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Phase I studies of treatment of malignant gliomas and neoplastic meningitis with ^{131}I-radiolabeled monoclonal antibodies anti-tenascin 81C6 and anti-chondroitin proteoglycan sulfate Mel-14 F $(ab')_2$ – a preliminary report

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Summary

The advent of monoclonal antibody (MAb) technology has made Ehrlich's postulate of the 'magic bullet' an attainable goal. Although specific localization of polyvalent antibodies to human gliomas was demonstrated in the 1960s, the lack of specific, high affinity antibody populations and of defined target antigens of sufficient density precluded therapeutic applications. Not until the identification of operationally specific tumor-associated antigens (present in tumor tissue but not normal central nervous system tissue); production of homogeneous, high affinity MAbs to such antigens; and the use of compartmental administration (intrathecal or intracystic), has the promise of passive immunotherapy of primary and metastatic central nervous system neoplasms been recognized. We report here preliminary data from Phase I studies of the compartmental administration of the anti-tenascin MAb 81C6 and $F(ab2)_2$ fragments of MAb Mel-14, which recognizes the proteoglycan chondroitin sulfate-associated protein of gliomas and melanomas, to patients with primary central nervous system tumors or tumors metastatic to the central nervous system.

Phase I dose escalation studies of intracystically administered ^{131}I-labeled anti-tenascin MAb 81C6 to either spontaneous cysts of recurrent gliomas or surgically created cystic resection cavities have resulted in striking responses. Of five patients with recurrent cystic gliomas treated, four had partial responses, clinically or radiographically. Similarly, in patients with surgically created resection cavities, a partial response at the treatment site and extended stable disease status has been obtained following intracystic administration of ^{131}I-labeled 81C6. No evidence of hematologic or neurologic toxicity has been observed in either patient population, with the exception of transient exacerbation of a pre-existing seizure disorder in a single patient. Dosimetry calculations indicated high intracystic retention for four to six weeks with little or no systemic dissemination; estimated total doses intracystically ranged from 12,700–70,290 rad.

Intrathecal administration of labeled MAbs to patients with neoplastic meningitis is more difficult to assess in terms of clinical responsiveness. Of patients so treated with either ^{131}I-labeled 81C6 or ^{131}I-labeled Mel-14 $F(ab)_2$, cerebrospinal fluid and radiographic responses have been achieved, and survival prolongation through maintenance of stable disease has been observed in several cases.

Initial results from Phase I dose escalation trials are encouraging in terms of the proportion of cases of disease stabilization and partial and complete responses obtained. Importantly, neurotoxicity has been virtually nonexistent, and hematologic toxicity rare and rapidly responsive to treatment. In the intracompartmental setting, then, the promise of chimerized MAb molecules or of dimeric or monomeric single-fragment

chains, either radiolabeled or drug- or toxin-conjugated, is great. The possibilities of MAb-mediated, targeted therapy for tumors of the central nervous system are many and promising. Future work will be with newly defined antigens of exquisite tumor specificity, such as the variant epidermal growth factor receptor III molecule. New labeling technology will allow halogens such as ^{131}I and ^{211}At to be used for internalized or membrane-localized antigens. Internalized MAbs will be able to be used as immunotoxins or labeled with chemotherapeutic agents.

Introduction

Since the original concept of targeted therapy of tumors was introduced by Ehrlich [1–3] near the turn of the century, specific delivery of cytocidal agents to tumors, sparing normal tissues, has been a long sought goal. With regard to using antibodies for targeted therapy, the pioneering studies of Landsteiner showing specificity obtainable with polyclonal antibodies were reported also in the early part of the 20th century [4]. The first studies of targeted therapy of brain tumors with antibodies were those carried out as a follow-up to the Pressman procedure [5] of paired label evaluation of injection of ^{125}I-labeled polyclonal antibody and ^{131}I-labeled control immunoglobulin and evaluation of the amount of polyclonal antibody specifically targeted to tumor. After a series of feasibility studies, Mahaley and Day [6–8] performed clinical studies in the mid-1960s with radiolabeled polyclonal antibodies against brain tumors and demonstrated that sufficient uptake by tumor was obtained for scanning purposes. The absolute dose localized to tumor, however, was insufficient to deliver therapeutic amounts of radioactivity to brain tumors. Since only heterogeneous polyclonal antibodies, probably reactive with multiple antigens and containing antibodies of multiple affinities, were available, and the specific antigens involved could not be identified to prepare specific reagents, this work, although elegant for its time in demonstrating the feasibility of the approach, did not lead to clinical advances in brain tumor targeted therapy.

For a number of years thereafter, targeted therapy with antibodies for brain tumors and neoplastic meningitis was neglected until the technical advance by Kohler and Milstein [9] in which murine monoclonal antibody (MAb) technology was developed. The great advantage of the Kohler and Milstein procedure was twofold. First a homogeneous preparation of antibody of a single immunoglobulin class of uniform affinity that could be produced in unlimited amounts was available, and the antibody reactivity was limited to a very specific epitope on the tumors being studied. Nevertheless, the vast majority of the first generation of murine MAb produced, although helping to detect many new molecules, turned out to be against tumor-associated and not tumor-specific antigens. As clinical studies began with MAbs, both with systemic malignancies and with brain tumors, it was found that either no or very low levels of specific antibody or no specific localization was occurring in tumors. This lack of efficacy of radioimmunotherapy is not surprising since, in both systemic tumors and brain tumors, only small fractions of injected dose (0.001 to 0.01% ID/g) have been found to be retained in tumors [10–12].

