# Local control of experimental malignant pancreatic tumors by treatment with a combination of chemotherapy and intratumoral $^{224}$Radium-loaded wires releasing alpha-emitting atoms

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**We developed $^{224}$Ra-loaded wires that when inserted into solid tumors, release radioactive atoms that spread in the tumor and irradiate it effectively with alpha particles (diffusing alpha-emitters radiation therapy (DaRT)). In this study, we tested the ability of intratumoral $^{224}$Ra-loaded wires to control the local growth of pancreatic tumors and the enhancement of this effect by chemotherapy. Pancreatic mouse tumors (Panc02) were treated with $^{224}$Ra-loaded wire(s) with or without gemcitabine. The tumor size and survival were monitored, and autoradiography was performed to evaluate the spread of radioactive atoms inside the tumor. Mouse and human pancreatic cancer cells, irradiated *in vitro* by alpha particles with or without chemotherapy, were evaluated for cell growth inhibition. The insertion of $^{224}$Ra-loaded wires into pancreatic tumors in combination with gemcitabine achieved significant local control and was superior to each treatment alone. A dosimetric analysis showed the spread of radioactive atoms in the tumor around the wires. Alpha particles combined with gemcitabine or 5-FU killed mouse and human cells *in vitro* better than each treatment alone. DaRT in combination with gemcitabine was proven effective against pancreatic tumors *in vivo* and *in vitro*, and the process may be applicable as a palliative treatment for patients with pancreatic cancer. (Translational Research 2012;159:32–41)**

**Abbreviations:** 5-FU = 5-fluorouracil; DaRT = diffusing alpha-emitters radiation therapy; DSB = double strand breaks; DMEM = Dulbecco's modified eagle medium; LET = linear energy transfer; OD = optical density; PBS = phosphate-buffered saline; SCC = squamous cell carcinoma; SSB = single stranded break

Alpha radiation is a high linear energy transfer (high-LET) radiation, which can serve as an attractive alternative to photon or electron-based radiation treatments (energy in the range of 6–9 MeV and LET of 100–200 keV/$\mu$m).[1] The efficacy of alpha particles against cancer cells is well established. Typically, a single alpha particle hit to the nucleus has a 20% to 40% probability of killing the cell, and only a few hits are required to ensure cell lethality, mostly because of complex, irreparable, DNA double strand breaks (DSBs).[2,3] In addition, unlike photons or electrons, the effect of alpha radiation is largely

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## AT A GLANCE COMMENTARY

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#### Background

We envision that the alpha radiation-based diffusing alpha-emitters radiation therapy (DaRT) treatment will be used to destroy primary tumors and large metastatic foci. In the case of disseminated cancer, it will be combined with chemotherapy or other agents to affect regional and distant metastases.

#### Translational Significance

A clinical indication considered for the use of DaRT is the palliative treatment of pancreatic cancer. Pancreatic cancer involves fast growing, metastatic tumors, and in many cases, it is inoperable. Intratumoral radiation of the tumor by alpha particles may increase local tumor control and reduce colateral damage caused by conventional radiation therapy.

insensitive to the cell oxygenation state, making hypoxic cells just as vulnerable to treatment as cells with normal oxygen levels. The short range of alpha particles in tissue (40–90 $\mu$m) ensures that cells lying outside of the targeted region are spared, provided that the alpha-emitting atoms are brought to the immediate vicinity of the target cancer cell.

Currently, alpha radiation is used in radioimmunotherapy,[1] targeting single cancer cells or small cellular clusters, and in $^{223}$Ra-based palliative treatments for skeletal metastases in breast and prostate cancer patients, relying on the bone-seeking properties of radium.[4] Thus, although alpha radiation is a highly lethal form of radiation, it has a limited use for treatment of solid tumors because of its short range in tissue.

In previous publications, we presented a practical solution that potentially allows the treatment of the entire tumor volume with this short-range radiation using intratumoral wires, carrying radium-224 ($^{224}$Ra) atoms fixed below their surface. As $^{224}$Ra decays it releases, by recoil, its short-lived daughter atoms into the tumor. These spread inside the tumor, delivering, through their alpha decays, cytotoxic dose levels in a region measuring several millimeters about each wire. We termed this treatment diffusing alpha-emitters radiation therapy (DaRT).[5]

Once the wire has been inserted into the tumor and for as long as it stays active in it, (its activity falls exponentially by a factor of 1000 within a few weeks), radioactive atoms are released and spread into the biologic environment. The region accessible to these atoms is irradiated effectively by alpha particles causing tissue damage, local tumor destruction, and prolongation of survival of animals bearing squamous cell and lung derived tumors.[5-8]

Pancreatic cancer is the fourth leading cause of cancer deaths in the developed world. Six percent of the cancer death cases are a result of pancreatic cancer, in both genders.[9,10] Surgical resection is the only potentially curative treatment for patients with pancreatic cancer, although many patients are not candidates for resection. External beam gamma radiation, chemotherapy, mainly gemcitabine and 5-FU, immunotherapy, and other therapies are also used to treat patients with pancreatic cancer.[11-15] Several studies have shown that both survival rate and palliative benefit can be improved when radiotherapy is combined with chemotherapy.[12,16,17]

The obvious need for novel treatment modalities for pancreatic cancer prompted us to test in this study the ability of intratumoral $^{224}$Ra-loaded wires to control the local growth of experimental pancreatic tumors and the enhancement of this effect by combining the treatment with chemotherapy.

