Tumor Cure Probability During α-RIT of Ovarian Cancer with Different Radiation Sensitivity

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Abstract. Purpose: To calculate the tumor cure probability (TCP) and metastatic cure probability (MCP) during α-radioimmunotherapy (α-RIT) of small ovarian cancer tumors with cells of different radiation sensitivity. Materials and Methods: An in-house-developed biokinetic model and a Monte-Carlo program were used to calculate the cumulative activity on tumor cell surfaces and the specific energy to tumor cell nuclei, respectively. An in-house-developed computational model was used to calculate the TCP and MCP as a function of assumed radiation sensitivities, expressed as $D_{37}$, of the tumor cells. The calculations were performed using various assumptions regarding the activity distribution in measured tumors and used the α-particle energies emitted from astatine-211 ($^{211}\text{At}$). Regarding the calculations of the cumulative activity on each cell surface, the number of antigenic sites expressed by NIH:OVCAR-3 cells for the mAb MX35 F(ab')2 was used. To illustrate the tumor growth at the peritoneum in nude mice, scanning electron microscopy images were used. Results: In the case of a maximum diffusion depth of 30 μm for the activity in the tumors, the TCP was high for $D_{37}$ values not exceeding ~4.3, ~2.9, ~1.8, and ~0.8 Gy for 200, 100, 50, and 25 kBq $^{211}\text{At}$-MX35 F(ab')2 four weeks after cell inoculation, respectively. In order to achieve complete remission of the metastatic disease in mice (i.e. MCP=1), the $D_{37}$ value should not exceed ~2.2, ~1.3, ~0.6, and ~0.3 Gy when injecting 200, 100, 50, or 25 kBq $^{211}\text{At}$-MX35 F(ab')2, respectively, assuming a maximum diffusion depth of 30 μm for the activity in the tumors. Conclusion: The radiation sensitivity, expressed as $D_{37}$, of tumor cells subjected to α-RIT could be decisive for therapeutic outcome, expressed as TCP or MCP, when treating small tumors of ovarian cancer.

Ovarian cancer is frequently lethal because of residual disease metastasized to the peritoneal surfaces, notwithstanding complete clinical remission after surgery and systemic chemotherapy. Conventional platinum-based chemotherapy is often ineffective as a result of cellular resistance mechanisms. External whole-abdominal irradiation has only shown limited success because of the toxic effects on the intestines. However, monoclonal antibodies (mAbs) have made targeted radiation therapy possible, offering the chance to increase the absorbed dose to the tumor, while remaining below the toxicity level of surrounding normal tissues. Several genes are known to be involved in the development of ovarian cancer. In hereditary cases, the most common mutated genes are the tumor suppressor genes $\text{BRCA1}$ and $\text{BRCA2}$, and the DNA repair genes $\text{MSH2}$ and $\text{MLH1}$ (1-2). The most frequently studied tumor suppressor genes in sporadic cases of ovarian cancer encode the proteins p53 and CDKN2A (3-4). Tumor suppressor p53 is a transcription factor that plays an essential role in the cell cycle regulation. It is however often mutated to a defective form, which may be highly expressed in cancer cells, and contributes to cell transformation and malignancy. Mutations and deletions in such tumor suppressor genes may cause loss of protective functions in the regulation of the cell through the events of replication and division and results in altered response to radiation damage, resulting in either radiosensitive or radioresistant phenotypes. The radiosensitivity of tumor cells varies and is a critical determinant of the probability of cure in patients receiving radiotherapy for cancer (5). For example, the relationship between different types of p53 mutations and the degree of radiation sensitivity in vitro was recently published (6). The authors showed that the sensitivity of Saos-2 cells to ionizing radiation varied greatly with the type of mutation. Findings like these make it of outmost importance to investigate how the therapeutic outcome after targeted radiotherapy will be affected by the difference in radiosensitivity of ovarian cancer cells.

