Treatment of Neuroblastoma Meningeval Carcinomatosis with Intrathecal Application of α-Emitting Atomic Nanogenerators Targeting Disialo-Ganglioside GD2

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ABSTRACT

Labeling of specific antibodies with bifunctional chelated Actinium-225 (225Ac; an α generator) allows the formation of new, highly potent and selective α-emitting anticancer drugs. We synthesized and evaluated a radioimmunoconjugate based on 3F8, an IgG3 antibody that specifically binds to ganglioside GD2, which is overexpressed by many neuroectodermal tumors including neuroblastoma. The 225Ac-1,4,7,10-tetra-azacyclododecane (DOTA)-3F8 construct was evaluated for radiochemical purity and sterility, immunoreactivity, cytotoxicity in vitro, induction of apoptosis on GD2-positive cells, as well as for pharmacological biodistribution and metabolism of the 225Ac generator and its daughters in a nude mouse xenograft model of neuroblastoma. The 225Ac-3F8 showed an IC50 of 3 Bq/ml (80 pCi/ml) on the neuroblastoma cell line, NMB7, in vitro. Apoptosis of these cells was not observed. Biodistribution in mice showed specific targeting of a subcutaneous tumor; there was redistribution of the 225Ac daughter nuclides mainly from blood to kidneys and to small intestine. Toxicity was examined in cynomolgus monkeys. Monkeys injected with 1 to 3 doses of intrathecal 225Ac-3F8 radioimmunoconjugate (80 to 150 kBq/kg total dose) did not show signs of toxicity based on blood chemistry, complete blood counts, or by clinical evaluations. Therapeutic efficacy of intrathecal 225Ac-3F8 was studied in a nude rat xenograft model of meningeal carcinomatosis. The 225Ac-3F8 treatment improved survival 2-fold from 16 to 34 days (P = 0.01). In conclusion, in vivo α generators targeted by 3F8 warrant additional study as a possible new approach to the treatment of carcinomatous meningitis.

INTRODUCTION

The 3F8 is a murine IgG3 monoclonal antibody reactive with disialo-ganglioside G02, which is overexpressed by several solid tumors of neuroectodermal origin, including neuroblastoma, melanoma, and small cell lung cancer. Neuroblastoma affects mostly children under the age of 5 years. More than 90% of children diagnosed at an early stage can be cured, but late stage disease responds poorly to treatment (1). Leptomeningeval carcinomatosis in these patients is an increasingly recognized life-threatening complication with few curative approaches, in part because of the blood brain barrier. The leptomeninges are a common site of recurrence. Locoregional therapy with specific monoclonal antibodies directed at GD2 is one of the more promising new approaches, as reported recently (2, 3). Although GD2 is expressed on normal neural cells, primarily in cortical gray matter, spinal cord, cerebellum, and peripheral nerves, it is not found in the normal leptomeninges (3). Because of its cross reactivity with peripheral pain fibers, one major side effect of intravenous 3F8 is antibody dose-dependent pain. However, extravasation of intravenous 3F8 into normal human brain is rarely detected in cerebrospinal fluid or by γ imaging, primarily because of the blood brain barrier (4, 5). Thus, both native antibody and 131I-labeled 3F8 have been safely infused in patients in clinical trials, with no acute or long-term adverse central nervous system effects (6).

Typical leptomeningeval carcinomatosis consists of 5 to 10 tumor cell layers, which presents a suitable geometry for a high-energy α emitter (7). The Actinium-225 (225Ac) is a potent α-emitting element generator (8). It has a half-life of 10 days and decays through 6 new elements with a total of four net α emissions in its decay chain to stable 209Bi. At the currently used specific activities, resulting in a ratio of 225Ac to antibody of about 1:1,000, the high expression of G02 (up to 5 × 106 molecules on each tumor cell) could allow delivery of up to 5,000 225Ac atoms per cell at saturating antibody concentrations. An additional small (40 to 80 μm) radiation field effect from α radiation might also affect tumor cells in close proximity, where the antibody binding to antigens is not possible or available. We hypothesized that antibody 3F8, tagged with 225Ac atomic generators, would be capable of safely killing neuroblastoma cells in a xenograft carcinomatous meningitis model.

