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Growth retardation and destruction of experimental squamous cell carcinoma by interstitial radioactive wires releasing diffusing alpha-emitting atoms

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In the present study, we examined the antitumoral effects caused by the release of alpha emitting radioisotopes into solid squamous cell carcinoma (SCC) tumors. Using a novel method termed DART (Diffusing Alpha-emitters Radiation Therapy), we assessed the efficacy of short-lived daughters of ^{224}Ra releasing alpha particles, dispersing in the malignant tissue, to cause tumor growth retardation and destruction. It was carried out using specially designed wires loaded with ^{224}Ra activities in the range of 7–42 kBq in a set of experiments performed on BALB/c and nude mice bearing metastatic SCC tumors derived from either mouse SQ2 or human CAL27 cell lines. The insertion of a DART wire to the center of 6–7 mm primary tumors, retarded tumor growth, reduced lung metastatic load, prolonged life expectancy and in some cases caused tumor eradication. These effects were enhanced either when treating smaller tumors or treating identical tumors with 2 DART wires. Similar experiments on human-derived SCC tumors in nude mice were consistent with the outcomes of the murine model. Histological assessments revealed the tissue damage pattern, and indicated a role for the tumor vasculature in the dispersion of the atoms and the propagation of the damage. Our findings indicate that Diffusing Alpha-emitting Radiation Therapy is effective in a model system using SCC primary tumors. The *in situ* destruction of primary solid tumors by DART is evidently a necessary step toward curing cancer and might be augmented by chemotherapy and other modalities such as immunotherapy or anti-growth factors agents.

Key words: interstitial radiotherapy; alpha radiation; squamous cell carcinoma

Brachytherapy is continually progressing as a viable form of radiation therapy, allowing the delivery of a higher radiation dosage to the tumor with potentially less damage to surrounding tissue. Brachytherapy procedures, employing either permanent low dose rate seeds (primarily ^{125}I and ^{103}Pd) or temporary high dose rate ^{192}Ir wires, are already in widespread use for the treatment of different solid malignancies, including the prostate, breast, lung, uterus, cervix uteri, head and neck and brain.[1–5] Like EBRT, brachytherapy relies on the use of photons (X- and gamma-rays), with an additional short-range contribution of beta particles in the case of ^{192}Ir.

Unlike photons and beta particles, which are characterized by a low linear energy transfer (LET) and considerable range in tissue (millimeters for beta particles, centimeters for photons), alpha particles are a form of high-LET radiation, depositing several MeV over a range of 40–90 μm. This makes them highly effective against cancer cells, with only a few alpha particle traversals through the nucleus required to induce cell death.[6,7]

The therapeutic potential of alpha particles in the treatment of cancer has long been recognized. Its high-LET and short range combine to suggest an effective and safe mode of treatment, where a large dose is delivered to the targeted region, while neighboring healthy cells are spared, provided that the alpha emitting atoms are brought to the immediate proximity of the targeted cells. Motivated by this potential, a large number of parallel efforts have been initiated in the last 2 decades to promote the concept of targeted alpha therapy.[8] These efforts have recently reached the stage of clinical trials, including ^{223}Ra treatments for bone metastases[9] and alpha-radioimmunotherapy treatments with monoclonal antibodies labeled with ^{213}Bi or ^{211}At for myeloid leukemia, intralesional and systemic melanoma and glioma.[10,11] Nevertheless, the growing experience with alpha-radioimmunotherapy has led to the generally accepted view that while this method has a promising potential for the treatment of single cells, small cell clusters and micrometastases, it is generally not suitable for the treatment of solid tumors.[12]

In a recent work,[13] we proposed a new approach for treating solid tumors with alpha particles. The method, termed as DART—Diffusing Alpha-emitters Radiation Therapy—consists of treating solid tumors with interstitial radioactive wires that continually release short-lived alpha emitting atoms into the tumor. Wires impregnated with small activities of ^{224}Ra (3.66 d half-life) are inserted *via* fine-gauge needles into the tumor, releasing, by recoil, ^{220}Rn (55.6 s half-life), ^{212}Po (0.15 s half-life) and ^{212}Pb (10.64 h half-life) atoms, while ^{224}Ra itself remains fixed below the wire surface. ^{224}Ra is itself the decay product of ^{228}Th (1.91 y half-life), and the preparation of ^{224}Ra DART wires is based on a ^{228}Th generator (Fig. 1).

The released atoms disperse inside the tumor by the combined effects of thermal diffusion and vascular convection, leading through their alpha decays, to the formation of a high-dose region measuring several millimeters in size about each wire. In our previous study,[13] we demonstrated, by both physical and histological measurements, the formation of a 5–7 mm high-dose necrotic region about single ^{224}Ra-loaded DART wire inserted into murine squamous cell carcinoma (SCC) tumors.

The experimental SCC model was also chosen to assess the curative potential of DART. This choice was motivated, in part, by the need for improvement of conventional radiation therapy (either external beam or brachytherapy) for human SCC tumors, primarily of the head and neck, where SCC is the main histological subtype (80–90%) and where treatments are usually complicated by the proximity of normal tissues and organs such as the spinal cord, brain stem, parotid glands and optic pathway structures, which are inevitably exposed to high-radiation dose and manifest side effects including chronic radiation toxicities like mucosal fibrosis and atrophy.[14–16] Not only can these effects compromise optimal treatment delivery, they can lead to a lifetime risk with profound effects on patient's quality of life.[17,18]

In this article, we focus on the effect of ^{224}Ra-loaded DART wires on murine and human-derived SCC tumors *in vivo*, regarding both tumor development and animal survival, with accompanying *in vitro* experiments, which demonstrate the effect of alpha particles on cultured SCC cells.

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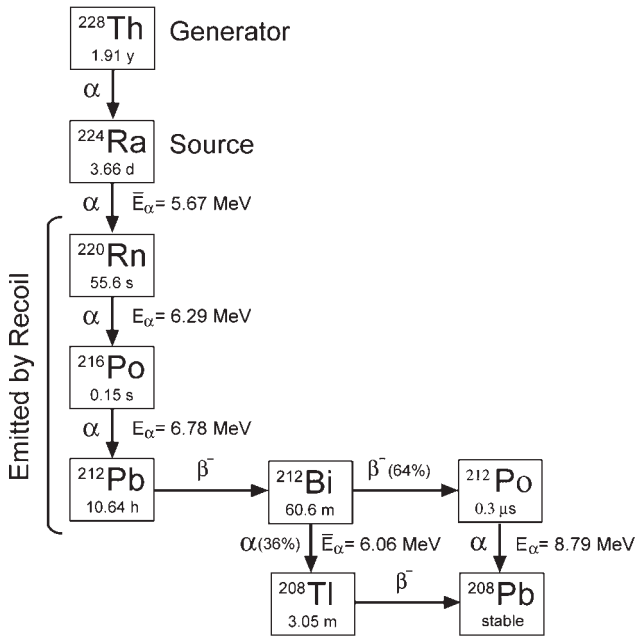

FIGURE 1 – ^{228}Th decay chain.

