Clinical Experience with α-Particle–Emitting ^{211}At: Treatment of Recurrent Brain Tumor Patients with ^{211}At-Labeled Chimeric Antitenascin Monoclonal Antibody 81C6

Michael R. Zalutsky[1,2], David A. Reardon[2,3], Gamal Akabani[1], R. Edward Coleman[1], Allan H. Friedman[2,4], Henry S. Friedman[2,4], Roger E. McLendon[2,5], Terence Z. Wong[1], and Darell D. Bigner[2,5]

[1]Department of Radiology, Duke University Medical Center, Durham, North Carolina; [2]The Preston Robert Tisch Brain Tumor Center, Duke University Medical Center, Durham, North Carolina; [3]Department of Medicine, Duke University Medical Center, Durham, North Carolina; [4]Department of Surgery, Duke University Medical Center, Durham, North Carolina; and [5]Department of Pathology, Duke University Medical Center, Durham, North Carolina

α-Particle–emitting radionuclides, such as ^{211}At, with a 7.2-h half-life, may be optimally suited for the molecularly targeted radiotherapy of strategically sensitive tumor sites, such as those in the central nervous system. Because of the much shorter range and more potent cytotoxicity of α-particles than of β-particles, ^{211}At-labeled agents may be ideal for the eradication of tumor cells remaining after surgical debulking of malignant brain tumors. The main goal of this study was to investigate the feasibility and safety of this approach in patients with recurrent malignant brain tumors. **Methods:** Chimeric antitenascin monoclonal antibody 81C6 (ch81C6) (10 mg) was labeled with 71–347 MBq of ^{211}At by use of *N*-succinimidyl 3-[^{211}At]astatobenzoate. Eighteen patients were treated with ^{211}At-labeled ch81C6 (^{211}At-ch81C6) administered into a surgically created resection cavity (SCRC) and then with salvage chemotherapy. Serial γ-camera imaging and blood sampling over 24 h were performed. **Results:** A total of 96.7% ± 3.6% (mean ± SD) of ^{211}At decays occurred in the SCRC, and the mean blood-pool percentage injected dose was ≤0.3. No patient experienced dose-limiting toxicity, and the maximum tolerated dose was not identified. Six patients experienced grade 2 neurotoxicity within 6 wk of ^{211}At-ch81C6 administration; this neurotoxicity resolved fully in all but 1 patient. No toxicities of grade 3 or higher were attributable to the treatment. No patient required repeat surgery for radionecrosis. The median survival times for all patients, those with glioblastoma multiforme, and those with anaplastic astrocytoma or oligodendroglioma were 54, 52, and 116 wk, respectively. **Conclusion:** This study provides proof of concept for regional targeted radiotherapy with ^{211}At-labeled molecules in oncology. Specifically, the regional administration of ^{211}At-ch81C6 is feasible, safe, and associated with a promising antitumor benefit in patients with malignant central nervous system tumors.

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For correspondence or reprints contact: Michael R. Zalutsky, PhD, Department of Radiology, Box 3808, Duke University Medical Center, Durham, NC 27710.
E-mail: zalut001@mc.duke.edu

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The majority of malignant brain tumors recur with an extremely poor prognosis (*1*). Because most gliomas recur locally (*2*), the administration of radiolabeled monoclonal antibodies (mAbs) into a surgically created resection cavity (SCRC) to deliver cytotoxic radionuclides to residual tumor cells represents an innovative intervention for augmenting local tumor control and thereby improving overall outcomes for patients with malignant brain tumors. Promising results have been obtained with antitenascin mAbs labeled with β-particle–emitting ^{131}I or ^{90}Y injected into the SCRC of patients with malignant central nervous system (CNS) tumors (*3–5*).

Radiolabeling of antitenascin mAbs with an α-emitter, such as ^{211}At, is an attractive extension of this approach because α-emitters exhibit unique features that may be optimally suited for the elimination of focal pockets of tumor cells located within the milieu of predominantly normal neural tissue in the CNS. ^{211}At has a half-life of 7.2 h, and each decay of ^{211}At results in the generation of an α-particle (*6*). These α-particles have a short particle pathlength in tissue that is equivalent to only a few cell diameters. Studies with human glioma cells have demonstrated the exquisite cytotoxicity of tumor-targeted ^{211}At, with effective killing achieved with only a few α-particle traversals per cell (*7*). These attributes are attractive for targeted radionuclide therapy because of the possibility of enhancing efficacy while avoiding the adjacent normal tissue toxicity inherent in the use of β-emitters, considerations of particular importance in attempts at

therapeutic interventions in sensitive areas, such as the CNS.

Despite the conceptual appeal, the translation of targeted α-particle therapy into the clinical domain has been slow, in part because of limited radionuclide availability and the dearth of α-emitters with physical half-lives compatible with clinical use (*8*). Clinical literature describing the behavior of targeted α-particle radiotherapy in human cancer patients is limited to the use of a ^{213}Bi-labeled humanized anti-CD33 mAb, with a half-life of 46 min, in the treatment of patients with recurrent acute myeloid leukemia (*9*) and the administration of ^{223}Ra-radium citrate, with a half-life of 11.4 d, to patients with breast and prostate carcinoma and skeletal metastases (*10*). The lack of serious adverse effects observed in these trials provided encouragement for the present study with ^{211}At, an α-emitter with an intermediate half-life and different chemical properties.

We evaluated the feasibility, safety, and efficacy of the administration of chimeric antitenascin mAb 81C6 (ch81C6) labeled with ^{211}At (^{211}At-ch81C6) into the SCRC of patients with recurrent malignant brain tumors. This setting was appealing for the initiation of clinical investigations with ^{211}At not only because of the need for more effective brain tumor treatments but also because of our prior experience with ^{131}I-labeled 81C6 in patients with malignant gliomas (*3,4,6,11,12*). These studies demonstrated that the treatment was well tolerated and associated with encouraging survival results and delayed escape of the labeled mAb from the SCRC. Because it has greater in vivo stability than its murine parent (*13*), ch81C6 was used as the carrier molecule for ^{211}At delivery. The stability and maximum tolerated dose of ^{211}At-ch81C6 after intravenous administration were defined in mice before the initiation of this clinical feasibility study (*14,15*).

