Astatine-211-labeled Antibodies for Treatment of Disseminated Ovarian Cancer: An Overview of Results in an Ovarian Tumor Model[1]

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Abstract

Purpose: **The aim of the study was to establish and refine a preclinical model to α-immunoradiotherapy of ovarian cancer.**

Experimental Design: **At-211 was produced by cyclotron irradiation of a bismuth-209 target and isolated using a novel dry distillation procedure. Monoclonal antibodies were radiohalogenated with the intermediate reagent *N*-succinimidyl 3-(trimethylstannyl)benzoate and characterized in terms of radiochemical yield and *in vitro* binding properties. *In vitro* OVCAR-3 cells were irradiated using an external Cobalt-60 beam, as reference, or At-211-albumin and labeled antibody. Growth assays were used to establish cell survival. A Monte Carlo program was developed to simulate the energy imparted and the track length distribution. Nude mice were used for studies of WBC depression, with various activities of Tc-99m antibodies, as reference, and At-211 antibodies. In efficacy studies, OVCAR-3 cells were inoculated i.p., and animals were treated 2 weeks later. The animals were either dissected 6 weeks later or followed-up for long-term survival.**

Results: **A rapid distillation procedure, as well as a rapid and high-yield, single-pot labeling procedure, was achieved. From growth inhibition data, the relative biological effectiveness of the α-emission for OVCAR-3 cells was estimated to be approximately 5, which is in the same range as found *in vivo* for hematological toxicity. At-211 MOv18 was found to effectively inhibit the development of tumors and ascites, also resulting in long-term survival without significant toxic effect.**

Conclusions: **Use of the short-range, high-linear energy transfer α-emitter At-211 conjugated to a surface epitope-recognizing monoclonal antibody appears to be highly efficient without significant toxicity in a mouse peritoneal tumor model, urging a Phase I clinical trial.**

Introduction

Micrometastatic disease is the origin of most cancer deaths, despite macroscopic removal of the visible tumor bulk and, frequently, addition of systemic adjuvant medical treatment.

Additional treatment modalities are urgently needed, and for many decades, oncologists have been looking for antibodies to be directed against tumor-associated antigens, killing only tumor cells. Not until recently have naked antibodies been developed exerting a cytotoxic effect via the immune system. Antibodies also have been considered suitable carriers of cytotoxic conjugates, primarily radionuclides, aimed at emitting a radiation dose high enough for complete tumor cell kill but sufficiently localized to prevent adverse effects such as bone marrow toxicity.

The definition of micrometastatic disease is vague. Essentially, the term means that conventional imaging techniques or tumor markers do not disclose residual tumor tissue in the range of single cells or small cell clusters to several millimeters or sometimes larger in size. After conventional chemotherapy, the residual occult disease can be assumed to have low dimensions but can still have a wide range in size.

It is clear that high-energy electron emitters deposit most of the energy far away from the site of decay (1), as shown in Fig. 1. Consequently, short-range emitters are better suited for microscopic disease. Also, it is desirable to choose an emission with a higher LET[3] and RBE than achieved with low-LET electrons because frequently the binding of atoms/cell is too low to permit enough cell kill. The possibility of using Auger emitters is limited by the demand to bring them within or close to the DNA. Consequently, the choice is between α-particle emitters, such as actinium-225, bismuth-213, or astatine-211. In the present study, because we aimed at conjugation with antibodies with reasonable pharmacokinetics and decay characteristics, At-211 was chosen to best fit the requirements.

Ovarian carcinoma was studied primarily due to its clinical importance. One of 50 women develops the disease, and 60% of these patients succumb to uncontrolled peritoneal growth. Ovarian carcinoma is radio- and chemosensitive. Surgical debulking

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[3] The abbreviations used are: LET, linear energy transfer; MAb, monoclonal antibody; RBE, relative biological effectiveness; PEEK, polyetheretherketon; m-MeATE, *N*-succinimidyl 3-(trimethylstannyl)benzoate; NIS, *N*-iodosuccinimide; NS[*I]IB, *N*-succinimidyl 3-[*I]iodobenzoate; NS[At-211]AtB, *N*-succinimidyl 3-[At-211]astatobenzoate.

