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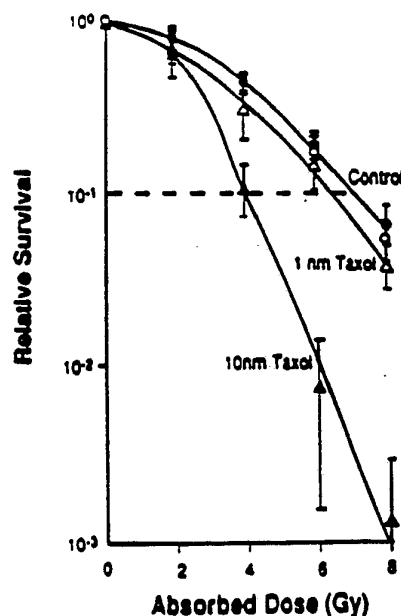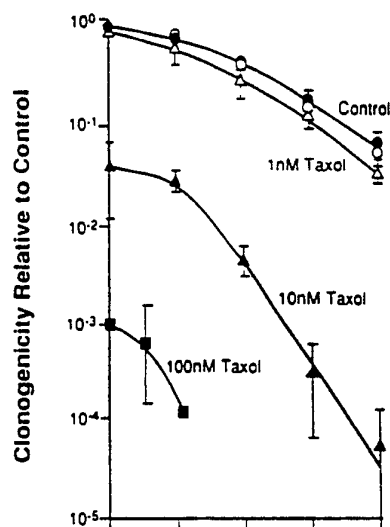
(57) Abstract

This invention provides a method of increasing sensitivity of cells to cytotoxic effects of ionizing radiation which includes incubating the cells with spindle poison in suitable carrier at a concentration effective to inhibit the cells from progressing through G2 or M phases of the cell cycle for a time effective to inhibit division of the cells and then administering a cytotoxic dose of ionizing radiation to the cells. This invention also provides a method of treating a cancer patient which comprises administering to patient a spindle poison in suitable carrier in an amount effective to inhibit tumor cells in the patient from progressing through G2 or M phases of the cell cycle for an amount of time effective to inhibit division of the tumor cells and then administering a cytotoxic dose of ionizing radiation to the patient. This invention further provides a method for increasing the sensitivity of cells to bleomycin and a kit useful for treating cancer patients.

5   **TAXOL AS A RADIATION SENSITIZER**

Background of the Invention

Throughout this application, various publications are
10   referred to within parenthesis.   Full bibliographic
citations for these references may be found at the end of
the specification, immediately preceding the claims.   The
disclosures for these publications in their entireties are
hereby incorporated by reference into this application in
15   order to more fully describe the art to which this invention
pertains.

Taxol is the prototype of a new class of antineoplastic
agents that targets microtubules.   It is a natural product
20   isolated from the bark of the western yew, Taxus brevifolia.
Its structure, a novel diterpene compound, and antitumor
activity in rodents were reported in 1971 (16). The drug's
unique mechanism of action has generated considerable
interest, both for its use to probe the function of the
25   cytoskeleton in basic science, and as a chemotherapeutic
agent in oncology.   Taxol is known to be a potent cytotoxic
agent against a range of human malignancies using cell
culture and xenographic model systems (9).   Human studies
have demonstrated taxol's ability to increase the mitotic
30   index in a variety of tissues (4).   Clinical trials have
demonstrated that taxol is an active agent in salvage
treatment for epithelial ovarian malignancies (2, 5, 15) and
has activity against breast cancer (3) as well as melanoma
(7, 18).
35
Unlike agents that bind to tubulin, the subunit of
microtubules, and inhibit microtubule formation (vinca

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alkaloids, podophyllotoxin, and colchicine), taxol induces
in vitro formation of exceptionally stable microtubules
(10). Tissue and culture studies have shown the ability of
taxol to block and/or prolong cells in the G2 or M phase of
5   the cell cycle (11). The microtubule cytoskeletons of
taxol-treated cells are exceedingly stable to
depolymerization, as are isolated drug-treated microtubules.
In addition, electron microscopy reveals an abnormal
microtubular cytoskeleton in drug-treated cells. The
10   inability of these cells to pass through the G2 and M phases
of the cell cycle probably results from the inability of
these cells to form a competent mitotic spindle or to
disassociate a drug-treated spindle. Taxol additionally
blocks the migration behavior of cells in culture. These
15   observations may explain the observed antitumor activity of
the drug.

