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# RADIOIMMUNOTHERAPY WITH $^{90}$Y-LABELED MONOCLONAL ANTIBODIES IN A NUDE MOUSE OVARIAN CANCER MODEL

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**Tumor stroma contains much fibrin, and so monoclonal antifibrin antibody can accumulate in tumors. We treated nude mice bearing human ovarian carcinoma xenografts with $^{90}$Y-labeled monoclonal antifibrin antibody Fab fragments administered intratumorally. The survival time vs. a control group was significantly prolonged and tumor growth rate was decreased. Another group of animals was treated with $^{90}$Y-labeled OC 125-monoclonal antibody; these mice received the antibodies intratumorally, intraperitoneally or intravenously. The survival time was longest in the intratumorally treated group. There was no significant difference in survival between $^{90}$Y-labeled OC 125 and antifibrin in the intratumorally treated animal groups. The tissue activity distribution studies revealed that bone marrow is the critical organ. Intratumorally injected monoclonal $^{90}$Y-antifibrin antibodies were retained at least 36 h (up to 50% of injected activity per gram tumor tissue) in the xenograft after one treatment, causing cell death. Beta-camera imaging and immunohistochemistry were performed for studies of the correlation between $^{90}$Y activity and fibrin distribution in tumor specimens. These results were in concordance. In conclusion, intratumoral administration seems suitable for radioimmunotherapy, with an antibody that targets stromal structures. The accumulation can be successfully monitored by a beta-camera.**

Radioimmunotherapy has so far had limited clinical success, mainly because of low tumor uptake (<0.01%/g tumor in patients) (1). There is a need for new methods for radioimmunotherapy, such as alternative routes of administration. Higher tumor uptakes might be achieved e.g. by using intratumoral injections. There are very few completely tumor-specific antibodies. However, non-specific localization in tumors has been obtained both in human and animal experiments with various antibodies not directly related to the tumor cells (2, 3). Our previous study showed tumor localization of monoclonal antifibrin antibodies, which was verified immunohistochemically (4).

For the present radioimmunotherapy study a monoclonal antifibrin antibody was chosen, since tumor stroma contains fibrin, and tumor uptake in a mouse cancer model has been reported (4, 5). Ovarian cancer was selected as experimental tumor model, because there are numerous antigens characterizing these malignancies (6, 7). The aim of the present study was to test intratumoral injection of a $^{90}$Y-labeled tumor-nonspecific antibody in a nude mouse model. We selected OC 125 (8) as antibody for this model, since it has been clinically used under various auspices (9–13) and also carefully studied in mouse

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models (14, 15). The effect of the administration route was studied with special reference to intratumoral injections, because this modality has been used in clinical experiments (16, 17). The tissue distribution was studied with immunohistochemistry and the radioactivity distribution with a beta-camera (18). $^{90}Y$ was chosen since its half-life (2.7 days) is long enough for accurate tumor localization, and short enough to minimize toxicity in organs playing an important role in catabolism (19); it has no gamma emission and its beta emission has a range in tissue corresponding to 100 to 1 000 cell diameters (20). $^{90}Y$ decays to a stable daughter nuclide, and $^{90}Y$ labeled tumor-specific antibodies have also been used by others (21, 22).

## Material and Methods

*Monoclonal antibodies.* For the therapeutic purposes we used two antibodies: Fab-fragment of the monoclonal antibody 59D8 (Centocor Europe, Leiden, the Netherlands) which recognizes a synthetic heptapeptide characteristic for fibrin but not for fibrinogen (23), and $F(ab')_2$-fragment of the monoclonal antibody OC 125 (CIS, Gif-sur-Yvette, France), which reacts specifically with the tumor marker CA 12-5, present in epithelial ovarian carcinoma (8). Carrier-free $^{90}Y$ was eluted from a sterile and pyrogen-free $^{90}Sr/^{90}Y$ generator. The eluate was purified with ion exchange chromatography and in the final solution of 0.5 mol/l acetate the $^{90}Sr/^{90}Y$ concentration ratio was $< 1 \times 10^{-5}$ at the time of labeling. The antibodies were labeled with $^{90}Y$ using cyclic DTPA-chelation and the incubation varied from 30 min to 12 h depending on the antibody to be labeled. The labeling efficiency was checked by thin layer chromatography (TLC). Specific activities $>370$ MBq/mg proteins were achieved.