This lack of accumulation of large amounts of MAbs has been attributed to many factors such as high interstitial pressure in tumors [13], lack of specificity of MAbs, and in some instances, dehalogenation or removal of the radioactive label from MAbs.

Our laboratory began working in the 1980s with MAbs, attempting to obtain operationally specific MAbs against brain tumor-associated antigens. The rationale for the approach was that toxicity was most likely to come from reactivity with normal brain. Even if there was some reactivity of antibody with normal systemic tissues, the use of compartmental therapy – i.e., injection of radiolabeled antibodies either into the intrathecal space for treatment of leptomeningeal disease and recurrent tumor that communicates with the ventricular system, or into spontaneously or surgically created cysts in malignant gliomas or metastatic brain tumors – would render operational specificity. This strategy

with radiolabeled antibodies has also been investigated by other groups [14–18].

The first antibody and molecular target that we chose to approach in the laboratory was MAb 81C6, originally produced against a human glioma cell line that expressed glial fibrillary acidic protein, and subsequently found to react with the polymorphic extracellular glycoprotein tenascin. Tenascin is distinct from other extracellular matrix proteins and is composed of a radially arranged hexamer joined at the amino terminal central knob by disulfide bonds. Each arm contains a single subunit with a molecular weight ranging from 200–300 kilodaltons. The complete nucleotide and deduced amino acid sequence of full length cDNA for tenascin has been published [19, 20]. The subunit consists of a linear array of repeated structural domains including an amino terminal cysteine-rich region involved in oligomerization, a linear segment of 14 to 15 epidermal growth factor-like repeats, a domain of 8 to 15 repeats of fibronectin type 3, and a fibrinogen-like region at the carboxy terminal end. Different subunits are generated by alternate splicing and a fibronectin type 3 repeat domain by one common primary transcript encoded by a single gene. The expression of tenascin-splicing variants can vary in different tissues, cell types, and cell lines [21, 22]. Expression of tenascin is distinct and highly regulated. Its expression is widespread during embryonic development, but it is restricted to a much smaller range of structures within normal tissues [see review of ref. 23]. It is prominent in mesenchymal tumors and carcinomas including gliomas, fibrosarcomas, osteosarcomas, melanoma, Wilms tumor, colon carcinoma, mammary and lung carcinomas, and squamous carcinomas. Tenascin is more prominent in the most anaplastic forms of glioma, namely glioblastoma multiforme, and is seen in up to 99% of glioblastoma multiforme [24]. Balza *et al.* [25] and Siri *et al.* [26] have produced several antibodies against tenascin, the most prominent of which, BC-2, binds to an epitope within the fibronectin type 3 repeat region. These antibodies have been used by Riva *et al.* [27, 28] for intralesional radioimmunotherapy of gliomas.

Our studies have focused on the use of MAb 81C6 [24], which recognizes alternatively spliced extra repeats and does not bind with all tenascin variants, imparting greater specificity. 81C6 also does not bind with normal brain and has been shown to bind at domain 12 of the alternatively spliced fibronectin type 3 repeat of the molecule [29]. Prior to the clinical studies described herein, we investigated tenascin in depth in preclinical studies. Selective uptake of ^{131}I-labeled 81C6 in D-54 MG human glioma subcutaneous and intracranial xenografts has been demonstrated in paired label studies [30, 31]. The immune reactivity and tumor localizing capacity of 81C6 has been shown to be superior to other anti-glioma MAbs [32]. Radioimmunotherapeutic trials with ^{131}I-labeled 81C6 in athymic mice with subcutaneous glioma xenografts [33] and in athymic rats with intracranial xenografts [34] have shown significant tumor growth delay, survival prolongation, and a few apparent cures. Evaluation of intrathecal therapy in rodent animal models is difficult with ^{131}I because of the extremely small size of the cerebrospinal fluid space and the spinal cord relative to the path length of ^{131}I-labeled β-particles. Thus, we evaluated the efficacy and lack of toxicity in a human xenograft model in athymic rats with neoplastic meningitis using MAb 81C6 with ^{211}At, which is an α-emitter with a shorter path length (55–80 μm). These studies demonstrated significant survival prolongation without significant toxicity [35]. Furthermore, dosimetry calculations suggest that it might be possible to treat cystic brain tumors with cyst fluid concentrations as low as 6 μCi per ml of ^{211}At [36].

These promising results in preclinical animal studies led to paired label human studies covering a range of 81C6 dosages, with administration prior to biopsy and gamma camera imaging one to three days after injection. Biopsy specimens of tumor and normal brain showed average uptake from 0.6 to 4.3×10^{-3}% ID/g, with tumor-to-normal brain ratios up to 25:1. Localization indices showed an up to fivefold higher accumulation of 81C6 compared with control IgG_2 murine immunoglobulin [11]. A SPECT imaging study in recurrent glioma patients was then carried out over a broad range of protein doses and demonstrated an inverse relationship between tumor-to-normal-tissue radiation dose ratios and MAb protein dose [37]. Excellent images of the

accumulation of the radiolabeled MAb in the tumor were obtained in all 18 patients. Because tenascin is expressed in normal liver, dose-limiting toxicity to this organ limited projected dosimetry to brain tumors to levels that were likely only to have delivered less than 1,000 rad to the brain tumors. Therefore, nonintravenous routes of administration or compartmental administration of 81C6 was initiated.

Three different Phase I protocols are now in progress that include delivery of ^{131}I-labeled 81C6 compartmentally to several different CNS locations: into recurrent gliomas with spontaneous cysts, into surgically created cystic resection cavities in patients with primary and metastatic malignant brain tumors, and into the intrathecal space for the treatment of patients with neoplastic meningitis or leptomeningeal disseminated tumors.