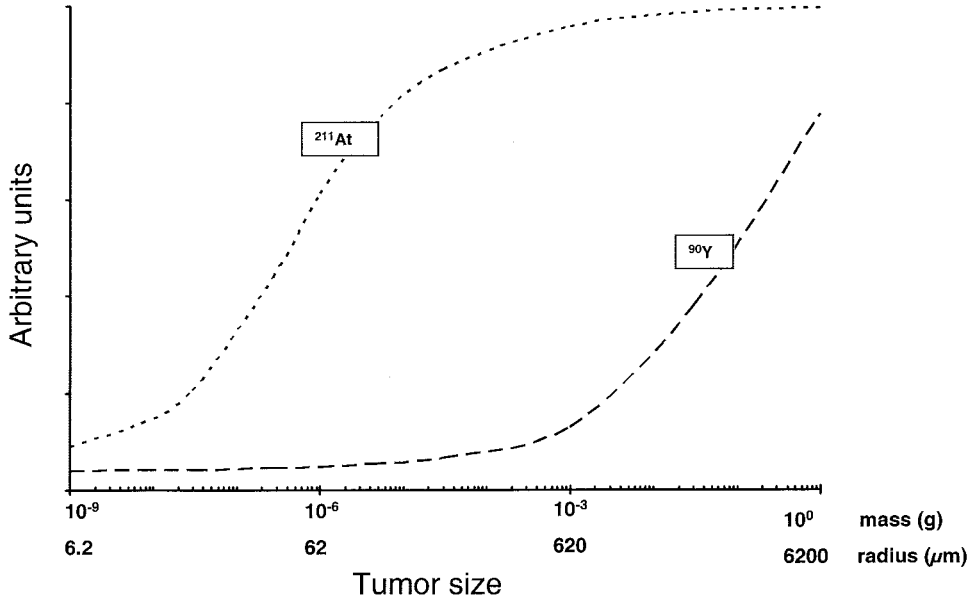

Fig. 1 Tumor:normal tissue mean absorbed dose ratio for Y-90 β-emitters and At-211 α-emitters distributed uniformly in spheres of unit density matter. The tumor:normal tissue activity concentration ratio was assumed to be 25.

and chemotherapy frequently result in macroscopic and even microscopic freedom from the disease; however, in the majority of cases, the carcinoma recurs in the peritoneal cavity. Consequently, an intracavitary (*i.e.*, i.p.) treatment assumed to have fewer systemic effects in a stage with minimal tumor burden was desired.

An interdisciplinary group comprising nuclear physicists and chemists, radiation physicists, immunobiologists, and oncologists was constituted to represent the whole chain, from nuclide production, extraction, and conjugation to *in vitro* and *in vivo* dosimetry, cell survival, bone marrow toxicity, and efficacy. At the same time, a human colon cancer cell line was investigated, as well as a pretargeting approach, with the latter being presented elsewhere in this publication (2).

Materials and Methods

Astatine Production

Astatine was produced in Denmark at the Positron Emission Tomography and Cyclotron Unit, Copenhagen University Hospital, using the nuclear reaction Bi-209(α, 2n)At-211 at a beam energy of 28 MeV. The target was prepared on 30 × 28 × 5-mm aluminum backings at the Department of Physics, Chalmers University of Technology (Göteborg, Sweden). The thickness of the highly enriched (99.999%) bismuth-209 target layer was in the range of 18–20 mg/cm^2 (19–24 μm), and to stabilize the bismuth layer and prevent diffusion of At-211 produced during irradiation, an additional layer of aluminum [2 mg/cm^2 (4–7 μm) thick] was added on top of the bismuth target layer. The target was irradiated with beam currents of 15–20 μA for 2–4 h using a Scanditronix MC32-NI cyclotron. The target was delivered by car from Copenhagen to Göteborg within 4 h (3).

Distillation of Astatine-211 from Irradiated Bismuth-209 Targets

A novel, dry distillation procedure for isolation of At-211 from irradiated Bi targets was developed to optimize recovery yields (3). The system consists of a quartz still, which was heated using a tube furnace (Thermolyne, type F21100). On the outlet side, a loop of PEEK tubing, cooled to –77°C with a mixture of ethanol and dry ice, is connected as a condensing unit. At the end of the system, a gas wash bottle containing a reducing agent is joined to the PEEK tubing to capture any astatine that may escape in uncondensed form. The nuclide is isolated by promptly evacuating the still using reduced pressure on the outlet side of the system.

The system is preheated to 670°C and flushed with carrier gas (N_2) 1 h before distillation. The bismuth layer of the irradiated target is mechanically removed from the aluminum backing, placed in a quartz boat, and immediately inserted into the center of the quartz still. Distillation is allowed to proceed until a maximum level of radioactivity has condensed in the PEEK tubing, as monitored online by measuring the Po-211 X-rays after the electron capture decay of astatine. The dry residue of astatine in the PEEK trap is recovered using 0.2–0.6 ml of chloroform.

