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^{166}Ho and ^{90}Y labeled 6D2 monoclonal antibody for targeted radiotherapy of melanoma: comparison with ^{188}Re radiolabel

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Abstract

Introduction—An approach to radioimmunotherapy (RIT) of metastatic melanoma is the targeting of melanin pigment with monoclonal antibodies to melanin radiolabeled with therapeutic radionuclides. The proof of principle experiments were performed using a melanin-binding antibody 6D2 of IgM isotype radiolabeled with a β emitter ^{188}Re and demonstrated the inhibition of tumor growth. In this study we investigated the efficacy 6D2 antibody radiolabeled with two other longer lived β emitters ^{90}Y and ^{166}Ho in treatment of experimental melanoma, with the objective to find a possible correlation between the efficacy and half-life of the radioisotopes which possess the same high energy β emission properties.

Methods—6D2 was radiolabeled with two other longer lived β emitters ^{90}Y and ^{166}Ho in treatment of experimental melanoma in A2058 melanoma tumor-bearing nude mice, with the objective to find a possible correlation between the efficacy and half-life of the radioisotopes which possess the same high energy β emission properties.

Results—When labeled with the longer lived ^{90}Y radionuclide – the 6D2 mAb did not produce any therapeutic effect in tumor bearing mice and while the slowing down of the tumor growth by ^{166}Ho -6D2 was very similar to the previously reported therapy results for ^{188}Re -6D2. In addition, ^{166}Ho -labeled mAb produced the therapeutic effect on the tumor without any toxic effects while the administration of the ^{90}Y -labeled radioconjugate was toxic to mice with no appreciable anti-tumor effect.

Conclusions—We concluded that it is very important to match the serum half-life of the carrier antibody with the physical half-life of the radionuclide to deliver the tumoricidal absorbed dose to the tumor.

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Keywords

Radiotherapy; melanoma; antibody; radioisotopes

INTRODUCTION

The incidence of melanoma is increasing worldwide, with a concomitant rise in mortality from metastatic disease. Patients who progress to stage IV metastatic melanoma (MM) have a median survival of less than 1 year.^[1] In the United States, 8,790 deaths from melanoma occurred in 2011 (American Cancer Society, 2013). Until recently, treatment options for patients with stage IV disease were limited and offered marginal, if any, improvement in overall survival. This situation changed with the FDA approval of Yervoy® (anti-CTLA4 monoclonal antibody), an immunomodulator that in a phase III trial was shown to improve overall survival.^[2] In addition, Zelboraf® that inhibits mutated B-RAF protein offers hope for 40–60% melanoma patients carrying this mutation.^[3,4] However, the responses to the latter have been relatively short-lived and followed by recurrences.

Most melanomas are pigmented by the presence of melanin and many of amelanotic melanomas do contain some melanin. Melanin is an intracellular pigment that is normally found in the melanosome. Melanomas, like many rapidly growing tumors have a large cell turnover resulting in cell lysis and release of pigment. Malignant tumors also contain permeable degenerating cells, which make feasible targeting of monoclonal antibodies (mAbs) to intracellular antigens. This approach is currently used for detection of prostate cancer, where the intracellular epitope of a prostate-specific membrane antigen (PSMA) is targeted with ¹¹¹In-labeled 7E11 mAb (ProstaScint; Cytogen, Princeton, NJ). Thus, provided such therapies spare other melanotic tissues – melanin presents a promising target for drugs targeting extracellular pigment and carrying a cytotoxic “payload” of radiation capable of killing cells via the “cross-fire” effect. To make “cross-fire” possible, radionuclides with a long β radiation range in tissue are needed.

