Radioimmunotherapy of Experimental Human Metastatic Melanoma with Melanin-Binding Antibodies and in Combination with Dacarbazine

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Abstract

Purpose: Melanin has emerged as an attractive target for radioimmunotherapy (RIT) of melanoma, and a radiolabeled monoclonal antibody (mAb) 6D2 to melanin is currently in clinical evaluation. We investigated two approaches to improve the targeting of radiation to tumors using melanin-binding mAbs: (*a*) the use of an additional mAb to melanin could provide information on whether using antibodies to melanin can serve as a general approach to development of therapeutics for melanoma, and (*b*) as melanin targeting involves the antibody binding to extracellular melanin released from necrotic melanoma cells, we hypothesized that the administration of a chemotherapeutic agent followed by RIT would facilitate the delivery of radiation to the tumors due to the increased presence of free melanin.

Experimental Design: We evaluated the therapeutic efficacy of two melanin-binding IgM mAbs labeled with ^{188}Re (6D2 and 11B11). We compared the efficacy of RIT with ^{188}Re-6D2 to chemotherapy with dacarbazine (DTIC) and to combined chemotherapy and RIT in human metastatic melanoma-bearing nude mice.

Results: Therapeutic efficacy of ^{188}Re-labeled 6D2 and 11B11 was comparable despite differences in their affinity and binding site numbers. Comparison of chemotherapy with DTIC and RIT revealed that RIT was more effective in slowing tumor growth in mice. Administration of DTIC followed by RIT was more effective than either modality alone.

Conclusions: These results provide encouragement for the development of RIT for melanoma with melanin-binding mAbs and suggest that combining chemotherapy and RIT may be a promising approach for the treatment of metastatic melanoma.

Melanoma is an increasing health problem that affects ~40,000 new patients each year in the United States and an estimated 100,000 patients worldwide (1). Melanoma is particularly notable as an important cause of cancer among individuals ages 30 to 50 years, which imposes economic losses to society that further compound the human loss and suffering caused by this disease. Although primary tumors that are localized to the skin can be successfully treated by surgical removal, there is no satisfactory treatment for metastatic melanoma, a condition that currently has an estimated 5-year survival of only 6% (2). Unfortunately, there has been little improvement in the prognosis for metastatic melanoma in the past 25 years. More recently, targeted radionuclide therapy has evolved into an efficient modality for cancer patients in whom standard antineoplastic therapies have failed (3). One type of targeted radionuclide therapy, radioimmunotherapy (RIT), takes advantage of the specificity of the antigen-antibody interaction to deliver tumoricidal doses of radiation to target cells using radiolabeled antibodies (4, 5). The clinical success of Food and Drug Administration-approved drugs such as Zevalin and Bexxar [anti-CD20 monoclonal antibodies (mAb) labeled with ^{90}Y and ^{131}I] for the treatment of relapsed or refractory B-cell non-Hodgkin's lymphoma shows the potential of RIT as an antineoplastic strategy.

Melanoma owes its name to the presence of the pigment melanin. Given that even amelanotic melanomas contain some melanin, this pigment presents a potential target for development of radionuclide therapy of metastatic melanoma. Historically, melanin was not considered a target for RIT because it is an intracellular pigment contained in organelles called melanosomes, which are outside the reach of an extracellular melanin-specific antibody. However, because melanomas are rapidly growing tumors, cell necrosis releases melanin into the extracellular space where it can be targeted for delivery of cytotoxic radiation by radiolabeled melanin-binding antibodies. Several antibodies to fungal melanin such as 6D2 and 11B11,

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Translational Relevance

Metastatic melanoma is currently an incurable disease with 5-year survival rate of only 6%. Several years ago, we showed the efficacy of treating experimental melanoma by targeting melanin pigment with radiolabeled melanin-binding antibody (Dadachova E, et al. Proc Natl Acad Sci U S A 2004;101:14865–70). After several years of preclinical development, this approach was translated into the clinic; phase I trial in patients with metastatic melanoma was completed in May 2008 with encouraging results (Klein M, et al. J Nucl Med 2008;49:52P), and phase II trial is currently in preparation. In preparation for phase II trial, we embarked on a search for strategies to improve the targeting of cytocidal radiation to the tumors using melanin-binding monoclonal antibodies (mAb). First, the use of an additional (non-6D2) mAb to melanin could provide information on whether using antibodies to melanin can serve as a general approach to development of therapeutics for melanoma. Second, as melanin targeting involves the antibody binding to the extracellular melanin released from necrotic melanoma cells, we hypothesized that the administration of a chemotherapeutic agent followed by radioimmunotherapy (RIT) would facilitate the delivery of cytocidal radiation to the tumors due to the increased presence of free melanin. Here, we report on the results of the RIT in a mouse model of experimental human metastatic melanoma with (*a*) ^{188}Re-labeled 11B11 mAb to melanin and (*b*) combination treatment the chemotherapeutic agent dacarbazine and RIT with ^{188}Re-6D2 mAb.