The second MAb, which underwent similar evaluation for potential clinical use primarily in melanoma but also in malignant glioma, is Mel-14, a MAb originally developed by Carrel *et al.* [38] that is reactive against the melanoma and glioma proteoglycan chondroitin sulfate-associated protein of gliomas and melanomas. Similar preclinical studies were carried out with Mel-14, but because Mel-14 is an IgG_{2a} molecule with significant Fc receptor binding, great diversity was observed in tumor uptake among animals in the same studies [39]. Moreover, when Lashford *et al.* [16] administered intact Mel-14 intrathecally to patients, significant hematologic toxicity was observed, again presumably because of Fc receptor binding to bone marrow. In preclinical studies it was found that the Fc receptor binding could be obviated by enzymatically preparing Mel-14 $F(ab')_2$, and significant localization in both subcutaneous and intracranial xenografts was obtained as well as successful treatment of intracranial and subcutaneous xenografts. Nevertheless, in paired label patient studies, similar low levels of antibody in tumor were observed, presumably due to low level expression of the antigen on normal tissue [12]. With ^{131}I-labeled Mel-14 $F(ab')_2$, under Federal Drug Administration (FDA) BB-IND-3344, intrathecal injection of patients with melanoma or neoplastic meningitis is being done in one Phase I study, and injection into surgically created cystic resection cavities is being performed in a second Phase I study.

It should be noted with all of the diseases being treated by these approaches, namely malignant gliomas and solid metastases to the brain, that there are no truly effective treatments and, as reported by Vick *et al.* [J. Neurooncology, these Proceedings, 1995], external beam radiation is only of limited effect and has dose-limiting toxicity to the normal nervous system. Survival of glioblastoma multiforme patients is generally less than 12 months [40] and, of patients with neoplastic meningitis, is on the average of three months from diagnosis [41, 42].

In this manuscript we report the preliminary results and present stages of five Phase I radioactivity dose escalation studies of ^{131}I-labeled 81C6 and ^{131}I-labeled Mel-14 $F(ab')_2$ used intracompartmentally in malignant glioma patients and in patients with systemic cancer metastatic to the parenchyma of the brain or to the leptomeninges. To date, there has been little toxicity with these studies except for one or two patients at higher dose levels who developed major hematologic toxicity (MHT). In all cases, MHT has responded to treatment with hematopoietic growth factors or blood or platelet transfusions. No significant neurotoxicity has been observed. In several of the studies, complete responses have been obtained and significant extension of survival, including high quality clinical survival, has been observed.

Materials and methods

Preparation and labeling of antibody

81C6 is grown in athymic mice in ascites form and purified over a Sepharose-Staphylococcal protein-A column in pyrogen-free buffers and glassware, following which a secondary purification takes place with PEI ion-exchange chromatography. All requisite studies in the FDA 'Points to Consider' guidelines are met for each batch of antibody, and appropriate sterility and general safety tests are carried out on each clinical batch. 81C6 has a shelf-life of greater than 12 months.

Mel-14 is purified over a Sepharose protein-A

column and dialyzed against 0.1 M sodium citrate buffer (ph 4.0). Digestion of antibody by pepsin is monitored by gel filtration on a 7.5 × 600 mm TSK-3000 column. When digestion to $F(ab')_2$ is approximately 75%, reaction is stopped by the addition of 1 M Tris (pH 8.0). After dialysis against 115 mM phosphate buffer (pH 7.4), Me1-14 is passed over a protein-A Sepharose column to remove intact immunoglobulin. The remaining $F(ab')_2$ fragment is then further purified over an AB_x ion-exchange column. As with 81C6, all FDA criteria in their Points to Consider guidelines are met, and general safety and sterility tests are performed with each clinical batch of Me1-14 $F(ab')_2$. The shelf-life of Me1–14 $F(ab')_2$ is significantly shorter than 81C6 and is only about six months.

Radiolabeling of antibody is carried out by a modification of the Iodogen procedure [11]. Immunoreactivity greater than 50% must be demonstrated by a Lindmo assay; high pressure liquid chromatography analysis routinely must be greater than 95% of the label under the immunoglobulin area of the curve; and there must be greater than 95% precipitability with trichloroacetic acid.

Patient eligibility and treatment plan

To be eligible for the study, patients must have had no prior external beam radiation therapy for at least three months and no anti-neoplastic chemotherapy for six weeks before MAb treatment unless there is evidence of progressive disease. Either cerebrospinal fluid tumor cells or solid tumor must be shown to be reactive with the antibody to be used for treatment. For the intrathecal studies, patency of the subarachnoid pathways is evaluated by administering ^{99m}Tc-albumin through an Ommaya reservoir surgically placed in the ventricles followed by gamma camera imaging through 24 hr. For the brain tumor patients, an Ommaya reservoir is placed in the tumor cyst or surgically created cystic resection cavity, and integrity of the cavity is evaluated by injecting ^{99m}Tc-albumin and imaging with a gamma camera through 24 hr.

Injection of patients

All five Phase I studies were designed with fixed protein doses of 10 mg of each antibody or antibody fragment with 20-mCi dose escalations in cohorts of 3–6 patients per dose until dose limiting toxicity is reached. It was found early in the study that one pediatric patient experienced MHT when treated with adult doses, and the protocol was amended so that pediatric patients are treated with an equivalent dose adjusted to the patient's body surface area. After performing the above quality control evaluations, the reservoir is accessed, several cubic centimeters of fluid are removed, and 1–3 ml of Isovue™ (Squibb) is administered during fluoroscopy to demonstrate delivery of the injected material into the cyst cavity or ventricle. The radioactive dose is administered after demonstration that the contrast is in the cerebrospinal fluid (CSF) or cyst fluid.