*Mice.* Female nude mice/nu/nu-BALB/cABom; (Bomholtgaard, Denmark), aged 7–9 weeks at the beginning of the experiments, were used. The animals were maintained under sterile conditions in a barrier unit, with autoclaved equipment and food.

*Cells and tumor growth.* A human ovarian carcinoma cell line HOV-007 (Farmos-Group, Turku, Finland) originating from a serous cystadenocarcinoma (FIGO stage IV), was used. Tumors were established in nude mice by subcutaneous injection in the neck of $10^5$–$10^6$ cells in 0.25 ml of medium. After 10–12 days the mice were killed and fragments of these primary tumors were transplanted subcutaneously in the left flank of nude mice. At the beginning of the therapy experiments the tumor volumes were approximately 2–5 $cm^3$; this size was achieved within 2 weeks after inoculation. The tumors grew very rapidly, and the animals died within additional 1–2 weeks without any management.

*Therapy.* For therapy trials the mice were divided into two categories. In the first category three groups of animals (5 in each group) were tested using different routes of administration with $^{90}Y$-DTPA-OC 125. The first group received the radiolabeled antibody intratumorally by a single injection (group IT); the second group received it intraperitoneally by a single injection (group IP); and the third group by an intravenous infusion during 1 min (group IV). In the second category the mice were divided into two groups (7 in each group). One group of animals received $^{90}Y$-DTPA-antifibrin antibodies intratumorally (antifibrin group). The other group (control group) received $^{90}Y$ in saline solution intratumorally; the administered volumes varied from 0.15 to 0.20 ml in all these experiments. The administration of $^{90}Y$-antibodies and $^{90}Y$-solutions were repeated every 2–3 days 2 to 7 times. The treatment activities varied from 3.0–4.5 MBq and the total activities administered were 15–23 MBq. The therapy effect was evaluated by survival times and tumor diameters.

*Activity distribution studies.* Two additional animals treated as those in the antifibrin group were used for studies of the activity distribution. One animal was sacrificed 36 h after the first antifibrin treatment and the other 36 h after the fourth antifibrin treatment. The distribution of $^{90}Y$ in the tissues was studied in all animals after therapy (the 7th treatment was the last one in all tests); the organs were dissected, weighed and counted for $\beta$-radioactivity in a liquid scintillation counter.

*Morphological studies.* The histology of the tumor xenografts was studied, and they were stained immunohistochemically for fibrin and CA 125; immunoperoxidase staining with avidin-biotin amplification was used (24). In addition, the tissue sections were stained with three monoclonal antibodies for carcinoembryonic antigen, CA 19-9 and cytokeratin respectively.

*Beta-camera imaging.* The tissue specimens (the same as used for immunohistochemistry) were studied for radioactivity distribution with a beta camera. The beta camera is described in detail elsewhere (18, 25). Briefly it consisted of a thin plastic scintillator and a light sensitive detector which contained a fiberoptic window, a photocathode, two microchannel plates and a resistive anode. The plastic scintillator was 0.9 mm thick and was mounted directly onto the fiberoptic window on the light sensitive detector. The detector system was kept at $-20°C$ to cool the photo cathode and facilitate handling of non-fixed samples.

## Results

In the category of animals treated with $^{90}Y$-labeled OC 125 the averages of survival times in the three groups with different routes of administration were as follows: IP group $8.3 \pm 4.3$ d; IV group $5.0 \pm 3.0$ d; IT group $11.0 \pm 4.0$ d. The differences between the IT and IV groups was statistically significant ($p < 0.001$, Student's t-test). The tumor growth was reduced in the IT group, but in the other two categories the tumors grew except in one animal in the IP group (Fig. 1).