both of IgM isotype, were generated in our laboratories in the process of studying cryptococcal melanogenesis *in vivo* and were shown to bind to melanins of different origins (6). Experimental results have established the feasibility of targeting melanin released from dead melanoma cells in tumors with melanin-binding antibodies (7) and peptides (8, 9) labeled with β-emitting radionuclide ^{188}Re. ^{188}Re is a generator-produced powerful β-emitter with E_{max} of 2.1 MeV, half-life of 16.9 h, and imageable γ-ray of 155 keV. Furthermore, this strategy is attractive because melanin in normal tissues is not accessible to the antibody by virtue of its intracellular location. Additional preclinical development of melanin-binding ^{188}Re-6D2 mAb, including pharmacokinetics, efficacy, and acute hematologic toxicity studies in a metastatic human melanoma model in mice (10), preceded recently completed phase I trial in patients with metastatic melanoma (11) that has shown targeting of ^{188}Re-6D2 mAb to the tumors, thus providing encouragement for further trials of RIT with melanin-binding mAbs.

Two approaches to improve the targeting of cytocidal radiation to the tumors using melanin-binding mAbs seem worthwhile of investigation. First, the use of an additional (non-6D2) mAb to melanin could provide information on whether using antibodies to melanin can serve as a general approach to development of therapeutics for melanoma. Second, given that the mechanism of melanin targeting involves the antibody binding to the extracellular melanin released from necrotic melanoma cells, we hypothesized that the administration of a chemotherapeutic agent followed by RIT would facilitate the delivery of cytocidal radiation to the tumors due to the increased presence of free melanin. Here, we report on the results of the RIT in a mouse model of experimental human metastatic melanoma with (*a*) ^{188}Re-labeled 11B11 mAb to melanin and (*b*) combination treatment of the chemotherapeutic agent dacarbazine (DTIC) and RIT with ^{188}Re-6D2 mAb.

Materials and Methods

Antibodies and melanin ELISA

mAb 6D2 was produced by Goodwin Biotechnology and purified using a multicolumn purification system (10). Purity of the 6D2 from this process was >95% via high-performance liquid chromatography-size exclusion chromatography. mAb 11B11 was obtained from supernatant made by growing the 11B11 hybridoma cells in standard DMEM with 5% FCS. The antibody was captured on a column using agarose beads with anti-mouse IgM (Sigma), eluted using acid, and then neutralized to pH 7. The antibody concentration was determined by ELISA by comparison with a commercial standard, and melanin ELISA was done as in ref. 12.

Radioisotope and radiolabeling

^{188}Re as sodium perrhenate Na^{188}ReO$_4$ was eluted from ^{188}W/^{188}Re generator (Oak Ridge National Laboratory). mAbs 6D2 and 11B11 were radiolabeled with ^{188}Re "directly" via generating -SH groups on mAbs with DTT as described previously (7).

Cells lines and *in vitro* binding experiments

MNT1 is a highly pigmented human melanoma cell line (a gift from Dr. V. Hearing, NIH) and was used for *in vitro* binding experiments. The MNT1 cells were cultivated in MEM supplemented with 20% fetal bovine serum; the lightly pigmented melanoma cell line A2058 (American Type Culture Collection) was grown in DMEM supplemented with 10% fetal bovine serum (with 5% penicillin-streptomycin, 37°C temperature and 5% CO_2 being the same for both cell lines). A2058 cells were used in the animal model of human metastatic melanoma. Both cell lines were harvested using 0.25% (w/v) trypsin-EDTA solution. The cells were washed in serum-free DMEM before use.

For *in vitro* binding experiments 0.1 nmol/L ^{188}Re-11B11 mAb was added to the increasing number of the whole or osmotically lysed MNT1 cells. After 1 h incubation at 37°C, the radioactivity in the tubes was measured in a γ-counter, the cells were collected by centrifugation, and the pellets were counted again. Percentage binding to the cells was determined from the ratio of counts in the pellet to the counts in the tube. For Scatchard binding determinations, increasing amounts (0.053-0.256 nmol/L) of ^{188}Re-11B11 mAb were added to osmotically lysed MNT1 cells (4×10^6 per sample). Scatchard analysis was used to compute the mAb binding constant K_a to melanin and number of binding sites per cell as in ref. 13.

Animal model and therapy studies

All animal studies were carried out in accordance with the guidelines of the Institutes for Animal Studies at the Albert Einstein College of Medicine. For RIT studies, 6- to 12-week-old female nude mice on BALB/c background were implanted subcutaneously with 8×10^6 A2058 human melanoma cells into the right flank and used for therapeutic experiments 12 days after tumor volumes were ~0.15 cm^3 (0.02-0.4 cm^3).

RIT with ^{188} Re-11B11. For investigating the ability of 11B11 mAb to deliver therapeutic doses of ^{188}Re to the tumors, the mice were randomized into three groups of five animals. The RIT group received one intraperitoneal injection of 1 mCi ^{188}Re-11B11 (100 μg; "hot" mAb). The control groups received intraperitoneal injections of either 100 μg unlabeled ("cold") 11B11 or PBS. Mice were weighed and tumor volumes were measured immediately before administration of mAbs

and every 3 to 4 days thereafter. Tumors were measured in three dimensions with calipers, and tumor volume was calculated by multiplying the product of the three perpendicular diameters by 0.5, assuming an elliptical geometry.