## MATERIALS AND METHODS

**Tumors cells.** Panc02 cells (murine pancreatic carcinoma) and MIA PaCa (human derived pancreatic carcinoma) were grown in Dulbecco's modified eagle medium (DMEM; GIBCO, Carlsbad, Calif) supplemented with 10% fetal calf serum (Biological Industries, Kibbutz Beit-Haemek, Israel), L-glutamine (2 mmol/L), penicillin (100 U/mL), and streptomycin (100 $\mu$g/mL) sodium pyruvate (1 mmol/L), nonessential amino acid solution (1%). The cell lines were stored in a humid incubator at a temperature of 37°C, $CO_2$ 7%. The doubling time of Panc02 cells is 17.2 $\pm$ 0.4 h.

**Anticancer chemotherapy drugs.** The nucleoside analog, gemcitabine (2′,2′-difluoro-2′-deoxycytidine; dFdC) (Gemzar; Eli Lilly and Co., Indianapolis, Ind) was dissolved in phosphate-buffered saline (PBS) and administrated *in vivo* at a concentration of 60 mg/kg by a single injection into the mice tail vein.[18]

The antimetabolite, 5-Fluorouracil (5-FU) (Ebewe Pharma, Unterach am Attersee, Austria), was dissolved in PBS for use.

***In vitro* assays for tumor cell damage. In vitro *clonogenic assay using Kapton wells setup.*** The effect of alpha particles on the ability of Panc02 cells to proliferate was studied using a broad beam $^{228}$Th irradiator as described in reference 7. The cells were seeded on a Kapton foil at a density of $2.5 \times 10^5$ cells/well, and after 24 h the cells were exposed to alpha particles for short periods of 0, 1, 2, 3, 4 and 6 min at an average dose rate of approximately 0.6 Gy/min as described.[7]

Immediately after irradiation in Kapton wells, the cells were harvested using trypsin (0.25% trypsin and 0.05% ethylene diamine tetraacetic acid disodium salt solution, stored at 4°C; Biological Industries). Their viability was examined by the colony formation assay as described.[8]

***Effect of alpha particles without or with chemotherapy on the viability of cells in culture.*** Panc02 ($5 \times 10^3$/well) and MIA PaCa ($10^4$/well) cells were seeded in microplates implanted with escalating $^{224}$Ra activities (radioactive microplates were prepared as described in Reference 7), ranging from 0.06 to 2 Bq/mm$^2$ and were grown for 48 h. When the combination of alpha particles and chemotherapy was tested, Panc02 and MIA PaCa cells ($10^4$ cells/well) were seeded in radioactive 96-well plates in 100 $\mu$L DMEM medium for each well. After 30–60 min, the chemotherapy was added in 100 $\mu$L DMEM and the plates incubated for an additional 48 h. The wells seeded with cells treated with radioactive or chemotherapy alone or nontreated served as controls.

At the end of the incubation, the remaining adherent viable cells were fixed and stained by hemacolor reagents, and the plate was measured with a microplate reader at 630 nm.[19] Viability was expressed as the ratio between the measured optical density (OD) of irradiated cells and the average OD of the nonirradiated controls (% viability = [OD treatment/OD control] × 100).

Cell growth inhibition was calculated using the following formula:

$$\text{cell growth inhibition } (\%) = 100 - \%\text{viability}$$

## *IN VIVO* EXPERIMENTS

**Animals.** Female C57BL/6 mice (8–12 weeks old) were obtained from the breeding colony of Tel-Aviv University, Israel. Animal care and experiments were carried out in accordance with the guidelines of the Israeli National Council for Animal Experimentation (permit no. M-05-082).

**Tumor cell inoculation.** Subcutaneous tumors were induced by i.d injection of $10^5$ Panc02 cells in 0.1 mL Hanks balanced salt solution buffer (Biological Industries) into the low lateral side of the mice back. Tumors appeared after 3 weeks. Local tumor growth was determined by measuring 3 mutually orthogonal tumor diameters with a digital caliper. The volume of the tumor was calculated using the formula: $V = D1 \times D2 \times D3 \times (\pi/6)$, where $D1$, $D2$, and $D3$ stand for the 3 mutually orthogonal tumor diameters.