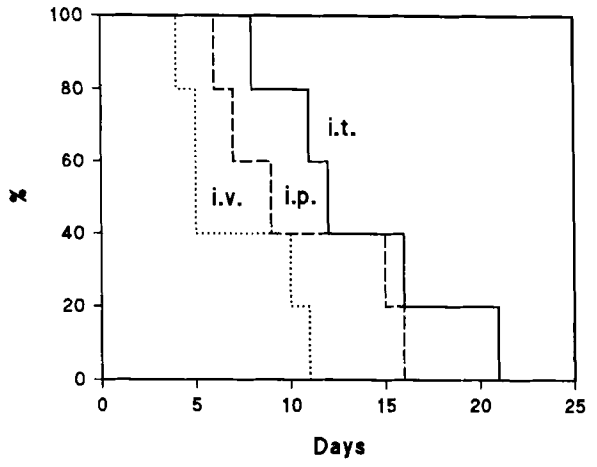


*Fig. 1.* Survival of $^{90}$Y-labeled OC 125 treated nude mice bearing human ovarian cancer. The effect of routes of administration is demonstrated. i.t. = intratumoral; i.v. = intravenous; i.p. intraperitoneal.

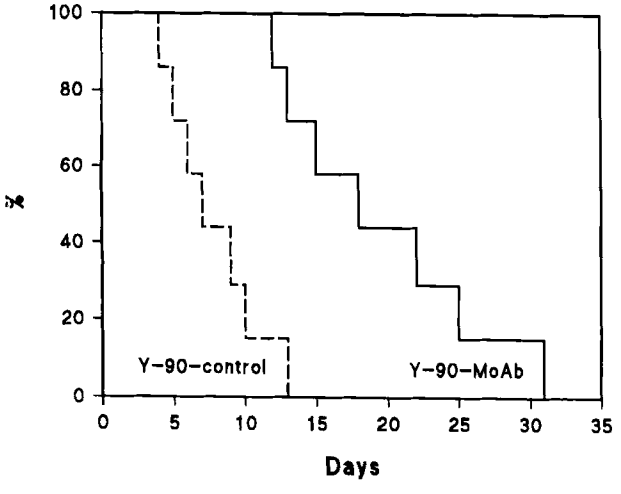


*Fig. 2.* Survival of $^{90}$Y-labeled antifibrin MAb-treated nude mice bearing human ovarian cancer using intratumoral injections compared to survival of animals that received intratumorally injected $^{90}$Y-saline solution.

The average survival time of the mice treated intratumorally with $^{90}$Y-labeled antifibrin was $17.4 \pm 5.8$ d. It was significantly longer in the $^{90}$Y-antifibrin group than in the $^{90}$Y-saline treated group ($p < 0.001$, Student's t-test), where it was $8.0 \pm 2.8$ d (Fig. 2).

In the histological samples tissue necrosis was observed in all $^{90}$Y-antibody-treated tumors. The activity distribution data with $^{90}$Y-antifibrin is presented in the Table. The living tumor cells were immunohistochemically stainable with antifibrin and anti-CA 125 antibodies. The tissue sections were also stained with three monoclonal antibodies for carcinoembryonic antigen, CA 19-9, and cytokeratin. Only the cytokeratin staining was weakly positive. Semiquantitative immunohistochemistry and quantitative beta camera imaging showed good correlation (Spearman's rank test $r = 0.94$).

**Table**

*Tissue activity after the 1st and 4th $^{90}$Y-antifibrin antibody treatment. The animals were sacrificed 36 h after intratumoral treatment.~ % of inj. dose/g tissue*

| Organ | 1st treatment | 4th treatment |
|---|---|---|
| Tumor | 50.2 | 19.9 |
| Liver | 0.23 | 0.48 |
| Thyroid | 0.01 | 0.02 |
| Bowel | 0.05 | 0.05 |
| Kidney | 13.8 | 5.9 |
| Bone marrow | 12.0 | 16.8 |
| Spleen | 3.6 | 6.0 |
| Lung | 3.0 | 4.7 |
| Blood | 2.1 | 2.9 |

## Discussion

This study shows that intratumoral injection enhanced the tumor accumulation and the therapeutic effect of both antifibrin and OC125, labeled with $^{90}$Y. It is known that the binding of $^{90}$Y-DTPA to the antibodies is not stable in vivo (22), and the free $^{90}$Y accumulates in the bone marrow. The critical organ in human studies has been the bone marrow (21). Our data is in concordance with that observation as the biodistribution studies showed high uptake in the bone marrow. We demonstrated a rather high in vivo stability of $^{90}$Y-DTPA-MAb complexes. The radionuclide and the immunohistochemical distributions were very similar. Tumor uptake up to 50%/ID/g tissue at 36 h after the first treatment and tumor uptake up to 20%/ID/g at 36 h after the fourth treatment respectively were observed.