Combination of chemotherapy and RIT with ^{188}Re-6D2. For combined chemotherapy with DTIC (Sigma) and RIT of A2058 melanoma-bearing nude mice, we initially performed a preliminary experiment aimed at determining the tolerability of DTIC by tumor-bearing mice and the ability of DTIC treatment to release some melanin from the melanoma cells. For this purpose, 10 mg/mL DTIC in citrate buffer (pH ~5.5) was prepared by dissolving 100 mg DTIC in 9.5 mL citrate buffer (pH 3.9) and 0.5 mL of 0.1 mol/L HCl. Five mice with A2058 tumors were treated for 5 days with daily intraperitoneal injections of 50 mg/kg DTIC (1.1 mg/mouse). Three days after completion of 5-(3,3-dimethyl-1-tri-azeno)imidazole-4-carboxamide (DTIC) treatment, two mice were sacrificed and their tumors were removed, fixed in 10% buffered formalin, routinely processed, paraffin embedded, cut into 5 μm slices, and analyzed histologically for the presence of melanin by staining with H&E, by iron staining, or by melanin bleach. The slides were viewed under 40× magnification. The remaining three mice were observed for their tumor size and body weight for 25 days.

To compare the efficacy between chemotherapy and RIT and the combination of chemotherapy and RIT in A2058 melanoma-bearing mice, mice were inoculated with A2058 melanoma cells, randomized into groups of five after tumor volumes reached 0.15 cm^3 (0.02-0.4 cm^3), and on day 0, the treatment of groups 1 and 2 with 50 mg/kg DTIC intraperitoneally daily for 5 consecutive days was initiated. Mice in group 3 received single intraperitoneal injection of 1 mCi ^{188}Re-6D2 mAb on day 0. Twenty-four hours after completion of pretreatment with DTIC (day 6), mice in group 2 received single intraperitoneal injection of 1 mCi ^{188}Re-6D2 mAb. Group 4 was given PBS intraperitoneally on day 0. Mice were observed for their body weight and tumor size as described above.

Micro-positron emission tomography of RIT or chemotherapy-treated melanoma-bearing mice

Positron emission tomography (PET) uses the accumulation of ^{18}F-fluorodeoxyglucose ([^{18}F]FDG) in the tumors to image cancer patients before and after initiation of treatment. To understand the effects of RIT with melanin-binding mAbs and chemotherapy with DTIC on A2058 tumor metabolism, we performed microPET of mice treated with either DTIC alone or RIT alone. Before administration of either DTIC or RIT, tumor-bearing mice were fasted for 3 h and then placed in an anesthesia chamber with 1.5% isoflurane-oxygen mixture. Anesthesia was continued until the completion of the imaging portion of the procedure. Each mouse was placed near a heating pad before scanning to maintain normal body temperature. Mice were injected via tail vein with 11.1 to 14.8 MBq (300-400 μCi) [^{18}F]FDG and 1 h later imaged in a R4 microPET scanner manufactured by CTI Concorde. The full width at half maximum for the R4 is ~2.1 mm with a field of view of 120 mm and a depth of field of 78 mm. Images were acquired for 10 min with a lower-level discrimination of 350 keV and upper-level discrimination of 650 keV. The timing window set to 6 ns. All of the default settings were selected during the histogram process. Reconstruction was done in Ordered Subsets Expectation Maximization Two Dimension reconstruction algorithm. Images were reconstructed in iterative reconstruction in a 128 × 128 × 64 (0.82 × 0.82 × 1.2 mm) pixel array. Data corrected for dead time counting losses, arc correction, random coincidences, and measured uniformity of detector responses (normalized) but not corrected for attenuation. The axial cutoff (Nyquist) in scatter settings was applied at 0.5. Twenty-four hours after imaging, the mice were treated with either RIT (a single intraperitoneal injection of 1 mCi ^{188}Re-6D2 mAb) or DTIC (50 mg/kg) for 5 consecutive days intraperitoneally daily or given PBS intraperitoneally. The mice were reimaged 1 week after the initial PET scan under identical imaging conditions.

Statistical analysis

The Wilcoxon rank-sum test was used to compare tumor sizes between different treatment groups in therapy studies. Differences were considered statistically significant when P values were < 0.05.

Results

^{188}Re-11B11 showed high-affinity binding constant for melanin. Melanin-binding ELISA showed that 11B11 was binding to fungal melanin, which confirmed its immunoreactivity (Fig. 1A). Binding of ^{188}Re-11B11 to MNT1 highly melanized cells was significantly enhanced by lysing the cells to release intracellular pigment consistent with the specificity of the mAb for melanin (Fig. 1B). Scatchard plot (Fig. 1C) revealed an affinity constant of 2.8×10^8 L/M for 11B11 mAb and 1.2×10^5 binding sites per lysed MNT1 cell. The affinity constant for ^{188}Re-11B11 was 1.5 times higher than for ^{188}Re-6D2 mAb, which was previously determined to be 1.8×10^8 L/M (14). In contrast, the number of binding sites per cell for 11B11 was almost three times less in comparison with 3.1×10^5 binding sites for 6D2 (14), which might explain the somewhat lower binding of ^{188}Re-11B11 to MNT1 cells when compared with earlier data for ^{188}Re-6D2 (7).