Studies performed to investigate the therapeutic efficacy of β-particle emitters, mostly yttrium-90 ($^{90}\text{Y}$) and iodine-131 ($^{131}\text{I}$) labeled mAbs, in animals and humans with ovarian cancer have shown limited success (7-17). Some studies with α-particle emitters have also been carried out.
In the present study, the tumor cure probability (TCP) and metastatic cure probability (MCP) of the α-particle emitter 211At, with a half-life of 7.21 h, mean range in tissue of ~62 μm, and a mean linear energy transfer (LET) of ~111 keV/μm, were calculated. The role of the radiation sensitivity of the tumor cells, expressed as $D_{37}$, on the therapeutic outcome was also calculated. We have previously investigated therapeutic efficacy using both intact IgG1 mAbs MOv18 and MX35, as well as fragmented mAbs (MX35 F(ab′)2 and nonspecific Rituximab F(ab′)2) in the treatment of microscopic disease of ovarian cancer in nude mice (20-24). In a previous study (25), we investigated the TCP for a fixed value of the radiation sensitivity of the tumor cells.

In a metastatic disease such as ovarian cancer, the tumors may vary in size, and to obtain complete remission all tumors must be eradicated. In the present study, we have further developed an in vivo TCP model based on measured tumor sizes (24). Based on a previous estimation of the tumor size distribution (25), the MCP was also calculated and plotted as a function of the radiation sensitivity, expressed as $D_{37}$, of the tumor cells in mice, $D_{37}$ being the absorbed dose required for a growth inhibition corresponding to 0.37.

**Materials and Methods**

**Radiation dosimetry.** Specific energy values of radiation to the nuclei of tumor cells, regarding various assumptions of the tumor size and activity distribution, were used from published data (25). A previously described computer program (26) was used for the dosimetry of the tumors. The program uses stopping power values of α-particles in liquid water (27) for Monte-Carlo-derived dosimetry and was designed to calculate the energy deposition in a defined target volume, a 7.2-μm radius sphere, simulating a single tumor cell nucleus embedded at various depths in the tumor. For each tumor size, five target positions were selected along the central axis of the tumor. Tumor sizes at the time of treatment four weeks after cell inoculation were estimated by analyzing scanning electron microscopy (SEM) images. The cell packing ratio was assumed to be 1.0 in each tumor, i.e., assuming no intercellular space. The calculations were performed for spherical tumors with radii, $r_{tumor}$, equal to 30, 45, and 95 μm. Four activity distributions were assumed: (i) activity homogeneously distributed at the antigenic sites throughout the whole tumor, (ii) activity homogeneously distributed at the antigenic sites within a maximum diffusion depth of 30 μm, (iii) activity distributed only at the antigenic sites at those cell surfaces defining the surface of the tumor, and (iv) activity freely circulating around the tumor without specific binding to antigenic sites. For the calculation of the number of antigenic sites exposed on the tumor surface, a ‘cobblestone’ surface was assumed, giving twice the tumor surface area compared with calculations for a smooth surface. The cumulative activity on a tumor cell was calculated with an in-house developed compartmental model (23). Regarding the mAb distribution described above in which limited penetration of the activity into the tumors was assumed, a diffusion model was adopted. This model was incorporated because a previous study had indicated that a homogeneous distribution of the activity in the tumors was inconsistent with the therapeutic results (24). The diffusion model was based on the assumption of a binding site barrier (28). A maximum diffusion depth ($d_{diff}$) of 30 μm in the tumors was assumed in those cases. The activity was assumed to diffuse freely to $d_{diff}$ momentarily after the injection into the abdominal cavity.

**Tumor cure probability model.** Based on measured tumor sizes four weeks after intraperitoneal inoculation of ~$1 \times 10^7$ NIH-OVCAR-3 cells in mice, cores and shells were defined for three tumor sizes, with a radius equal to 30, 45, or 95 μm (25). The 95-μm tumor corresponds to the largest tumors found at the time of the intraperitoneal treatment four weeks after the cell inoculation. The reason for defining cores and shells was that we expected an inhomogeneous absorbed dose distribution in the tumors, and this definition enabled us to calculate the probability for cell survival for different tumor sizes, activity distributions, and amounts of injected activity.