MATERIALS AND METHODS

Chimeric 81C6 mAb

Murine 81C6 is an IgG2b mAb that reacts with tenascin, an extracellular matrix glycoprotein ubiquitously expressed in high-grade gliomas but not in normal brain tissue (*16*). Genomic cloning was used to combine the murine 81C6 variable-region genes with the human IgG2 constant-region genes (*17*). ch81C6 was grown in a Mini-Max hollow-fiber bioreactor (Biovest) with CD hybridoma medium (Invitrogen) without serum or protein additives. Purification was achieved by affinity chromatography with a Sepharose–staphylococcal protein A column and then polyethyleneimine ion-exchange chromatography. Each clinical batch of ch81C6 was prepared in accordance with U.S. Food and Drug Administration (FDA) manufacture and testing guidelines (*18*).

^{211}At Production and Radiolabeling

^{211}At was produced on the Duke University Medical Center CS-30 cyclotron via the ^{209}Bi(α,2n)^{211}At reaction by bombarding natural bismuth metal targets with 28.0-MeV α-particles using the MIT-1 internal target; ^{211}At was separated from the target by dry distillation (*19*). Labeling of ch81C6 was accomplished by first synthesizing *N*-succinimidyl 3-[^{211}At]astatobenzoate (SAB) and then reacting SAB with the mAb in pH 8.5 borate buffer (*20*). Purification of the labeled mAb was achieved with a Sephadex G-25 column. SAB and mAb conjugation yields declined at higher ^{211}At activity levels, as discussed in a previous publication (*20*). Size-exclusion high-performance liquid chromatography indicated that 96.0% ± 2.5% (mean ± SD) of the ^{211}At activity eluted with a retention time corresponding to that of ch81C6. The immunoreactive fraction measured with recombinant tenascin fragments was 83.3% ± 5.3%. All preparations were determined to be pyrogen free and sterile before and after patient administration, respectively.

Patient Eligibility and Treatment

Eligible patients had a confirmed histologic diagnosis of recurrent supratentorial primary malignant tumors and were candidates for surgical resection. Patients with tumors that were infratentorial, diffusely infiltrating, or multifocal, with tumors that had intraventricular access, or with tumors that showed subependymal spread were ineligible. Histopathologic samples from the initial surgery were reviewed at Duke University Medical Center. The overexpression of tenascin in tumors was confirmed by positive staining of either fresh or paraffin-embedded tissue with 81C6 or affinity-purified polyclonal rabbit antitenascin serum, respectively. Patients were more than 18 y old and had a Karnofsky performance status (KPS) of at least 60%. Pregnant or lactating patients were ineligible. Other eligibility requirements were previously described (*4*).

Patients underwent a gross total resection and placement of a Rickham reservoir and catheter into the SCRC. An MRI with contrast medium was obtained within 48 h of resection. The protocol stipulated that residual tumor could not enhance measurably more than 1.0 cm beyond the margin of the SCRC. Rickham catheter patency and SCRC integrity were confirmed by injecting ^{99m}Tc-labeled albumin or diethylenetriaminepentaacetic acid into the Rickham reservoir and obtaining γ-camera images immediately and 4 and 24 h later. Patients with subgaleal leakage of radioactivity from the SCRC or with SCRC communication with the subarachnoid space (i.e., intrathecal communication) were not eligible for treatment. A baseline ^{18}F-FDG PET scan was obtained after resection. Before and 30–120 d after treatment, patients were tested for the generation of circulating antibodies to murine 81C6 and ch81C6 with a double-antibody radioimmunoassay or an enzyme-linked immunosorbent assay (*3*).

Because ^{211}At is a radiohalogen exhibiting thyroid accumulation in anionic form (*21*), eligible patients received 4 drops of a saturated solution of potassium iodine and 75 μg of liothyronine sodium (Cytomel) daily from 48 h before to 16 d after ^{211}At-ch81C6 administration. Patients were admitted to Duke University Medical Center for ^{211}At-ch81C6 administration. The Rickham reservoir was accessed with a 25-gauge butterfly needle using sterile technique, and up to 6 mL of SCRC cyst fluid was removed when possible. Ten milligrams of ch81C6 labeled with 71–347 mBq of ^{211}At were injected into the reservoir in a volume of ≤6 mL. The reservoir and catheter were flushed after ^{211}At-ch81C6 injection with the previously aspirated sterile SCRC fluid. Because of the nature of ^{211}At emissions and the low administered activity levels, patient radiation isolation was not required. Before discharge, brain MRI was performed.

The Duke Investigational Review Board (IRB) approved this investigation. Informed consent in a manner approved by the Duke IRB was obtained from each patient or the patient's guardian. The study was conducted under FDA investigational new drug number BB-IND-7516.

Biodistribution and Pharmacokinetics

The 77- to 92-keV polonium K x-rays emitted during ^{211}At decay were used to monitor ^{211}At-ch81C6 distribution in the patients. By use of a dual-head γ-camera fitted with low-energy, high-resolution collimators, anterior and posterior serial whole-body images were obtained immediately after injection of the labeled mAb and approximately 2, 4, 8, 12, and 24 h thereafter. A reference source of ^{211}At was used to set the camera photopeak window at 79 keV with a 20% width and was placed near the right ankle at the time of imaging. The positions of the patient's head and camera were duplicated for each acquisition to minimize artifacts and count variability. Regions of interest were drawn around the SCRC to determine cavity residence time and the percentage of ^{211}At decays occurring in the SCRC. γ-Camera imaging and SCRC pharmacokinetic data were obtained for all 18 patients. Whole-body images also were displayed with a 1% window to facilitate the visualization of low levels of ^{211}At present outside the SCRC. The 1% window represents an upper threshold of 0.01 times the maximum pixel count over the entire image; that is, every pixel with counts above this threshold is displayed at full intensity, highlighting regions of activity that otherwise would be difficult to visualize because of the much higher level of activity remaining in the SCRC.

Blood was sampled at approximately 1, 2, 4, 6, 12, 16, and 24 h from 10 patients after ^{211}At-ch81C6 injection. Counts in aliquots (1 mL) were obtained with an automated γ-counter. The percentage injected dose (%ID) of ^{211}At present in the blood pool was determined by comparison with an injection standard and decay correction and by assuming that blood represented 7% of the total body mass.