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MATERIALS AND METHODS

Antibody, Cells, and Immunohistochemistry. The 3F8 is an IgG3 antibody reactive with disialoganglioside GD2 (9). The clinical grade native antibody was stored in 0.1 mol/L citrate phosphate buffer at pH 4.5. NMB7, an adherent human neuroblastoma cell line with ~3 million GD2 antigen sites per cell, was cultured in RPMI 1640 supplemented with heat-inactivated, 10% fetal FCS, 2 mmol/L glutamine, penicillin (10 units/ml), and streptomycin (100 μg/ml). Anti-idiotype antibody was made previously (10). Cells were maintained in the logarithmic growth phase by changing medium every 3 days and seeding into new flasks when 50% confluency was reached. Cells were detached by incubating for 5 to 10 minutes at 37°C in 2 mmol/L EDTA/PBS.

Immunohistochemistry of xenotransplanted rat brains was done on 8-μm frozen sections with 1 μg/ml 3F8 as primary antibody and a mouse on mouse immunohistochemistry kit (Mouse-On-Mouse Peroxidase kit, Vector Laboratories, Burlingame, CA).

Preparation of 225Ac-Labeled 3F8. The preparation of the radioimmunoconjugate was done via a two-step labeling method (11). Briefly, 225Ac (in 25 μL 0.2 mol/L HCl; Oak Ridge National Laboratory, Oak Ridge, TN) was added to 2-(p-isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA-NCS; 10 g/l, 50 μL; Macrocyclcs, Dallas, TX) in a 0.6 mol/L tetramethylammonium acetate (Aldrich Chemical Co., Milwaukee, WI) buffer at pH 5.5. The reaction was allowed to proceed for 30 minutes at 60°C. The conjugation of the 225Ac-DOTA-NCS to the antibody was done by adding chelated radiometal to the antibody solution (2 mg/ml, 0.5 ml) and incubating for 40 minutes in a 0.3 mol/L carbonate carbonate buffer at pH 8 to 9. Free metals were removed by adding chelated radiometal to the antibody solution (2 mg/ml, 0.5 ml) and incubating for 40 minutes in a 0.3 mol/L carbonate buffer at pH 8 to 9. Free metals were removed by adding chelated radiometal to the antibody solution (2 mg/ml, 0.5 ml) and incubating for 40 minutes in a 0.3 mol/L carbonate buffer at pH 8 to 9. Free metals were removed by adding chelated radiometal to the antibody solution (2 mg/ml, 0.5 ml) and incubating for 40 minutes in a 0.3 mol/L carbonate buffer at pH 8 to 9. Free metals were removed.

Radioiodination of 3F8. The radioiodination of 3F8 was done by incubating with live cells (12). Briefly, 3 ng-labeled 3F8 were added to a 1% human serum albumin/PBS washed pellet of 5 × 10^6 NMB7 cells. The cells were then incubated for 20 minutes on ice followed by two washings with 1% human serum albumin/PBS. The washes were added along with the cell pellet to a 10-fold excess of liquid scintillation fluid and measured the following day when total secular equilibrium of the nuclides was reached.

Immunoreactivity was also assayed by high-performance liquid chromatography (Beckman model 135, 168, 508 HPLC system, Beckman Coulter, Fullerton, CA) equipped with a Gamma Radio detector (IN/US, Tampa, FL) with an anti-idiotype antibody for 111In-3F8. This method allows testing of reactivity of the radioimmunoconjugates without cells. Briefly, the radiolabeled antibody is mixed with a 2- to 3-fold excess of anti-idiotype antibody and injected into a high-performance liquid chromatography with a size exclusion column (TSK-Gel, SWXL3000, Supelco, Bellefonte, PA). The mobile phase was 0.15 mol/L NaCl/0.02 mol/L sodium acetate solution, pressure was 640 psi; the column was calibrated with a range of molecular weight standards (Sigma Chemical Company, St. Louis, MO). Because of its larger molecular weight, the complex of 3F8 and anti-idiotype results in shift of the retention time, which can be detected by UV absorption (280 nm) or by gamma counting for 111In-labeled IgG. This method was not applicable for the 225Ac-3F8 because of low specific activities yielding labeling below the sensitivity of the radiodetector.