^{188}Re-11B11 was therapeutic in experimental human metastatic melanoma. To assess if the high binding affinity constant of ^{188}Re-11B11 would affect the therapeutic efficacy of this radiolabeled antibody, we conducted a RIT experiment in nude mice (BALB/c background) implanted with A2058 lightly pigmented human melanoma cells. RIT with ^{188}Re-11B11 was well tolerated and mice did not lose weight as a result of the treatment. Treatment with 1 mCi ^{188}Re-11B11 resulted in significant inhibition of tumor growth (volume) in comparison with untreated controls or mice given "cold" 11B11 ($P < 0.05$; Fig. 2). Interestingly, "cold" 11B11 impaired tumor growth from day 18 in comparison with untreated controls ($P = 0.04$). The therapeutic results with ^{188}Re-11B11 were similar to those reported earlier with ^{188}Re-6D2 (10).

Pretreatment of A2058 tumor-bearing mice with DTIC releases some melanin from the cells. To test the hypothesis that pretreatment of melanoma-bearing mice with DTIC would result in the release of melanin from the necrotic cells, thus providing additional targets for subsequent RIT, mice were treated with 50 mg/kg DTIC for 5 consecutive days. On histologic evaluation of the tumors from untreated mice, the melanin pigment was not evident in viable tumor cells, except for occasional cells along the junction between viable and necrotic tumors (Fig. 3A), whereas tumors from DTIC-treated mice sacrificed 3 days after completion of DTIC regimen revealed a finely granular, uniform golden brown pigment within the lesions, particularly at the junction of the necrotic and viable tissues, which was assumed to be melanin (Fig. 3B). To prove that the brown pigment was extracellular melanin, the consecutive slides from DTIC-treated tumors were stained with H&E, iron stain, and melanin bleach. The absence of blue hemosiderin and refractile acid hematin (Fig. 3C, *middle*) and disappearance of brown coloration of granules post-bleaching (Fig. 3C, *right*) indicated the identity of the pigment released from the cells in DTIC-treated tumors as melanin.

Combination chemotherapy and RIT with ^{188}Re-6D2 was more effective in treating melanoma than chemotherapy or RIT with ^{188}Re-6D2 alone. The exposure of melanin in the tumors after