Patient follow-up and evaluation

Patients are followed in the hospital until radioactivity levels are less (30 mCi) than required by the state Radiation Safety Office. Planar gamma scintigraphy is performed at the time of discharge from the hospital and at weekly intervals thereafter up to four weeks after discharge for dosimetry calculations. Patients are then followed with serial general and neurologic exams, blood work, magnetic resonance imaging and positron emission tomography scanning, and CSF analyses (in patients on the intrathecal protocols) for up to one year.

Maximum tolerated dose definition

The maximum tolerated dose in each protocol is defined as the highest dose that produces no more than 2 instances of serious toxicity out of 6 patients treated at that dose. A serious toxicity is a grade 3 or grade 4 nonhematologic toxicity or an MHT (defined as > 28 days of absolute neutrophil count, < 500 cells per μl, or platelet count < 20,000 cells/μl).

Response evaluation

Response evaluation is defined as follows:
Complete response (CR) is disappearance of all radiographically detectable contrast enhancing lesions;
Partial response (PR) is greater than 50% reduction in tumor size as measured by the product of the largest perpendicular diameters of the measurable lesion;
Stable disease (SD) is less than 50% reduction in tumor size and no clinical progression;
Progressive disease (PD) is greater than 25% increase in the size of the tumor, appearance of a new radiographically demonstrable lesion, or SD with evidence of clinical progression.

Results

A phase I study of intracystic anti-tenascin MAb^{131}I-labeled 81C6 in the treatment of patients with recurrent cystic gliomas – preliminary results (Table 1)

The most striking responses have been observed in patients with recurrent gliomas that have spontaneous cysts. Although this is a rare type of glioma recurrence, there is no truly effective treatment for spontaneous cystic gliomas. The first patient treated had a recurrent cystic glioblastoma. Treatment was done on a compassionate plea basis. He had a partial clinical and radiographic response and was able to return to work and successfully run his business for a period of 12 months. The patient, with permission from the FDA, underwent another resection and was retreated when he experienced recurrence distal to the original site 13 months after original treatment. He lived for a total of 33 months after initial diagnosis and 26 months after the initial ^{131}I-labeled 81C6 treatment (adult patient No. 1 and 2, Table 1). All three of the other patients treated with recurrent cystic gliomas have had either partial responses or prolonged disease stability, clinically and radiographically. Dose escalation levels in this group of patients have now reached the 40-mCi level and will continue in increments of 20 mCi until the maximum tolerated dose is observed. It is significant in several of the patients with spontaneous cysts that little to no blood levels of antibody or radioactivity have been detected in blood sampling after dosing, and significant retention of activity within the cyst has been observed from four to six weeks after administration of antibody.

A phase I study of anti-tenascin MAb ^{131}I-labeled 81C6 via surgically created cystic resection cavity in the treatment of patients with primary or metastatic malignant brain tumors – preliminary results (Table 2)

The second study, in which a number of responses or stable disease status have been obtained has been is the Phase I study of ^{131}I-labeled anti-tenascin MAb 81C6 injected into a surgically created cystic resection cavity in the treatment of patients with primary and metastatic brain tumors. Sixteen evaluable patients have been treated to date, and the dose escalation has now extended to the 60-mCi dose level. There have been no toxicities observed, although one patient with a pre-existing seizure disorder had seizures immediately after administration of antibody, which were controlled by adjustment of anticonvulsant medication dosage. This study will continue the dose escalation until maximum tolerated dose is reached.

A phase I study of intrathecal anti-tenascin MAb ^{131}I-labeled 81C6 in the treatment of patients with leptomeningeal neoplasms – preliminary results (Table 3)

The largest number of patients we have treated, 8 children and 23 adults, and the highest dose escalation we have achieved have been in the study of intrathecal administration of ^{131}I-labeled 81C6 for neoplastic meningitis. This study is more difficult to evaluate in terms of both CSF and clinical responsiveness; but disease stabilization, one complete, and one partial response by objective radiographic criteria, and some apparent significant survival prolongations have been obtained. Most importantly,

again no significant toxicity has been observed except in one pediatric patient who had been heavily pretreated with chemotherapy and external beam radiation therapy. That patient had MHT that responded to treatment with hematopoietic growth factors and platelet transfusions; this led to a change in the protocol to adjust the pediatric dosage and radioactivity to per square meter of body surface. Since that adjustment, no hematologic toxicities have been observed except in one adult patient at the 80-mCi dose and the first patient at the 100-mCi dose. Because the protocol requires treatment of an additional three patients, or up to six patients if a hematologic toxicity occurs, eight patients were treated with the 80-mCi dose with no further toxicities before escalation to the 100-mCi dose level. At the 100-mCi dose level, it was also necessary to introduce a protocol amendment to increase the protein dose to 20 mg in order to maintain immunoreactivity at greater than 50% with that high level of activity. It is anticipated that the 100-mCi dose is likely to be the maximum tolerated dose and that hematologic toxicity will be the dose limiting factor with individual dosing. No neurotoxicity has been observed in any of the patients treated on this protocol.