For the 30-μm tumor, the core was defined as a sphere with a radius of 12 μm. Two shells were defined for this tumor size, one ranging from 12 to 21 μm from the center, and one ranging from 21 to 30 μm from the center. For the 45-μm tumor, the core was defined as a sphere with a radius of 9 μm. Four shells were defined 9-18, 18-27, 27-36, and 36-45 μm from the center. For the 95-μm tumor, the core was defined as a sphere with a radius of 23 μm. Eight shells were defined 23-32, 32-41, 41-50, 50-59, 59-68, 68-77, 77-86, and 86-95 μm from the center.

Polynomial fits were made to the specific energy calculations, representing the three tumor sizes (30, 45, and 95 μm), the four activity levels (200, 100, 500, and 25 kBq), and the four irradiation geometries (see above). The mean absorbed dose was assigned to the corresponding tumor core and shell.

The number of surviving cells in each tumor ($n_i$), consisting of $m$ compartments (i.e., the core + the shells), was calculated as:

$$n_i = n_{cell} \cdot \sum_{i=1}^{m} VFi \cdot Si = n_{cell} \cdot \sum_{i=1}^{m} VFi \cdot e^{-\alpha Pi}$$

(Eqtn. 1)

where $n_{cell}$ is the total number of cells in the tumor, $VFi$ is the volume fraction of the $i^{th}$ core or shell, $Si$ is the surviving fraction of cells in this $i^{th}$ core/shell receiving the mean absorbed dose $D_{37}$ and $\alpha$ is the parameter describing the cells’ radiation sensitivity ($1/D_{37}$ [Gy$^{-1}$]). $D_{37}$ being the absorbed dose required for a growth inhibition corresponding to 0.37.

Because a packing ratio of 1.0 was assumed, the $VFi$ represents the fraction of $n_{cell}$, for each tumor size, belonging to each core and shell.

The TCP for each tumor, consisting of $m$ compartments (i.e., the core plus the shells) and $n_i$ cells in each core or shell, was calculated for the different activity levels and irradiation geometries from:

$$TCP = \prod_{i=1}^{m} (1 - Si)^{ni}$$

(Eqtn. 2)
Figure 1. Computational model enabling the calculation of TCP and MCP. Compartment 1 calculates the cumulative activity at a tumor cell surface using the parameters $N_{mAb}$=the number of antibodies injected in the abdominal cavity, $B_{max}$=the number of antigenic sites on one tumor cell, $A_{sp}$=the specific activity of the injected radioimmuno complex, $k_{on}$=the rate at which the antibodies bind to the antigenic sites, and $k_{off}$=the rate at which the antibodies will be released from the antigenic sites. Compartment 2 calculates the mean specific energy delivered to tumor cell nuclei situated at various depths in the tumor using the parameters $E_\alpha$=the energies of the emitted α-particles from $^{211}$At, $r_{tumor}$=the radius of the relevant tumor, $r_{nucleus}$=the radius of the tumor cell nucleus, $s_{max}$=the maximum path length of the emitted α-particles from $^{211}$At. Compartment 3 calculates the TCP using the parameters $D_{37}$=absorbed dose resulting in 37% of the tumor cells surviving, $S_i$=surviving fraction, $r_{tumor}$=the radius of the relevant tumor. Compartment 4 calculates the MCP using $F_{tumor}$=the estimated distribution function of the number of tumors of different sizes present in an animal. Compartment 3 and 4 also enables the computation of $n_i$ (the number of surviving cells in a particular tumor) and $N_i$ (the total number of surviving cells in an animal), respectively.

where $S_i$ is the surviving fraction of cells in the $i^{th}$ core-shell receiving the mean absorbed dose $D_i$.

The total number of 30-, 45-, and 95-μm tumors present in each tissue specimen was estimated by analyzing the SEM images and used to calculate the MCP (Equation 3) for an animal, for each activity level and irradiation geometry, for a disease containing $k$ different tumors with $m_h$ number of 30-, 45-, and 95-μm tumors, respectively:

$$MCP = \prod_{h=1}^{k} (TCP_h)^{m_h}$$  
(Eqtn. 3)

where $TCP_h$ is the tumor cure probability for the $h^{th}$ tumor size.