Material and methods

Tumors

SQ2 cell line[19] was kindly provided by Dr. Gad Lavie from the Sheba medical center, Tel-Hashomer, Israel. This is a murine anaplastic cell line which was generated from a SCC tumor that has developed spontaneously in a male BALB/c mouse. The tumor was characterized and determined as SCC at the pathology institute of Sheba Medical Center. CAL 27 (Human SCC) cell line[20] was purchased from the American Type Culture Collection (CRL-2095, VA). The cells were grown in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal calf serum (Biological Industries, Israel), L-glutamine (2 mM), Penicillin (100 U/ml) and Streptomycin (100 μg/ml) (supplemented DMEM).

Cell survival

The *in vitro* effect of alpha particles on SQ2 cells was studied by exposing cells seeded on a thin Kapton (polyimide) foil to alpha particles traversing the foil from below. Plastic Petri dishes (60-mm diameter, Corning, NY, USA) were prepared by drilling a 9-mm hole at their center and gluing a 7.5-μm Kapton foil (Dupont, Luxemburg, Belgium) above the hole by a 10-min epoxy adhesive (Akapol, Argentina). Prepared dishes were sterilized by UV light for at least 1 hr. Cells were seeded on the foil at a density of 10^4 cells/dish and exposed to the alpha particle flux 24 hr later. Exposure was performed by positioning the dishes 10 mm above a silicon wafer coated with a thin layer containing ^{228}Th in secular equilibrium with its daughters (collimated by a 10-mm circular hole) in air. The average alpha particle flux across the foil was measured by an EG&G solid-state alpha particle detector. Exposure times were 0, 3, 6, 9 and 12 min, with an average flux of 1.1 $\times$ 10^4 alpha particles/mm^2 min across the exposed area. The calculated average dose rate, based on a Monte-Carlo calculation (not shown) performed using the SRIM-2003 code,[21] was 0.28 Gy/min. The experiment was repeated twice, with each exposure step repeated in 3 dishes. Cell development was monitored daily, with Hemacolor staining[22] performed on the 5th day after plating. Dishes were subsequently photographed by a light microscope (Olympus IX50, DP70 camera). Cell survival was scored using Matlab image processing tools to count the total number of pixels darker than a specified threshold. The compiled data (surviving fraction f as a function of the calculated dose) was fitted, using Matlab's curve fitting tool, with an exponential function $f(D) = \exp(-D/D_0)$ to estimate D_0—the average dose required to reduce survival to 37%.

Animals

Male BALB/c mice (8–12 weeks old) were obtained from the breeding colony of Tel-Aviv University, Israel. Male athymic nude mice (8–12 weeks old) were purchased from Harlan Pharmacological and Biological Monitoring, Israel. Animal care and experimentation was carried out in accordance with Tel-Aviv University guidelines. All surgical and invasive procedures were held under anesthesia by intraperitoneal inoculation of imalgen (100 mg/kg) and xylazine hydrochloride (10 mg/kg) solution in 0.25 ml of PBS.

Tumor cell inoculation

Animals were inoculated intracutaneously with $5\cdot\times$ 10^5 SQ2 cells or 2 $\times$ 10^6 CAL 27 cells in 0.2-ml HBSS buffer (Biological industries, Israel) into the low lateral side of the back. Local tumor growth was determined by measuring 3 mutually orthogonal tumor diameters with a digital caliper. The volume of tumor was calculated using the formula: $V = (\pi/6)\, D_1 D_2 D_3$, where D_1, D_2, D_3 stand for the measured diameters. SQ2 Tumors reached a size of 3–4 mm after 6 days, 6–7 mm after 10 days and 10 mm after 16 days.

^{224}Ra wire preparation

^{224}Ra wires were prepared using a ^{228}Th generator (as described in detail in Ref. 13). In this setup, positive ^{224}Ra ions emitted by recoil from a surface layer containing ^{228}Th are electrostatically collected near the tip of a thin conducting wire (in this case, 0.3-mm stainless steel acupuncture needle [Golden Needle, China]). The wires are then heat-treated to induce radium diffusion away from the surface, to a typical depth of 10–20 nanometers. The ^{224}Ra-impregnated wires are then characterized by an alpha particle detector to account for their ^{224}Ra activity and release rate of ^{220}Rn. The sources used in the *in vivo* experiments had ^{224}Ra activities in the range 7–42 kBq, with ^{220}Rn desorption probabilities of 25–43%.

Wire insertion

Wires, either loaded with ^{224}Ra or inert, cut to a length of 3–6 mm, were placed near the tip of a 23G needle attached to a 2.5-ml syringe (Picindolor, Italy) and manipulated into the tumor by a plunger placed internally along the syringe axis.

Histology

Histological analysis was performed on SQ2 tumors, both treated and untreated. Immediately after their removal, tumors were fixed by a 4% formaldehyde solution for at least 24 hrs. The preserved specimens were embedded in paraffin, and sections (5–10 μm) were stained using hematoxylin-eosin (H&E) (Surgipath, Richmond, IL, USA) and analyzed for tissue damage detection. Quantification and assessment of necrotic domains were done by delineating the damaged areas obtained by the staining and calculating the areas of the resulting polygons using Matlab. Metastatic burden quantification in lung sections was done by summing the gray values of all the pixels in the sample divided by the number of pixels using image J free software (http://rsb.info.nih.gov/ij/).

Statistical analysis

The statistical significance (p-value) of the differences between tumor volumes in the various groups was assessed by applying Student's 2-sided t-test. Survival analysis (Mantel-Cox test) was carried out using Statsoft Statistica 7.0.