Storage Stability of Actinium-225-3F8. Storage properties were investigated by comparing immunoreactivity of one batch of 225Ac-labeled antibody stored at either 4°C or -20°C. For this assay the radioimmunoconstruct (0.2 to 0.4 mg/ml) was kept in 1% human serum albumin at pH 7, and immunoreactivity and radiopurity was assessed on day 1, 3, and 7. Immunoreactivity was measured with live cells, and radiopurity was measured with thin-layer chromatography as described above.

Apoptosis of NMB7 Cells. Three samples of NMB7 cells (40 ml, 1 × 10^5 cells/ml) were prepared with 300 Bq (8 nCi; resulting in an activity concentration of 200 pCi/ml), 15 kBq (400 nCi; activity concentration of 10 nCi/ml) 225Ac-3F8, and no activity. Aliquots of 10 ml were transferred into different cell culture flasks. For a positive control, a cell suspension of HL60 cells (a GD2 negative CD33+ human leukemia cell line) was treated with 370 Bq/ml (10 nCi/ml) 225Ac.

Cells were harvested from one flask for each activity level, and an aliquot of cells was taken out of the HL60 cell suspension daily. The cells were washed twice with PBS, resuspended in 200 μL PBS, and then added dropwise to a 1% paraformaldehyde solution (3 ml) for fixation. The fixation was allowed to continue for 20 minutes on ice. Then, cells were washed once again with PBS and finally resuspended in 70% EtOH. Cells were kept at −20°C until all of the time points were sampled, and FACs analysis with a Tunnel assay (Apoalet DNA fragmentation kit, Clontech, BD Biosciences, Palo Alto, CA) was done after a final washing step with PBS.

Cytotoxicity Assay In Vitro. NMB7 cells (4 × 10^4) in 200 μL complete medium were seeded into wells of a 96-well plate. To prevent uneven evaporation, the wells at the edge of the plates were filled with 300 μL of water. A 1:2 geometric dilution with 1% human serum albumin/PBS of 225Ac-3F8 was prepared, and 50 μL of activity were added to the cells. After 5 days incubation, 50 μL of 370 kBq/ml [3H]thymidine (Perkin-Elmer Life Science, Boston, MA) was added, and incorporation was allowed for 5 hours at 37°C. The plates were processed with a Skatron cell harvester. Cells were collected on fiberglass filters, lysed with deionized water, and rinsed from any low molecular weight fractions. Filters were dried overnight, sealed in a polyethylene bag with scintillation fluid, and measured in a liquid scintillation counter.

Biodistribution in Subcutaneously Xenografted Mice. Housing and care were in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals.
The animal protocols were approved by the Institutional Animal Care and Use Committee at Memorial Sloan-Kettering Cancer Center.

To assess the ability of the constructs to target tumor cells in vivo and to evaluate the redistribution of the released $^{225}$Ac daughters, biodistribution studies in xenotransplanted nude mice were conducted in 6-week-old female athymic nude mice (Taconic, Germantown, NY). Matrigel containing $10^6$ NMB7 cells (0.2 ml) was implanted s.c. into both flanks of each mouse 19 days before injection of $^{225}$Ac-3F8. On injection day, the tumors measured $\sim 6 \times 6$ mm. The need for immediate counting required radioactivity detection of the $\gamma$-emitting daughter nuclides Francium-221 ($^{221}$Fr) and Bismuth-213 ($^{213}$Bi) by direct gamma counting (Packard Cobra Gamma Counter, Packard Instrument Co., Meriden, CT). To achieve high enough count rates, injections of $\sim 37$ kBq/mouse, which would be lethal after $\sim 10$ days, were used. At 22, 30, and 150 hours after injection, groups of 3 to 5 mice each were sacrificed by CO$_2$ asphyxiation, and their blood, normal organs (heart, lung, stomach, spleen, small intestine, liver, kidney, and bone), and tumor were removed. The tissues were rinsed in PBS, blotted dry, and weighed; activity levels of $^{221}$Fr (185 to 250 keV window) and $^{213}$Bi (360 to 480 keV window) were measured in each specimen in a gamma counter. At 22 and 150 hours, mice were evaluated for the fate of the daughter radionuclides by counting blood, small intestine, kidney, liver, and tumor within 7 minutes after sacrifice. At this time the secular equilibrium between $^{221}$Fr ($T_{1/2} = 4.5$ minutes) and $^{225}$Ac ($T_{1/2} = 10$ days) is not reached yet. A second counting at 30 minutes (at $^{221}$Fr equilibrium) reveals the new equilibrium. $^{225}$Ac counts and the activity from the third daughter $^{213}$Bi. The $^{225}$Ac counts are in equilibrium with $^{221}$Fr after 25 minutes and with $^{213}$Bi after 6 hours.