**$^{224}$Ra-loaded wire preparation.** $^{224}$Ra-loaded wires were prepared using a $^{228}$Th generator as described in detail.[5] In this application, positive $^{224}$Ra ions emitted by alpha decay induced recoil from a surface thinly coated with $^{228}$Th were collected electrostatically on stainless steel wires (3–5 mm long and 0.3 mm diameter; Wujiang Jia Chen Acupuncture Device Co., Wujiang, China). The collected radium ions are situated on the surface of the wire and would be quickly washed away if brought in direct contact with live tissue fluids. To prevent this, the wires were subjected to heat treatment (450°C for approximately 1 h) so as to induce the diffusion and the intercalation of the radium ions in the solid matrix of the wire. The processed wires were measured by solid-state detector alpha counting system to determine the total $^{224}$Ra activity and the desorption probability of the $^{220}$Rn daughters. The retention of the radium on the wires throughout the treatment was verified *in vivo* by counting the tumor activity through a collimated Geiger counter. Alternatively, the wires removed from tumors were found to contain essentially the initial $^{224}$Ra activity, reduced by the temporal radioactive decay. Note that failing to perform the heat treatment resulted indeed in quick loss of activity from the wire and its removal from the tumor. The wires were inserted into the tumors a short time (typically 1 h) after their preparation.

**DaRT wire implantation.** Immediately after a DaRT wire was prepared and its activity was measured ($^{224}$Ra activity 16.8-43 kBq), it was implanted as described previously.[7]

**Anesthesia.** Intraperitoneal inoculation of 0.25 mL (solution in PBS) of anesthetic compound (100 mg/kg imalgen + 10 mg/kg xylazine hydrochloride solution) was given 10 min before starting the treatment. All surgical and invasive procedures were carried out under anesthesia.

**Autoradiography and histology of treated tumors.** The intratumoral radionuclide ($^{212}$Pb) spread was measured by autoradiography in 8 Panc02 tumors, each treated with a single DaRT wire ($^{224}$Ra activity ranging from 14 to 67 kBq, $^{220}$Rn desorption probability 25 % to 44%). The tumors were excised 3–4 days after wire insertion. In each case, the wire was extracted from the tumor 10–15 min after its excision, and the excised tumor was subsequently processed and analyzed as described.[5,8] Tumor samples were also stained with hematoxylin-eosin (Surgipath, Richmond, Ill) and analyzed for tissue damage as described.[6]

**Statistical analysis.** The statistical significance ($P \leq 0.05$) of the differences between volumes of tumors in the various groups was assessed by applying analysis of variance with repeated measures, by SPSS software (SPSS Inc, Chicago, Ill). The survival times plotting (Kaplan-Meir test) and survival comparison between

groups (Mantel-Cox test) were carried out by using StatSoft "Statistica" statistical software (StatSoft Inc, Tulsa, Okla).

## RESULTS

**Survival curves of Panc02 cells.** To estimate the sensitivity of Panc02 cells to alpha radiation, the cells were exposed to a flux of alpha particles when seeded on Kapton foil, and their clonogenicity was measured. The results of 2 experiments performed on Panc02 cells are shown in Fig 1. In both experiments, each data point represents an average value of 3–4 different cell samples irradiated in different Kapton foil wells. The surviving fraction was calculated as the ratio between the number of viable colonies in a given petri dish (containing irradiated cells) and the average number of colonies in the control dishes. As it is commonly done for high-LET radiation over the dose range we studied, the data were fitted with the function $f(D)=e^{-D/D_0}$ using Matlab's curve fitting tool to estimate $D_0$ (the mean lethal dose), yielding $D_0 = 1.2 \pm 0.1$ Gy for the first experiment and $D_0 = 1.1 \pm 0.1$ Gy for the second.

The fit to Exp1 was less ideal compared with that of Exp2 because of the larger error bars in this experiment. A more general survival curve, such as $e^{-\alpha \cdot D-\beta \cdot D^2}$, is possible and better fits the data with $\alpha$ and $\beta$ values of $0.35 \pm 0.15$ Gy$^{-1}$ and $0.23 \pm 0.10$ Gy$^{-2}$, respectively.

**Alpha radiation impact on Panc02 and MIA PaCa cells *in vitro*.** To examine the sensitivity of pancreatic cancer cells to alpha radiation, the cells (Panc02 or MIA PaCa) were seeded for 48 h in 96-well plates preimplanted with $^{224}$Ra atoms, and the cell growth was assessed as described in the Materials and Methods.

Compared with nontreated cells, cell proliferation was inhibited in direct correlation with the increase in the $^{224}$Ra activity, for both cell lines, Panc02 and MIA PaCa (Fig 2). At the highest level of activity (2 Bq/mm$^2$), inhibition rates of 75% (Panc02 cells) and 35% (MIA PaCa cells) were measured.