Evaluation of Adverse Events and Response

After ^{211}At-ch81C6 administration, patients were monitored for toxicity and survival. Initial follow-up occurred within the first month after treatment. Complete blood counts with differential were obtained weekly for the first 8 wk. Adjuvant chemotherapy was prescribed for 1 y beginning approximately 4 wk after ^{211}At-ch81C6 administration. Because of variability in chemotherapy regimens administered before study enrollment, chemotherapy after ^{211}At-ch81C6 administration was prescribed on an individualized, "best-clinical-management" basis with standard dosing schedules for conventional salvage chemotherapeutic agents, such as temozolomide, lomustine, irinotecan, and etoposide. Patients were reevaluated before the initiation of chemotherapy and every 8–12 wk during chemotherapy. Patients were evaluated every 3 mo for the first year, every 4 mo for the second year, and biannually thereafter. Each follow-up appointment included a complete general and neurologic examination, KPS rating, complete blood counts, chemistry panel evaluation, and MRI with contrast medium. ^{18}F-FDG PET scans were obtained as clinically indicated. Thyroid function was assessed within 1–2 mo of ^{211}At-ch81C6 administration and every 6–12 mo thereafter.

CTC version 2.0 (Common Toxicity Criteria, National Cancer Institute) was used to score toxicity. Although the occurrence of seizures was recorded, seizures were not considered an indication of neurologic toxicity because of their expected frequency in this disease setting. The precise etiology of nonseizure neurologic toxicity after ^{211}At-ch81C6 therapy was difficult to define. Neither clinical features nor findings from either MRI or PET reliably distinguished between recurrent tumors and treatment-induced radiation necrosis. Although stereotactic needle biopsy is limited with regard to volume sampling, it remains the definitive tool for the diagnosis of focal brain lesions. Therefore, the etiology of observed neurologic toxicity was determined on the basis of stereotactic needle biopsy results whenever possible.

Progressive disease was defined by the occurrence of at least one of the following: greater than 25% increase in enhancing tumor cross-sectional area or the appearance of radiographically new lesions that were also hypermetabolic on PET scans, evidence of clinical deterioration and greater than 25% increase in enhancing tumor size or the appearance of radiographically new lesions on MRI, or biopsy-proven recurrent tumor.

Statistical Analysis

A single-center phase I study with a classical "3 + 3" format was designed to determine the maximum tolerated dose of ^{211}At-ch81C6. However, difficulties in preparing elevated radioactivity levels (>250 MBq) of the labeled mAb necessitated departure from this design and resulted in more than 3 patients being evaluated at doses lower than those required on the basis of the observed toxicity. The Kaplan–Meier method (*22*) was used to estimate survival distributions; survival was measured from the date of ^{211}At-ch81C6 administration to death. All patients were monitored until death.

RESULTS

Patient Characteristics

The study population included 18 patients with recurrent malignant brain tumors treated at Duke University Medical Center between April 1998 and June 2001. Nine patients (50%) were women, and the median age was 50 y (range, 27–76 y). Fourteen patients had glioblastoma multiforme (GBM) (78%), 3 patients had anaplastic oligodendroglioma (AO) (17%), and 1 patient had anaplastic astrocytoma (AA) (5%). All patients had a KPS over 70%. The median number of prior episodes of progressive disease was 1 (range, 1–2). All patients had received external-beam radiotherapy before ^{211}At-ch81C6 administration, and 8 (44%) had received prior chemotherapy. The median time between initial diagnosis and ^{211}At-ch81C6 administration was 8.3 mo (range, 3.2–278 mo). One potential patient was excluded from treatment because of subgaleal leakage on the postoperative flow study; this leakage appeared to be related to SCRC proximity to the ventricular system rather than SCRC size. Thus, more than 90% of potential patients received treatment.

Five patients received 71–104 MBq of ^{211}At, 7 patients received 135–148 MBq, 5 patients received 215–248 MBq, and 1 patient received 347 MBq. All ^{211}At doses were conjugated to 10 mg of ch81C6. After ^{211}At-ch81C6 treatment, 14 patients (78%) received systemic chemotherapy. The specific post–^{211}At-ch81C6 treatment chemotherapy agents, doses, and schedules were determined by the primary neurooncologist for each patient.

Adverse Events

No patient enrolled in the present study experienced dose-limiting toxicity. There were no episodes of grade 2 or higher hematologic toxicity attributable to ^{211}At-ch81C6

(Table 1). However, one patient with recurrent GBM developed aplastic anemia after a single dose of lomustine (110 mg/m^2) administered 3 mo after treatment with 74 MBq of ^{211}At-ch81C6. Of note, the patient had normal blood counts after ^{211}At-ch81C6 administration until approximately 5 wk after lomustine administration, at which point persistent grade 4 neutropenia and thrombocytopenia developed. Evaluation revealed hypocellular bone marrow (<5% cellularity) with a 46, XX, t(1;20)(p13.2;q13.2) karyotype noted in 2 of 36 metaphases. The patient was treated with transfusions, hematopoietic growth factors, antithymocyte globulin, prednisone, and cyclosporine, with minimal hematologic improvement, and died of recurrent GBM approximately 20 mo after ^{211}At-ch81C6 treatment.

Nonhematologic toxicity included neurologic and nonneurologic events. Neurologic events occurred in 6 patients who experienced seizures (grade 2, $n = 2$; grade 3, $n = 3$; grade 4, $n = 1$); however, these were not considered dose limiting because seizures are an expected event in patients with brain tumors. Furthermore, each of these events occurred at the time of progressive disease, and all but one of these patients also had seizures before ^{211}At-ch81C6 administration. Six patients experienced grade 2 neurologic events at least possibly attributable to ^{211}At-ch81C6, including 3 patients with headache, 1 patient with expressive aphasia, 1 patient with hand numbness, and 1 patient with left inferior quadrantanopsia. Each of these events resolved within a few days or weeks and a short course of corticosteroids, except for the visual field deficit. All remaining neurologic events occurred at the time of progressive disease. There were no grade 3 or higher neurologic events related to ^{211}At-ch81C6, and none of the patients required repeat surgery for radionecrosis.

Nonneurologic events possibly attributable to the study regimen involved single patients who experienced grade 2 nausea and grade 2 fatigue. Two patients experienced infections, including 1 patient with a grade 2 episode of bronchitis and 1 patient with *Pneumocystis carinii* pneumonitis. Both of these infections resolved with appropriate antibiotic therapy. There was one death from a pulmonary embolism.