The radioactive purity of At-211 after isolation from the irradiated target was verified using a high-purity germanium detector. The radioactivity during distillation was monitored using a collimated Geiger Müller counter.

Labeling of m-MeATE

The intermediate reagent m-MeATE, first synthesized by Zalutsky *et al.* (4), was used for radiohalogenation with iodine or astatine. The reaction parameters were set so that the labeled product could be used without purification in the subsequent binding to proteins and polymers (5, 6).

For iodination, the m-MeATE reagent (0.5 nmol) was evaporated in a borosilicate microvial. Thereafter, chloramine T in a mixture of ethyl acetate, dimethylformamide, and acetic acid (at a volume ratio of 94:5:1) and Na^{125}I were added. The reaction was allowed to proceed for 5–15 min at room temperature during gentle agitation. The reaction was stopped by

addition of a reducing agent, $Na_2S_2O_5$, and the organic phase of the solvent was evaporated.

Initially, this procedure was also adopted for astatine labeling of the m-MeATE precursor. Due to probable overoxidation of the astatine when using the same reaction conditions as for iodination, the method for astatination of m-MeATE was adapted, and NIS was used instead of chloramines T as the oxidizing agent.

For astatination, an aliquot of the recovered astatine from the distillation of the irradiated target was transferred to a microvial, and the solvent was evaporated. To the dry astatine residue, NIS (0.1–0.5 nmol) and the m-MeATE precursor (0.5–1.0 nmol, in methanol containing 1% acetic acid by volume) were added, and the reaction was allowed to proceed for 15–20 min. The reaction was stopped using the reducing agent $Na_2S_2O_5$. To calculate labeling efficiencies in the labeling of m-MeATE, a small aliquot of the reaction mixture was removed and analyzed with high-performance liquid chromatography. This procedure was also applied to iodination, with the exception that *N*-bromosuccinimide was used as the oxidizing agent.

The labeled reagent, NS[*I]IB or NS[At-211]AtB, was then immediately used unpurified for conjugate binding to antibodies.

Labeling of Antibodies and Albumin

The Iodogen Method. Conventional iodination of proteins was performed using the sparingly water-soluble oxidation agent Iodogen (1,3,4,6-tetrachloro-3α,6α-diphenylglycouril), obtained from Pierce Chemical Co. Iodogen was immobilized in polypropylene Eppendorf tubes. Radioiodine (I-125 or I-131) and protein were added, and the reaction was proceeded at neutral pH for 2 min at room temperature. The reaction was stopped by withdrawal of the reacting solvent from the tube. The antibody fraction in the labeling mixture was isolated by gel filtration using disposable PD-10 columns (6).

Conjugate Labeling Using NS[*I]IB or NS[At-211]AtB. For conjugate labeling, the antibody was prepared in 0.2 M carbonate buffer (pH 8.5) and added to the crude labeling mixture. The unprotonated primary amines on the antibody were then reacted with the radiohalogenated reagent, NS[*I]IB or NS[At-211]AtB, for 20–30 min during gentle agitation. Antibodies were isolated by gel filtration using disposable G-25 PD-10 columns (6).

Technetium Labeling of Antibodies. Antibodies were labeled with a direct labeling method using stannous chloride as reductant and Tc-99m-sodium-pertechnetate. The antibody fraction was isolated by gel filtration using Sephadex G-25 in disposable columns (PD-10; AmershamBiosciences).

Tumor Cells and Experimental Models in Vitro

The ovarian cancer cell line OVCAR-3 (NIH) was established by Hamilton *et al.* (7) from ascites of a patient with a poorly differentiated serous ovarian cancer. In nude mice, it grows s.c. and i.p., and when inoculated i.p., the growth closely mimics the human disease, with peritoneal carcinomatosis and ascites. In the present study, the cell line was obtained from the American Type Culture Collection (Manassas, VA).

The cells were cultured in T-75 cell culture flasks at 37°C in a humidified atmosphere. RMPI 1640 was supplemented with 10% FCS, 1% L-glutamine, and 1% streptomycin-penicillin. The medium was changed twice weekly, and the cells were passaged at confluence.