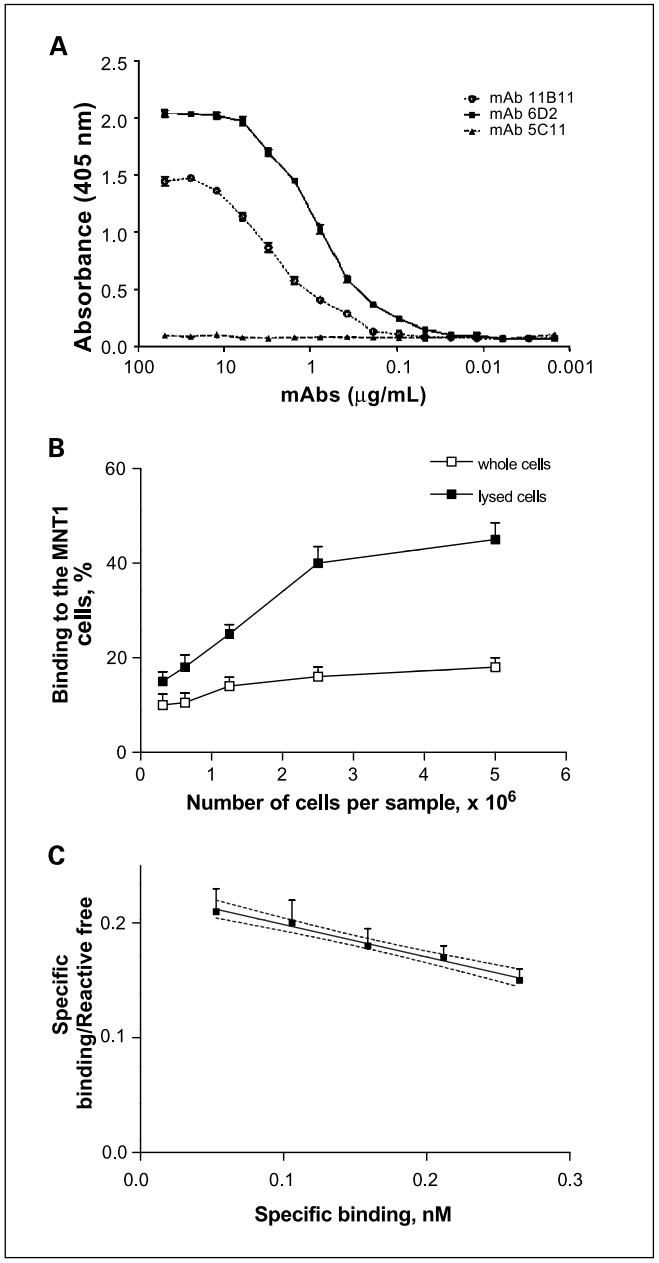

Fig. 1. Binding of ^{188}Re-11B11 mAb to fungal melanin and to MNT1 highly pigmented human melanoma cells: *A,* immunoreactivity of 11B11 as assessed by fungal melanin ELISA. Melanin-binding 6D2 mAb and irrelevant IgM 5C11 were used as positive and negative controls, respectively; *B,* binding to whole and osmotically lysed MNT1 cells. Y-axis shows percentage of ^{188}Re-11B11 mAb in sample which is bound to the MNT1 cells; *C,* Scatchard analysis of binding to MNT1 cells.

treatment with DTIC provided encouragement for conducting combination therapy studies in melanoma-bearing mice. Given the relative equivalence of mAbs 6D2 and 11B11 in binding and efficacy studies, we opted for limiting the *in vivo* combination studies to ^{188}Re-6D2 as this mAb is currently in clinical trials. Figure 4A shows the influence of the treatment on tumor progression in various groups. Tumors in control mice given only PBS grew aggressively such that by day 15 all mice had to be sacrificed. During the early time points post-therapy (up to day 15), both chemotherapy alone and chemotherapy plus RIT with ^{188}Re-6D2 were more effective in impairing tumor growth than RIT alone. However, from day 15, the tumor growth trend changed such that the tumors in chemotherapy only group started to grow much faster than in either the combined treatment or RIT alone group (P = 0.02). The combined treatment was more effective in stabilizing the tumors than RIT alone up to day 25 (P = 0.03). By day 27, the efficacy of combined treatment and RIT equalized (P = 0.06), whereas, during the last days of observation, stabilization of tumors in RIT only group was more pronounced than in combined treatment group. The histologic analysis of RIT-treated tumors at the completion of the study revealed significant infiltration of the tumors by inflammatory cells (Fig. 4B).

MicroPET of tumor-bearing mice before treatment showed pronounced [^{18}F]FDG uptake in the tumors (Fig. 5A). The [^{18}F]FDG uptake and the tumor sizes in untreated mice on the follow-up scans 1 week later increased significantly (Fig. 5B). When the effects of chemotherapy or RIT with ^{188}Re-6D2 on tumor metabolism were compared, the [^{18}F]FDG uptake in RIT-treated tumors almost disappeared (Fig. 5C) whereas in chemotherapy-treated tumors it became less diffuse and concentrated in the center of the tumor (Fig. 5D).

Discussion

The encouraging results of RIT with ^{188}Re-labeled melanin-binding mAb 6D2 in two different models of experimental melanoma (7, 10), followed by localization of ^{188}Re-6D2 in tumor sites in patients with metastatic melanoma (11), provided impetus to seek ways to improve the targeting of cytocidal radiation to the tumors using melanin-binding mAbs. We first investigated whether another mAb to melanin had advantages over mAb 6D2, which is currently in clinical development. mAb 11B11 was hypothesized to bind mammalian melanin similar to 6D2, as fungal and mammalian melanins share many chemical and structural similarities (15). Binding of ^{188}Re-11B11 to MNT1 highly melanized cells

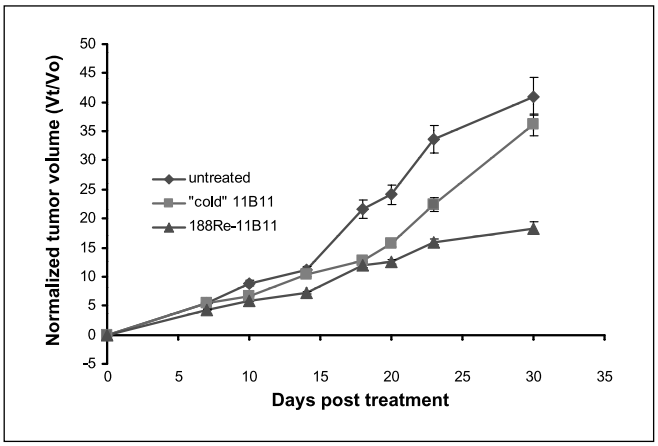

Fig. 2. Radioimmunotherapy of A2058 melanoma-bearing mice with ^{188}Re-11B11 melanin-binding mAb. Mice were given IP either 1 mCi ^{188}Re-11B11 mAb ("hot" mAb), 100 μg 11B11 mAb ("cold" mAb), or PBS. The change in tumors volume is shown with Vo being a tumor volume on the day of treatment, and Vt - tumor volume on the day of measurement. The difference between "hot" mAb and PBS groups became significant (P < 0.05) on Day 14, and between "cold" mAb and PBS groups - on Day 18.