The accumulation of the Y-labeled antibody was at least partly responsible for the therapeutic effect, which was confirmed by the immunohistochemical staining. Our findings show that these samples can be successfully imaged with a beta camera. The beta camera registration is fast. Its spatial resolution is about 0.5 mm (FWHM) for $^{90}$Y. Beta camera imaging took only 5–10 min whereas corresponding autoradiography exposures will take several days (26). After i.v. injection, the tumor uptake in this model was approximately 10%/ID/g tumor (OC 125), which was much less than reported by Anderson-Berg et al. (19) in an erythroleukemia model. At 36 h after intratumoral administration the uptake was 5–7 times higher than in i.v. injected animals. Intratumoral injection might therefore be useful for clinical practice, also when a cell skeleton binding antibody (e.g. associated with intermediate filaments) is used. This phenomenon has previously been studied by Riva et al. (17) in brain glioblastoma patients using an antitenascin antibody, which binds to tumor stroma. Intra-

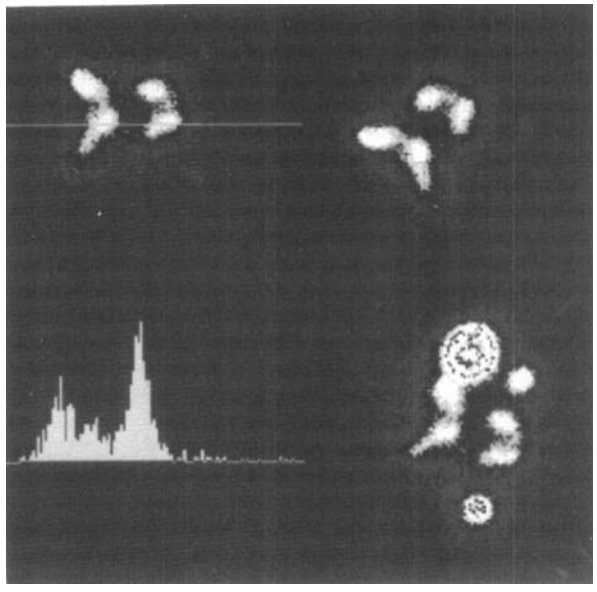

*Fig. 3.* Beta camera study showing the $^{90}Y$ activity distribution in a tumor cross section. The animal was sacrificed 36 h after last treatment. Intense intratumoral binding of monoclonal $^{90}Y$-labeled antifibrin antibody can be noted. The images on the right demonstrate this binding in two separate areas (corresponding to two tumor stromal areas). In the lower right image the three additional dots represent $^{90}Y$ standards and markers for correct orientation in the histological slices. The images on the left demonstrate the exact activity profile in one level (shown in upper left part of the image). The imaging time was 10 min.

tumoral administration was found to be an effective way to cause cell death, which was histologically verified.

Systematic therapy with a non-specific antibody is as a rule not possible, because of poor tumor seeking properties and high accumulation in critical organs. Our findings, however, suggest that a monoclonal antifibrin MoAb is suitable for antitumor targeting and could be a candidate for human radioimmunotherapy trials. More attention has to be paid to other non-specific antibodies for radioimmunotherapy trials. However, the diffusion of such radiolabeled antibodies into the target tissues will probably be too slow for treatment of macroscopic tumors (27). From a dosimetric point of view, calculations show that $^{90}Y$ is superior to $^{131}I$ for treatment of larger tumors when a homogenous activity distribution cannot be achieved. A 10-fold increase in the uptake has to be obtained in order to achieve tumor erradication in this model with $^{131}I$ according to the calculations in the literature (28). Antibodies raised against fibrin, vimentin, tenascin, laminin and cytokeratin may provide new tools, since higher concentrations might be obtained in the tumor.

The experimental tumor model described here, may be modified for future purposes by, for instance, in vivo biotin-avidin amplification (29, 30). The antibody uptake can also be enhanced by use of smaller $F_v$-fragments (31) or by biological response modifiers, such as IFN and IL-2 (32, 33). Extracorporeal immunoadsorption (34) and plasmapheresis (35) can also increase the tumor-to-non-tumor ratio. Better in vivo absorbed dose estimates are possible by using positron labeled antibodies (36). An ovarian cancer nude rat model has already been used for PET studies (37).

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