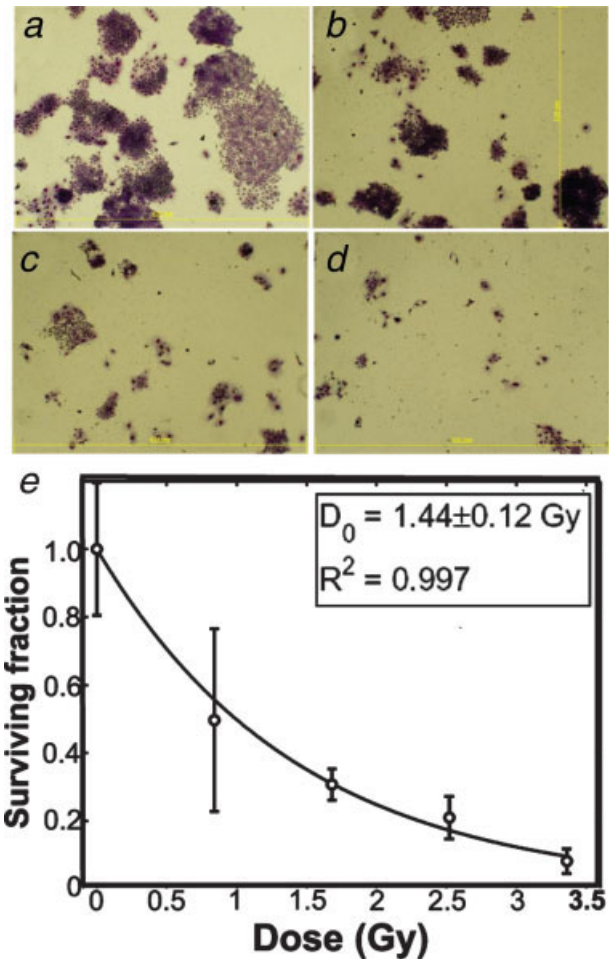

Figure 2 – SQ2 cell cultures exposed to alpha particles flux-cells (10^4) seeded in Kapton covered dishes: (*a*) 0 Gy, (*b*) 0.85 Gy, (*c*) 1.7 Gy, (*d*) 2.55 Gy. Images were analyzed for dye signal detection and the ratio between the unexposed average signals to irradiated groups is pronounced in (*e*) with fitted curve and standard deviations. R^2 represent goodness of fit. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

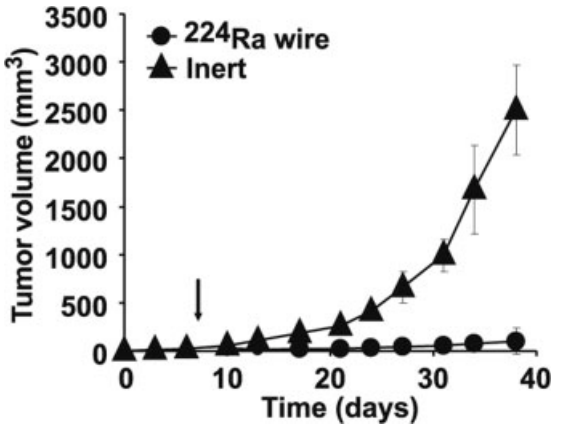

Figure 3 – SQ2 tumors of 3 mm in diameter treated by single DART insertion—Dart treatment applied to animals bearing 3-mm tumors (average diameter) and monitored for tumor growth. Inert: Tumor bearing mice treated with inert wires ($n = 8$). ^{224}Ra wire: Tumor bearing mice treated with DART wires ($n = 5$).

Results

Cell survival

These experiments were performed to verify that direct hit by alpha particles kill SCC tumor cells. Figure 2 shows a survival curve obtained in the *in vitro* experiments (SQ2 cells), fitted with the exponential $f(D) = \exp(-D/D_0)$, where D is the calculated dose and D_0 the sought parameter. The average dye signal detected in dishes exposed to 0.8 Gy was about 50% compared to control dishes, dropping to about 8% in dishes exposed to 3.4 Gy. The range of numerical values of D_0 (for different threshold levels used in the analysis) was 1.3–1.6 Gy. The R^2 value (representing the goodness of fit) was larger than 0.995 in all cases. To examine the proliferative capacity of the treated tumor cells, we also resuspended the cells and recultured them. It was observed that these cells were not able to proliferate even if transferred to fresh culture media. These findings led to the conclusion that when SCC cells are exposed to high LET radiation delivered by alpha particles they are killed.

Effect of a single ^{224}Ra wire on the development of SCC tumors in BALB/c mice

The effect of a single ^{224}Ra wire on tumor development and animal survival was studied in BALB/c mice bearing SQ2 tumors, in 3 lines of experiments: (*i*) An experiment assessing the effect of a single ^{224}Ra wire on small tumors (3–4 mm diameter) to evaluate the ability to destroy the whole tumor, (*ii*) A set of experiments in which SQ2 tumors were treated upon reaching a typical lateral diameter of 6–7 mm, (*iii*) A dose response experiment, where the effect of increasing ^{224}Ra wire activity on tumor development and life expectancy was investigated.

In the 1st line of experiments, randomized mice with 3–4 mm tumors were treated with a single ^{224}Ra wire (^{224}Ra wire, $n = 5$) or treated with a nonradioactive wire identical in shape to the radioactive ones (Inert, $n = 8$). In each group, tumor volumes and survival times were monitored. Out of the 5 tumors treated (with ^{224}Ra activities in the range 17–22 kBq and ^{220}Rn desorption probability of 24–33%), 4 tumors displayed temporary regression with 1 of the 4 completely eradicated (with no recurrence). Thirty-six days after treatment, the average volume of the treated tumors was 19-fold smaller than the inert wire group ($p < 0.001$, Fig. 3).

In the 2nd line of experiments, randomized mice with 6–7 mm tumors were either not treated (NT, $n = 20$), treated with a single ^{224}Ra wire (^{224}Ra wire, $n = 22$) or treated with a nonradioactive wire identical in shape to the radioactive ones (Inert, $n = 22$). In each of the 3 groups, tumor volumes and survival times were monitored. The results presented in Figure 4*a* indicate that while the inert wires had no effect on tumor volume, ^{224}Ra wires considerably retarded tumor development. The effect became evident 10 days after the treatment and became more pronounced as time passed. After 20 days, the average tumor volume of the ^{224}Ra group was 8 times smaller compared to untreated control group ($p < 0.01$ on Day 20). In about 22% of the ^{224}Ra treated mice, the primary tumor completely regressed, (*i.e.*, the observable tumor temporarily disappeared) and there was 1 case of complete cure with no tumor recurrence. Treatment by a single ^{224}Ra wire also considerably prolonged life expectancy as shown in Figure 4*b*. Thirty-five days after tumor inoculation, the survival rate of the 2 control groups, was less than 10%. In contrast, at the same time the survival rate of the ^{224}Ra treated mice, was above 90% ($p < 0.01$). The difference in survival between the 2 control groups on Day 35 (after inoculation) was statistically insignificant ($p = 0.87$). Figures 8*a* and 8*b* show photographs of a representative BALB/c mouse treated with a single Ra-224 wire, in which tumor eradication was observed, compared to a control mouse from the same experiment. Evidently small SQ2 tumors (3–4 mm diameter) were apparently more susceptible to treatment than 6–7 mm tumors.