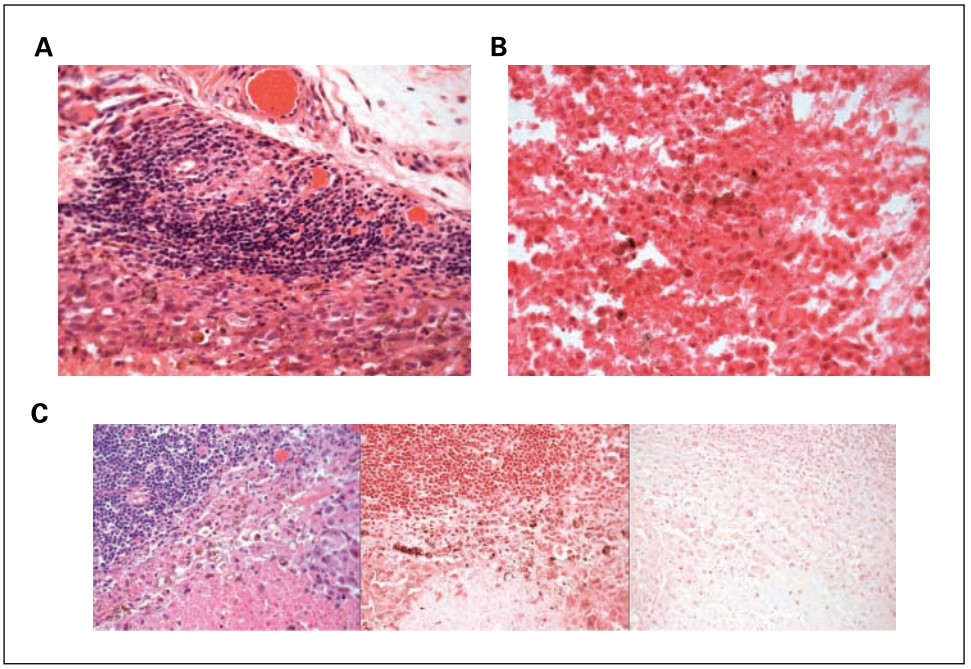

Fig. 3. Histology of A2058 melanoma tumors from DTIC-treated mice: *A*) H&E-stained untreated control A2058 tumor. Melanin pigment is not evident in viable cells except for scattered cells along the junction between viable-necrotic tumor; *B*) melanin granules in necrotic tumor tissue of A2058 human metastatic melanoma-bearing mice treated for 5 days with DTIC and sacrificed 3 days after. A finely granular uniform golden brown pigment within the lesions, particularly at the junction of the necrotic and viable tissue is assumed to be melanin; *C,* consecutive slides from DTIC-treated tumors stained with H&E (left panel), iron stain (middle panel) and melanin bleach (right panel).

was melanin-specific as lysing of the cells resulting in melanin release leads to increase in mAb binding. Although the absolute binding of ^{188}Re-11B11 to melanoma cells was lower than for ^{188}Re-6D2, when administered as 1 mCi dose to A2058 melanoma-bearing mice, its therapeutic efficacy was very similar to that of ^{188}Re-6D2 mAb. We recently showed that high-affinity constants of radiolabeled mAbs for their respective antigens are more important contributors to the efficacy of RIT than the number of binding sites (16). We note that the results obtained with ^{188}Re-11B11 (Fig. 2) were similar in magnitude to those observed with ^{188}Re-6D2 (Fig. 4A) despite the facts that the experiments were done at different times. Hence, the