Taxol has undergone several Phase I trials at many
institutions (9). Plasma concentrations of 1 nM to 5 μM
20   taxol at safe therapeutic doses are comparable to those
required for the antiproliferative and microtubule-
stabilizing effects of the drug in vitro. Mitotic arrest
has been observed in the esophagus, stomach, small
intestine, colon, liver, skin, bone marrow, and testes of
25   patients biopsied within 11 days after receiving taxol (4).
Dose-limiting toxicity includes leukopenia,
thrombocytopenia, alopecia, nausea and vomiting, diarrhea,
stomatitis, peripheral neuropathy, rashes, elevated serum
triglyceride levels, and severe hypersensitivity (most
30   likely related to the cremophor vehicle) (1, 5, 6, 17, 18).
Partial responses have been reported in patients with non-
small cell lung cancer, melanoma, and ovarian cancer. One
Phase II study has reported significant activity against
standard drug protocol refractory ovarian cancer (8).

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It is well known from radiobiological principles that G2/M is the most radiosensitive phase of the cell cycle (13). However, use of taxol, related compounds or pharmaceutically acceptable salts thereof to enhance the cytotoxic effect of ionizing radiation has not previously been described.

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Summary of the Invention

This invention provides a method of increasing the
sensitivity of cells to the cytotoxic effects of ionizing
radiation which comprises first incubating the cells with a
spindle poison in a suitable carrier at a concentration
effective to inhibit the cells from progressing through the
G2 or M phases of the cell cycle for an amount of time
effective to inhibit division of the cells and then
administering an effective cytotoxic dose of ionizing
radiation to the cells.

This invention also provides a method of treating a cancer
patient which comprises first administering to the patient
a spindle poison in a suitable carrier in an amount
effective to inhibit tumor cells in the patient from
progressing through the G2 or M phases of the cell cycle for
an amount of time effective to inhibit division of the tumor
cells and then administering an effective cytotoxic dose of
ionizing radiation to the patient.

This invention further provides a method of increasing the
sensitivity of cells to the cytotoxic effects of bleomycin
which comprises first incubating the cells with a spindle
poison in a suitable carrier at a concentration effective to
inhibit the progression of the cells through the G2 or M
phases of the cell cycle for an amount of time effective to
inhibit cell division and then exposing the cells to an
effective cytotoxic concentration of bleomycin.

This invention still further provides a kit useful for
treating a cancer patient which comprises a spindle poison
in an amount sufficient to establish a concentration of the
poison in the body of the patient effective to inhibit the

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progression of tumor cells through the G2 and M phases of
the cell cycle and an effective cytotoxic dose of a
radioisotope in aqueous solution, an antibody-conjugated
radioisotope or bleomycin.

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<u>Figures</u>

Figure 1   Cell   surviving   fraction   versus   drug
            concentration following a 24 hr treatment of
            human astrocytoma cells with taxol. Control
            plating efficiencies ranged from 52 to 61%.
            Standard error bars are shown when greater
            than the size of the symbols.

Figure 2   Nomarski-interference   contrast
            photomicrographs of human astrocytoma cells
            following a 24 hr treatment with various
            concentrations of taxol: (a) 0 nM; (b) 1 nM,
            (c) 10 nM; and (d) 100 nM. The effect of
            the drug on cellular morphology is apparent
            as is nuclear multi-micronucleation at the
            higher concentrations. Cells progressively
            accumulate at the G2/M phase of the cell
            cycle.