A phase I study of intrathecal MAb fragment ^{131}I-labeled Mel-14 F(ab')$_2$ in patients with neoplasms metastatic to the leptomeninges – preliminary results (Table 4)

Malignant melanoma has a propensity for metastasis to the nervous system, and an extremely high percentage of patients with melanoma have either solid metastases or neoplastic meningitis at the time of autopsy. We have treated a total of 11 patients with ^{131}I-labeled Mel-14 F(ab')$_2$, 1 child with melanosis, 8 patients with melanoma, 1 with an oligodendroglioma, and 1 with a glioblastoma with leptomeningeal disease. After treatment, 3 patients had complete CSF responses (2 consecutive negative CSF cytologies after initial positive cytology), 2 others had partial radiographic response, and the range in survival following treatment has been from 1 to 11 months. Since the average survival with melanoma neoplastic meningitis is 3 months we believe, but of course cannot prove in a Phase I study, that survival prolongation and high quality clinical survival have been obtained in many patients. At the 80-mCi dose level, one patient was observed to have MHT, which responded to treatment with growth factors and blood product transfusions. Protocol requirements are that four additional patients be treated at that dose, and it is possible that the

Table 1. Phase I study of intracystic anti-tenascin monoclonal antibody ^{131}I-labeled 81C6 in the treatment of patients with recurrent cystic gliomas

Patient	Diagnosis	Gender/age/ race	mCi of ^{131}I 81C6/protein dose in mg	Best response			Survival since DX (in months)	Survival since RX (in months)	Toxicity	Cause of death
				CSF	CLIN[a]	RAD				
Pediatric										
1	GBM	M/16/W	40/7.2	NA	ST	PR	Alive (18)	Alive (9)	No	
Adult										
1	GBM	M/45/W	15.2/10.0	NA	CR	PR	33	16	No	PD (CNS)
2[b]	GBM	M/46/W	20/7.9	NA	PD	PD	33	10 (26)	No	PD (CNS)
3	A	F/29/W	21.7/10.0	NA	PR	SD	Alive (41)	Alive (31)	No	
4	AA	F/56/W	20/9.9	NA	PR	PR	Alive (51)	Alive (14)	No	

Abbreviations: DX, diagnosis; RX, treatment; CSF, cerebrospinal fluid response; CLIN, clinical response; RAD, radiographic response; GBM, glioblastoma; NA, not applicable; ST, clinically stable; PR, partial response; CR, complete response; PD, progressive disease; A, astrocytoma; SD, stable disease; NE, nonevaluable (studies not done); AA, anaplastic astrocytoma.

[a] Clinical responses are preliminary estimates.

[b] Retreated adult patient No. 1.

80-mCi dose level will be the maximum tolerated dose.

A phase I study of MAb fragment ^{131}I-labeled Mel-14 F(ab')$_2$ via a surgically created resection cavity in the treatment of patients with primary and metastatic malignant melanoma and other brain tumors – preliminary results (Table 5)

One patient had a left parietal melanoma brain metastasis that was resected and treated with external beam radiation. The tumor recurred locally, and a resection cavity was created with the second surgical removal of the patient's intracerebral melanoma. A dose level of 37 mCi on 10 mg of Mel-14 F(ab')$_2$ was administered to a virtually completely sealed surgical resection cavity and, although the patient died from a subdural hematoma two and one-half months after treatment, there was no recurrent tumor at the treatment site, although there was tumor at other sites.

Radiation dosimetry

Dosimetry is being carried out in all patients in both the cystic antibody studies (Tables 1, 2, and 5) and in

Table 2. Phase I study of anti-tenascin monoclonal antibody ^{131}I-labeled 81C6 via surgically created cystic resection cavity in the treatment of patients with primary or metastatic malignant brain tumors

Patient	Diagnosis	Gender/age/ race	mCi of ^{131}I 81C6/protein dose in mg	Best response			Survival since DX (in months)	Survival since RX (in months)	Toxicity	Cause of death
				CSF	CLIN[a]	RAD				
Pediatric										
1	GBM	M/13/W	20/10.0	NA	ST	SD[b]	17	4	No	PD (CNS)
Adult										
1	GBM	F/53/W	15/10.0	NA	ST	PD	21	12	No	PD (CNS)
2	GBM	M/41/W	10/2.0	NA	ST	SD[b]	18	14	No	PD (CNS)
3	ANA OLIGO	M/39/W	20/10.0	NA	ST	SD[c]	Alive (41)	Alive (15)	Wound inf	
4	GBM	M/53/W	20/9.9	NA	ST	SD[b]	Alive (21)	Alive (15)	Seizures[d]	
5	GBM	M/41/W	20.6/11.8	NA	PD	PD	13	3.5	No	PD (CNS)
6	Melanoma	M/36/W	40/9.8	NA	PD	PD	8.5	2.5	No	PD (CNS)
7	GBM	M/25/W	40/8.4	NA	ST	SD	Alive (14)	Alive (11)	No	
8	AA	M/47/W	40/10.0	NA	ST	PD	Alive (23)	Alive (7)	No	
9[e]	GBM	M/26/W	40/9.9	NA	ST	SD	Alive (13)	Alive (4)	No	
10	GBM	M/41/W	40/10.0	NA	ST	SD	Alive (3)	Alive (1)	No	
11	GBM	M/57/W	40/9.6	too	early		Alive (10)	Alive (1)		
12	GBM	M/50/W	60/9.8	too	early		Alive (6)	Alive		
13	GBM	M/45/W	60/10.0	too	early		Alive (18)	Alive		
14	GBM	M/48/W	20/10.0	NA	ST	SD	14.5	14	No	PD (CNS)
15	GBM	M/21/W	20/10.3	NA	ST	SD	10	5.5	No	PD (CNS)
16	GBM	F/19/W	20/9.9	NA	ST	SD	Alive (11)	Alive (10)	No	
17	ANA OLIGO	M/31/W	40/10.0	NA	ST	SD	Alive (8)	Alive (7)	No	
18	GBM	M/71/W	40/10.0	NA	ST	SD	Alive (3)	Alive (2)	No	
19	GBM	M/61/W	40/10.0	NA	ST	SD	Alive (3)	Alive (1)	No	

Abbreviations: DX, diagnosis; RX, treatment; CSF, cerebrospinal fluid response, CLIN, clinical response; RAD, radiographic response; GBM, glioblastoma; NA, not applicable; ST, clinically stable; SD, stable disease; PD, progressive disease; ANA, anaplastic; OLIGO, oligodendroglioma; AA, anaplastic astrocytoma.