The cells, at passage 15–25, were harvested during the exponential growth phase by rinsing the monolayer with 0.02% EDTA solution in PBS or with versene. After removing the rinsing liquid, the cells were incubated with 0.25% trypsin for approximately 3 min and then resuspended in culture medium and centrifuged at 170 × *g* for 3 min. The supernatant was removed, and the cells were resuspended in fresh medium. The cells were then filtered through a 27-μm PET membrane to acquire a single-cell suspension without cell aggregates. A hemocytometer was used for cell counting, after which 500,000 cells were suspended in 50 ml of fresh medium in 50-ml cylindrical (diameter = 2.8 cm) centrifuge tubes. For preparation of the At-211 MAb therapy, the initial cell concentration was 5×10^6 cells/ml, used with 20-min incubation (8). For certain dosimetric and growth assay experiments, the human colon cancer cell line Colo-205 was used.

MAbs

MOv18 is a murine MAb of the IgG1 class first characterized by Miotti *et al.* (9). It was raised against a poorly differentiated ovarian carcinoma. It recognizes a membrane folate-binding glycoprotein of 38 kDa (10) and reacts with about 90% of human ovarian carcinomas but also reacts with endometrial and cervical carcinoma (9, 11–13). The antigen was detected in ascites of 60% of ovarian cancer patients and in 29% of patients with other carcinomas (13). There are a soluble form and a membrane-bound form of the antigen, with the soluble form probably originating from the cell surface-anchored form through shedding (14). The antibody was kindly provided by Centocor B/V (Leyden, the Netherlands).

The immunoreactivity of the labeled antibodies was determined by a cell binding assay. The immunoreactivity fraction was established according to Lindmo *et al.* (15). Initially, the immunoreactivity of MOv18 was low and varied considerably, probably due to shedding of the antigen (16, 17). The OVCAR-3 cells were successfully cultured in a medium without folic acid. Furthermore, the immunoreactive tests were performed on fixed cells. After these improvements, the immunoreactive fraction was about 70% (6).

In Vitro Procedures

Irradiation. All experiments were carried out to achieve high dosimetric accuracy. Because α-particle cross-fire (*i.e.*, when a nucleus is hit by an α-particle originating from a neighboring cell) was expected to complicate the dosimetry, much dispersed single-cell suspensions (10,000 cells/ml) were used, making any cross-fire probability negligible. As irradiation vessels, 50-ml centrifuge tubes were chosen. The volume was sufficient for the irradiation of a relatively large number of cells, necessary for statistical reasons. The centrifuge tubes were rotated slowly (at 2 rpm) at 37°C. All irradiation was performed for 4 h, after which the cells were centrifuged for 3 min, the supernatant was removed, and the cells were resuspended in 50 ml of medium. This procedure was repeated three times for both

the irradiated and the control cells. No cells were found to adhere to the tubes. Simultaneously with each cell irradiation, the corresponding control cells were sham-irradiated in a twin phantom (18, 19).

Cells were exposed to At-211 albumin and At-211 MAb and the external radiation from a Co-60 source.

Theoretical Dosimetry. A Monte Carlo program was developed in the language C, specifically designed for the simulation of At-211 irradiation of single cells. Stopping power values for α-particles in water (20) were fitted to polynomials. The program was designed to simulate the energy imparted, ϵ, as well as the α-particle track length, *c*, for each 0.01 μm in a target. The target could be any defined volume but was defined as the average volume of the cell nuclei used in the experiments. The program made it possible to separate the position of an At-211 decay from that of its daughter, Po-211, decay.

The program was tested in several ways. One was to compare the analytically solved mean chord length of traversals of a centric inner sphere of tracks originating from an outside sphere (21) with that given by the Monte Carlo calculations. Another was to calculate the mean absorbed dose to a large volume and compare it with that found from a large number of individual hits. Yet another way of testing the program was to compare the number of cell membrane-originating α-particles that hit a cell nucleus with the number calculated from solid angle measurements. In all of these tests, the deviation from the expected result was $<0.2\%$.

Without accurate means of irradiating a single cell with controlled high-LET radiation, the single-cell radiosensitivity must be found from the average dose to all cells, resulting from the irradiation of a single-cell suspension. This was done in the following manner: a cell survival curve was established for the irradiation in question. From an exponential fit to this curve, the required added activity for *e.g.* 37% cell survival could be estimated. The additional activity and the irradiation time were then used for cumulated activity calculations. The Monte Carlo program provided the average number of cell nuclei hits (taking into account different distributions of At-211 and Po-211 decays) as well as the distribution of single-event and multievent energy imparted to those cell nuclei being hit. From the average number of α-particle hits, the Poisson distribution was used to find the fraction of cells receiving zero hits, one hit, two hits, and so on.