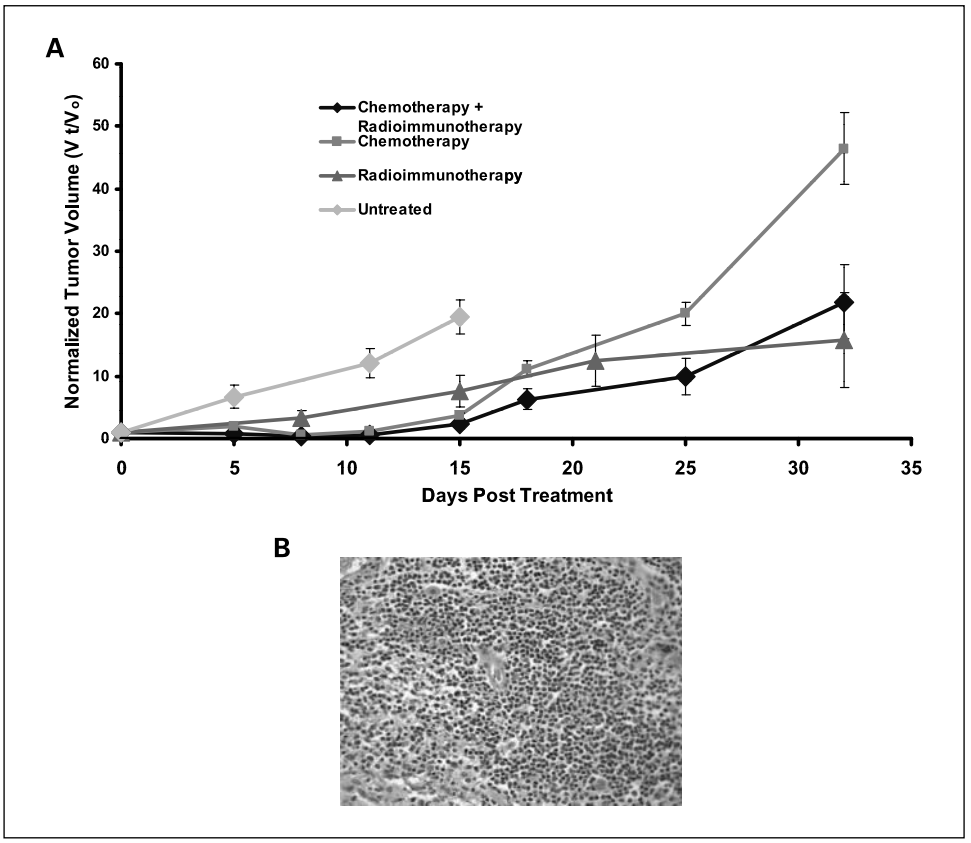

Fig. 4. Comparison of the efficacy of chemotherapy alone, RIT alone with ^{188}Re-6D2 mAb and combination of chemotherapy and RIT in mice with A2058 melanoma. *A,* change in tumor volume in control mice in comparison with mice given chemotherapy alone (50 mg/kg DTIC IP for 5 days), or RIT alone (1 mCi ^{188}Re-6D2 mAb), or combination of chemotherapy and RIT (50 mg/kg DTIC IP for 5 days followed by 1 mCi ^{188}Re-6D2 mAb on Day 6). Vo - a tumor volume on the day of treatment, Vt - tumor volume on the day of measurement. Day 0 means the start of a therapy regiment for each particular group; *B*) H&E-stained tumor from RIT alone treated mouse showing the infiltration with inflammation-related cells.

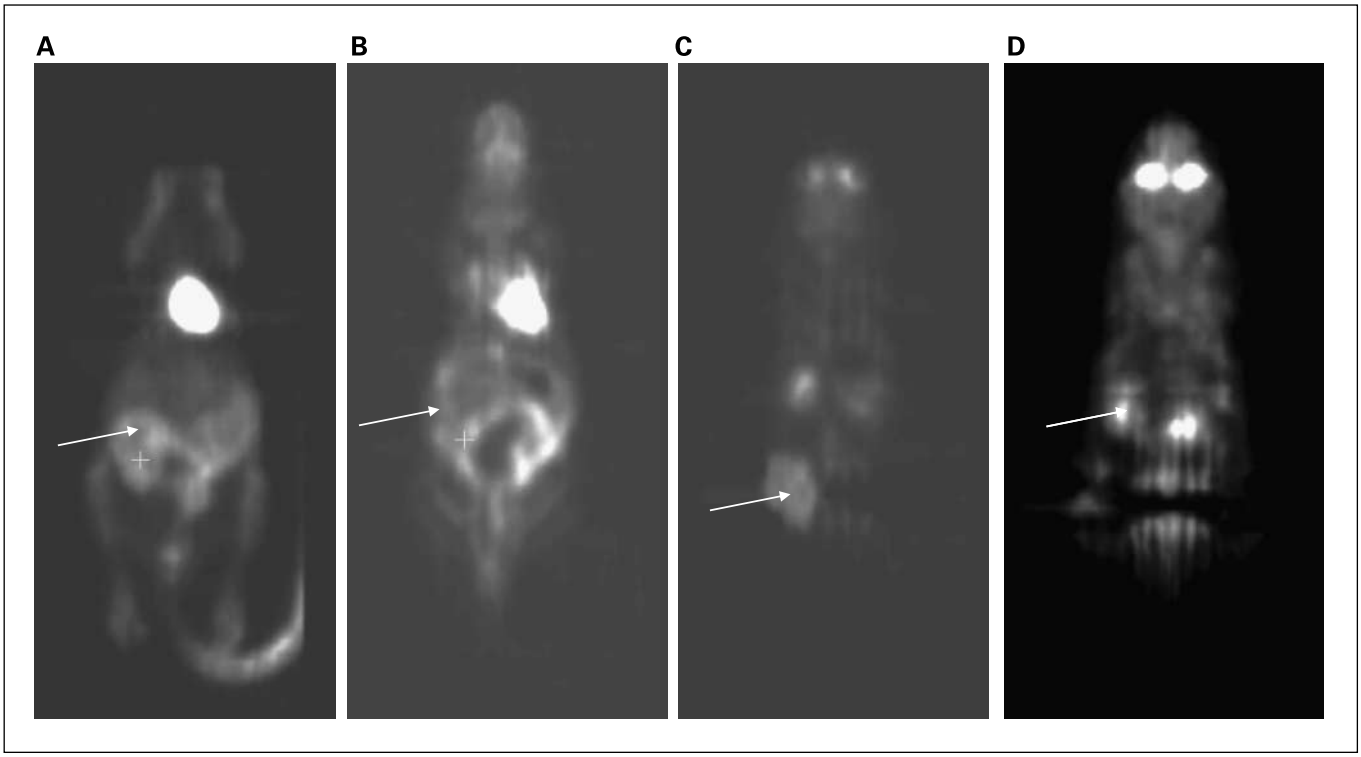

Fig. 5. Coronal ^{18}F-FDG microPET images of A2058 melanoma-bearing mice treated with chemotherapy or RIT. Mice were imaged on Days 0 and 7. White arrows point to the tumors. *A,* untreated mouse on Day 0; *B*) mouse treated with 1 mCi ^{188}Re-6D2 mAb on Day 1 and imaged on Day 7; *C*) untreated mouse on Day 7; *D*) mouse treated with 50 mg/kg DTIC IP for 5 days and imaged on Day 7.

higher constant of ^{188}Re-11B11 relative to ^{188}Re-6D2 could have compensated for the fewer binding sites and contributed to comparable efficacy of treatment.