**Cytotoxic effects of alpha radiation combined with gemcitabine or 5-FU.** To examine the cytotoxic effect of combined chemotherapeutic agents and radiation, Panc02 cells and MIA PaCa cells were exposed to alpha radiation in the presence of gemcitabine or 5-FU at submaximal cytotoxicity concentrations. Gemcitabine was used at a concentration of 0.001 $\mu$g/mL and 5-FU at 0.5 $\mu$mol/L (calibration data not shown).

Panc02 cells treated with either 5-FU (0.5 $\mu$mol/L) or with radiation (0.2 Bq/mm$^2$), demonstrated 26% and 15% cell growth inhibition rates, respectively (Fig 3). The combined treatment with 5-FU (0.5 $\mu$mol/L) and radiation (0.2 Bq/mm$^2$) achieved 41% cell growth inhibition.

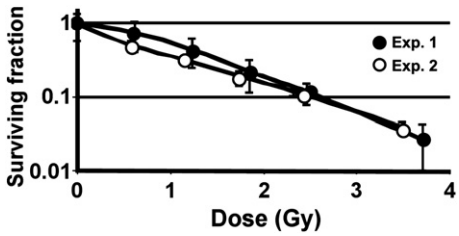


**Fig 1.** Survival curves of Panc02 cells exposed to alpha particles emitted from a sealed $^{228}$Th source. The curves represent data from 2 different experiments (3–4 repetitions at each dose level, in each experiment). The data were fitted with the function $f(D)=e^{-D/D_0}$ and the resulting values for $D_0$ are shown in the legend. The error bars represent standard deviations.

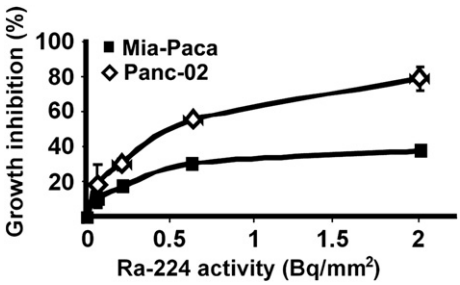


**Fig 2.** Inhibition of tumor cell growth by alpha particles. Tumor cells ($5 \times 10^3$ Panc02, and $10^4$ MIA PaCa) were incubated for 48 h in a radioactive plate. Cell growth was monitored by staining the adherent cells.

Treatment of panc02 cells with either Gemzar (0.001 $\mu$g/ml) or alpha radiation (0.2 Bq/mm$^2$) alone resulted in 13% and 19% cell growth inhibition, respectively. A combination of the 2 treatments increased the level of cell growth inhibition to 31%.

Incubation of MIA PaCa cells for 72 h with either Gemzar (0.001 $\mu$g/mL) or radiation (0.63 Bq/mm$^2$) alone resulted in 23% and 26% cell growth inhibition, respectively. The combination of the 2 cytotoxic modalities resulted in 39% cell growth inhibition (results not shown).

***In vivo* studies to determine the effects of $^{224}$Ra wires on pancreatic tumors.** *In vivo* experiments were performed to examine the efficacy of radioactive $^{224}$Ra wires in causing an antitumoral effect and local control of pancreatic carcinoma tumors in C57BL/6 mice. At the second stage, we examined the combined treatment of $^{224}$Ra wires and Gemzar on tumor development.

**Inhibition of the growth of pancreatic tumors by treatment with $^{224}$Ra-loaded wires.** The first series of experiments was done to assess the effect of a $^{224}$Ra wire inserted in a pancreatic tumor in comparison with the effect of an inert wire. Mice with tumors (37 mm$^3$ average volume), were randomized into 1 of

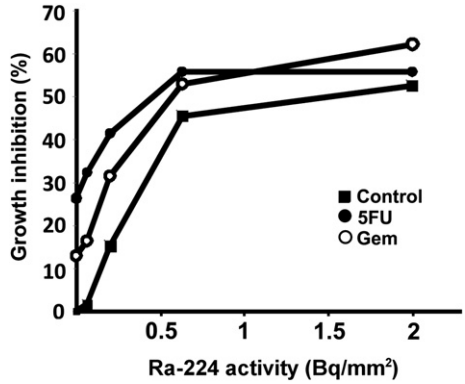


**Fig 3.** Growth inhibition of Panc02 cells after 48 h of incubation in a radioactive plate, combined with 5-FU or gemcitabine. Cell growth was monitored by staining the adherent cells.