Figure 1 shows a schematic diagram of the computational model used to calculate the TCP and MCP, including its most important parameters.

Scanning electron microscopy of tumors in mice. Specimens for ultrastructural analysis were obtained from mice anesthetized with Metofane (Mallinckrodt Veterinary Inc., Mundelein, IL, USA) four weeks after cell inoculation. The thoracic cavity was exposed and specimens, including peritoneal cavity and jejunum (including mesenteries), were harvested by dissection. Specimens were further fixed overnight in the aldehyde mixture. After rinsing in 0.15 M cacodylate, specimens for electron microscopy were subjected to the osmium-thiocarbohydrazide-osmium (OTOTO) postfixation technique (29). Dehydration followed in a series of ethanol, finally replaced by two changes of hexamethyldisilazane, which was allowed to evaporate under a fume hood. The dried specimens were mounted on aluminum stubs and were examined in a Zeiss 982 Gemini field-emission scanning electron microscope after coating with palladium in an Emitech 550 sputter coater. Digital images were collected at a resolution of 1024×1024 pixels. Each tissue specimen (~16 mm²) was examined in the electron microscope.

Results

Tumor cure probability (TCP). In Figure 2, the TCP as a function of $D_{37}$ for each tumor size, irradiation geometry, and activity level is plotted. It can be seen that for the smallest tumor investigated ($r_{tumor}$=30 μm) the TCP was generally high for all values of $D_{37}$, activity levels, and irradiation geometries, except for the case of unbound activity. In that case, the limit of the $D_{37}$ for achieving TCP=1 (and hence $n_i=0$) was ~1.8, ~1.3, ~0.3, and ~0 Gy for 200, 100, 50, and 25 kBq $^{211}$At-MX35 F(ab’)², respectively. The corresponding $D_{37}$ values for the 45-μm tumor were ~0.9, ~0.3, ~0.1, and ~0 Gy. Regarding the largest tumors found four weeks after cell inoculation ($r_{tumor}$=95 μm) the situation was worse. Due to the relatively short path length of the emitted α-particles in tissue (~71 μm), the irradiation geometries in which the activity, was only situated on the surface of the tumor or completely unbound circulating around the tumor, the TCP would be equal to zero for all $D_{37}$ values. In the case of limited diffusion of the activity the TCP was high (i.e. $n_i=0$) for $D_{37}$ values not exceeding ~4.3, ~2.9, ~1.8, and ~0.8 Gy for 200, 100, 50, and 25 kBq $^{211}$At-MX35 F(ab’)², respectively. In the case of a homogeneous activity distribution in the tumors the TCP started to decline apparently only at the 25 kBq level and for $D_{37}$ values exceeding ~4 Gy.

Metastatic cure probability (MCP). In Figure 3, the MCP as a function of $D_{37}$ for each irradiation geometry and activity level is plotted. The curves for the cases in which the activity...
was distributed only at antigenic sites on the surface of the tumor or unbound and freely circulating around the tumor were omitted due to the fact that the MCP is zero for all values of $D_{37}$ in those cases. This is explained by the limited path length of the $\alpha$-particles (~71 μm) giving unirradiated cells in the core of those tumors with radii larger than that (i.e. $r_{\text{tumor}} = 95 \mu m$), and hence an MCP equal to zero. It can be seen in Figure 3, that in the case of a limited diffusion depth of 30 μm for the activity in the tumors, the limit of the $D_{37}$ for achieving MCP=1 was ~2.2, ~1.3, ~0.6, and ~0.3 Gy for 200, 100, 50, and 25 kBq $^{211}$At-MX35 F(ab')$_2$, respectively. The corresponding values for when the activity was homogeneously distributed in the tumor were ~7.5, ~4.8, ~2.8, and ~1.2 Gy.