In vitro studies have demonstrated that the melanin-binding 6D2 mAb bound only to nonviable melanoma cells that had released their melanin or had permeable cell membranes that allowed the mAb access to melanin.⁵ When treated with ¹⁸⁸Re-6D2 mAb – mice with MNT1 human metastatic melanoma tumors showed a marked reduction in tumor volume compared to controls.^[5] Detailed mouse biodistribution data showed that the effective half-life of ¹⁸⁸Re-6D2 in mice was around 6.5 hrs.^[6] The novelty of this approach is that the target, melanin, is an abundant tumor-associated pigment which is very resistant to degradation and persists at the site of the tumor, thus providing more target sites for the targeting molecules with every RIT cycle. Furthermore, since most of the melanin in healthy tissues is intracellular, it is not likely that antibody therapy targeting melanin would harm other pigmented cells such as normal melanocytes or melanin-containing neurons. Recently, ¹⁸⁸Re-6D2 has been evaluated in Phase I clinical trials where no adverse effects were observed and in fact, targeted tumors remained stable for several months in more than half of the patients.^[7]

The radiolanthanides offer diverse β – energies, penetration depths and half-lives that could be matched with the residence times of antibody and peptide carriers.^[8–12] The radiolanthanides also possess imageable γ rays that will facilitate *in vivo* tracking and calculation of radiation dosimetry. Chelation chemistry varies only in a subtle fashion across the lanthanide series and therefore, to a first approximation, the radiolanthanides are interchangeable in aminocarboxylate ligands, allowing fine tuning of the nuclear properties required for a disease target.^[9, 11, 13] This study examines the potential use of Holmium-166 (¹⁶⁶Ho) (β_{max} : 1.8 MeV, $t_{1/2}$: 27 hrs) and Yttrium-90 (⁹⁰Y) (β_{max} : 2.2 MeV, $t_{1/2}$: 2.7 d) for radiotherapy of melanoma. Both ¹⁶⁶Ho and ⁹⁰Y are hard β emitters with energies close to that of ¹⁸⁸Re which was used in our previous RIT of melanoma work. The major difference between these radionuclides is in their physical half-lives with ¹⁶⁶Ho half-life of 27 hrs being relatively short while ⁹⁰Y half-life of 2.7 d is significantly longer thus allowing us to assess the influence of radioisotope half-life on radiotherapy efficacy.

MATERIALS AND METHODS

Antibodies and cell lines

Highly pigmented human melanoma cells MNT1 (a kind gift from Dr. V. Hearing, NIH, Bethesda, MD) were grown in MEM/20% FBS medium. Lightly pigmented human melanoma cell line A2058 was purchased from ATCC (Manassass, VA) and grown as described in.^[6] The melanin-binding mAb 6D2 (IgM) was produced by Goodwin Biotechnology (Plantation, FL). The IgM UNLB, binding to lipopolysaccharide (clone 11E10, Southern Biotechnology Associates), were used as irrelevant isotype-matched controls.

Reagents

Buffers were made using the highest grade reagents available and using Ultrapure Millipore water to avoid any metal contamination. In addition, all buffers were incubated with 2 grams of CHELEX-100 (Bio-Rad) per liter of buffer for 24 hrs. The Conjugation Buffer, 1M NaHCO₃ (10X), was combined with 0.01 M EDTA, pH 8.2. The resulting Conjugation Buffer (10X) was diluted ten fold to obtain the final conjugation buffer (1X). The final EDTA concentration in the buffer was 1 mM and the pH remained 8.2. The Purification Buffer consisted of 0.15 M Ammonium acetate (1X) pH 6. The Saline Purification Buffer consisted of 0.15 M Ammonium acetate (1X) + 0.4 NaCl, pH 6. This buffer was incubated with CHELEX-100 for 48 hrs. CHX-A'' DTPA was purchased from Macrocyclics Inc (Dallas, TX).

Radionuclides

⁹⁰Y was purchased from MDS Nordion (Fleurus, Belgium) as ⁹⁰YCl₃ in 0.04 N HCl with 31,822 MBq/mL specific activity. High Specific activity ¹⁶⁶Ho was produced at the University of Missouri Research Reactor (MURR) from the neutron irradiation of 1 mg of isotopically enriched ¹⁶⁴Dy to produce ¹⁶⁶Dy which β decays to ¹⁶⁶Ho. As “cold” ¹⁶⁵Ho is produced as well – an initial separation is performed by absorption chromatography at the end of the irradiation to isolate the ¹⁶⁶Dy from the ¹⁶⁵Ho. The ¹⁶⁶Dy is then allowed to

decay for 40 hrs to produce the no carrier added ^{166}Ho which is then separated by absorption chromatography. The final product was concentrated and redissolved with 0.05 M HCl.