Interestingly, from day 18 post-treatment, administration of "cold" 11B11 modestly inhibited the increase in tumor volume in comparison with untreated controls. The same effect was previously observed for "cold" 6D2 in both MNT1 and A2048-melanoma bearing mice (7, 10). A possible explanation for this could be the induction of proinflammatory effects by murine IgM, which is a potent activator of the complement system (17). The activation of complement elevates levels of some cytokines such as tumor necrosis factor-α, which can result in inflammation (17). Thus, "cold" antibody bound to extracellular melanin at the tumor site provides synergistic immunologic support for the cytocidal effects of RIT. Overall, the experiments with melanin-binding 11B11 mAb confirmed the results previously obtained with mAb 6D2, supporting the conclusion that the antitumor effects observed with these mAbs are due to their specificity for melanin.

Given the relative therapeutic equivalence of mAbs 6D2 and 11B11, we focused further work on mAb 6D2 and evaluated the efficacy of combining chemotherapy and RIT with ^{188}Re-6D2. A rationale for combining chemotherapy and RIT was the premise that chemotherapy would kill some tumor cells and liberate intracellular melanin to provide more target for RIT. We chose DTIC because it is considered the gold standard for treatment of patients with metastatic melanoma, despite only having a response rate of 15% to 20%, with most responses not being sustained (2). It has also been used previously in mouse melanoma models (18, 19), with 50 mg/kg/d × 5 days being effective in slowing tumor growth with little toxicity. Histologic analysis of A2058 tumors taken from mice with DTIC showed an increased amount of melanin in the extracellular space, consistent with the notion that cell death liberated intracellular melanin. A similar effect on the amount of extracellular melanin was noted in a prior study that analyzed histology after RIT (10).

The most obvious result of the therapy study was clear superiority of RIT over chemotherapy in the ability to control the tumor growth after day 15 as well as the superiority of combination therapy in the early stages of tumor growth. In patients with many different cancers including melanoma, the early response to chemotherapy correlates with the significant decrease in [^{18}F]FDG tumor uptake during PET due to an inability of dying or dead cells to actively take up glucose and predicts the overall response to therapy (20). Likewise, in tumor-bearing mice treated with chemotherapy, the decrease in [^{18}F]FDG tumor uptake in comparison with the baseline value was much less pronounced than in mice treated with RIT alone. Importantly, the combination therapy was more effective than either RIT or chemotherapy alone during the early and intermediate stages of treatment, but the effect appeared additive in the sense that, early in the course of the experiment, the inhibition of tumor growth was largely due to chemotherapy, whereas, later in the course of the experiment, the effect was largely due to RIT. During the last days of observation period, RIT alone was superior to the combined treatment, underscoring possible involvement of immune system that was suppressed by DTIC component in the combined treatment group. Hence, it is possible that improved results could be obtained by treating with DTIC first to release melanin followed by RIT after immune recovery has

occurred, although testing of such regimens would require a different animal model. We note that much of the therapeutic effect of RIT is observed at a time when the radiolabeled antibody should be eliminated given the rapid clearance of IgM and the half-life of ^{188}Re. Given that DTIC has significant effects on leukocytes, it is conceivable that some of the difference between tumor volume in the DTIC and RIT groups early in the course of the experiment reflected increased inflammation in the setting of IgM administration caused by complement activation with consequent tissue edema. Clearly, the potential involvement of the immune system in the therapeutic effect of RIT is an extremely interesting facet of this experimental therapy that will require additional future studies.

In conclusion, we investigated the potential of improving the efficacy of RIT in melanoma with melanin-binding antibodies by using an alternative antibody to 6D2 and by treatment of melanoma-bearing experimental mice with a chemotherapeutic agent before administration of RIT. Melanin-binding mAb 11B11 was effective in an animal model of melanoma using an aggressive lightly melanized melanoma cell line, thus underscoring the effectiveness of melanin-binding vehicles in delivering cytotoxic radiation to melanoma. Treatment of tumor-bearing mice with DTIC before administration of RIT resulted in more effective reductions in tumor size in the early stages of post-treatment than RIT alone. These latter observations might be useful for selection of patients for clinical trials, as those patients who have recently undergone a course of chemotherapy might respond more favorably to anti-melanin RIT.

Disclosure of Potential Conflicts of Interest

The authors have filed a patent application and have also served as consultants for Pain Therapeutics.

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