**Tumor growth.** Figure 4 shows SEM images of ovarian tumors on the peritoneum in nude mice. The biopsies were taken from the upper left quadrant of the abdominal wall at the time of treatment, i.e. four weeks after the intraperitoneal tumor cell inoculation. To simplify the dosimetric and TCP calculations, spherical and cobblestone-surfaced tumors were assumed in all calculations.

**Discussion**
Calculations of the specific energy delivered to tumor cell nuclei originating from specific binding of the radiolabeled mAbs to the antigenic sites of the tumor cells were first performed for optimal conditions, i.e. assuming that all...
tumor cells and antigenic sites in the tumor were available to the mAbs. This situation most probably does not reflect the situation in vivo, but was assumed for the purpose of estimating the maximum attainable specific energy delivered to cell nuclei. The specific energy delivered to cell nuclei was also chosen to be calculated for the other extreme, no diffusion at all into the tumor, resulting in a \(^{211}\)At-mAb distribution only on the surface of the tumor. This means that the radiation did not reach the inner part of the tumor when \(r_{\text{tumor}} > s_{\text{max}}\) (i.e. the maximum path length of the \(\alpha\)-particles, \(\sim 71 \mu m\)). Among the four irradiation geometries considered in an earlier study, the one assuming a limited diffusion depth of 30 \(\mu m\) agreed best with the therapeutic outcome (24). In that study, a large increase of the MCP (from 0.33 to 0.98) between the 50 and 100 kBq level agreed with an increase in the TFF (from 22\% to 50\%), between the same activity levels.

In a previous in vitro study investigating the radiation sensitivity of NIH:OVCAR-3 cells using \(^{211}\)At, a \(D_{37}\) value of 0.56 Gy was reported (30). In another study of the same cell line, an in vivo \(D_{37}\) value of 1.59 Gy was reached also using \(^{211}\)At (31). As clearly indicated in the results of the present study, the role of the radiation sensitivity of the tumor cells has to be considered during therapy. Molecular and cellular in vitro and in vivo studies have increased our knowledge regarding the radiosensitivity properties of different tumor cells. It is apparent that no single factor/gene or even cluster of factors/genes determines the degree of radiosensitivity. However, research has demonstrated a variety of factors rendering cells towards either radiosensitive or radioresistant phenotypes. Some of these are already under evaluation as prognostic factors in the...
in this study, the TCP was high for an assumed limited diffusion depth of 30 μm of the activity such/identified biological and physical properties. In the case in the design of successful treatment strategies based on different degrees of radiosensitivity. Increased radioresistance was exhibited among mutants with point mutations in the hotspot regions, most commonly mutated in ovarian cancer cells. The presented theoretical model may aid in the design of successful treatment strategies based on such/identified biological and physical properties. In the case of an assumed limited diffusion depth of 30 μm of the activity in the study, the TCP was high for D37 values not exceeding ~4.3, ~2.9, ~1.8, and ~0.8 Gy for 200, 100, 50, and 25 kBq injected 211At-MX35 F(αb′)2, respectively. Regarding the MCP in the case of the limited diffusion depth of the activity in the tumors, the limit of D37 for achieving MCP=1 was ~2.2, ~1.3, ~0.6, and ~0.3 Gy for 200, 100, 50, and 25 kBq 211At-MX35 F(αb′)2, respectively. In humans, these activity levels correspond to ~400, ~200, ~100, and 50 MBq 211At-MX35 F(αb′)2, respectively.

Since the research group led by Professor Ragnar Hultborn (Department of Oncology, Sahlgrenska Academy, University of Gothenburg) and Professor Lars Jacobsson (Department of Radiation Physics, Sahlgrenska Academy, University of Gothenburg) just recently published the result of a phase I study on nine women with refractory ovarian cancer using 211At-MX35 F(αb′)2 (37), and are now planning to a phase II study, we find it important to investigate different aspects potentially influencing the therapeutic outcome, e.g. the radiation sensitivity of the tumor cells investigated in this study.

Conclusion

The radiation sensitivity, expressed as D37, of cells subjected to α-RIT could be decisive for the therapeutic outcome, expressed as TCP or MCP, when treating small tumors of ovarian cancer in mice.

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References


