
AA	15,20	NA	NA	--	11	14	14	N	
AA	60	23	56	79	10	23	26	N	
GBM	20	20	58	78	12	35	36	N	
AA	20	7	59	66	2	14	15	N	
GBM	20	4	61	65	3	5	12	N	
AA	120	38	64	102	17	21	22	N	
GBM	20,120	26	61	87	123	156	158	Y	

* GBM, glioblastoma multiforme; AO, anaplastic oligodendroglioma; AA, anaplastic astrocytoma

Table 2
Time-line reflecting the occurrence of histologic findings among biopsies taken following ¹³¹I-labeled 81C6 mAb therapy.

	Time (months)															26-37
	1	3	4	5	6	7	8	9	10	11	12	13	15	17	23	
Tumor Present	-	-	+	+	-	-	+	+	-	-	+	+	-	-	-	+
Coagulative Necrosis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
Liquefactive Necrosis	+	-	-	+	+	+	+	+	+	-	+	+	+	-	-	+
Reactive Gliosis	-	+	+	-	+	+	+	-	+	+	+	-	-	+	+	+
Blood Vessel Hyalinization	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Blood Vessel Thrombosis	+	-	-	-	-	+	±	+	-	-	+	+	+	+	-	+
Macrophage Infiltrate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Each mark (+ or -) represents a single biopsy obtained from a single patient at each time interval and the mark represents the presence (+) or absence (-) of the histologic finding.