One patient developed a second malignancy after ^{211}At-ch81C6 administration. This patient had recurrent AO and developed an undifferentiated, anaplastic small-cell neoplasm with neuroblastic features (World Health Organization grade IV) in the neck, diagnosed by lymph node biopsy 8 wk after the administration of 215 MBq of ^{211}At-ch81C6. A brain MRI at that time revealed evidence of recurrence at the primary tumor site. The patient underwent re-resection, which confirmed recurrent malignant glioma. The patient opted for no further therapy and died from progressive tumor approximately 6 mo after ^{211}At-ch81C6 administration. Of note, this patient had previously received extensive cytotoxic therapy, including conventional external-beam

TABLE 1
Pharmacokinetics and Overall Survival and Toxicity Results for Patients Treated with ^{211}At-ch81C6

Patient	Histologic findings	Administered activity (MBq)	Cavity volume (cm^3)	Cavity residence time (h)	% of decays occurring in cavity	%ID in blood pool at:		Overall survival (wk)	Toxicity*
						6 h	12 h		
1	AO	72.7	6.0	10.3	99.0	0.018	0.055	235	
2	GBM	74.0	21.7	10.0	96.0	0.020	0.064	59	
3	GBM	70.7	3.7	9.7	93.3	0.106	0.261	82	Aplastic anemia (grade 4); seizures (grade 3)
4	GBM	72.2	2.4	10.4	100.0	NA	NA	42	Hand numbness (grade 2; resolved)
5	AO	103.6	10.0	10.2	98.0	NA	NA	116	Seizures (grade 3); headache (grade 2; resolved)
6	GBM	144.3	0.2	10.3	99.0	NA	NA	150	Seizures (grade 3)
7	GBM	144.7	15.3	10.3	99.0	0.044	0.093	151	
8	GBM	135.4	9.5	10.3	99.0	0.023	0.038	46	
9	GBM	148.0	29.5	9.8	94.1	NA	NA	54	Seizures (grade 2); headache (grade 2; resolved); visual field loss (grade 2)
10	GBM	148.0	15.2	10.2	98.0	NA	NA	51	Aphasia (grade 2; resolved)
11	GBM	148.0	16.0	10.1	97.1	NA	NA	14	
12	GBM	245.3	37.2	9.8	94.1	0.010	0.019	25	
13	GBM	236.4	2.4	9.6	92.2	0.174	0.430	53	
14	GBM	247.9	7.4	9.6	92.2	0.013	0.019	32	
15	GBM	236.8	11.9	9.1	87.4	0.077	0.122	15	Seizures (grade 4)
16	AO	214.6	28.3	10.4	100.0	NA	NA	71	
17	GBM	347.1	33.9	10.4	100.0	0.027	0.037	76	Headache (grade 2; resolved)
18	AA	148.0	4.8	10.3	99.0	NA	NA	78	Seizures (grade 2)

*Toxicity grade in accordance with CTC version 2.0.
NA = not available.

radiotherapy and chemotherapy, which consisted of carmustine-impregnated biodegradable wafers and 8 cycles of procarbazine, lomustine, and vincristine chemotherapy.

Human Antimouse Antibody

Thirty-nine serum samples obtained from 15 patients were evaluated for reactivity with ch81C6. Positive reactivity was seen in 8 samples (21%) and from 5 patients (33%). With the exception of one sample obtained from each of 2 patients, the response was confined to murine variable regions. No observed toxicity was related to human antimouse antibody reactivity.

Biodistribution and Pharmacokinetics

Serial whole-body images of patient 1 are shown in Figure 1; 100% and 1% windows were used to best visualize ^{211}At activity in the SCRC and the remainder of the body, respectively. A region of interest was set around the SCRC, and the clearance of ^{211}At activity from the cavity was determined (Fig. 2). Complete retention of ^{211}At in the cavity (no biologic clearance, only physical decay) would correspond to a residence time of 10.4 h. As summarized in Table 1, the residence time for ^{211}At in the SCRC after the administration of ^{211}At-ch81C6, 10.05 $\pm$ 0.37 h (mean $\pm$ SD), reflected excellent retention of ^{211}At in the SCRC. Correcting the clearance curves in Figure 2 for ^{211}At physical decay revealed that 96.7% $\pm$ 3.6% of ^{211}At decays occurred in the SCRC. Even in the images displayed with a 1% window, discernible localization of ^{211}At activity in specific anatomic structures was generally not observed. In some patients, enhanced but transient accumulation of ^{211}At in the liver, spleen, and possibly the thyroid and bone marrow was seen (Fig. 1B). Consistent with the high retention of ^{211}At-ch81C6 in the SCRC, the %ID of ^{211}At in the blood was low and appeared to only gradually increase with time (Fig. 3). The %ID values for ^{211}At in the blood pool (n = 10) 6 and 12 h after