Protein Conjugation to CHX-A'' DTPA

6D2 antibody at 4.9 mg/mL or IgM UNLB antibody at 1 mg/mL (volume up to 0.1 mL) was transferred to dialysis tubing (Slide-A-Lyzer MINI Dialysis Units, 20K MWCO, Thermo Scientific, IL) and was dialyzed against 1X conjugation buffer (1 L) for 2 hrs at room temperature. The protein solution was transferred to a metal free 2.0-mL Eppendorf tube and CHX-A'' DTPA (approx. 1 mg/1 mL) in conjugation buffer was added to the protein solution. The volumes of CHX-A'' DTPA added were adjusted to vary the CHX-A'' DTPA / protein ratios. The reaction mixture was allowed to stand at room temperature overnight (18 hrs). The mixture was then transferred to Centricon-50 filtration units. Saline purification buffer was added to give a final volume of 1.5 mL and then centrifuged at 3,000 RPM to approximately 0.2 mL. The purification procedure was repeated 6 times. The samples (~200 μL) were transferred to dialysis tubing (Slide-A-Lyzer MINI Dialysis Units, 20K MWCO) and were dialyzed against purification buffer (1 L) for 2 hrs at room temperature to obtain the samples free of NaCl. The concentration of the CHX-A'' DTPA-6D2 antibody construct was measured using Bradford assay^[14] and the total protein recovery was always higher than 80%. The average number of chelators per antibody was determined by the yttrium-arsenazo III spectrophotometric method.^[15] Immunoreactivity of the CHX-A'' DTPA-6D2 was measured by melanin ELISA, performed as in.^[6]

Radiolabeling procedure

For radiolabeling with ^{90}Y 203.5 MBq (5.5 mCi) in 0.04 M HCl was added to 1.1 mg of CHX-A'' DTPA-6D2 in ammonium acetate buffer. The solution was gently mixed and allowed to incubate at 37°C for 60 min, after which 0.1 mol/L EDTA was added to the tube (50 μL) to chelate any free radiometal. The radiolabeling yield was measured by silica gel instant thin layer chromatography (SG ITLC) developed in ammonium acetate. In this system, radiometal EDTA complex moves with the solvent front while radiolabeled protein stays at the application point. The strips were cut into 2 equal segments and the radioactivity of each segment was counted in a γ counter (Wallac, Finland).

For radiolabeling with ^{166}Ho , 200 μL of ^{166}Ho (100 MBq (2.7 mCi)/0.5 mL in 0.05M HCl) was added to 250 μg of CHX-A'' DTPA-6D2 antibody conjugate (500 μL , 0.5 mg/mL) in purification buffer (pH 6). The solution was gently mixed and allowed to incubate at 37°C for 60 min, after which 50 μL of 0.1 mol/L EDTA was added to chelate any free radiometal. The resulting solution was incubated for 25 min after which ITLC assay revealed that the radiolabeling yield was above 80%. The ITLC for ^{166}Ho - CHX-A'' DTPA-6D2 was performed using cellulose (Whatman 3MMCHR) strips and a mobile phase of 60% ethanol, 40% water using a BioScan 2000. Under these conditions, the ^{166}Ho -EDTA elutes at the solvent front and ^{166}Ho - CHX-A'' DTPA -6D2 remains at the origin. For *in vitro* cell binding studies and biodistribution, the ^{166}Ho - CHX-A'' DTPA -6D2 mAb was purified from free ^{166}Ho using HiTrap desalting column (GE HealthCare, Pittsburg, PA). First, the column was washed with 25 mL purification buffer (pH 6). Next, the sample was introduced

to the column and purification buffer was used to elute the sample off the column. ^{166}Ho -CHX-A'' DTPA-6D2 was identified by ITLC assay and used in further experiments.