The various distributions were linked and weighted in an Excel spreadsheet in accordance with the Poisson distribution. The specific energy, *z*, was calculated from the energy imparted. It was possible to simultaneously set and change the single-cell radiosensitivity, z_{37}, for all distribution intervals (22).

Growth Assay. A growth assay using 96-well plates was used for cell survival estimates (23). Each irradiated cell suspension was diluted with fresh medium to a cell concentration of 5000 cells/ml, using fourteen 96-well plates. On each plate, 30 wells were used for identically irradiated cells (1000 cells in 200 μl medium/well), and 30 wells for control cells. The outer rows were used as blanks.

Each growth assay allowed calculation of an apparent cell survival at any time posttreatment, simply by dividing the mean absorbance of the 30 wells containing irradiated cells by that of the 30 wells containing identically treated, nonirradiated cells.

Table 1 Efficiency of and preparation time for conjugated astatination of antibodies

Results of six experiments are given as means ± SD.

Process	Efficiency (%)	Time (min)
Isolation and workup of At-211 from irradiated Bi-209 target	79 ± 3	20
At-211 labeling of m-MeATE	92 ± 3	20
Conjugation to antibodies and purification	58 ± 7	40

The lowest fraction of each growth assay in the 240–310-h postirradiation interval was used as a cell survival estimate for each particular experiment (18, 19).

In Vivo Procedures

For the *in vivo* studies of i.p. growing ovarian cancer, 1×10^7 cells of the cell line OVCAR-3 were injected i.p. in 6–8-week-old female BALB/c *ν/ν* mice weighing approximately 20 g. The mice were kept under clean but not fully pathogen-free conditions, with free access to ordinary sterilized laboratory food and acidified water (pH 2.5–2.7) *ad libitum*. The tumor progression after the inoculation of cells was studied by weekly dissection of mice. After 2 weeks, microscopic peritoneal tumor growth was seen, but no ascites were present. Two months after the injection of the cells, the disease was well established in untreated mice, with macroscopic tumor nodules and ascites in at least 90% of the animals. At the end of the experiments, or when the animals' general condition deteriorated, the mice were sacrificed and dissected. The amount of tumor and ascites was estimated. From animals that were macroscopically tumor free, multiple biopsies were taken for histological examination from the parietal peritoneum, intestine, mesenterium, and diaphragm and from any suspicious area.

In the short-term and long-term therapy studies, the mice were treated 2 weeks after tumor cell inoculation, in other words, while the disease was still in the microscopic stage. The mice were given At-211-MOv18 (300–400 kBq) either i.p. or i.v., and controls received i.p. injection with either phosphate buffer, unlabeled MOv18, or At-211 bound to unspecific antibodies (C242). In all groups, a volume of 1 ml was injected (24–26).

For the biodistribution studies, 1×10^6 OVCAR-3 cells were injected s.c. in both flanks of 6–8-week-old mice. The tumors were serially transferred to subsequent mice by implanting fragments of tumor tissue with a diameter of 2–3 mm s.c. into the flanks. At the end of the experiments, the animals were sacrificed. Blood was collected, and tumor and organs were dissected and weighed. Biodistribution and pharmacokinetics were also studied in tumor-free mice at various times postinjection.

Toxicity in tumor-free mice after i.v. or i.p. injection of At-211 MAb, 0.4–1 MBq, was measured as leukocyte depression in peripheral blood. After pilot experiments, 5 days postinjection was considered suitable. For comparison and RBE determination, an electron emitter, Tc-99m, 0.5–1 GBq, was used labeled to MAb and injected i.p or i.v. The choice of Tc-99m was based on its proper physical characteristics; a half-life similar to that of At-211 (6.0 h and 7.2 h, respectively) and a