**Therapeutic Efficacy in Meningeal Carcinomatosis.** A suitable model of meningeval carcinomatosis is only available in rats. Therefore, efficacy of $^{225}$Ac-3F8 at delaying tumor growth was determined in a nude rat model for meningeval carcinomatosis (13). Meningeval carcinomatosis was induced by xenografting NMB7 neuroblastoma cells directly onto the meninges of 4- to 6-week-old animals weighing between 50 and 110 g. Rats were anesthetized by i.p. injection of 0.2 ml of 1% human serum albumin/PBS flush after establishing the midline incision. When hind limb pareses was the only neurologic deficit. Pathological evaluation of the central nervous system was done on 10 animals.

Survival curves were constructed with a Prism software package (Graphpad Software Inc., San Diego, CA), based on the method of Kaplan-Meier. Statistical comparisons among the different treatment groups were done with the Mantel-Haenszel log-rank test.

**Safety Studies after Intrathecal Administration into Nonhuman Primates.** The $^{225}$Ac-3F8 radioimmunoconjugate was administered via lumbar puncture to three cynomolgus monkeys, between 3 and 4 years of age, weighing between 3 and 5 kg. They had not previously received radioimmunoconjugates. Animals were anesthetized by intramuscular ketamine before administration for routine weighing, examination, and phlebotomy. Monkeys received 0.5 ml of radioimmunoconjugate and 1 ml of 1% human serum albumin/PBS flush after establishing secure access to the spinal cavity between L4/L5.

Three animals received 1, 2, or 3 injections of intrathecal $^{225}$Ac-3F8. Monkey #1 received a single 111 kBq/kg (3 $\mu$Ci/kg) $^{225}$Ac-3F8 dose. Monkey #2 and monkey #3 each received dose applications with cumulative doses of 150 kBq/kg (4.1 $\mu$Ci/kg) and 80 kBq/kg (2.2 $\mu$Ci/kg). Routine blood chemistry and blood activity levels were measured approximately once or twice per week for 6 weeks before dose escalation was done.

**RESULTS**

Radioiuparity, Sterility, Pyrogenicity, Stability, and Immunoreactivity of Actinium-225–3F8. The radioimmunoconstruct with specific activities between 0.37 and 2.6 MBq/mg (0.01 and 0.07 mCi/mg) was sterile ($n = 4$) and contained <8 EU/ml of pyrogen ($n = 4$). Mean radiochemical purity was 91.7% ($n = 19$). The immunoreactive fraction was similar to previous $^{111}$In conjugates (14). Live cells in an antigen to antibody excess were able to bind between 50% and 75% of $^{225}$Ac-3F8 ($n = 7$). High-performance liquid chromatography analysis of the radiolabeled immunoglobulin anti-idiotypic complexes showed the expected shift from 8 to 6 minutes for 100%...
of the activity for $^{111}$In-3F8. Storage of the antibody in 1% human serum albumin for 7 days at 4°C and −20°C did not change radiopurity or immunoreactivity.

**Radioisotopic and Apoptosis of NMB7 Neuroblastoma Cells.** The proliferation capacity of NMB7 cells was reduced to 50% at an activity concentration of 3 Bq/ml (80 pCi/ml, ED50). When the binding sites were blocked with excess cold antibody, the ED50 value shifted upwards to 30 Bq/ml (800 pCi/ml; Fig. 1).

Tunnel assay did not show apoptosis in these neuroblastoma cells treated with $^{225}$Ac-3F8, measured on each of 4 days, whereas in the positive control experiments, HL60 cells treated with tumoricidal doses of $^{225}$Ac underwent apoptosis on day 2 (data not shown).