[a] Clinical responses are preliminary estimates.

[b] Partial response at treatment site.

[c] Scans showed increased enhancement; biopsy (7/13/93) showed inflammatory cells – no tumor cells.

[d] Transient focal seizures 3 hr after treatment.

[e] Retreated adult patient No. 7.

the intrathecal studies (Tables 3 and 4). The most reliable dosimetry estimation has been obtained in the cystic cases. Examples from two patients illustrate that extremely large radiation doses can be obtained. For example, adult Patient 1 in Table 1 had a cyst volume, determined from the computer tomography scan, of 28 ml and received an antibody dose of 10 mg and 15.9 mCi of ^{131}I. Estimating dosimetry

Table 3. Phase I study of intrathecal anti-tenascin monoclonal antibody ^{131}I-labeled 81C6 in the treatment of patients with leptomeningeal neoplasms

Patient	Diagnosis	Gender/age/ race	mCi of ^{131}I 81C6/protein dose in mg	Best responses			Survival since DX (in months)	Survival since RX (in months)	Toxicity of death	Cause
				CSF	CLIN[a]	RAD				
Pediatric										
1	AA	M/13/W	40/10.0	NA	PD	PD	2	1	No	PD (CNS)
2	GBM	F/14/W	20/10.0	NA	PD	SD	19	4	No	PD (CNS)
3	Medulloblastoma	F/14/A	40/10.0	NA	ST	SD	Alive (24)	Alive (14)	MHT	
4	Medulloblastoma	M/9/W	30/7.5	NA	ST	SD	3.5	2	No	PD (CNS)
5	A	F/5/W	40[b]/9.2	NA	ST	SD	Alive (18)	Alive (11)	No	
6	Ependymoma	M/12/W	40[c]/9.8	NA	ST	PD	Alive (12)	Alive (10)	No	
7	Ependymoma	M/4/W	40[d]/9.7	NA	ST	SD	Alive (25)	Alive (9)	No	
8	Ependymoma	M/3/W	40[e]/9.9	NA	ST	NA	Alive (13)	Alive (8)	No	
Adult										
1	Breast cancer	F/45/B	30.8/10.0	NE	PD	NE	1	0.5	No	PD (CNS)
2	Ependymoma	M/18/W	40/10.0	NA	ST	SD	Alive (29)	Alive (27)	No	
3	Spinal cord GBM	M/49/W	59/6.8	NE	PD	NE	1.25	0.25	No	PD (CNS)
4	GBM	M/67/W	40/4.8	NA	ST	PD	13.5	12.5	No	PD (CNS)
5	ANA glioma	F/35/W	40/10.2	NA	ST	PD	4	3	No	PD (CNS)
6	ANA glioma	M/45/W	40/6.7	NA	ST	SD	Alive (14)	Alive (14)	No	
7	GBM	F/46/W	40/10.0	NA	ST	PD	3.75	3.5	No	PD (CNS)
8	GBM	M/66/W	40/10.0	NE	PD	PD	1.25	1	No	PD (CNS)
9	GBM	M/35/W	60/9.7	NA	ST	SD	Alive (12)	Alive (10)	No	
10	GBM	M/41/W	60.9/9.8	NA	ST	CR[f]	18	5	No	PD (CNS)
11	GBM	F/63/W	80.0/9.7	NA	ST	SD	6	5	No	PD (CNS)
12	GBM	M/38/W	80/9.9	NA	PD	PD	7	5	No	PD (CNS)
13	GBM	F/18/W	80/9.9	NA	ST	PR	Alive (18)	Alive (6)	No	
14	GBM	M/47/W	80.4/9.9	NA	ST	PD	Alive (7.5)	Alive (7)	MHT	
15	GBM	M/50/W	76.8/10	NA	ST	PD	Alive (5.5)	Alive (5)	No	
16	GBM	F/74/W	80/10.0	NA	ST	PD	Alive (6)	Alive (4)	No	
17	AA	F/49/W	80/9.9	NA	PD	PD	Alive (49)	Alive (4)	No	
18	GBM	F/62/W	80/9.8	NA	ST	SD	Alive (3.5)	Alive (3)	No	
19	GBM	M/36/W	80/10.0	NA	ST	PD	Alive (3)	Alive (2)	No	
20	GBM	M/54/W	100/20.0	NA	PD	PD	2	1	MHT	PD (CNS)
21	GBM	M/41/W	100/19.5	too	early		Alive (1)	Alive (1)		
22	GBM	M/32/W	100/19.3	too	early		Alive (1)	Alive		
23	AA	F/52/W	80/10.0	too	early		Alive (63)	Alive		

Abbreviations: DX, diagnosis; RX, treatment; CSF, cerebrospinal fluid response; CLIN, clinical response; RAD, radiographic response; AA, anaplastic astrocytoma; NA, not applicable; PD, progressive disease; GBM, glioblastoma, SD, stable disease; ST, clinically stable, MHT, major hematologic toxicity = 28 days with absolute neutrophil count < 500 or platelets < 20,000; A, astrocytoma; NE, nonevaluable (studies not done); ANA, anaplastic.