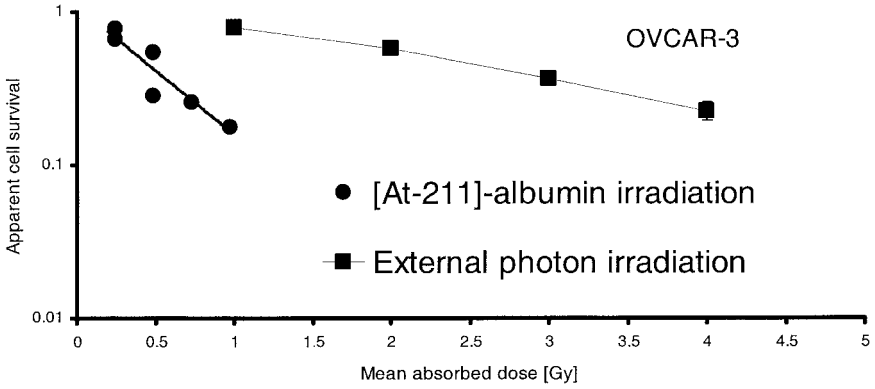

Fig. 2 Apparent cell survival of OVCAR-3 cells irradiated with At-211-albumin (●) or external Co-60 photons (■). *Error bars* for the external irradiations (when larger than the symbols) show the SD of mean results of at least three experiments.

particle range of the same magnitude as for the α-particles (150 μm and 61 μm, respectively). These facts make the absorbed dose rates and absorbed fractions in tissues comparable for the two nuclides, simplifying the RBE determination.

Results

Astatine Production

Upon arrival in Göteborg, target activities ranged from 150 to 300 MBq At-211.

Distillation of Astatine-211 from Irradiated Bismuth Targets

Distillation was almost instantaneous, with yields of >90% of the initial target activity. The tube furnace was turned off after a 1–2 min distillation. The isolated activity was captured in the PEEK cryotrap and eluted with 400–600 μl of chloroform, resulting in recovery yields in the range of 75–85%. The total time needed to transform the activity into a chemically useful form was 20–30 min (3).

Labeling of m-MeATE

Iodination of m-MeATE. Optimization was performed according to the molar ratio between the reacting species, I-125 and m-MeATE, in combination with different solvents. High yields, 85–95%, of the desired product, *N*-succinimidyl 3-[^{125}I]iodobenzoate, were obtained even at very low molar ratios between the precursor and the nuclide when using a solvent mixture of ethyl acetate, dimethylformamide, acetic acid, and water (5).

Astatination of m-MeATE. Initially, similar reaction conditions to those in the iodination procedure were investigated for the At labeling of m-MeATE, but the yields were found to be low. The oxidizing agent NIS was later introduced and chemically evaluated with regard to electrophilic astatination. The results showed that in a polar protic solvent, such as methanol, with a low concentration of m-MeATE, the labeling efficiency was very high, with yields of >90% of the final product NS[At-211]AtB. A similar, highly efficient labeling system was also adopted for iodination using *N*-bromosuccinimide as the oxidizing agent (5).

Labeling of Antibodies

A summary of the efficiencies obtained and the time required for the astatination of antibodies, including the isolation of the nuclide, is given in Table 1. The binding of radiohalogenated C215 to living Colo-205 cells resulted in immunoreactive fractions of 85–90% for the iodinated and 70–90% for the astatinated antibodies. The apparent affinity derived from Scatchard analysis gave binding constants of 3–4 n_M^{-1} for the iodinated and 2 n_M^{-1} for the astatinated C215 (6). The specific activity of labeled antibodies is low, approximately 1 At atom per 1000 molecules.

Initially, the binding fractions and affinities to living OVCAR-3 cells for the iodinated MOv18 could not be established because of problems associated with shedding of the antigen. To circumvent this problem, the cells were cultivated in folate-free medium and carefully fixed for the immunoreactive fraction and affinity assay. Binding to the fixed cells showed immunoreactive fractions of 75–85% and 50–60%, with apparent heterogeneous affinity constants of 2–77 and 2–20 n_M^{-1}, respectively, for the astatinated and Iodogen ^{125}I-labeled MOv18. The astatinated MOv18 always exhibited better binding to the fixed OVCAR-3 cells than did the Iodogen-iodinated MOv18 (6).

In Vitro Irradiation

Because irradiation geometry in the dilute cell suspensions was well characterized, At-211-labeled albumin was used to estimate RBE. No cellular uptake of these conjugates could be identified. The apparent cell survival in relation to the calculated mean absorbed dose is shown in Fig. 2. At 37% survival, the RBE was 5.3 ± 0.7 for OVCAR-3 cells using Co-60 as reference, with a dose of 3.0 ± 0.3 Gy (19).

Attempts were made to establish cell survival curves for At-211-MOv18 on OVCAR-3 cells. At first, no cellular uptake could be detected. Cell survival around 100% confirmed that the At-211-MOv18 had not bound to the cells. The use of a cell medium without folic acid, however, did produce binding. Preliminary uptake data of 3 kBq of At-211-MOv18 on OVCAR-3 cells showed that 5% of the total activity (3500 times the concentration of the surrounding medium) was cell-bound.