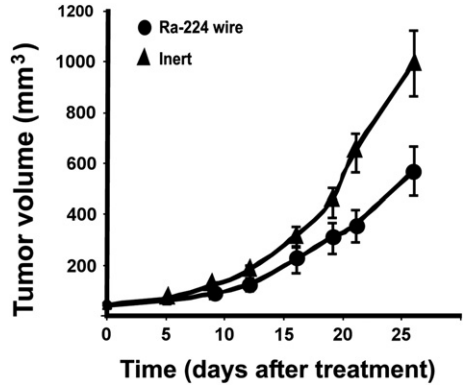


**Fig 4.** Tumor growth retardation by a single $^{224}$Ra wire treated mice compared with inert wire group. Insertion of a single Ra-224 loaded wire ($^{224}$Ra activity; 17-43 kBq) to the center of murine pancreatic tumors with an average volume of 37 mm$^3$ ($^{224}$Ra wire) relative to the control group (inert wire).

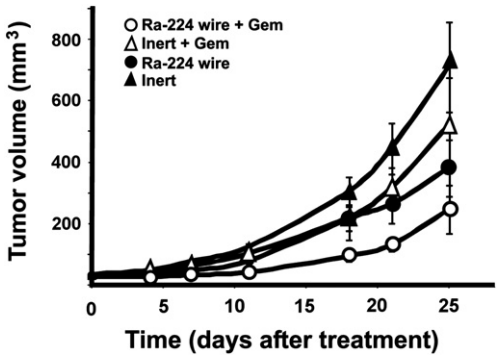


**Fig 5.** Tumor growth retardation by a single $^{224}$Ra wire combined with Gemzar compared with the $^{224}$Ra wire group, inert wire group, and Gemzar+ inert wire group. The tumor length is 4.9 ± 0.1 (STE). Radioactive wire activities ranged from 17 to 43 kBq per wire.

2 treatment groups: tumor-bearing mice treated with a $^{224}$Ra-loaded wire ($^{224}$Ra wire) or tumor-bearing mice treated with a non radioactive wire (Inert wire). At the time of treatment no necrosis was observed by histologic examination in tumors with a similar size.

The results presented in Fig 4 indicate that a significant difference (Pv = 0.02) was found between tumor volumes of the DaRT-treated group ($^{224}$Ra wire) as opposed to the control group (inert wire). The effect was evident during the whole inspection period and becomes more substantial with time.

**Inhibition of tumor development by $^{224}$Ra wires in combination with gemcitabine.** Next, we examined the combined treatment of a single $^{224}$Ra wire and the chemotherapeutic drug gemcitabine (Gemzar), compared with an inert wire alone, inert wire and Gemzar, or $^{224}$Ra wire alone.

Mice with intradermal Panc02 tumors (27 mm$^3$ average volume) received $^{224}$Ra wire treatment with or without chemotherapy. The drug (Gemzar, 60 mg/kg) was injected intravenously 2–3 h after wire insertion, and the animals were inspected for tumor progression.

The results presented in Fig 5 demonstrate that the combined treatment was the most effective modality in local tumor control compared with the effect of inert wire + Gemzar (Pv < 0.001) or the $^{224}$Ra wire treatment (Pv = 0.033).

**Intratumoral distribution of radioactive atoms.** The $^{212}$Pb activity distribution was high near the source, in this case dropping by a factor of 20–30 per millimeter with increasing radial distance. The spatial spread of $^{212}$Pb was found to be correlated partially with tissue damage, as can be shown in Fig 6. The spread was somewhat anisotropic, with an elliptical cross section. The typical ratio between the major and minor axes of the ellipse was 1.1–1.7. For the sources used in the experiments (having an initial $^{220}$Rn release rate of 6–19 kBq [0.16–0.5 $\mu$Ci]), dose levels exceeding 10 Gy were found over a region with an average diameter of ∼2.5 mm, with the size of the high dose region increasing logarithmically with the source activity. When normalized to a standard source $^{220}$Rn release rate of 37 kBq (1 $\mu$Ci), the average diameter corresponding to doses in excess of 10 Gy was ∼3 mm.

**Leakage of radioactive atoms from the tumor.** We define the $^{212}$Pb leakage probability as the probability that a $^{212}$Pb atom released from the wire decays outside the tumor. The leakage probability was estimated experimentally for tumors treated with DaRT wires by measuring the $^{212}$Pb and $^{224}$Ra activities on the wire before its insertion to the tumor and at tumor removal time and by measuring $^{212}$Pb and $^{224}$Ra activities in the tumor at tumor removal time. $^{224}$Ra and $^{212}$Pb activities on the wire before insertion were calculated from alpha spectroscopy measurements of the wire. The calculation of $^{212}$Pb and $^{224}$Ra activities, which remained