The correlation between ^{224}Ra wire activity and observed effects on tumor volume and animal survival was studied in an

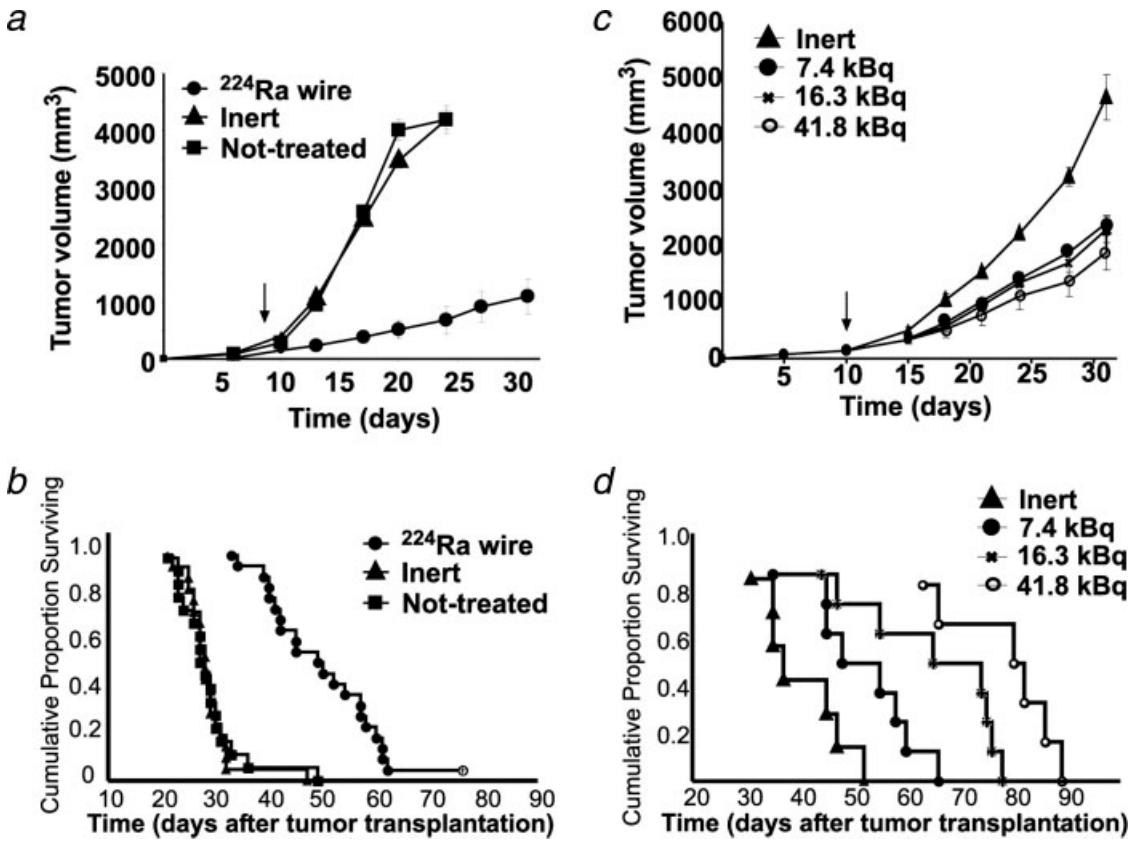

Figure 4 – Single DART wire experiments: BALB/c mice bearing SQ2 tumors, treated with a single DART wire and monitored for tumor growth and survival. (a-b) Nontreated: Nontreated tumor bearing mice ($n = 20$). Inert: Tumor bearing mice treated with inert wires ($n = 26$). ^{224}Ra wire: Tumor bearing mice treated with DART wires ($n = 22$). Treatment day varied between 7 and 10 days after tumor inoculation and was determined by the time tumors reached the size of 6–7 mm (average diameter). (*a*) Tumor development (standard errors are distinguished by bars). (*b*) Survival curve. (c-d) Dose-response curve—Inert: Tumor bearing mice treated with inert wires ($n = 7$). 7.4 KBq: Tumor bearing mice treated with DART wires delivering the average dose of 7.4 KBq ($n = 8$). 16.3 KBq: Tumor bearing mice treated with DART wires delivering the average dose of 16.3 KBq ($n = 8$). 41.8 KBq: Tumor bearing mice treated with DART wires delivering the average dose of K41.8 KBq ($n = 6$). (*c*) Tumor growth (treatment day is marked with an arrow and standard errors are distinguished by bars). (*d*) Survival curve.

experiment comprising 4 groups, treated with either inert wires ($n = 7$) or wires with increasing ^{224}Ra activities: 7.4 ± 1.1 kBq ($n = 8$), 16.3 ± 2.2 kBq ($n = 8$) and 41.8 ± 2.2 kBq ($n = 6$). The ^{220}Rn desorption probability was 28 ± 2% for all 3 groups. Tumors were treated upon reaching an average lateral diameter of 6–7 mm with a single ^{224}Ra wire each. Significant reduction in tumor volume was found in all ^{224}Ra treated groups compared with the group in which inert wires were used ($p < 0.001$ on Day 31 for all treated groups). There was a direct correlation between radiation intensity and tumor growth retardation, yet the differences were not statistically significant ($p = 0.71$, 0.195, 0.35) between successive groups (Fig. 4*c*). However, when examining life expectancy (Fig. 4*d*), increasing ^{224}Ra activities resulted in significantly prolonged survival ($p = 0.039$, 0.001, 0.021 between successive groups).

Histological assessment of necrotic damage caused by a single intratumoral ^{224}Ra wire

To determine the size of the destruction area caused by the radioactive wire, we used larger tumors to define the damage contour formed around a single ^{224}Ra wire. SQ2 tumors were transplanted in BALB/c mice and treated with a single ^{224}Ra wire when tumors reached the size of 1 cm (average diameter). Seven to 10 days later, tumors were removed and processed for histological examination. Additionally, 2 control groups (untreated and inert wires) underwent the same procedures.

Histological analysis of the ^{224}Ra-treated tumors revealed a widely spread pattern of necrosis around the wire insertion point (Fig. 5), while in the control samples small necrotic domains could be identified randomly. The radioactivity-induced damage spread out to the edge of the tumor up to 5 mm away from the ^{224}Ra wire, covering 14–59% of the total area of the tumors. Similar measurements on control specimens, showed very few necrotic domains (0–3%) that could not be attributed to the inert wires. Necrotic domains in the treated tumors displayed, in the majority of cases, a high degree of damage at the outer perimeter of the tumor.