Figure 3   Radiation survival curves with different
            concentrations of taxol. Clonogenicity for
            G18 cells is expressed as a function of
            taxol concentration and radiation dose. A.
            Survival is expressed on an absolute scale,
            relative to unirradiated controls without
            taxol. The effect of taxol alone on cell
            survival can be seen as the change in
            survival at 0 Gy. The interaction of taxol
            and radiation on cells is demonstrated by
            the change in the shape of the curves at
            higher taxol concentrations. B. Survival
            curves are presented with survival at a
            particular radiation dose expressed relative

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to unirradiated controls with the same concentration of taxol. This emphasizes the change in the shape of the survival curves.

Figure 4.   Cytotoxicity and enhancement of radiation sensitization as a function of time of exposure to 10 nM taxol. Cells were exposed to 10 nM taxol for the indicated time periods and clonogenicity assessed with and without 6 Gy irradiation.   A. Plating efficiency, or effect of taxol alone on cell survival.   For DMSO-treated controls, the plating efficiency is roughly stable, perhaps increasing slightly as a function of time, whereas for the taxol-treated cells, there is clearly a decrease in plating efficiency as a function of time.   B. Survival of cells treated with 10 nM taxol and 6 Gy of radiation.   Survival of irradiated cells is expressed relative to unirradiated cells treated with taxol for the same period of time.   Therefore, the change in cell survival seen as a function of time of taxol exposure is a measure of the increased interaction between these two treatments.   A relative decrease in cell survival, and therefore an increase in the level of interaction between taxol and radiation is seen for cells treated for 16 and 24 hours.   C. Percent of cells in the G2 or M phases of the cell cycle as determined by flow cytometry for the times indicated.

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Detailed Description of the Invention

This invention provides a method of increasing the
sensitivity of cells to the cytotoxic effects of ionizing
radiation which comprises first incubating the cells with a
spindle poison in a suitable carrier at a concentration
effective to inhibit the cells from progressing through the
G2 or M phases of the cell cycle for an amount of time
effective to inhibit division of the cells and then
administering an effective cytotoxic dose of ionizing
radiation to the cells.

Suitable cells for use in accordance with the practice of
this invention may be tumor cells. The tumor cells may be
brain tumor cells, e.g., astrocytoma, glioblastoma
multiforme or medulloblastoma cells. The tumor cells may
also be ovarian tumor cells, e.g., epithelial, sex-chord
stromal, lipid, germ or gonadoblastoma cells. The tumor
cells may further be lung tumor cells, e.g., adenocarcinoma,
large cell, small cell or squamous cell tumor cells. The
tumor cells may still further be breast tumor cells, e.g.,
invasive duct carcinoma, medullary carcinoma or muscinous
carcinoma cells. The tumor cells may also be melanoma
cells.

The term "spindle poison" as used herein means any agent
capable of interfering with the functioning of the
microtubules in a cell with the result that cell division is
inhibited and the cell is blocked in the G2 or M phases of
the cell cycle. Spindle poisons may be agents which
interfere with microtubule formation and thereby inhibit
cell division, e.g., vinblastin and vincristin. Spindle
poisons may also be agents which stabilize microtubules and
prevent their disassembly during cell division. Examples of

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such microtubule-stabilizing agents suitable for use in the practice of this invention include taxol-related compounds. The term "taxol-related compound" as used herein encompasses compounds possessing a microtubule-stabilizing activity similar to taxol. In the presently preferred embodiment of this invention, the taxol-related compound is taxol. Taxol may be a natural product from the bark of the yew _Taxus_ Sp. L. One species of this genus useful as a source of taxol in the practice of this invention is the yew _Taxus_ _brevifolia_. However, the term "taxol" as used herein encompasses not only taxol from this source, but taxol produced by any method, including chemical synthesis or tissue culture production.

Suitable carriers for a spindle poison may be any of a number of aqueous solutions well known to those skilled in the art.   Presently preferred are aqueous solutions of dimethyl sulfoxide.