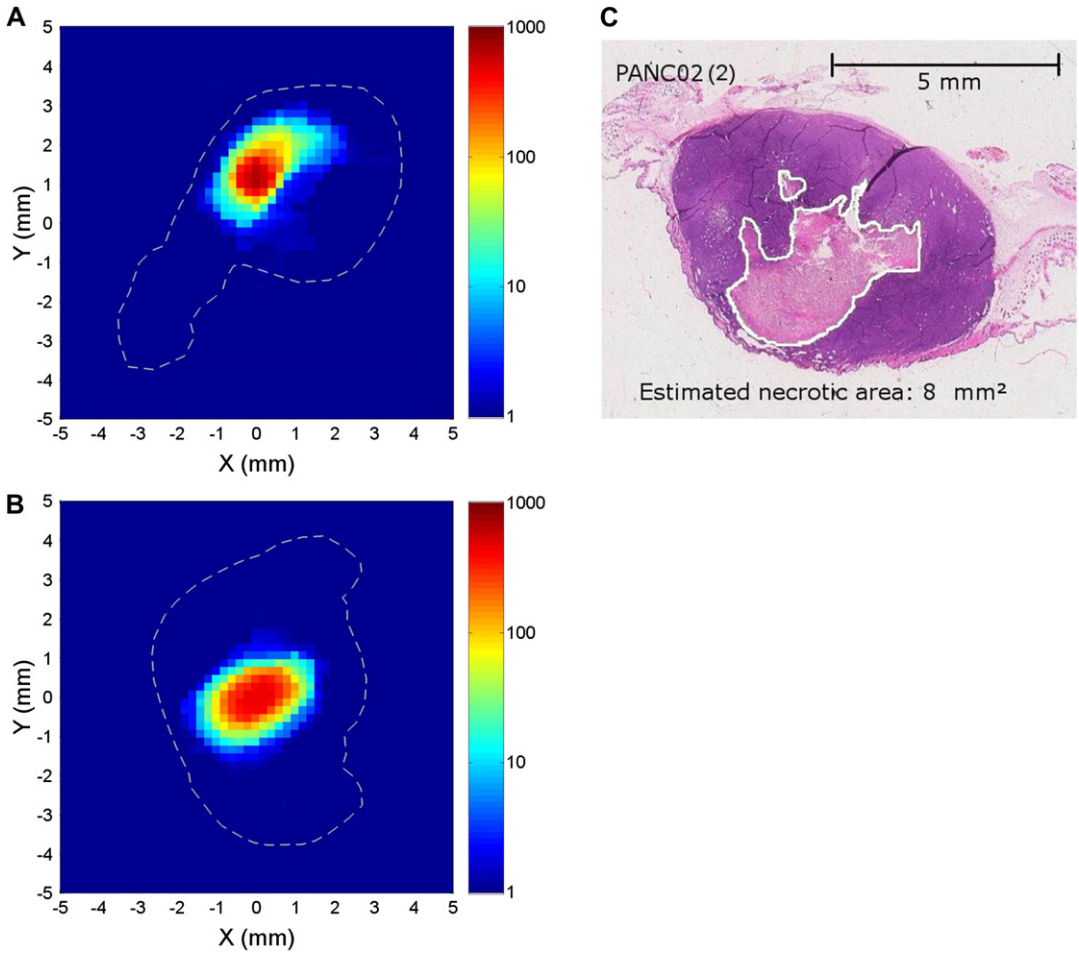


**Fig 6.** Autoradiography and histology of Panc02 tumors treated with a single DaRT wire each. The $^{224}$Ra activities were 37 kBq (**A**) and 17 kBq (**B**) with respective $^{220}$Rn desorption probabilities of 25% and 39% (ie, $^{220}$Rn release rates of 9.3 kBq and 6.6 kBq, respectively). Both tumors were excised 4 days after wire insertion. (**C**), A display of tumor sections stained by hematoxylin & eosin. Panc02 tumor treated with a DaRT with a total section area as follows: 34 mm$^2$, estimated necrotic area: 8 mm$^2$, necrotic fraction: 25%, and initial Rn release rate: 21.5 kBq. (Color version of figure is available online.)

on the wire after treatment, was done by analyzing gamma measurements (taken with a NaI gamma counter) of the wire after tumor removal.

The $^{212}$Pb leakage probability was measured for nine Panc02 tumors with masses in the range of 0.02–1 g, 4 days after being treated with a single wire inserted to their center. The leakage probability value calculated ranged between 55% and 85% and was not dependent on the tumor mass.

**Distribution of radioactive atoms in body organs.** Several organs (kidney, spleen, liver, and leg) were taken out of the mice for measurement in the gamma counter in addition to the tumor and the wire. The excised organs were weighed and inserted into a capped scintillation vial taken for gamma measurements. Each sample was measured for several times over a period of 24–72 h. The data were analyzed to yield the sample $^{224}$Ra and $^{212}$Pb activity content at tumor removal time.

The uptake probability of $^{212}$Pb in an organ at time ($t$) represents the instantaneous probability that the $^{212}$Pb atom is found in an organ after leaving the tumor. The highest uptake probability of $^{212}$Pb atoms was found in the kidneys. The next organ with a relatively high uptake is the liver. The other organs showed relatively low uptake. The $^{212}$Pb uptake probability per unit mass measured in the kidney ranged between 0.018 and 0.37, and in the liver between 0.005 and 0.017.