***In vitro* serum stability studies**

The *in vitro* stability of ^{166}Ho -CHX-A'' DTPA-6D2 was performed by incubating the complex with fetal bovine serum (a kind gift of the Foster Lab at Hunter College, NY) at 37°C. At various time points (0 and 12 hrs) aliquots were taken and analyzed by HPLC using Pharmacia Superose 12 HR 10/30 column (mobile phase was 100 mM sodium phosphate/0.05% sodium azide 1 mL/min pH 6.2). The detection was performed using on-line UV detector (Agilent) and radioactivity detector (home-built). The antibody peak eluted at 7.8 min.

Cell lines and *in vitro* binding experiments

The binding of the ^{166}Ho -CHX-A'' DTPA-6D2 to highly pigmented human melanoma MNT1 cells was evaluated by incubating 40 ng/mL of the radiolabeled mAb with $0.25\text{--}2.0 \times 10^6$ osmotically lysed cells. After incubation for 1 hr at 37°C with gentle shaking the cells were collected by centrifugation, the supernatant was removed, the cell pellet washed with PBS, and the pellet and the supernatant were counted in a γ counter to calculate the percentage of antibody binding to the cells. As a control for proving the specificity of ^{166}Ho -CHX-A'' DTPA-6D2 binding, the MNT1 cells were also pre-incubated with an excess (50 $\mu\text{g/mL}$) of "cold" 6D2 for 1 hr before the addition of ^{166}Ho -CHX-A'' DTPA-6D2.

Biodistribution of ^{166}Ho -CHX-A'' DTPA-6D2 in A2058 melanoma tumor-bearing nude mice

All animal studies were carried out in accordance with the guidelines of the Institute for Animal Studies at the Albert Einstein College of Medicine. Four six-eight week old female nude mice BALB/c were implanted subcutaneously into the right flank with 8×10^6 A2058 human metastatic melanoma cells and used for the biodistribution after 10 days, when the tumor volumes reached on average 0.15 cm^3 ($0.02\text{--}0.4 \text{ cm}^3$). In order to increase the amount of 6D2 per mouse to 100 μg to match the results with ^{188}Re -6D2 described in [6] – 275 μL (1.35 mg) 6D2 was added to Sample 4. Mice were injected IV with 1.85 MBq (50 μCi) ^{166}Ho -CHX-A'' DTPA-6D2 in 100 μL volume and the biodistribution was performed 2 hrs later.

Comparative therapy studies in tumor bearing mice

For the therapy study, A2058 tumor-bearing mice (5 mice per group) with tumors of the size described above were treated with 3 different activities of ^{90}Y -CHX-A'' DTPA-6D2 – 4.625, 9.25 and 18.5 MBq (0.125, 0.250 and 0.5 mCi) with values being selected based on the literature data describing ^{90}Y -labeled IgGs in experimental RIT of cancer [6,17] as no ^{90}Y -labeled IgM data was found), or with 37 MBq (1.0 mCi) ^{166}Ho -CHX-A'' DTPA-6D2 or given PBS IP. Tumor sizes were measured weekly for 4 weeks and the results were compared with ^{188}Re -6D2 data. [6]