Effect of double ^{224}Ra wire insertion on the development of squamous cell carcinoma in BALB/c mice

The patterns of necrosis observed in the histopathological analysis suggested that damage to remote parts of the tumor might be a result of either directional distribution of radioactive atoms through the blood or disrupted blood supply to these areas due to destruction of blood vessels at the base of the tumor, or both.

To examine the possible role of the blood flow on tumor destruction, 2 ^{224}Ra wires were inserted horizontally at the base of the tumor. Applying 2 wires per treatment resulted in extensive reduction in tumor volumes (45-fold compared to the controls 29 days after treatment), with complete primary tumor eradication in 10 out of 14 animals and no recurrence in 5 out of the 10 animals (Fig. 6).

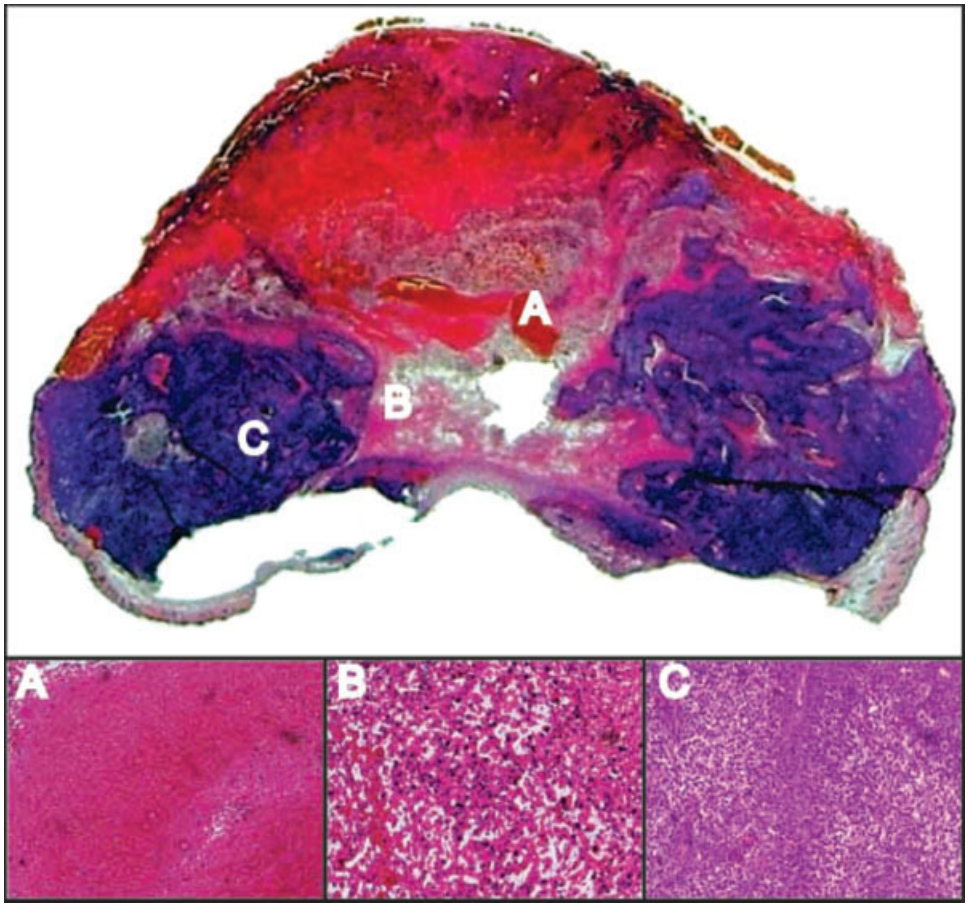

FIGURE 5 – Tissue damage applied by DART wires-H&E cross section of SQ2 tumor treated by a single DART wire at its center. Three fields' enlargements (×10) are presented below: a necrotic region nearby the wire location (*a*), a region distant 3.5 mm from the wire (*b*) and a viable region distant 7 mm from the wire (*c*).

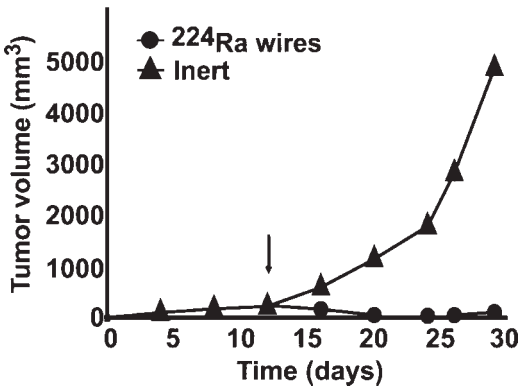

FIGURE 6 – Double DART insertion: 2 DART wires inserted to the low basis of each SCC tumor, to the proximity of the tumor bed. Inert: Tumor bearing mice treated with inert wires ($n = 14$). ^{224}Ra wire: Tumor bearing mice treated with DART wires ($n = 14$). Treatment day is marked with an arrow. Standard errors are distinguished by bars.

Effect of a single intratumoral ^{224}Ra wire on the development of human squamous cell carcinoma in nude mice

The next level was to investigate the effect of ^{224}Ra wires on SCC tumors derived from a human cell line. Nude mice inoculated with human-derived CAL 27 tumor cells were treated with either ^{224}Ra or inert wires. ^{224}Ra wires had a pronounced effect on tumor development. Fifteen days post treatment, the average volume of the ^{224}Ra treated tumors ($n = 10$) was 72–80% smaller than that of the control group ($n = 10$). These results are shown in Figure 7 and were consistent in 2 separate experiments that differed by the initial average tumor size at the day of the treatment (6 and 8–9 mm). Figures 8*c* and 8*d* depict photographs of a representative nude mouse treated with a single ^{224}Ra wire, in which tumor eradication was observed, as opposed to an untreated mouse.

Spread of lung metastases in BALB/c mice bearing SQ2 tumors, treated with intratumoral ^{224}Ra wires

Histological assessment of lung sections was conducted to question the effect of the destruction of the primary tumor by DART wires on the development of metastases. Tumor bearing BALB/c mice were divided into 2 groups containing 5 animals each. The 1st group was treated with intratumoral insertion of 2 inert wires (*Inert*) while the 2nd group received an intratumoral insertion of 2 ^{224}Ra wires (*^{224}Ra wires*). Twenty-six days later animals were sacrificed and lungs were harvested and processed for histological analysis in respect to normal lung tissues taken from healthy BALB/c mice (*Normal*). Figure 9 describes the inhibition of lung metastatic load in ^{224}Ra wires treated mice when compared to lungs of mice treated with inert wires. A small increase in the lung metastatic burden was detected in the samples of DART treated animals as shown in Figure 9*c* when compared to healthy lungs (Fig. 9*d*). Evidently, lung samples of inert treated animals were almost completely full of metastatic cells (Fig. 9*b*), and there was a 6-fold increase in the metatstatic burden compared to DART treated animals (Fig. 9*a*).