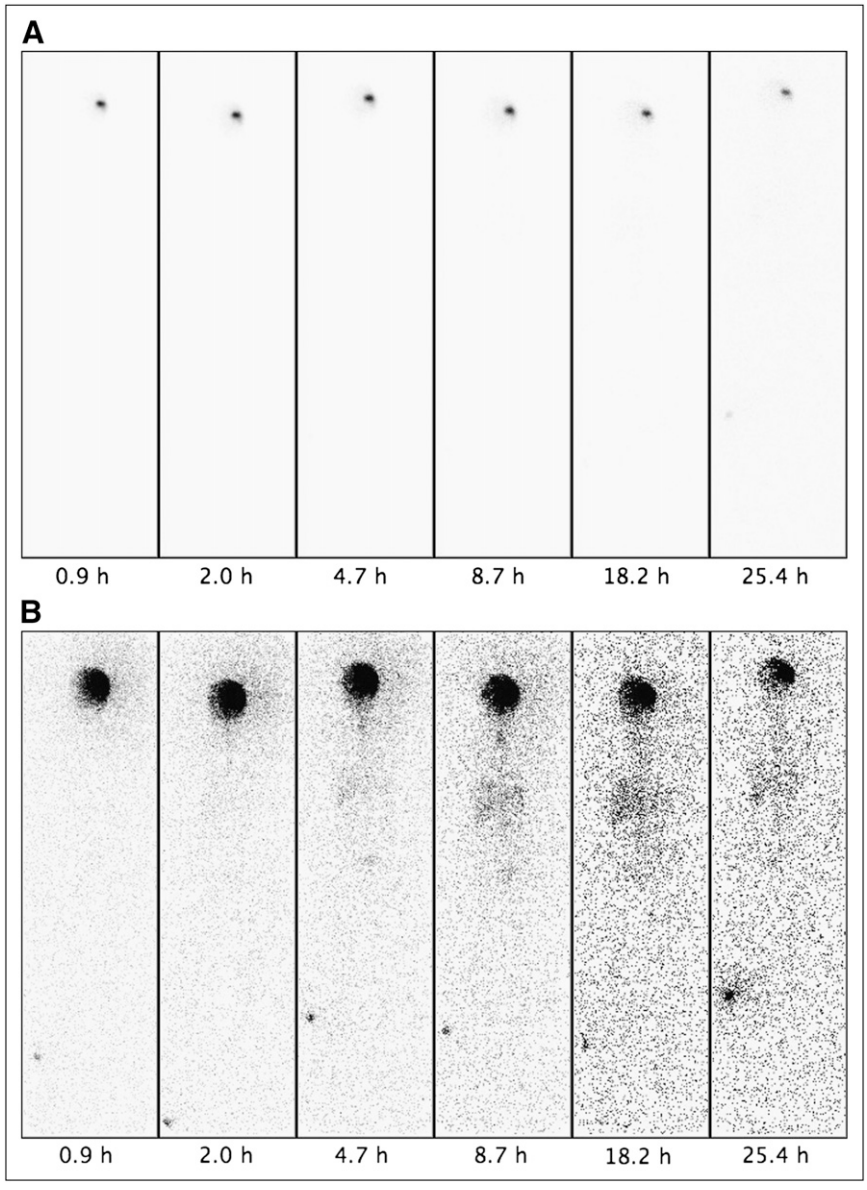

FIGURE 1. Serial whole-body anterior γ-camera images obtained after injection of 73 MBq of ^{211}At-ch81C6 into SCRC of patient 1. (A) 100% window. (B) 1% window set to enhance areas with low activity concentrations. Focal activity seen in lower part of image is imaging standard.

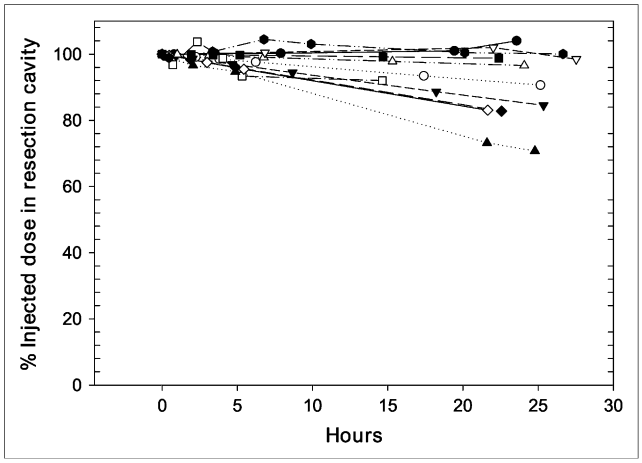

FIGURE 2. Clearance of ^{211}At activity from SCRC, determined by setting region of interest around cavity on serial γ-camera images obtained after administration of ^{211}At-ch81C6 into SCRC. Data are for patients 1 (○), 2 (▽), 3 (▼), 4 (⬢), 7 (△), 8 (●), 12 (□), 13 (♦), 14 (◇), 15 (▲), and 17 (■).

the administration of ^{211}At-ch81C6 into the SCRC were 0.044 ± 0.043 and 0.067 ± 0.069, respectively. Taken together, these results suggest limited catabolism and excellent stability of the labeled mAb in vivo.

Biopsies and Surgical Procedures

Twelve patients (67%) underwent 15 surgical procedures for progressive clinical or radiographic changes, including 14 stereotactic biopsies and 1 resection. Six biopsies revealed gliosis, and 8 biopsies confirmed recurrent tumors. Two patients had 2 biopsies separated by 14 and 22 mo. In both of these patients, the initial biopsy revealed gliosis, and the second biopsy confirmed recurrent malignant gliomas. One patient underwent stereotactic biopsy followed by resection 3 mo later. The biopsy specimen revealed gliosis, and the resection confirmed recurrent tumor. No patient required repeat surgery due to the development of radionecrosis.

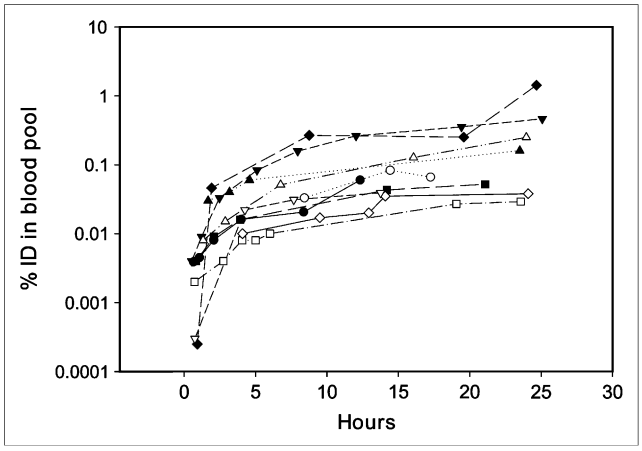

FIGURE 3. %ID of ^{211}At-ch81C6 in blood pool as function of time, determined from serial blood sample counts. Data are for patients 1 (○), 2 (▽), 3 (▼), 7 (△), 8 (●), 12 (□), 13 (♦), 14 (◇), 15 (▲), and 17 (■).

Pattern of Recurrence

Progressive disease was local in all cases but one. One patient with left temporal GBM developed a noncontiguous recurrence in the left frontal lobe 6.5 mo after ^{211}At-ch81C6 administration. Figure 4 shows serial MRI scans obtained from patient 5, who had recurrent AO and was treated with 104 MBq of ^{211}At-ch81C6. After gross total resection, there was a minimal enhancing rim; however, rim enhancement gradually became more prominent with time, and the cavity collapsed. By wk 57, a focal enhancing lesion was noted, and a biopsy revealed recurrent AO. The patient died from recurrent tumor 116 wk after ^{211}At-ch81C6 treatment.

Response and Survival Data

Survival was the most important criterion for efficacy because all patients underwent total or nearly total resection leaving little or no residual tumor. The median survival times for all patients, for those with GBM, and for those with AO or AA were 57 wk (95% confidence interval [CI]: 47–78 wk), 52 wk (95% CI: 33–76 wk), and 97.0 wk (95% CI: 72–235 wk), respectively (Fig. 5). The 1-y survival probabilities for all patients, for those with GBM, and for those with AO or AA were 61% (95% CI: 42%–88%), 50% (95% CI: 30%–84%), and 100%, respectively. Of note, 2 of 14 patients with recurrent GBM survived for nearly 3 y after ^{211}At-ch81C6 treatment.