Dosimetry calculations

To calculate the doses to the tumors and normal organs for ^{166}Ho -CHX-A'' DTPA-6D2 and ^{188}Re -6D2 the full published biodistribution data was reported in^[6] was utilized for calculations. The model^[19] which we used in this paper extended the general model developed by Hui¹⁸ beyond ^{90}Y to ^{188}Re , ^{166}Ho , ^{149}Pm , ^{64}Cu and ^{177}Lu using two Monte Carlo radiation transport codes, MCNP4C and PEREGRINE, to solve the radiation transport problem. Both transport codes utilize the Monte Carlo methodology, but MCNP4C's geometry is typically defined by combinations of simple geometrical shapes whereas PEREGRINE is voxel-based. Absorbed fractions in the liver, spleen, kidneys, lungs, heart, stomach, small and large bowel, thyroid, pancreas, bone, marrow (femur), and the whole body were estimated. Additionally, absorbed fraction values were determined for a range of tumor sizes. For tumors, a geometrical model is employed which more accurately reflects the tumors that are grown on the flanks of mice. This geometry simulates the tumors as spheres half embedded in the carcass of the mouse while the top half is protruding above the carcass surface and is covered by 0.5 mm of skin. These absorbed fractions, along with isotope specific isotope properties (yield, average B energy, etc.) were used to develop MIRD-type tables with units of mGy/MBq-sec for all possible combinations of source and target organs. Once the total number of decays (MBq-sec) in source organs are estimated from laboratory data, the doses to all target organs can be calculated by simple matrix multiplication.

RESULTS

Immunoreactivity of CHX-A'' DTPA-6D2

The immunoreactivity of CHX-A'' DTPA-6D2 constructs as a function of initial ratio, from 0.01:1 to 1:1, was determined. The results are illustrated in Figure 1. The immunoreactivity of the construct appeared to be independent of the initial ratios of the ligand to 6D2 mAb in the 0.01–1 range. At the ratio of 1:1 the immunoreactivity was preserved and this initial ratio was chosen for further experiments. The final ratio of ligand conjugated to the 6D2 antibody, determined using yttrium-arsenazo III spectrophotometric method,^[15] was found to be 0.2:1 (ligand to antibody).

Radiolabeling

For ^{90}Y the radiolabeling yield was >95% and no radiolabeled product purification was required. For ^{166}Ho specific activities of 74 MBq (2 mCi)/mg and 148 MBq (4 mCi)/mg CHX-A'' DTPA-6D2, the radiolabeling yields were generally above 90%. At higher specific activities of 296 and 370 MBq (8 mCi and 10 mCi)/mg CHX-A'' DTPA-6D2, the radiolabeling yields were lower, 80% and 13%, respectively. For *in vitro* and *in vivo* studies, the specific activity of 159 MBq (4.3 mCi)/mg was employed.

Stability in serum and *in vitro* binding to MNT1 cells

After the initial best ratios were chosen based on the immunoreactivity studies above, the CHX-A'' DTPA-6D2 construct was radiolabeled with ^{166}Ho ; the specific activity was determined to be 159 MBq (4.3 mCi)/mg of protein. The radiolabeling with ^{90}Y resulted in

5 mCi/mg specific activity of the protein. The serum stability of the ^{166}Ho -CHX-A'' DTPA-6D2 antibody was examined in serum fetal bovine at 37°C. Aliquots were taken initially and at 12 hrs and analyzed by HPLC. The antibody peak eluted at 7.8 min. The integrity of the radiolabeled antibody was conserved after 12 hr incubation at 37° C (Figure 2).

The *in vitro* melanin-binding properties were examined by evaluation of the binding of radiolabeled 6D2 to MNT1 cells. Since melanin is available for binding outside of the cells, the MNT1 cells were osmotically lysed and further release of melanin was induced. To accomplish the blocking experiment, the 6D2 antibody was added to the cells prior to the addition of the radiolabeled ^{166}Ho -CHX-A'' DTPA-6D2. The results presented in Figure 3 demonstrate the specific binding of ^{166}Ho -CHX-A'' DTPA-6D2 to melanin released from the melanoma cells.

Biodistribution studies

The biodistribution of the ^{166}Ho -CHX-A'' DTPA-6D2 was compared to that of ^{188}Re -6D2, previously reported by our group.^[6] The tumor localization for ^{166}Ho -CHX-A'' DTPA-6D2 at 2 hrs post injection was quite similar to that of ^{188}Re -6D2 while clearance of ^{166}Ho -CHX-A'' DTPA-6D2 from the major organs much faster than ^{188}Re -6D2. The data are shown in Table 1.