Similar studies on the colon cancer line Colo-205 using the MAb C215 resulted in a 15,000 ± 3,000 higher At-211 concentration in the cell fraction than seen in the medium. A number of 26 ± 5 At-211 decays/cell were needed to produce a 37% survival. A similar number of decays (19 ± 5) were found to be required for OVCAR-3 cells when exposed to free At,

Table 2 Number of animals free from macroscopic tumors 6 weeks after treatment

Treatment	No. of animals	Percentage of tumor-free animals
At-211-MOv18 i.p.	28/30	93
At-211-MOv18 i.v.	4/10	40
MOv18 unlabeled i.p.	1/10	10
At-211-colo242 i.p.	2/8	25
Phosphate buffer i.p.	0/10	0

resulting in a high cellular binding/uptake, 173 ± 22 times that of the surrounding medium (19).

In Vivo Studies

Biodistribution and Pharmacokinetics. Approximately 20% of the injected dose per gram of tissue was found in blood at 3 h postinjection, irrespective of whether the mice had received i.v. or i.p. injection. The femoral bone marrow/blood fraction was stable, approximately 0.27 between 3 and 72 h after i.p. injection (26).

Accumulation of i.v. administered antibody was slow in s.c. implanted OVCAR-3 tumors weighing between 0.05 and 1.4 g and inversely correlated with the tumor weight. The tumor:blood ratio was approximately 0.3 at 6 h postinjection and rose to approximately 2 at 72 h postinjection, indicating a slow accumulation as well as a slow release (27).

Bone Marrow Toxicity and RBE. When different amounts of At-211- and Tc-99m-conjugated MAbs were administered i.p. or i.v. to tumor-free nude mice, corresponding to absorbed doses to the bone marrow of 0.01–0.57 and 0.36–1.76 Gy, respectively, the WBC count was depressed by 12–90% and 23–88%, respectively, 5 days after injection, indicating a RBE of 3.2 ± 0.8 for the α-particles from At-211 relative to the electron emission from Tc-99m. The absorbed dose to the bone marrow was calculated for a uniform activity distribution in the bone marrow.

Treatment Efficacy

Short-Term Studies. The mice were inoculated with OVCAR-3 cells 2 weeks before i.p. treatment with At-211-MOv18 together with various control groups, as seen in Table 2. Six weeks later, all animals were dissected without information on treatment, and the presence of macroscopic tumors and/or ascites was recorded. As seen in Table 2, 93% of actively treated mice (28 of 30) were free from tumors, whereas the control mice (given PBS, unlabeled MOv18, or At-211-unspecific MAb) presented with tumor growth in 25 of 28 animals. The activity of At-211 injected was in the range of 400 kBq, resulting in no obvious side effects and an insignificant depression of WBC count.

Long-Term Studies. Mice treated as mentioned above (with 15 mice given At-211-MOv18 i.p., and 15 mice given PBS i.p.) were followed to imminent death when sacrificed and dissected or to 222 days after tumor cell inoculation. Survival is shown in Fig. 3. All animals succumbing before the end of follow-up disclosed macroscopic tumor growth. At day 222, the two surviving mice in the control group also disclosed macroscopic tumors, whereas five of the surviving animals in the treated group were free from tumors even at microscopic level.

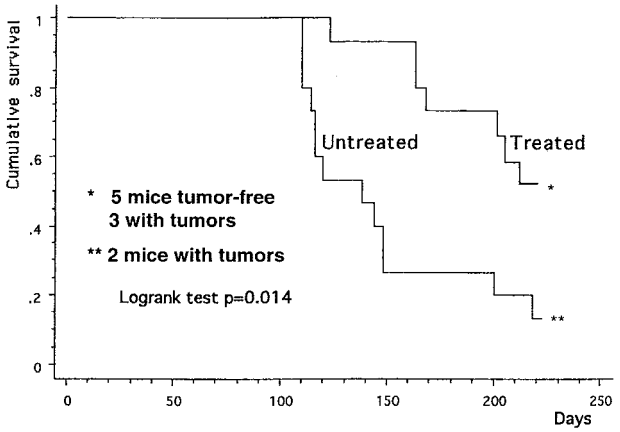

Fig. 3 Long-term outcome of At-211-MOv18 i.p. treatment of OVCAR-3-inoculated mice compared with outcome in an untreated control group.