DISCUSSION

This is the first study evaluating a ^{211}At-labeled targeted radiotherapeutic agent in cancer patients. Two clinical trials with other α-particle–emitting radionuclides, ^{213}Bi with a half-life of 46 min and ^{223}Ra with a half-life of 11.4 d, were reported previously (*9,10*). An attractive feature of ^{211}At is that its physical half-life is intermediate between those of ^{213}Bi and ^{223}Ra, thereby offering a better alternative for some of the most promising molecular carriers and clinical settings for targeted α-particle therapy. This is also the first clinical study exploring the administration of an α-particle–emitting radiotherapeutic agent via a nonintravenous route with the goal of treating minimum residual disease, a tactic long thought to be favorable for harnessing the treatment potential of this highly potent and short-range type of radiation (*6,8*).

We previously demonstrated that radioimmunotherapy with ^{131}I-labeled antitenascin mAb 81C6 injected into the SCRC of patients with malignant gliomas is well tolerated and associated with encouraging survival results (*3,4,12,23*). Although the results of these β-emitter radioimmunotherapy studies are promising, α-emitters offer several important advantages, including minimal dependence on tumor oxygenation for achieving efficient cell killing (*24*). Moreover, β-emitters offer no advantage over conventional external-beam therapy with regard to biologic effectiveness. In contrast, the linear energy transfer of ^{211}At α-particles is about 100 keV/μm, and the result is that the distance between ionizing events is about the same as the

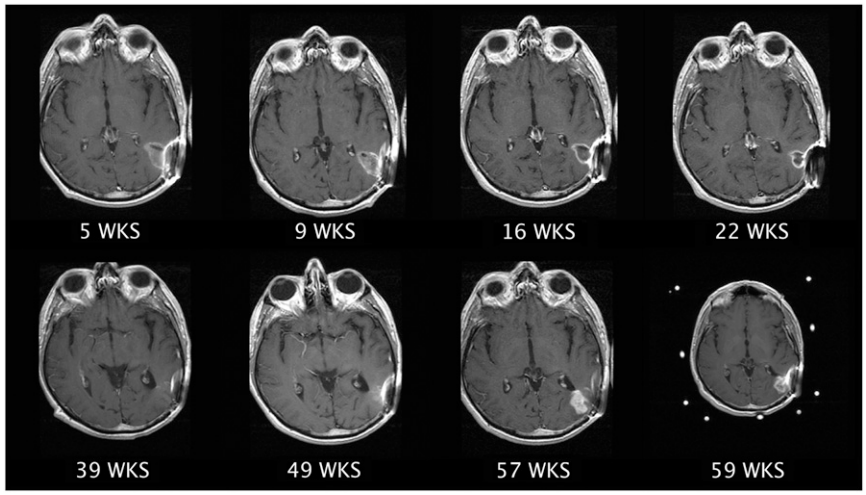

FIGURE 4. Serial MRI images (gadolinium-enhanced T1-weighted images, axial plane) of representative patient after ^{211}At-ch81C6 therapy. After gross total resection, SCRC rim was minimally enhanced. After ^{211}At-ch81C6 administration, rim enhancement gradually became more prominent as SCRC retracted. Focal nodular enhancement noted 57 wk after ^{211}At-ch81C6 administration was subsequently confirmed to be recurrent anaplastic oligodendroglioma.

distance between DNA strands, thus increasing the probability of inducing irreparable DNA strand breaks. For this reason, DNA damage induced by α-particles is less likely to be affected by DNA repair enzymes, such as methylguanine methyltransferase (MGMT), an important mediator of resistance to alkylators and methylators in patients with malignant gliomas (*25*). Furthermore, MGMT has been shown to compromise the effectiveness of the combined application of external-beam radiation and temozolomide, a treatment strategy shown to be of some benefit in glioma patients with low levels of this enzyme (*26*).

The distribution of ^{211}At activity during the 24-h period after the administration of ^{211}At-ch81C6 into the SCRC was determined by serial imaging and blood counting. The SCRC residence time, 10.05 ± 0.37 h, was not significantly different from that corresponding to an infinite biologic half-life (10.38 h) in this compartment. Consistent with this finding, the total activity in the blood pool was less than 0.5 %ID at all time points. Although these results were consistent with the excellent stability of ^{211}At-ch81C6, they also could reflect the generation of labeled catabolites of a particular molecular size and nature that remained sequestered in the SCRC. Analysis of the molecular weight profile of ^{211}At species in the blood was attempted; however, the activity concentration of ^{211}At was far too low for this to be successful.

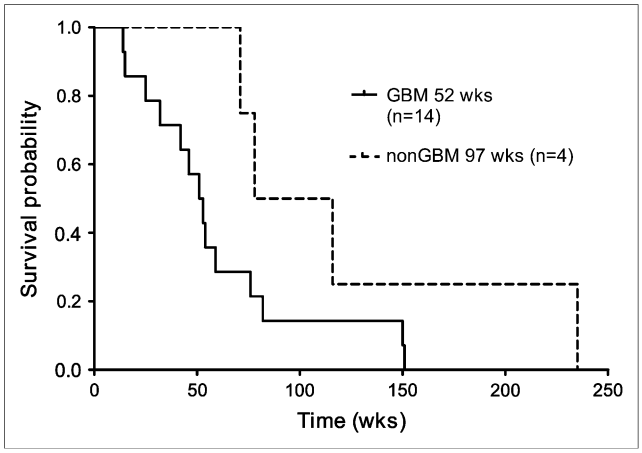

FIGURE 5. Kaplan–Meier overall survival estimates for patients receiving ^{211}At-ch81C6, stratified by histologic findings. GBM = glioblastoma multiforme.

The accumulation of ^{211}At in the liver and spleen and possibly in the thyroid and bone marrow was observed by whole-body γ-camera imaging in a few patients, but only when a 1% window was used to enhance regions receiving counts 2 orders of magnitude lower than peak SCRC counts. If deastatination of the labeled mAb had occurred, then uptake of ^{211}At-astatide in the thyroid, stomach, spleen, and lungs, in that order, would have been expected. (*21*). On the other hand, high levels of tenascin are present in normal human liver and spleen, and in previous studies in which radioiodinated murine 81C6 was administered intravenously, the highest levels of radioiodine accumulation were observed in these organs (*27,28*). Whatever its cause, the fact that ^{211}At uptake outside the SCRC was present only at levels that were difficult to detect is encouraging.