For the purposes of this invention, an "effective inhibiting concentration" of the spindle poison is any concentration of the spindle poison effective to inhibit the progression of the cells through the cell cycle and cause cells to accumulate in the G2 or M phases. Typically, the effective inhibiting concentration of the spindle poison is a concentration from about 1 nM to about 50 μM.

In the practice of this invention, an "effective inhibiting amount of time" is any amount of time for which incubation of the cells with the spindle poison at an effective inhibiting concentration will be effective to inhibit the progression of cells through the G2 or M phases of the cell cycle. Typically, the effective inhibiting amount of time is an amount from about 6 hours to about 24 hours, desirably

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an amount from about 8 hours to about 20 hours. More
desirably, the effective inhibiting amount of time is an
amount about 18 hours.

5   In one embodiment of this invention, administration of an
effective cytotoxic dose of ionizing radiation to the cells
comprises exposing the cells to a beam of ionizing
radiation. For the purposes of this invention, an effective
cytotoxic dose of ionizing radiation is any dose of ionizing
10   radiation effective to kill cells. Typically, the effective
cytotoxic dose of ionizing radiation is a dose from about 1
Gy to about 10 Gy, desirably a dose from about 2 Gy to about
8 Gy. Methods of producing a beam of ionizing radiation
suitable for the exposure of cells in accordance with the
15   practice of this invention are well known to those skilled
in the art. In the presently preferred embodiment of this
invention, the beam of radiation is produced by an
irradiator. The term "irradiator" as used herein is
intended to mean a device comprising a lead shield
20   surrounding a radioisotope which emits ionizing radiation
and a mechanism for exposing cells placed adjacent to the
irradiator to the ionizing radiation. Methods of using an
irradiator to expose cells to ionizing radiation are well
known to those skilled in the art. In the practice of this
25   invention, the irradiator may comprise the radioisotope
Cesium-137 or the radioisotope Iridium-192.

In another embodiment of this invention, the administration
of an effective cytotoxic dose of ionizing radiation to the
30   cells comprises contacting the cells with an aqueous
solution containing a radioisotope. For the purposes of
this invention, an "effective cytotoxic dose" of ionizing
radiation is any dose of ionizing radiation effective to
kill cells. Typically, the effective cytotoxic dose of

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ionizing radiation is a dose from about 1 Gy to about 10 Gy, desirably a dose from about 2 Gy to about 8 Gy. Suitable radioisotopes for use in accordance with the practice of this invention may be any of a number of radioisotopes well known to those skill in the art. In the presently preferred embodiment of this invention, the radioisotope is Phosphorous-32.

This invention also provides a method of treating a cancer patient which comprises first administering to the patient a spindle poison in a suitable carrier in an amount effective to inhibit tumor cells in the patient from progressing through the G2 or M phases of the cell cycle for an amount of time effective to inhibit division of the tumor cells and then administering an effective cytotoxic dose of ionizing radiation to the patient. Administration of a spindle poison to a patient may be by any of the means well known to those skilled in the art suitable for contacting cells in the body of a patient with a spindle poison, e.g., by intravenous injection. Preferably, the patient is a human patient.

As stated above, the term "spindle poison" as used herein encompasses any agent capable of interfering with the functioning of the microtubules in a cell with the result that cell division is inhibited and the cell is blocked in the G2 or M phases of the cell cycle. The spindle poison may be an agent which stabilizes microtubules, e.g., a taxol-related compound. In one embodiment of this invention, the taxol-related compound is taxol. Taxol may be a natural product of the yew Taxus Sp. L. However, the term "taxol" as used herein encompasses taxol produced by any method, including chemical synthesis or tissue culture production.

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Suitable carriers for administering a spindle poison to a
patient may be any of a number of aqueous infusion solutions
well known to those skilled in the art. Presently preferred
is an aqueous infusion solution comprising 5% dextrose
5   injection USP, i.e., D5W.