Discussion

In the present study we examined the *in vitro* and *in vivo* antitumoral effects associated with the release of the alpha emitting daughters of ^{224}Ra into solid tumors. The results of this work provide evidence that ^{224}Ra loaded DART wires considerably re-

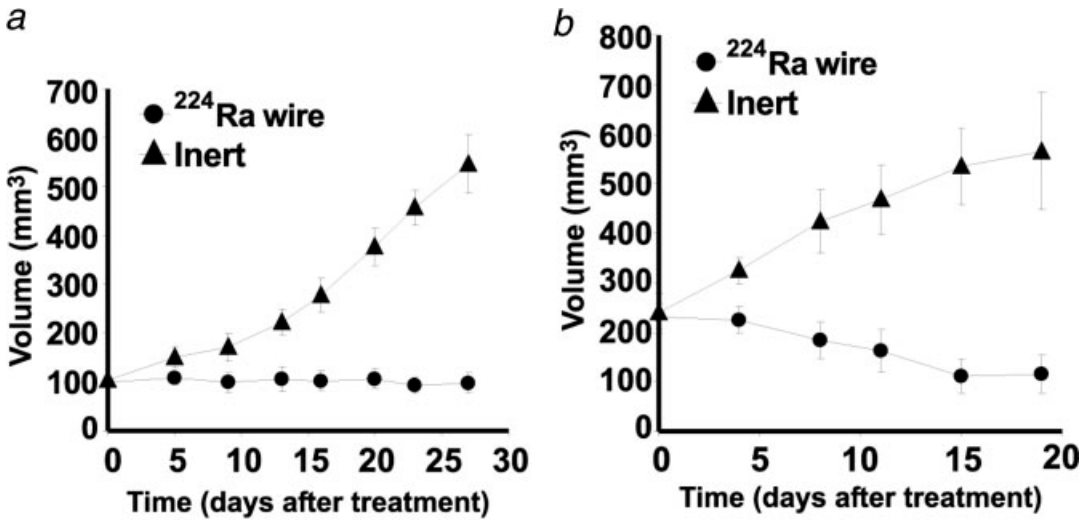

FIGURE 7 – Single DART wire insertion into human SCC tumors—Nude mice bearing human SCC tumors, treated with a single DART wire and monitored for tumor growth. Inert: Tumor bearing mice treated with inert wires. ^{224}Ra wire: Tumor bearing mice treated with DART wires. (*a*) Treatment day occurred 18 days after tumor inoculation and was determined by the time tumors reached the size of 6–7 mm (average diameter). (*b*) Treatment day occurred 29 days after tumor inoculation and was determined by the time tumors reached the size of 8–9 mm (average diameter). For both experiments, each group contained 10 animals (standard errors are distinguished by bars).

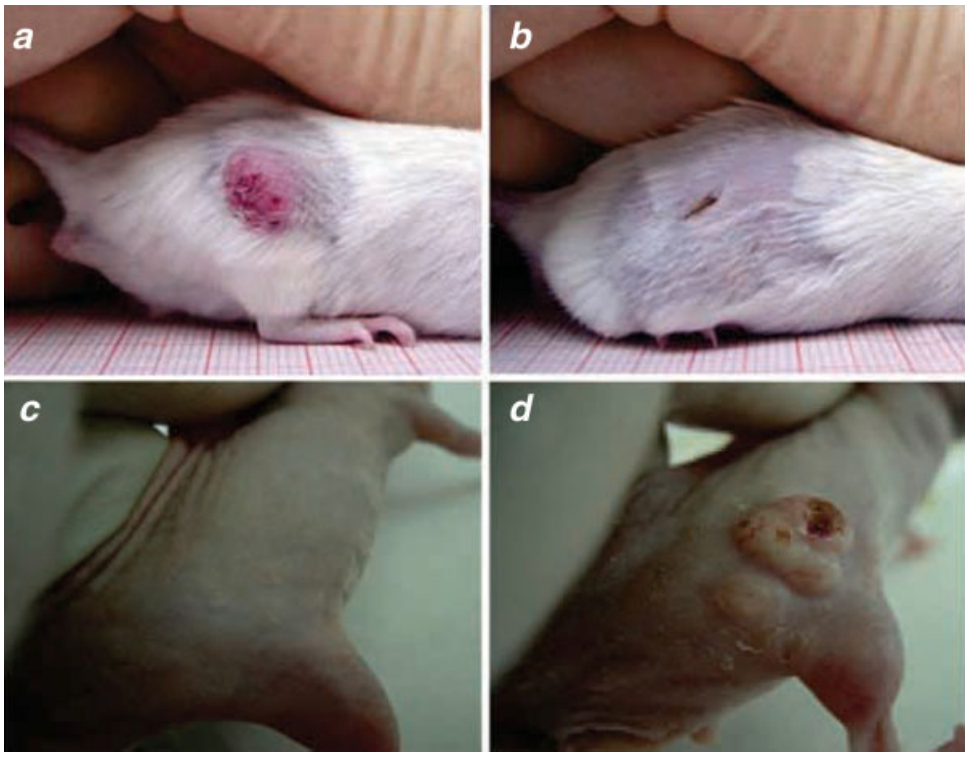

FIGURE 8 – Nontreated and treated animals: (*a*) nontreated BALB/c mouse 14 days posttumor transplantation; (*b*) ^{224}Ra-wire treated animal 14 days posttumor transplantation (30 KBq); (*c*) ^{224}Ra-wire treated nude mouse 45 days posttumor transplantation (24.6 KBq); (*d*) untreated nude mouse 45 days posttumor transplantation. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

tarded tumor development and prolonged life expectancy in the experimental SCC models studied.

The *in vitro* experiments described indicate that the proliferative capability of SQ2 cells is considerably inhibited by the passage of only a few alpha particles through each nucleus. On the basis of the calculated value for D_0, the surviving fraction at 2 Gy (SF2) is between 0.2 and 0.3, about 1.5–2-fold lower than typical SF2 values for human SCC cells.[23] These findings were supported by XTT assays testing irradiated cells seeded in fresh nonirradiated dishes (data not shown).