No dose-limiting toxicity was observed in the present study, and none of the patients required reoperation for radionecrosis. Consistent with the very low leakage of activity of labeled mAb from the SCRC, there were no episodes of grade 2 or higher hematologic toxicity. No grade 3 or higher neurologic events possibly attributable to radioimmunotherapy were observed, and the grade 2 episodes seen in 6 patients resolved quickly. The lack of significant neurologic toxicity observed with ^{211}At-ch81C6 is consistent with the short pathlength of α-particles in tissue and the low energy of associated photon emissions (polonium K x-rays). Unanticipated problems in synthesizing the levels of SAB required for further dose escalation prevented the determination of the maximum tolerated dose of ^{211}At-ch81C6. These difficulties, detailed previously

(*20*), were subsequently determined to be related to radiolysis-mediated alterations in the labeling chemistry that became more severe at higher ^{211}At activity levels. A revised labeling procedure that has substantially reduced these problems has now been developed (*29*).

Although our treatment strategy minimizes systemic exposure because of regional administration into the SCRC and the short half-life of ^{211}At, an increased risk of late, secondary cancers is of concern for patients receiving DNA-damaging agents such as α-particle emitters. Although one patient developed a second malignancy in the present study, it is unclear whether this event was related to ^{211}At-ch81C6 administration because this patient had also been treated previously with extensive cytotoxic therapy. Furthermore, the rapidity of secondary cancer development (8 wk after the administration of ^{211}At-ch81C6) diminished the likely relationship between this event and ^{211}At-ch81C6 administration because secondary cancers typically develop months to years after exposure to DNA-damaging agents (*30*). Nonetheless, diligent monitoring of α-particle radioimmunotherapy recipients for secondary cancer development is warranted in future studies.

Although the number of patients in the present study was small, the median survival time for patients with recurrent GBM and for patients with all recurrent brain tumors after ^{211}At-ch81C6 treatment was similar to that observed previously with ^{131}I-labeled murine 81C6 (*3*). In that study, dose-limiting toxicity was neurologic, defining a maximum tolerated dose of 3,700 MBq, and 47% of the patients were treated at or above the maximum tolerated dose. A potential advantage of treating brain tumors with shorter-range α-particle emitters, such as ^{211}At, is that it might be possible to achieve efficacy similar to that achieved with β-particle emitters but with a lower toxicity for normal brain regions in the vicinity of the SCRC. It is encouraging that 8 of 14 patients with recurrent GBM survived for 1 y and that 2 patients survived for nearly 3 y after receiving 144 MBq of ^{211}At-ch81C6. It is also encouraging that the median survival time of 52 wk observed in the present study compares favorably with the median survival times of 23 and 31 wk reported for patients with recurrent GBM treated with best care plus placebo and carmustine polymers, respectively (*31*). The number of patients in the present study was too small to discern whether there was a clear dose-response effect for ^{211}At-ch81C6 treatment. Furthermore, the variations in SCRC volumes among patients would be expected to play as important a role in determining the radiation dose delivered to the SCRC interface as the administered megabecquerels of ^{211}At (*11*).

An important consequence of the short range of α-particles in tissue is that variations in antigen expression and mAb delivery could result in heterogeneities in radiation dose deposition, which could compromise efficacy in tumors. Conventional MIRD methodology assumes a homogeneous distribution of the radionuclide in tissue and is not well suited to the stochastic nature of the energy deposition of α-particles in volume elements approximating cellular dimensions (*32*). In brain tumors, tenascin C expression increases and becomes more perivascular with increasing grade (*33*), features that would be expected to contribute to heterogeneous uptake of antitenascin mAbs, such as that used in the present study. We have developed a histologic image–based theoretic model that can be used to estimate GBM and normal brain radiation doses for α-particle–emitting mAbs (*34*). Studies are in progress to calculate tumor microdosimetry for the patients in the present study on the basis of the measured distribution kinetics of ^{211}At-ch81C6 and tenascin concentrations as well as morphologic findings from tissue obtained at surgery.

CONCLUSION

In the present pilot study, we demonstrated that the administration of α-particle–emitting ^{211}At-ch81C6 into the SCRC of patients with recurrent CNS tumors after resection was feasible. Furthermore, the toxicity associated with this approach was minimal. No enrolled patient experienced dose-limiting toxicity after the administration of single ^{211}At-ch81C6 doses of up to 347 MBq. In addition, overall outcomes were highly encouraging, with a median overall survival time of 54.1 wk. Our results suggest that further evaluation of ^{211}At-ch81C6 for patients with CNS tumors is warranted. Further radioimmunotherapeutic strategies under consideration for ^{211}At-ch81C6 include multiple SCRC dose administration schedules, use as part of a radiotherapeutic cocktail containing a β-emitter to modulate the dose profile, and intrathecal administration for patients with leptomeningeal carcinomatosis.

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REFERENCES

1. Wong ET, Hess KR, Gleason MJ, et al. Outcomes and prognostic factors in recurrent glioma patients enrolled onto phase II clinical trials. *J Clin Oncol.* 1999;17:2572–2578.
2. Gaspar LE, Fisher BJ, Macdonald DR, et al. Supratentorial malignant glioma: patterns of recurrence and implications for external beam local treatment. *Int J Radiat Oncol Biol Phys.* 1992;24:55–57.
3. Bigner DD, Brown MT, Friedman AH, et al. Iodine-131-labeled antitenascin monoclonal antibody 81C6 treatment of patients with recurrent malignant gliomas: phase I trial results. *J Clin Oncol.* 1998;16:2202–2212.
4. Reardon DA, Akabani G, Coleman RE, et al. Phase II trial of murine ^{131}I-labeled antitenascin monoclonal antibody 81C6 administered into surgically created resection cavities of patients with newly diagnosed malignant gliomas. *J Clin Oncol.* 2002;20:1389–1397.
5. Riva P, Franceschi G, Frattarelli M, et al. Loco-regional radioimmunotherapy of high-grade malignant gliomas using specific monoclonal antibodies labeled with ^{90}Y: a phase I study. *Clin Cancer Res.* 1999;**5**(suppl):3275s–3280s.
6. Zalutsky MR, Vaidyanathan G. Astatine-211-labeled radiotherapeutics: an emerging approach to targeted alpha-particle radiotherapy. *Curr Pharm Des.* 2000; 6:1433–1455.
7. Larsen RH, Akabani G, Welsh P, Zalutsky MR. The cytotoxicity and microdosimetry of astatine-211-labeled chimeric monoclonal antibodies in human glioma and melanoma cells in vitro. *Radiat Res.* 1998;149:155–162.