## DISCUSSION

Pancreatic cancer is one of the leading causes of cancer death. Pancreatic carcinoma tends to respond poorly to chemotherapy and carry a dismal prognosis.

Gemcitabine and 5-FU are most commonly used as treatment for this kind of cancer,[13,20,21,22] and gemcitabine is currently considered frontline treatment for pancreatic cancer.[23]

Several studies showed that both survival rate and palliative benefit can be improved when radiotherapy is combined with chemotherapy[12,16] before[17,24] or without surgery.[25]

In an attempt to maximize the dose administrated to a malignant tissue while minimizing the dose to other regions,[26] internal (interstitial) radiation brachytherapy has progressively become an established treatment modality. Brachytherapy allows a higher total dose of radiation in a shorter time than is possible with external treatment, and it lessens radiation damage to adjacent normal tissue. The radioactive material, mainly X-ray or gamma radiation emitters, is sealed in an implant and is placed directly into or adjacent to the affected tissue.[27] For several decades, brachytherapy with X-rays was applied in patients with carcinoma of the pancreas to achieve better local control of the tumor and palliation.[28-34] Beta emitting isotopes were also used in microbrachytherapy technique for treatment of malignant hepatic lesions secondary to pancreatic carcinoma.[35]

Photons and beta particles are characterized by a low-LET and a dose field, which may penetrate deep into the healthy tissue surrounding the tumor. The use of alpha particles, however, may lead to a much more localized dose distribution. In addition, the use of high-LET radiation (alpha particles in particular) has additional radiobiologic benefits, such as the nearly constant efficacy against hypoxic cells.[36,37] Yet, the short range of alpha particles in tissue (less than 0.1 mm) has so far limited their use in the treatment of cancer to radioimmunotherapy,[38-40] or radiopharmaceuticals such as Alpharadin ($^{223}RaCl_2$; Algeta, Oslo, Norway).[41]

Alpha irradiation has so far been considered unsuitable for the treatment of solid tumors, and brachytherapy using alpha-emitting sources was not feasible because of the lack of a practical way to cover the tumor volume effectively with these short-range particles. DaRT may provide, for the first time, an efficient and safe method for treating the entire volume of solid tumors with a therapeutic dose of alpha particles, by overcoming the basic limitation inherent to alpha radiation, namely, its exceedingly short range in human tissue.

The poor response of pancreatic cancer to treatment requires the development of novel treatment modalities. In the present study, we evaluated the potency of a new brachytherapy device, based on alpha radiation alone and in combination with chemotherapy, against pancreatic cancer-derived experimental tumors.

We examined the effect of alpha particles emitted from $^{224}Ra$ and its daughters on murine and human pancreatic cell lines *in vitro* as well as on solid tumors. This method, previously tested by our group on squamous cell carcinoma (SCC)[6] and lung carcinoma,[8] showed that $^{224}Ra$-loaded wires retarded tumor development and prolonged life expectancy considerably. The results of the current study demonstrated that $^{224}Ra$ wires retarded pancreatic tumor development alone and more so when combined with the drug gemcitabine.

The *in vitro* experiments revealed that both mouse (Panc02) and human (MIA PaCa) pancreatic cells lines are sensitive to alpha radiation. The proliferation of the cells was interrupted in cells hit by the alpha particles in a dose-dependent manner (Figs 1 and 2).

Furthermore, the treatment of Panc02 cells with alpha radiation in combination with either Gemzar or 5-FU achieved a higher growth inhibition rate than each treatment alone (Fig 3). A similar effect was observed for the human cell line MIA PaCa. In a previous study, alpha radiation and 5-FU worked better to kill SCC cells than each treatment alone.[7]

Some chemotherapeutic drugs destroy tumor cells by their own cytotoxic action and additionally enhance the effects of radiotherapy. Chemotherapeutic drugs that have the potential to produce substantial sensitization of tumor cells to radiation treatment are defined as radiosensitizers,[42-44] such is the chemotherapy drug gemcitabine.