In the practice of this invention, the amount of the spindle
poison effective to inhibit the progression of cells through
the G2 or M phases of the cell cycle is an amount of the
10   spindle poison sufficient to establish a concentration of
the agent in the blood of the patient effective to inhibit
the cells from progressing through the G2 or M phases of the
cell cycle.   For the purposes of this invention, "an
effective inhibiting concentration" of a spindle poison is
15   any amount of the poison effective to inhibit the
progression of cells through the G2 or M phases of the cell
cycle. Typically, the effective inhibiting concentration of
the spindle poison is a concentration from about 1 nM to
about 50 μM.   The effective concentration of the spindle
20   poison in the blood of the animal may be maintained by
readministering the poison to the patient at an interval of
time after the preceding administration.   Methods of
determining the appropriate length of the interval between
administrations of the spindle poison are well known to
25   those skilled in the art.   Presently preferred is an
interval of about one week between administrations.

For the purposes of this invention, an amount of time
effective to inhibit the progression of cells through the G2
30   or M phases of the cell cycle is any amount of time for
which incubation of the cells with a spindle poison will be
effective to inhibit the progression of cells through the
cell cycle and cause the cells to accumulate in the G2 or M
phases.   Typically, the "effective inhibiting amount" of

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time is an amount greater than about 24 hours.

In one embodiment of this invention, the effective cytotoxic dose of ionizing radiation is administered by exposing the patient to a beam of radiation produced by a linear accelerator. For the purposes of this invention, an "effective cytotoxic dose" of ionizing radiation is any dose of ionizing radiation effective to kill cells. Typically, the effective cytotoxic dose of ionizing radiation absorbed by a patient from exposure to a beam of radiation in accordance with the practice of this invention is a dose from about 4,000 cGy to about 8,000 cGy. Desirably, the effective cytotoxic dose of ionizing radiation is a dose from about 6,500 cGy to about 7,500 cGy. In the practice of this invention, the effective cytotoxic dose of ionizing radiation may be administered to the patient in a schedule of increments. Methods of establishing a schedule of increments for administering ionizing radiation to a patient are well known to those skilled in the art. An example of such a schedule contemplated by this invention comprises a dose of ionizing radiation of about 200 cGy per day for five days per week for a period from about six weeks to about eight weeks.

In another embodiment of this invention, an effective cytotoxic dose of ionizing radiation is administered by implanting a radioisotope in tumors in the body of the patient for an amount of time sufficient to expose the tumor to the effective cytotoxic dose. For the purposes of this invention, an "effective cytotoxic dose" of ionizing radiation is any dose of ionizing radiation effective to kill cells. Typically, the effective cytotoxic dose of ionizing radiation is a dose from about 8,000 cGy to about 20,000 cGy. Desirably, the effective cytotoxic dose is a

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dose about 16,000 cGy. Suitable radioisotopes for implanting in tumors in the body of a patient in accordance with the practice of this invention may be any of those well known to those skilled in the art. Presently preferred are

5 Iodine-125 or Iridium-192. The practice of this invention also contemplates exposing the patient to a beam of ionizing radiation. Methods of generating a beam of ionizing radiation suitable for application to a patient are well known to those skilled in the art. In the presently

10 preferred embodiment of this invention, the radiation beam is generated by a linear accelerator. One skilled in the art would be readily able to determine without undue experimentation whether the beam of radiation is to be applied to the patient before or after the radioisotope is

15 implanted. Typically, the effective cytotoxic dose of ionizing radiation to which the patient is exposed in accordance with the practice of this invention comprises a dose of ionizing radiation from about 4,000 cGy to about 6,000 cGy administered by the beam of radiation and a dose

20 of ionizing radiation from about 2,000 cGy to about 4,000 cGy administered by the radioisotope implant.

In a further embodiment of this invention, the effective cytotoxic dose of ionizing radiation is administered to the

25 patient by radiosurgery. The term "radiosurgery" as used herein is intended to mean a procedure for applying a dose of ionizing radiation to a narrowly defined area of tissue in the body of the patient while avoiding exposing surrounding tissue. Radiosurgery allows for the accurate

30 determination of target size and location, treatment planning and accurate delivery of radiation. A radiosurgery system includes a stereotactic frame, an appropriate radiation source and computer hardware and software. The appropriate radiation source may be a "gamma-knife", e.g.,

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a gamma knife containing Cobalt-60, or a linear accelerator. Typically, the dose of ionizing radiation a patient is exposed to by radiosurgery is a dose from about 1,200 cGy to about 2,800 cGy.