In vivo radiotherapy studies

The therapeutic effects of ^{166}Ho -CHX-A'' DTPA-6D2 were compared to those of ^{90}Y -CHX-A'' DTPA-6D2 in A2058 tumor bearing mice. Furthermore, the results were compared with those from the published ^{188}Re -6D2 study in the same tumor model^[6] (Figure 4). ^{90}Y -CHX-A'' DTPA-6D2, at activities of 4.625 and 9.25 MBq, did not produce any therapeutic effect in comparison with PBS treated controls while 18.5 MBq ^{90}Y -CHX-A'' DTPA-6D2 dose was toxic with all mice in that group dying by Day 14 (not shown on the graph). Thirty seven MBq ^{166}Ho -CHX-A'' DTPA-6D2 produced some slowing down of the tumor growth for 14 days post-injection and then the tumor growth in that group equalized with the controls. This effect on the tumor was very similar to that of 37 MBq of ^{188}Re -6D2 with the only statistically significant difference between two agents observed on Day 28 of observation period ($P < 0.05$).

Dosimetry to the tumor and organs

The dosimetry results for ^{166}Ho -CHX-A'' DTPA-6D2 and ^{188}Re -6D2 are shown in Table 2. Due to the comparable β energies and relatively close physical half-lives – the total doses to the tumor and the organs were very similar for both radionuclides.

DISCUSSION

RIT emerged as an alternative treatment modality to chemotherapy and external beam radiation therapy almost 3 decades ago. In 2003–04 FDA approved two radiolabeled monoclonal antibodies (mAbs) to CD20 antigen Zevalin® and Bexxar® (labeled with ^{90}Y and ^{131}I , respectively) for the treatment of relapsed or refractory B-cell NHL as a single

shot. As a result of its clinical success, Zevalin® was approved by FDA as part of first line therapy for Non-Hodgkins Lymphoma (NHL) and demonstrates the potential of RIT as an anti-neoplastic strategy.

Several years ago our group suggested targeting melanin, which gives melanoma its name, with a radiolabeled mAb to melanin for treatment of metastatic melanoma. [5,6] We demonstrated the feasibility of targeting melanin released from melanoma cells in tumors with melanin-binding mAb 6D2 labeled with β -emitting radionuclide ^{188}Re (^{188}Re). That approach was taken into the clinic for a Phase Ia/Ib clinical trial in patients with metastatic melanoma and shown to be safe as well as resulted in tumor stabilization and prolongation of survival in some patients. [7]

In general, radiopharmaceutical development based on radioactive rhenium (^{188}Re) is scant in comparison with radiolanthanides such as ^{177}Lu . The reasons for this is the lack of a stable *in vivo* ^{188}Re chelate that does not allow the oxidation to perrhenate ($^{188}\text{ReO}_4^-$, Re(VII)). Along with the lack of stable chelate technology, there are no convenient, efficient methods to introduce ^{188}Re into mAbs. Presently “direct labeling” (i.e., reduction of the mAb disulfide bonds) followed by reduction of $^{188}\text{ReO}_4^-$ and incorporation of reduced ^{188}Re into the disulfide bonds, is the only viable method for radiolabeling antibodies with ^{188}Re and the melanin-targeting mAbs were labeled in this fashion. This method has acknowledged major limitations, [20] including *in vivo* instability of the conjugates and compromised immunogenicity of the mAb due to the labeling procedure. This is why it is important to investigate the potential of other strong β emitters for radiolabeling of mAbs for melanoma therapy.