Chemotherapy and especially gemcitabine, has been shown to increase radiosensitivity to photon radiation in different cell lines including pancreatic cancer.[45-47] Gemcitabine can induce radiosensitization at concentrations 1000 times lower than typical plasma levels obtained with the drug[44] and can sensitize radioresistant cell lines to radiation.[48,49] Gemcitabine was also demonstrated as a potent radiosensitizer when used with photon irradiation in *in vivo* preclinical and clinical studies. Several studies revealed the efficacy of using gemcitabine and radiation against pancreatic cancer.[16,50,51]

Thus, we examined retardation of tumor growth by *in vivo* treatment with $^{224}Ra$-loaded wires with and without chemotherapy. The insertion of a single $^{224}Ra$-loaded wire to the center of murine pancreatic tumors had a pronounced retardation effect on tumor growth rate compared with inert wires (Fig 4). Growth retardation of tumors by $^{224}Ra$-loaded wires was strengthened by the concomitant use of Gemzar (Fig 5). After 25 days, the tumors treated by the combination were 4-fold smaller than the control tumors and 2.4-fold smaller than those treated by chemotherapy. The effect was strong during the first 12 days posttreatment when the activity of the wire ($^{224}Ra$ half life 3.66 days) and chemotherapy were still high.

Although we found that chemotherapy and alpha radiation together were more effective, we did not observe

a radiosensitizing effect as observed for gamma radiation. Gamma radiation produces relatively few direct DSBs, and most of the damage is single stranded breaks (SSB), with some SSBs close enough together to act as a DSB. So affecting repair can make a big difference, especially homologous recombination repair (HRR) that depends on a template and is critical after treatment with gemcitabine or other drugs, which affect DNA repair.[44]

After alpha radiation, most of the damage in DNA is DSBs, which are directly lethal and hard to repair. So it is expected that drugs affecting HRR would have no radiosensitizing effect, and other drugs would have just a modest effect. Similar results reported in another study point to a similar conclusion. Three multiple myeloma cell lines were irradiated with or without 10 nmol/L gemcitabine 24 h prior to radiation. Gemcitabine led to radiosensitization of LP1 and U266 cells with low-LET but did not radiosensitize any cell line when combined with high-LET.[52]

A comparison of the effects of DaRT wires on tumors of different histologic types revealed that SCC derived tumors can be better controlled[6,7] compared with pancreatic tumors as found in this study. The potency of DaRT may be dependent on the distribution of radioactive atoms inside the tumor, leakage of the radioactive atoms from the tumor, and the radiosensitivity of the cells to alpha particles.

Histologic and autoradiographic observations indicated that there is mortality of cells around the DaRT wire, with therapeutically significant doses over a region measuring 2–3 mm in diameter about the wire (area, 3–8 $mm^2$) (Fig 6). The spread of radioactivity in pancreatic tumors was smaller compared with what we observed in experimental tumors of SCC (diameter, 5–7 mm; area, 20–40 $mm^2$)[5] or lung carcinoma (diameter, 3–4 mm, area, 7–13 $mm^2$).[8] This may result from a lower diffusion coefficient in the pancreatic tumor caused by a denser tissue or caused by faster elimination of the radioactive atoms from the tumor. The leakage probability values calculated ranged between 55% and 85% and were not dependent on the tumor mass. This means that a large fraction of the radioactive atoms, which are released from the radioactive wire, escape from the tumor and do not kill tumor cells.

Thus, the spread of radioactive atoms was in inverse correlation with the extent of clearance of radioactive atoms from the tumor that was highest in pancreatic tumors and lowest in SCC. The sensitivity of cells to alpha particles was determined *in vitro* by measuring the mean lethal dose ($D_0$). It was observed that SCC cells are more radiosensitive to alpha radiation ($D_0 = 0.85 \pm 0.02$ Gy) compared with pancreatic cancer cells ($D_0 = 1.1 – 1.2$ Gy). However, the 2 cell lines exhibited similar responsiveness to $\gamma$-radiation (unpublished results). Because $\alpha$-radiation causes mainly DNA DSBs, currently we investigate whether the differences are controlled by DNA damage or repair mechanisms.

It may be assumed that the relatively low intratumoral damage in pancreatic cancer correlates with short-range intratumoral spread of alpha releasing atoms, low cell sensitivity to alpha radiation, and high clearance of the radioactive atoms from the tumor. The future task is to seek reagents that might affect these parameters and increase pancreatic tumor ablation by DaRT wires.

DaRT is a paradigm shift in the use of alpha radiation for the treatment of solid tumors. DaRT can be used to destroy solid tumors, and in the case of malignant metastasizing cancer, it will be augmented by chemotherapy or immune response stimulators to affect regional and distant metastases. The safety of the DaRT modality has been analyzed in detail in Ref.[53], taking into account not only the alpha radiation but also the beta and gamma emissions occurring in the entire decay chain. DaRT is expected to be safe and with few side effects, and it may serve as an important tool to achieve better local control of the tumor and palliation in patients with cancer. Currently, we plan a clinical trial with patients bearing head and neck SCC.

Although brachytherapy is not a common option for the treatment of tumors of the pancreas, the high killing efficiency of alpha radiation and the ability to localize the effect make DaRT an interesting and important future treatment modality. Yet, pancreatic tumors might be a difficult target for DaRT, and more studies are required to increase the effect of DaRT wires on pancreatic tumors so it can serve for increased local control and palliation.

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