**Biodistribution in Subcutaneously Xenotransplanted Mice.** Highly vascularized organs (lung, spleen, kidney, and liver) showed uptake over a 6-day period of the $^{225}$Ac-3F8 radioactivity because of their blood content and were generally far lower than tumor, except at early time points, because of blood activity. Of normal organs, only the spleen showed selective uptake of activity over time. The tumor to organ ratios generally exceeded 3, with the exception of the tumor to spleen ratios (Fig. 2). However, spleen values were grossly distorted at the later time points by substantial radiation damage, leading to markedly decreased organ weight and consequent increases in percentage injected dose per gram. Therefore, the percentage injected dose per gram in spleen exceeded the percentage injected dose per gram in tumor at 150 hours after administration of $^{225}$Ac-3F8. Although the stomach and small intestine showed only a small accumulation of activity, clinical acute toxicity was observed. At necropsy on day 6, animals had dilated stomachs, suggesting gastric outlet obstruction or a loss of motility of the upper gastrointestinal tract. However, this toxicity is particular to this experiment because of application of large doses to

**Fig. 1** Cytotoxicity of the $^{225}$Ac-3F8 radioimmunoconjugate assessed with $[^3H]$thymidine incorporation on NMB7 neuroblastoma cells. The results are plotted as percentage of untreated growth control. For a specificity control, the binding sites were blocked with excess cold antibody before incubation with the radioimmunoconjugate. Specific killing of NMB7 cells (■). Nonspecific killing on blocked cells (○).

**Fig. 2** Biodistribution of $^{225}$Ac-3F8 at 1, 2, and 6 days after i.v. administration in mice. The results were expressed as a percentage of injected dose per weight (organ activity divided by organ mass in grams divided by total activity injected, $n = 3$ to 5). Bars, ±SE.
measure organ activity. On average, the injected doses per weight were 25 times higher than those used for the nude rat therapy experiments described below.

The analyses of the redistribution of the 225Ac daughters showed specific accumulation of the released 225Ac daughters into kidney, small intestine, and to a lesser extent, the stomach (Fig. 3). Whereas the kidney accumulation was expected, the uptake of the daughters into the gastrointestinal tract might be attributed to 221Fr, which has a poorly understood distribution (15). The activity derived from daughters released from 225Ac elsewhere was calculated (Fig. 3). The baseline is 0%, in which all of the daughters decay at the site of release without a redistribution effect. Negative values represent the percentage of daughter atoms that were cleared from the primary place of production. This was observed mainly for the blood. Positive values represent the percentage of daughter dose that is added to the initially delivered dose, derived from other places in the body. This was observed for kidney, intestine, and muscle.

Therapeutic Efficacy in Meningeal Carcinomatosis. Median survival was 20 days for groups treated at day 4 either with 225Ac-3F8 (n = 7) or control 225Ac-HuM195 (n = 6), suggesting no specific radioimmunotherapeutic effects. The nonradioactive control groups treated with native 3F8 (n = 4) or 1% human serum albumin/PBS (n = 5) had median survivals of 15.5 and 18 days, respectively (Fig. 4A). These differences were not statistically significant. However, a comparison of radiolabeled conjugate-treated animals versus nonradioactively treated animals yielded a significant difference (P = 0.041). Thus, nonspecific irritation appeared to have a small positive impact on survival. The mean administered activity in this experiment was 8.5 kBq (230 nCi) and was given in a single, nontoxic dose.

In a second experiment, when treatment was delayed only 2 days after xenograft implantation, median survival was 33.5 days for 225Ac-3F8 (n = 16), 16 days for 225Ac-HuM195 (n = 6), 17.5 for native 3F8 (n = 10), and 16 days for 1% human serum albumin/PBS (n = 9; Fig. 4B). Mean administered activity was 6.5 kBq (175 nCi, 25% lower than in experiment #1) in an attempt to minimize the effect of nonspecific irritation from unbound isotopes seen in the first therapy experiment.

Specifically treated animals survived significantly longer than controls (P = 0.01).