This study examines the potential use of Holmium-166 (^{166}Ho) (β_{max} : 1.8 MeV, $t_{1/2}$: 27 hrs) and Yttrium-90 (^{90}Y) (β_{max} : 2.2 MeV, $t_{1/2}$: 2.7 d) for radiotherapy of melanoma. There are many advantages to these isotopes and the radiolanthanides in general. These include ease of conjugation of the bifunctional ligand, CHX-A'' DTPA – benzyl isothiocyanate can be conjugated to the 6D2 antibody with retention of immunoreactivity. Another advantage is that the radiolabeling procedure is relatively quick and facile compared to direct labeling necessary for ^{188}Re (β_{max} : 2.20 MeV, $t_{1/2}$: 16.7 hrs). The use of no carrier added ^{166}Ho was specifically chosen as the closest match to the half-life of ^{188}Re . Also, importantly, the bifunctional chelate chemistry for linking radiolanthanides to antibodies is mature. Moreover, ^{90}Y and ^{166}Ho are stable in the CHX-A'' DTPA chelate. The isotopes chosen possess energies and half-lives that encompass those of ^{188}Re and this study allows us to evaluate the effect of energy and half-life on therapy of melanoma using the 6D2 antibody. ^{166}Ho possesses a lower β^- energy and range in tissue than ^{188}Re with a slightly longer half-life. ^{90}Y possesses similar β^- energy but a much longer half-life.

Conjugation of the bifunctional ligand did not compromise the immunoreactivity of the resulting construct towards melanin. The development of high specific activity radiolanthanides is key to the development of lanthanide-based radiopharmaceuticals for RIT. The CHX-A'' DTPA-6D2 antibody construct was labeled with no carrier added ^{166}Ho with a specific activity of 148 MBq/mg of protein using standard techniques with the radiolabeling yields being greater than 80%. The serum stability of the ^{166}Ho -CHX-A''

DTPA-6D2 antibody, chosen as a representative for the radiolanthanides and ^{90}Y , suggests that the complex remains intact in serum: similar HPLC profiles at the initial time and at 12 hrs incubation were obtained. *In vitro* binding showed that the percentage of *in vitro* binding of $^{166}\text{Ho-CHX-A''}$ DTPA-6D2 to MNT1 cells increased with an escalating concentration of cells suggesting specific binding to MNT1 cells. Blocking with 6D2 antibody decreased the binding of $^{166}\text{Ho-CHX-A''}$ DTPA-6D2 10-fold which serves as additional proof of the specificity of $^{166}\text{Ho-CHX-A''}$ DTPA-6D2 binding to MNT1 melanoma cells.

The biodistribution of the $^{166}\text{Ho-CHX-A''}$ DTPA-6D2 was performed for comparison with the $^{188}\text{Re-6D2}$, previously reported by Dadachova et al.^[5,6] The tumor localization for $^{166}\text{Ho-CHX-A''}$ DTPA-6D2 and for $^{188}\text{Re-6D2}$, being around 1% ID/g at 2 hrs post injection, was similar. This observation, when taken together with the almost identical radiation doses to the tumors and the major organs derived from the dosimetric calculations, supports the use of ^{166}Ho as a potentially suitable radiotherapeutic nuclide for melanoma therapy.

The therapeutic effect of $^{166}\text{Ho-CHX-A''}$ DTPA-6D2 and $^{90}\text{Y-CHX-A''}$ DTPA-6D2 was studied in mice bearing tumors similar to the previously reported data for $^{188}\text{Re-6D2}$.^[6] Since the three radioisotopes have different half-lives, the importance of the half-life versus the clearance of the antibody from the body could be addressed and analyzed. When labeled with the longer lived ^{90}Y radionuclide – the 6D2 mAb did not produce any therapeutic effect in tumor bearing mice while reduced the tumor growth by $^{166}\text{Ho-CHX-A''}$ DTPA-6D2 was very similar to the previously reported therapy results for $^{188}\text{Re-6D2}$.^[6] Carrier, protein clearance, the radionuclide half-life and the residence time of the radiolabeled protein in the tumor should be considered for the choice of radionuclide and antibody combinations for optimal therapeutic efficiency. The biodistribution of $^{188}\text{Re-6D2}$ was characterized by fast blood clearance with a half-life of 6.5 hrs. This is in good agreement with the short half-lives of IgMs that may be completely cleared from the blood in 24 hrs. The rapid pharmacokinetics of the 6D2 antibody appears well matched with the shorter half-live radionuclides. As the residence time of 6D2 in the tumor is short, shorter lived radionuclides such as ^{166}Ho and ^{188}Re can deliver much higher absorbed dose to the tumor when compared to long lived ^{90}Y , resulting in a measurable therapeutic effect.