The insertion of a single DART source to the center of 6–7 mm murine SCC tumors had a pronounced retardation effect on tumor growth, leading, in over 45% of the cases to a scope of responses varying from temporary regression to complete eradication and no recurrent tumor. This is in line with the physical and histological results reported in our previous work,[13] which demonstrated the formation of a 5–7 mm region characterized by a high radiation dose (>10 Gy from source insertion to infinity) and extensive cell death about each wire. The effect was considerably more pronounced for smaller tumors (3–4 mm diameter), where the average volume of the treated tumors was 19-fold smaller than that of the controls 5 weeks after treatment. The spread of radioactive atoms may be a result of diffusion and convection through the blood, as indicated by the findings that ^{212}Pb atoms were detectable in peripheral blood and in major organs.[13]

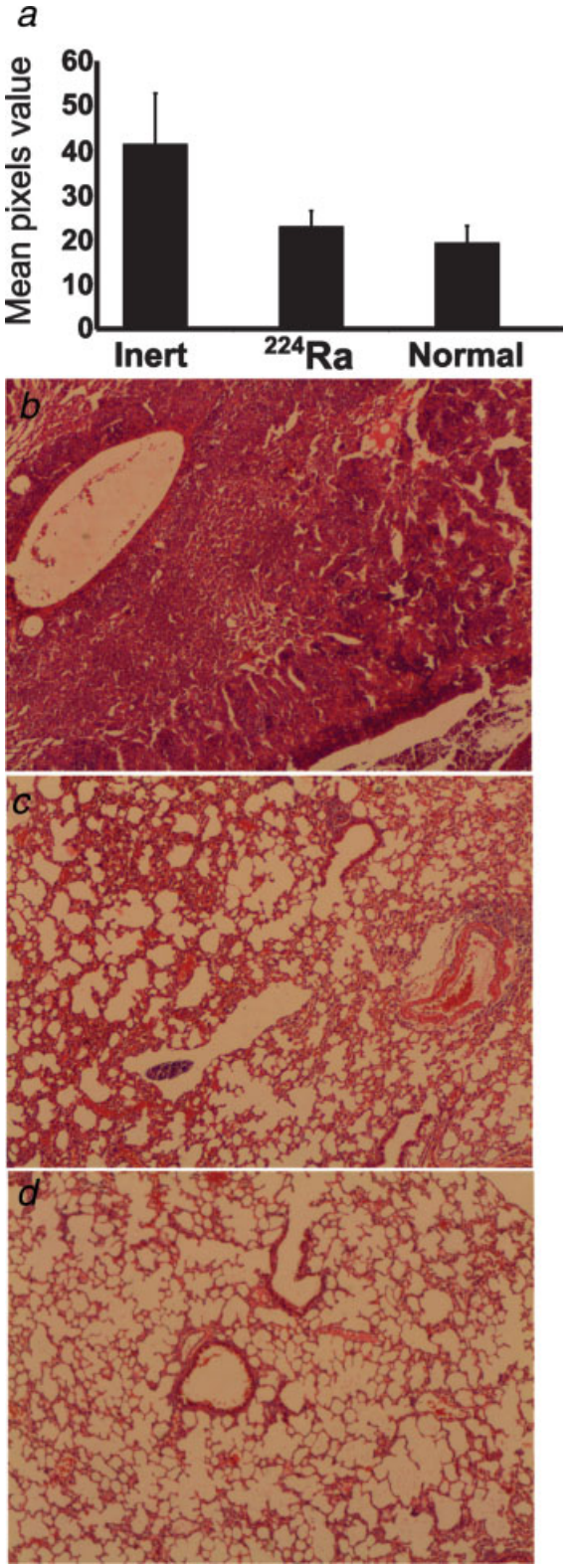

FIGURE 9 – Metastatic lung burden-H & E cross lung section analyzed by image J for the assessment of the presence of metastases. (*a*) Ratio between normal healthy lung sections (Normal) and section of animals treated with either radioactive wires (^{224}Ra) or nonradioactive wires (Inert). (*b*) lung section of inert treated mouse. (*c*) lung section of ^{224}Ra treated mouse. (*d*) Lung section of healthy normal mouse. All sections are in ×10 magnitude. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The impact on tumor volume increased moderately with increasing wire activity in the range 7.4–41.8 kBq (as demonstrated in the dose-response experiment). This is consistent with our physical measurements,[13] which indicated that the size of high-dose region rises steeply for source ^{224}Ra activities in the range of 0–10 kBq, but then continues to grow at a much milder slope with increasing source strength.

The observed effect on tumor development was dramatically enhanced when 2 DART wires were applied to 6–7 mm diameter tumors. One possible explanation for this observation is that the dispersion of radioactive atoms throughout the tumor volume was enhanced by a directional blood flow originating from the tumor bed (*i.e.*, the atoms were carried "downstream" by the blood flow into the tumor). The proximity of the wires to the tumor bed may have also disrupted the blood supply to farther regions inside the tumor. Both possibilities are consistent with the necrosis patterns observed in the treated tumors, which seemed to be "fanning out" from the bottom of the tumor toward its periphery.

The effect on tumor development was preserved when shifting from murine to human-derived SCC tumors. Up to 80% of the treated tumors regressed during the 1st 2 weeks after treatment, and 30% disappeared either temporarily or permanently during this period.

^{224}Ra wires administrated into the primary tumors led to dramatic prolongation of life expectancy (Figs. 4*b* and 4*d*). Since calibration studies indicated that excision of primary SQ2 cell-derived tumors resulted in lung metastases-related death of ~80–85% of the animals,[19] we assumed that radioactive treatment reduced metastatic spread and thus prolonged survival. Therefore, we examined the metastatic burden in the lungs of the treated mice, and found that ^{224}Ra wires treatment resulted in a significant reduction of 83% in the lung metastatic load compared to those of the inert treated animals. This reduced metastatic load may result from either vascular injury, as was also found for high-LET radiation,[24,25] or that higher dose levels caused larger necrotic areas, hence, lowered the number of viable cells able to metastasize or both. This is evidently a key question that deserves extensive additional experimental work.

Our results indicate that Diffusing Alpha-emitters Radiation Therapy could be used to cause intense damage to SCC primary tumors and may even lead to complete tumor destruction. In an era of improving systemic therapy, local control has become very important. Thus, the efficacy of this therapeutic strategy in experimental systems of malignant tumors that simulate clinical situations forms a basis for evaluating clinical relevance and for the development of an appropriate treatment of human cancer.