Immunohistochemistry of coronal mammary brain sections at day 4 after xenograft shows that many tumor cell clusters are not immediately accessible to the surface of the meninges (Fig. 5). This most likely explains the different results in rat survival when treatment was administered at 2 days versus 4 days after xenograft.

Subgroup analysis indicated that high, specific activity levels of the drug construct had a direct effect on survival time. In all of the animals treated with 225Ac-3F8 with survival times <20 days, specific activity was below 0.74 GBq/g (0.02 Ci/g), and in all of the animals treated with 225Ac-3F8 with survival times greater than 45 days, specific activity was greater 1.11 GBq/g (0.03 Ci/g). An analysis of animals who received 225Ac-3F8 at specific activity greater or lower than 1.04 GBq/g (0.028 Ci/g) with their corresponding control animals showed a decrease of significance level from 0.022 to 0.086 (Fig. 4, C and D). According to the described clinical score, ~70% of the animals in the three control groups had tumor growth near the brainstem, whereas only 33% of the animals treated with 225Ac-3F8 had symptoms suggestive of tumor growth near brainstem.

Toxicity and Drug Blood Half-Life in Nonhuman Primates. The blood half-life of 225Ac-3F8 in one monkey was measured in a single, first injection; the half-life was ~2 days and followed a biexponential curve (Fig. 6). This indicated both a relatively fast clearance and possible retention in tissue-expressing ganglioside GD2 at low levels.

Systemic toxicity was not observed in any monkey. Serum hepatic enzymes (serum glutamic-oxaloacetate transaminase, serum glutamic-pyruvic transaminase, alkaline phosphatase, γ-glutamyl transpeptidase, and bilirubin), renal values (blood urea, nitrogen, and creatinine), hematologic indices (hemoglobin, red blood cell count, and white blood cell count), and electrolytes (sodium, potassium, chloride, carbon dioxide, calcium, and phosphorus) were normal at all times.

Monkey #1 was treated with one injection of 225Ac-3F8 (111 kBq/kg = 3 μCi/kg) and has been observed for 20 months to date. He remains well and continues to be bright, alert, and
reactive. No long-term serologic abnormalities have been detected.

Monkey #2 (receiving a total of 150 kBq/kg) developed a neurogenic bladder 1 week after the second dose injection along with tail biting and was electively euthanized 40 days after the third injection. Histopathology in this animal showed myelin sheath vacuolization, axonal swelling, and disruption of neuronal fibers at the sacral level of the spinal cord at the injection site. This damage was more prominent in the dorsal white fiber tracts than in the lateral or ventral tracts. Technical difficulties in the attempted injection of the first dose led to failure of dose delivery, and damage likely occurred both to mechanical shearing and retention of $^{225}$Ac-3F8 at the injection site.

Monkey #3 received 3 injections of $^{225}$Ac-3F8 (receiving a total of 80 kBq/kg) and was observed for evidence of both acute and chronic toxicity for a 36-month period. He remained clinically well, bright, alert, and reactive. Serum chemistry and hematologic indices remained normal. Elective euthanasia for histopathology at 36 months revealed no focal central nervous system lesions or systemic abnormalities in any organ.

**DISCUSSION**

We investigated the pharmacology and activity of a GD2-targeted, $\alpha$-emitting, in vivo generator with an ultimate goal of developing a selective intrathecal therapy. First, we showed the ability to feasibly prepare this novel agent. The 3F8 has a tendency to form aggregates, especially above pH 7. Although the labeling procedure contains one reaction at pH 8, we were able to couple the $^{225}$Ac generator to 3F8 without loss of biological function, with previously described procedures (11). Moreover, storage for several days of the radioimmunoconjugate in 1% human serum albumin (pH 7 at 4°C or 20°C) at an antibody concentration of 0.5 mg/ml did not impair function. Acceptable radiopurities (above 90%) were achieved and are comparable with other methods of labeling 3$^1$ radiometals to mAb. Thus, production of this agent and its preclinical and clinical application are possible.