CONCLUSIONS

We have radiolabeled melanin binding mAb 6D2 with a short-lived radiolanthanide ^{166}Ho and with longer lived ^{90}Y and investigated the potential of these radioconjugates in experimental melanoma therapy. ^{166}Ho -labeled mAb to melanin produced some therapeutic effect on the tumor without any toxic effects while the administration of the ^{90}Y -labeled radioconjugate was toxic to mice with no appreciable anti-tumor effect. We concluded that it is very important to match the serum half-life of the carrier antibody with the physical half-life of the radionuclide to deliver the tumoricidal absorbed dose to the tumor.

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Figure 1. Immunoreactivity of complex CHX-A'' DTPA -6D2 antibody as a function of varying the initial ratios of CHX-A'' DTPA to 6D2 antibody.

Figure 2.
 ^{166}Ho -CHX-A'' DTPA-6D2 antibody serum stability at 0 and 12 hrs. The ^{166}Ho -CHX''ADTPA-6D2 mAb elutes at 7.8 min.

Figure 3.

In vitro binding of ¹⁶⁶Ho- CHX-A'' DTPA -6D2 to MNT1 human melanoma cells. The blocked MNT1 samples were incubated with 6D2 antibody prior to incubation with ¹⁶⁶Ho- CHX-A'' DTPA -6D2.

Figure 4. Therapy of A2058 tumor bearing mice with ^{166}Ho -CHX-A'' DTPA-6D2 and ^{90}Y -CHX-A'' DTPA 6D2. The ^{188}Re -6D2 therapy data from ⁶ is also included into the graph for comparative purposes.

Table 1Biodistribution of the ^{166}Ho -CHX''ADTPA-6D2 mAb in A2058 tumor bearing mice.

Organ	Injected dose per gram, % $\pm$ SD	
	^{166}Ho CHX''A 6D2	^{188}Re -6D2 ⁶
Blood	0.35 $\pm$ 0.15	20.56 $\pm$ 7.00
Lungs	0.27 $\pm$ 0.21	4.54 $\pm$ 0.80
Heart	0.64 $\pm$ 0.28	1.85 $\pm$ 0.40
Spleen	0.49 $\pm$ 0.36	8.42 $\pm$ 2.00
Liver	0.91 $\pm$ 1.03	8.46 $\pm$ 3.20
Kidney	3.28 $\pm$ 0.74	12.90 $\pm$ 4.20
Bone/Marrow	0.70 $\pm$ 0.52	4.50 $\pm$ 1.20
Tumor	0.97 $\pm$ 0.80	1.33 $\pm$ 0.50
Muscle	0.00 $\pm$ 0.00	0.02 $\pm$ 0.00

Table 2
Dosimetry calculations for the ^{166}Ho -CHX-ADTPA-6D2 and ^{188}Re -6D2 mAbs in A2058 tumor bearing mice.

Organs	Organ self dose, mGy/mCi		Dose from other organs, mGy/mCi		Total dose, mGy/mCi	
	^{166}Ho	^{188}Re	^{166}Ho	^{188}Re	^{166}Ho	^{188}Re
Liver	13,855	12,953	1260	1186	15,115	14,139
Spleen	8586	7723	4924	4625	13,510	12,348
Kidney	21,816	20,321	2111	1955	23,927	22,275
Lungs	3319	3275	9636	9019	12,955	12,294
Heart	3655	3237	2618	2528	6273	5765
Stomach	4874	4731	7518	6919	12,392	11,650
L. Bowel	1487	1423	1680	1572	3167	2995
Tumor	1959	1835	148	143	2108	1979
Bone	1833	1709	3869	3558	5702	5267
Marrow	23,163	21,286	7976	7436	31,139	28,722
Blood	7914	7719	4394	4071	12,308	11,791
Muscle	99	83	4220	4022	4319	4106