When considering the application of DART to human patients, the hazards involved in the use of alpha radiation should be considered. In our previous work,[13] we observed that a considerable fraction of the ^{212}Pb atoms (~30–50% for 1 g tumors, ~10% for 2.5 g tumors) released by the wire leaves the tumor probably through the blood and is absorbed in major organs, primarily the kidneys. In this respect, meaningful insight may be gained by considering that patients suffering from ankylosing spondylitis, received intravenously a total of 10 MBq of ^{224}Ra (1 MBq weekly over 10 consecutive weeks), with the entire quantity of ^{224}Ra, along with its daughters, allowed to circulate in the body. Studies including a large number of patients (>1,500) over about 30 years did not reveal damaging effects caused by those ^{224}Ra treatments.[26,27] In our proposed treatment, we expect the total administered ^{224}Ra activity to be of the order of a few MBq, with the entire ^{224}Ra activity confined to the wire. The only elements that will enter the bloodstream are ^{212}Pb and ^{212}Bi and the maximal dose to healthy organs is expected to be of the order of a few cGy.

To conclude, the DART modality combines the advantages of local intratumoral irradiation with the high killing efficacy of cancer cells by alpha particles. The introduction of DART to the treatment of cancer may add a powerful and safe tool, permitting the utilization of alpha particles in the treatment of solid tumors.

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A patent was filed by the Tel Aviv University on this treatment modality. Most of the authors are shareholders in the company (T.C., L.A., M.S., I.K., Y.K.) and have served as CTO (I.K.) or consultants (L.A., Y.K.).

References

1. Williamson JF. Brachytherapy technology and physics practice since 1950: a half-century of progress. Phys Med Biol 2006;51:R303–R325.
2. Sailer SL. Radiation therapy for prostate cancer: external beam, brachytherapy, and salvage. N C Med J 2006;67:149–53.
3. Kaufman SA, DiPetrillo TA, Wazer DE. Accelerated partial breast irradiation: current modalities and investigations. Med Health R I 2006;89:70–3.
4. Grimard L, Esche B, Lamothe A, Cygler J, Spaans J. Interstitial low-dose-rate brachytherapy in the treatment of recurrent head and neck malignancies. Head Neck 2006;28:888–95.
5. Pellizzon AC, dos Santos Novaes PE, Conte Maia MA, Ferrigno R, Fogarolli R, Salvajoli JV, Kowalski LP. Interstitial high-dose-rate brachytherapy combined with cervical dissection on head and neck cancer. Head Neck 2005;27:1035–41.
6. Roeske JC, Stinchcomb TG. The average number of α-particle hits to the cell nucleus required to eradicate a tumour cell population. Phys Med Biol 2006;51:N179–N186.
7. Beyer GJ, Miederer M, Vranjes-Duric S, Comor JJ, Kunzi G, Hartley O, Senekowitsch-Schmidtke R, Soloviev D, Buchegger F. Targeted α therapy in vivo: direct evidence for single cancer cell kill using ^{149}Tb-rituximab. Eur J Nucl Med Mol Imaging 2004;31:547–54.
8. Bethge WA, Sandmaier BM. Targeted cancer therapy using radiolabeled monoclonal antibodies. Technol Cancer Res Treat 2005;4:393–405.
9. Lewington VJ. Bone-seeking radionuclides for therapy. J Nucl Med 2005;46 (Suppl 1):38S–47S.
10. Jurcic JG, Larson SM, Sgouros G, McDevitt MR, Finn RD, Divgi CR, Ballangrud AM, Hamacher KA, Ma D, Humm JL, Brechbiel MW, Molinet R, Scheinberg DA. Targeted α particle immunotherapy for myeloid leukemia. Blood 2002;100:1233–9.
11. Jurcic JG. Immunotherapy for acute myeloid leukemia. Curr Oncol Rep 2005;7:339–46.
12. Kairemo KJ. Radioimmunotherapy of solid cancers: a review. Acta Oncol 1996;35:343–55.
13. Arazi L, Cooks T, Schmidt M, Keisari Y, Kelson I. Treatment of solid tumours by interstitial release of recoiling short-lived α emitters. Phys Med Biol 2007;52:5025–42.
14. Chung CH, Levy S, Yarbrough WG. Clinical applications of genomics in head and neck cancer. Head Neck 2006;28:360–8.
15. Seiwert TY, Cohen EE. State-of-the-art management of locally advanced head and neck cancer. Br J Cancer 2005;92:1341–8.
16. Reyes Lopez V, Navalpotro Yague B. Adjuvant treatment of locally-advanced head and neck tumours. Clin Transl Oncol 2005;7:183–8.
17. Dobrossy L. Epidemiology of head and neck cancer: magnitude of the problem. Cancer Metastasis Rev 2005;24:9–17.
18. Gunderson LL. Rationale for and results of intraoperative radiation therapy. Cancer 1994;74:537–41.
19. Blank M, Lavie G, Mandel M, Hazan S, Orenstein A, Meruelo D, Keisari Y. Antimetastatic activity of the photodynamic agent hypericin in the dark. Int J Cancer 2004;111:596–603.
20. Gioanni J, Fischel JL, Lambert JC, Demard F, Mazeau C, Zanghellini E, Ettore F, Formento P, Chauvel P, Lalanne CM. Two new human tumor cell lines derived from squamous cell carcinomas of the tongue: establishment, characterization and response to cytotoxic treatment. Eur J Cancer Clin Oncol 1988;24:1445–55.
21. Ziegler JF. Stopping and ranges in matter (SRIM). Available at http://www.srim.org.
22. Keisari Y. A colorimetric microtiter assay for the quantitation of cytokine activity on adherent cells in tissue culture. J Immunol Methods 1992;146:155–61.
23. Hoffmann W, Blase MA, Santo-Hoeltje L, Herskind C, Bamberg M, Rodemann HP. Radiation sensitivity of human squamous cell carcinoma cells in vitro is modulated by all-trans and 13-cis-retinoic acid in combination with interferon-α. Int J Radiat Oncol Biol Phys 1999;45:991–8.
24. Milliat F, Francois A, Isoir M, Deutsch E, Tamarat R, Tarlet G, Atfi A, Validire P, Bourhis J, Sabourin JC, Benderitter M. Influence of endothelial cells on vascular smooth muscle cells phenotype after irradiation: implication in radiation-induced vascular damages. Am J Pathol 2006;169:1484–95.
25. Thomas PA, Tracy BL, Ping T, Wickstrom M, Sidhu N, Hiebert L. Relative biological effectiveness (RBE) of 210Po α-particles versus X-rays on lethality in bovine endothelial cells. Int J Radiat Biol 2003;79:107–18.
26. Lassmann M, Nosske D, Reiners C. Therapy of ankylosing spondylitis with ^{224}Ra-chloride: dosimetry and risk considerations. Radiat Environ Biophys 2002;41:173–8.
27. Nilsson S, Larsen RH, Fossa SD, Balteskard L, Borch KW, Westlin JE, Salberg G, Bruland OS. First clinical experience with α-emitting radium-223 in the treatment of skeletal metastases. Clin Cancer Res 2005;11:4451–9.