The agent, which has been widely used in patients for neuroblastoma, showed specific cytotoxicity *in vitro* and anti-tumor activity in a rat meningeal carcinomatosis model (1, 6, 13). The probable mechanism of action of the native 3F8 include antibody-dependent cell-mediated and complement-mediated cytotoxicities. Additional preclinical and clinical trials with $^{131}$I-labeled 3F8 have shown that radioimmunotherapy with $\beta$-emitting isotopes can improve this targeted therapy approach (16, 17). An additional improvement of therapeutic potency by use of $\alpha$-emitting isotopes can be inferred from comparative *in vivo* experiments (8, 18, 19). The $\alpha$ particles have advantageous features for targeted therapy, such as emission path lengths of a few cell diameters and great potency because of a high, linear energy transfer. These advantages might be especially important when single cells or small tumor cell clusters are the dominant architecture of the malignant disease. The most potent, targetable, $\alpha$-emitting isotope system currently available is $^{225}$Ac. Recent advances in its radiochemistry opened a new approach of targeted, $\alpha$-emitting nanogenerators, which yield a cascade of new elements and $\alpha$ emissions (8).

Internalization of the $\alpha$-emitting drug into cells is not
required for this therapeutic approach and, therefore, radioimmunotherapy has an advantage over immunotoxins. We hypothesized that the combination of the highly potent, α-emitting isotope generator, $^{225}$Ac, with the clinically validated carrier molecule, 3F8, might have therapeutic applications.

NMB7 neuroblastoma cells appeared more sensitive to α radiation than carcinoma cell lines such as breast cancer or ovarian cancer; NMB7 was as radiosensitive as prostate cancer, leukemia, and lymphoma cell lines (19, 17). Despite this radiosensitivity, apoptosis after α irradiation could not be detected in the NMB7 cells with tunnel assays. This is similar to observations with other carcinomas and different from observations with hematopoietic cancers (20). The lack of apoptosis combined with the radiosensitivity of the NMB7 cell line might indicate that apoptosis is not the dominant mechanism determining radiosensitivity to α irradiation in all of the cells. In addition, there was a 10-fold increase in cytotoxicity with specifically bound isotope over nonspecific background radiation from unbound isotopes. Therefore, the geometry and path length of the radiation emission appear important to its activity.

This new, α-emitting radioimmunoconjugate remains stable in vitro and in vivo and is tolerated by primates after intrathecal administration. Comparable doses of drug when translated to a rat model of meningeal carcinomatosis showed a therapeutic effect. Despite the favorable characteristics for successful radioimmunotherapy in these xenografted nude rats, certain features of the system could reduce the efficacy of the 3F8 radioconjugate, namely impaired delivery of sufficiently high-radiation doses to the tumor cells. In single cells or small clusters, in which accessibility to the drug is both rapid and high, radiosensitivity, immunoglobulin affinities, and expression of target antigen-binding sites are major factors in efficacy. In contrast, the lack of internalization of the radioconstruct into the target cell cytoplasm might result in loss of radiation from the α-emitting daughter elements on decay of the generator (8). These daughter nuclides together with unbound radioimmunoconjugate will be cleared rapidly from the CSF. Unbound radioimmunoconjugate will also be cleared from the CSF within only a few hours (21). Additionally, pharmacological and pharmacokinetic hurdles may reduce the activity of $^{225}$Ac-3F8. This includes poor accessibility to thick layers of tumor cells, which are not well vascularized. Therefore, depending on the distribution and geometry of the tumor cells, the diffusion time might be inadequate for accumulation of sufficient radiation doses to achieve cytotoxicity before tumor growth or escape. Limited
field effects would be seen with these emitters. Such obstacles may reduce efficacy in patients as well, requiring repeated dosing or, possibly, intravenous dosing to reach better vascularized and larger tumors. Because higher $^{225}\text{Ac}$ values have been tolerated in other studies, we expect it is possible to deliver an even higher radiation dose to tumor cells with 3F8. This could be achieved by larger individual doses, by repeated dosing schedules, and by increases in isotope specific activity. Repeat dosing might lead to immunogenicity, which has not been seen with this antibody (6), or to greater toxicity. Cytotoxic efficacy correlates directly with isotope-specific activity, especially when small numbers of $\alpha$-emitting atoms are delivered to each cell. The data here suggest that additional studies of this therapeutic modality and possible advancement to Phase I studies are warranted.

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