[a] Clinical responses are preliminary estimates.

[b] Per meter squared = 18.8 mCi.

[c] Per meter squared = 28 mCi.

[d] Per meter squared = 15.6 mCi.

[e] Per meter squared = 16.0 mCi.

[f] Radiographically, and verified with multiple meningeal biopsies.

Table 4. Phase I study of intrathecal monoclonal antibody fragment ^{131}I-labeled Mel-14 F(ab')$_2$ in patients with neoplasms metastatic to the leptomeninges

Patient	Diagnosis	Gender/age/ race	mCi of ^{131}I 81C6/protein dose in mg	Best responses			Survival since DX (in months)	Survival since RX (in months)	Toxicity	Cause of death
				CSF	CLIN[a]	RAD				
Pediatric										
1[b]	Melanosis	F/10/W	40/9.8	NA	ST	PD	4	3	No	PD (CNS)
Adult										
1	Melanoma	M/60/W	54.5/9.2	CR	ST	PD	7	6	No	PD (CNS)
2	Melanoma	F/26/W	42.5/10.2	PD	PD	NE	2	1	No	PD (CNS)
3	Melanoma	F/55/W	44.0/9.0	CR	ST	NE	3.5	2.75	No	PD (CNS)
4	Melanoma	F/69/W	46.0/7.7	SD	ST	NE	5	4.5	No	PD (CNS)
5	Melanoma	M/48/W	60.0/10.0	SD	PR	PR	7	6	No	PD (CNS)
6	Melanoma	F/38/B	60.0/10.0	CR	CR	SD	10	8	No	PD (CNS)
7	Melanoma	M/26/W	59.8/10.0	SD	PR	PR	10	8	No	PD (CNS)
8[c]	Melanoma	M/27/W	50.2/9.0	SD	ST	PD	4	3 (11)	No	PD (CNS)
9	OLIGO	F/74/B	80.0/9.7	NA	ST	SD	Alive (13)	Alive (11)	No	
10	GBM	M/34/W	80.0/10.0	NA	ST	SD	4.25	4	MHT	PD (CNS)

Abbreviations: DX, diagnosis; RX, treatment; CSF, cerebrospinal fluid response; CLIN, clinical response; RAD, radiographic response; NA, not applicable; ST, clinically stable; PD, progressive disease; NE, nonevaluable (studies not done); CR, complete response; SD, stable disease; PR, partial response; MHT; major hematologic toxicity = > 28 days with absolute neutrophil count < 500 or platelets < 20,000; OLIGO, oligodendroglioma; GBM, glioblastoma.

[a] Clinical responses are preliminary estimates.

[b] Child treated with exception with FDA approval.

[c] Retreated adult patient No. 7.

from serial scans determined over a several-week period, assuming radioactivity was evenly distributed in cyst fluid and not diffused into tumor, indicated radiation to the tumor surface of 12,700 rad, diminishing at 2 mm within the cyst wall surface to 1,270 rad. Similarly, the patient with metastatic melanoma treated with ^{131}I-labeled-Mel-14 F(ab')$_2$, who had received 37 mCi of ^{131}I-labeled-Mel-14 F(ab')$_2$, had activity in the cyst determined for 38 days. The total dose (beta + gamma) to the cyst wall was calculated to be 70,290 rad and was decreased to 8,700 rad at 3 mm beyond the cyst wall.

Discussion

We and others, with a variety of MAbs, have not been able to obtain sufficiently high tumor doses of intravenously administered radiolabeled MAbs in either systemic malignancies or primary brain tu-

Table 5. Phase I study of monoclonal antibody fragment ^{131}I-labeled Mel-14 F(ab')$_2$ via surgically cystic resection cavity in the treatment of patients with primary or metastatic malignant melanoma and other brain tumors

Patient	Diagnosis	Gender/age/ race	mCi of ^{131}I 81C6/protein dose in mg	Best responses			Survival since DX (in months)	Survival since RX (in months)	Toxicity	Cause of death
				CSF	CLIN[a]	RAD				
1	Melanoma	M/37/W	37/10	NA	ST	SD[b]	7.5	2	No	PD (CNS) subdural hematoma

Abbreviations, CSF, cerebrospinal fluid response; CLIN, clinical response; RAD, radiographic response; NA, not applicable; ST, clinically stable; SD, stable disease; PD, progressive disease.

[a] Clinical Responses are preliminary estimates.

[b] Responses at treatment site; other lesions noted on image study.

mors to undertake therapeutic studies [11, 12, 43]. The factors responsible for the inadequate accumulation for therapeutic purposes are not entirely clear at present but may include such things as lack of tumor-specific antibodies so that systemically administered MAb is binding to normal organs and is not able to be available for accumulation in tumors. Several different antibodies have been shown to localize to very significant degrees in human tumor xenografts in subcutaneous or intracranial tumors in athymic rodents where the human specificity and lack of reactivity of the MAbs with normal rodent tissues provide an artificially tumor-specific MAb situation. Other factors that may be contributing to this lack of sufficient localization following systemic administration may include dehalogenation of the radiolabel; enzymatic removal of MAb from tumors; high interstitial pressure within tumors; and lack of MAb humanization or fragment preparation ($F(ab')_2$, Fab, sFv or dimeric sFVs) with subsequent selection of suboptimal MAb isotype or fragment and radionuclide used for labeling.