Discussion

Biologically targeted radiotherapy should be regarded as an optimal form of conformal radiotherapy. Targeting may be accomplished by exploiting some quality, characteristic for the tumor tissue, *e.g.* iodine uptake, receptor or antigen overexpression. In the latter cases, the targeting molecule is a polypeptide or an antibody, which a radionuclide is associated with. There are a vast number of options available as reviewed by Goldenberg (28) and Carlsson *et al.* (29).

Ovarian carcinoma most frequently is restricted to the peritoneal cavity. Because it is also chemo- and radiosensitive, it is a clinically relevant and methodologically attractive entity for developing regional radioimmunotherapy. Development in radioimmunoimaging often precedes therapy in the choice of antibodies, as reviewed by Kalofonos *et al.* (30). During the past 20 years, approximately 100 studies on radioimmunotherapy in ovarian cancer have been published, covering uptake, distribution, and efficacy *in vitro* and *in vivo*, mainly in mice with xenografted human ovarian carcinomas, to similar studies in women. Almost exclusively, electron emitters with a fairly long range have been used, *i.e.* I-131 and Y-90. Nonetheless, the Hammersmith group has reported promising results on minimal disease in human Phase I/II trials (31–33). As a consequence, a randomized trial using Y-90-HFMG1 has been initiated (34). Also with promising results, Crippa *et al.* (35) and Van Zanten-Prybysz *et al.* (36) used the MAb MOv18, as used in the present study, labeled with I-131 in Phase I/II studies. The Nantes-based group used the antibody OC-125 labeled with I-131 in a preclinical model of OVCAR-3 cell spheroids (37) as well as in a Phase I/II study (38), the latter with little therapeutic efficacy, however. The toxicity of the same compound was also studied by Haisma *et al.* (39) and Muto *et al.* (40), and moderate hematological and gastrointestinal morbidity were seen when given i.p. Juweid *et al.* (41) used ^{131}I-labeled MN-14 anti-CEA antibodies injected i.v. in women with macroscopic disease, with objective responses and a modest toxicity. Jacobs *et al.* (42) used Re-186-labeled NR-LU-10 antibodies i.p. in women with advanced disease, resulting in moderate response and mor-

bidity. Using a short-range electron emitter, Alvarez *et al.* (43) administered Lu-177 labeled to the CC49 antibody, i.p., in women with macro- and microscopic disease. Efficacy was restricted to patients with minimal disease, a finding that is in agreement with the expected results, given the distribution of absorbed dose.

The disperse results obtained in the human studies referred to above as well as in animal studies probably reflect differences in the tumor doses attained with the various antibodies and radionuclides as well as heterogeneity in the clinical stage of disease comprising both macro- and microscopic growth. The clinical setting where cure is most likely is the one when the disease burden is minimal. However, in this situation, the use of long-range electron emitters is inappropriate, as discussed by Rotmensch *et al.* (44), who made dosimetric calculations for β- and α-emitting nuclides deposited i.p. Horak *et al.* (45) used the α-emitter Pb-212 labeled to the anti-HER2/neu antibody AE1 in mice with various dimensions of SK-Ov-3 deposits and found the efficacy most pronounced in the minimal tumor size situations. Recently, McDevitt *et al.* (46) described the use of Ac-225 labeled to internalizing antibodies *in vitro* and *in vivo*, with a high therapeutic index. Zalutsky *et al.* (47) and Imam (48) have reviewed the use of α-emitters.

In this report, the achievements, from physics to treatment studies, of an interdisciplinary research group have been reviewed, indicating a rationale for Phase I clinical trials with At-211-labeled MAb to establish pharmacokinetics and toxicity among women following successful relapse chemotherapy of ovarian cancer. The treatment concept should be applied to patients with a minimal burden of the disease. However, a pretargeting approach, allowing a prolonged distribution of nonradioactive antibodies, as described by Paganelli *et al.* (49), Zhang *et al.* (50), and Lindegren *et al.* (2, 51), may prove useful also in macroscopic disease. If the procedures are feasible, other large tumor groups such as breast, prostatic, and colorectal cancer as well as lymphomas may be suitable candidates for the treatment reviewed. Use of a mixture of various radionuclides suitable for a diversity of tumor dimensions might also be successful.

Aspects that need attention are the availability of At-211, as well as upscaling using higher activities without introducing radiolysis, increasing specific activity, and development of pretargeting procedures. At the same time, a search for MAbs with suitable binding characteristics is necessary.

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