8. Mulford DA, Scheinberg DA, Jurcic JG. The promise of targeted α-particle therapy. *J Nucl Med.* 2005;46(suppl 1):199S–204S.
9. Jurcic JG, Larson SM, Sgouros G, et al. Targeted alpha particle immunotherapy for myeloid leukemia. *Blood.* 2002;100:1233–1239.
10. Nilsson S, Larsen RH, Fossa SD, et al. First clinical experience with alpha-emitting radium-223 in the treatment of skeletal metastases. *Clin Cancer Res.* 2005;11:4451–4459.
11. Akabani G, Reardon DA, Coleman RE, et al. Dosimetry and radiographic analysis of ^{131}I-labeled anti-tenascin 81C6 murine monoclonal antibody in newly diagnosed patients with malignant gliomas: a phase II study. *J Nucl Med.* 2005;46:1042–1051.
12. Reardon DA, Quinn JA, Akabani G, et al. Novel human IgG2b/murine chimeric antitenascin monoclonal antibody construct radiolabeled with ^{131}I and administered into the surgically created resection cavity of patients with malignant glioma: phase I trial results. *J Nucl Med.* 2006;47:912–918.
13. Reist CJ, Bigner DD, Zalutsky MR. Human IgG_2 constant region enhances in vivo stability of anti-tenascin antibody 81C6 compared with its murine parent. *Clin Cancer Res.* 1998;4:2495–2502.
14. McLendon RE, Archer GE, Larsen RH, Akabani G, Bigner DD, Zalutsky MR. Radiotoxicity of systemically administered ^{211}At-labeled human/mouse chimeric monoclonal antibody: a long-term survival study with histologic analysis. *Int J Radiat Oncol Biol Phys.* 1999;45:491–499.
15. Zalutsky MR, Stabin MG, Larsen RH, Bigner DD. Tissue distribution and radiation dosimetry of astatine-211-labeled chimeric 81C6, an alpha-particle-emitting immunoconjugate. *Nucl Med Biol.* 1997;24:255–261.
16. Bourdon MA, Wikstrand CJ, Furthmayr H, Matthews TJ, Bigner DD. Human glioma-mesenchymal extracellular matrix antigen defined by monoclonal antibody. *Cancer Res.* 1983;43:2796–2805.
17. Zalutsky MR, Archer GE, Garg PK, Batra SK, Bigner DD. Chimeric anti-tenascin antibody 81C6: increased tumor localization compared with its murine parent. *Nucl Med Biol.* 1996;23:449–458.
18. U.S. Food and Drug Administration. Points to consider in the manufacture and testing of monoclonal antibody products for human use. Washington, DC: U.S. Department of Health and Human Services; 1997.
19. Larsen RH, Wieland BW, Zalutsky MR. Evaluation of an internal cyclotron target for the production of ^{211}At via the ^{209}Bi(α,2n)^{211}At reaction. *Appl Radiat Isot.* 1996;47:135–143.
20. Zalutsky MR, Zhao XG, Alston KL, Bigner D. High-level production of α-particle-emitting ^{211}At and preparation of ^{211}At-labeled antibodies for clinical use. *J Nucl Med.* 2001;42:1508–1515.
21. Larsen RH, Slade S, Zalutsky MR. Blocking [^{211}At]astatide accumulation in normal tissues: preliminary evaluation of seven potential compounds. *Nucl Med Biol.* 1998;25:351–357.
22. Kaplan E, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc.* 1958;53:457–481.
23. Reardon DA, Akabani G, Coleman RE, et al. Salvage radioimmunotherapy with murine iodine-131-labeled antitenascin monoclonal antibody 81C6 for patients with recurrent primary and metastatic malignant brain tumors: phase II study results. *J Clin Oncol.* 2006;24:115–122.
24. Hall EJ. *Radiobiology for the Radiologist.* 5th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2000.
25. Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med.* 2005;352:997–1003.
26. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med.* 2005;352:987–996.
27. Schold SC Jr, Zalutsky MR, Coleman RE, et al. Distribution and dosimetry of I-123-labeled monoclonal antibody 81C6 in patients with anaplastic glioma. *Invest Radiol.* 1993;28:488–496.
28. Zalutsky MR, Moseley RP, Coakham HB, Coleman RE, Bigner DD. Pharmacokinetics and tumor localization of ^{131}I-labeled anti-tenascin monoclonal antibody 81C6 in patients with gliomas and other intracranial malignancies. *Cancer Res.* 1989;49:2807–2813.
29. Pozzi OR, Zalutsky MR. Radiopharmaceutical chemistry of targeted radiotherapeutics, part 2: radiolytic effects of ^{211}At α-particles influence *N*-succinimidyl 3-^{211}At-astatobenzoate synthesis. *J Nucl Med.* 2005;46:1393–1400.
30. Andre M, Henry-Amar M, Blaise D, et al. Treatment-related deaths and second cancer risk after autologous stem-cell transplantation for Hodgkin's disease. *Blood.* 1998;92:1933–1940.
31. Brem H, Piantadosi S, Burger PC, et al. Placebo controlled trial of safety and efficacy of intraoperative delivery by controlled biodegradable polymers of chemotherapy for recurrent gliomas. *Lancet.* 1995;345:1008–1012.
32. Humm JL. A microdosimetric model of astatine-211 labeled antibodies for radioimmunotherapy. *Int J Radiat Oncol Biol Phys.* 1987;13:1767–1773.
33. Herold-Mende C, Mueller MM, Bonsanto MM, Schmitt HP, Kunze S, Steiner HH. Clinical impact and functional aspects of tenascin-C expression during glioma progression. *Int J Cancer.* 2002;98:362–369.
34. Akabani G, McLendon RE, Bigner DD, Zalutsky MR. Vascular targeted endoradiotherapy of tumors using alpha-particle-emitting compounds: theoretical analysis. *Int J Radiat Oncol Biol Phys.* 2002;54:1259–1275.