With compartmental administration and the use of MAbs that are operationally tumor-specific within the compartment and that are reactive with brain tumors or leptomeningeal disease but nonreactive with normal brain, both our group, as reported herein, and several others [16, 18, 27, 28] are reporting some success in pilot studies or Phase I studies. For example, as shown in Table 1, we have had a significant number of responders, both clinically and radiographically, with administration of ^{131}I-labeled 81C6 to recurrent tumors with spontaneous cysts, extended disease stability in patients with surgically created resection cavities treated with ^{131}I-labeled 81C6 as shown in Table 2 in surgically created resection cavities, and complete responders, and possibly survival prolongation, in intrathecal administration of ^{131}I-labeled Me1-14 $F(ab')_2$, as shown in Table 4.

Our studies, which have been performed under Investigational New Drug permits, have been done as rather classical Phase I studies with a fixed protein dose and escalation of the radioactivity dose to determine the maximum tolerated individual dose in terms of toxicity. We did not anticipate seeing the number of responses that have occurred in the Phase I studies (Tables 1, 2, 4, and 5), because the Phase I studies were predominantly carried out to evaluate toxicity and to determine maximum tolerated individual dose. Toxicities have been minimal and have largely been related to bone marrow suppression, none of which have been life threatening.

As with any new studies, and particularly Phase I studies, more questions have been raised than have been answered with this group of studies. First of all, it has been apparent that accurate dosimetry estimation with the intrathecal studies is difficult, and better methods for dosimetry calculation need to be used. Because of the complex geometry of the CSF spaces and the arbitrary nature of tumor geometry, the simple S-factor technique introduced by the Medical Internal Radiation Dosimetry Committee generally cannot be used for dosimetry calculations for intrathecal administration for leptomeningeal disease. Instead we have developed a computer program that presents an arbitrary tumor radioactivity distribution as a $512 \times 512 \times 512$ digital bit map and numerically convolves this distribution with the appropriate electron point kernel for the radionuclide of interest. The time course of concentration may be characterized by a single exponential function or by a measured time activity curve derived from imaging data or CSF concentration based on CSF sampling. The component dose due to any simultaneous gamma rays may also be included.

On the other hand, more accurate dosimetry has been obtained in the spontaneous and surgically created cyst patients, and the design of the Phase II studies might very well be considered on a projected radiation dose rather than done in a simple dose escalation manner by taking into account the volume of the cavity. Other considerations in design of the Phase II studies will be the added effect on efficacy of multiple dosing. At least two patients, numbers 1 and 2 under Table 1 and numbers 2 and 5 under Table 2, have been retreated. This retreatment took place in the presence of positive human antimouse antibody responses (HAMA) with no allergic or untoward effects from the HAMA responses. From a theoretical standpoint, HAMA responses could actually be of value in helping clear MAb from systemic circulation more rapidly than in the initial treatment of patients who have no HAMA

responses. It is yet to be determined whether significant amounts of HAMA are present in CSF and tumor cyst fluid and whether HAMA would interfere with MAb distribution in tumor.

It is likely that HAMA responses may be reduced by the use of human/mouse chimeric MAbs, which are now available both for 81C6 and Me1-14 $F(ab')_2$ [44, 45]. For both anti-tenascin 81C6 and Me1-14, human/mouse chimeric MAbs of specificity and affinity, virtually identical to the parent murine molecules, have been generated in human IgG_2 constant framework regions. In *in vivo* localization studies with human gliomas in athymic rodents, these antibodies exhibit longer half-life than the murine molecule. With the 81C6 human/mouse chimeric MAb, a greater than twofold increase in tumor localization has been observed with the human/mouse chimeric MAb compared with the murine MAb [44, 45]. An additional advantage of the human IgG_2 constant region is the reduced Fc receptor binding. It will have to be determined in a clinical setting whether the longer half-life of the humanized antibody and decreased Fc receptor binding provides greater efficacy and less toxicity than the murine counterpart. Another approach that may aid in producing better constructs which would overcome tumor penetration and that would have a shorter half-life which might reduce toxicity is the construction of either dimeric or monomeric single-chain MAb fragments from these antibodies [46, 47].

We still believe that the most significant advance in using MAbs successfully for treatment of brain tumors and leptomeningeal disease will be obtaining antibodies with the greatest tumor specificity. Toward that goal, we have identified a mutant molecule designated EGFRvIII (epidermal growth factor receptor variant III), which is expressed in between 30% and 50% of malignant astrocytic gliomas [48]. This mutant epidermal growth receptor contains a tumor-specific epitope because of a glycine introduced at the fusion junction of the in-frame deletion of the rearranged epidermal growth factor receptor gene in these tumors. Recently, we have been successful in preparing high affinity MAbs against this receptor, which MAbs bind well to this clonally expressed, potentially tumor-specific epitope. At least two of the antibodies internalize very rapidly and would be ideal candidates for an immunotoxin preparation. Moreover, by using radiolabeling methodology other than Iodogen, which results in rapid loss of label from tumor cells after internalization, we have been able to achieve significant localization in human glioma xenografts in athymic mice with these new, specific epidermal growth factor variant molecules. Thus, combination therapy, both with immunotoxins and radiolabeled MAbs, would be of great potential significance in the treatment of brain tumors.

Other approaches that are likely to improve tumor targeting are improved labeling methodologies that will maximize retention of radioactivity in tumor and the appropriate matching of radionuclides with MAb isotype or MAb fragment. In particular, we are actively involved in the investigation of ^{211}At, a powerful α-emitter with a shorter particle range than ^{131}I and much greater relative biological effectiveness.

Even though the data in this manuscript must be regarded as preliminary because these are only partially completed Phase I studies, we are sufficiently encouraged to continue the above approaches aggressively and believe that, ultimately, various forms of MAb-mediated therapy will have a role in treatment of both primary and metastatic brain tumors as well as in the treatment of neoplastic meningitis.

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