This invention further provides a method of increasing the sensitivity of cells to the cytotoxic effects of bleomycin which comprises first incubating the cells with a spindle poison in a suitable carrier at a concentration effective to inhibit the progression of the cells through the G2 or M phases of the cell cycle for an amount of time effective to inhibit cell division and then exposing the cells to an effective cytotoxic concentration of bleomycin. For the purposes of this invention, an "effective cytotoxic concentration of bleomycin" is any concentration of bleomycin effective to kill cells. Methods of determining effective cytotoxic concentrations of bleomycin are well known to those skilled in the art or are readily determinable by routine experimentation.

This invention still further provides a kit useful for treating a cancer patient which comprises a spindle poison in an amount sufficient to establish a concentration of the poison in the body of the patient effective to inhibit the progression of tumor cells through the G2 and M phases of the cell cycle and an effective cytotoxic dose of a radioisotope in aqueous solution, an antibody-conjugated radioisotope or bleomycin. The kit of this invention may include instructions for administering the spindle poison and the aqueous solution of the radioisotope, the antibody-conjugated radioisotope or bleomycin in accordance with the practice of this invention.

This invention will be better understood from the Results

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and Discussion which follow. However, those skilled in the
art will readily appreciate that the specific results
discussed are only illustrative of the invention as
described more fully in the claims which follow thereafter.

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Materials and Methods

The astrocytoma cell line (G18) was established in culture
from a surgical specimen obtained from the Neurological
Institute of New York, Columbia-Presbyterian Medical Center
(12). Cells were grown in Modified Eagles Medium (MEM) with
Hanks Balanced Salts (HBS, obtained from Gibco) and 10%
Fetal Calf Serum (Hyclone) with 12.5 ml SerXtend (Hana
Biological) per 500 ml of serum. Medium was supplemented
with MEM L-glutamine, essential amino acids, non-essential
amino acids, vitamins, and gentamicin. For survival curve
incubations, the medium was supplemented with
penicillin/streptomycin. Cells were routinely grown in
flasks with loosely capped tops, in incubators with 5% CO_2
and subcultured 1-2 times per week at a ratio of 1:20 (12).

Taxol (NSC 125973) was obtained from the NCI drug program.
A stock solution of 10^{-2} M was prepared in dimethyl sulfoxide
(DMSO), kept at -40°C and thawed for use. The quantity of
drug and DMSO added per dish was between 0.1%-1.0% of the
total volume of medium.

Cells were incubated in slide flasks. Approximately 2 x 10^4
cells and 2 ml of medium were added to each flask. These
were allowed to incubate for approximately 24 hr prior to
removal of the medium and photography. Nomarski
interference contrast photomicrography was carried out using
an Olympus Vanox AH4 microscope.

Cells were acutely irradiated with a ^{137}Cs irradiator (Atomic
Energy of Canada Model GC40) operating at a dose rate of
1.12 Gy/min. Irradiation times ranged from 1.8 to 7.1
minutes.

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Cells were plated in 100 mm dishes with 10 ml of medium and
allowed to attach for approximately 20 hr. The number of
cells per dish was chosen such that 100-200 colonies would
survive after a specified treatment. Taxol or DMSO was then
5   added and the cells allowed to incubate for another 20-24 hr
with drug present. Following the incubation period, cells
were acutely irradiated, medium removed by aspiration, and
10 ml of medium was added back. Samples with unirradiated
controls were also prepared in the same way. Dishes were
10   then incubated for 10 days. Three plates were prepared for
each concentration and dose of irradiation. Cells were
fixed in 75% methanol/25% acetic acid and stained with
crystal violet. Colonies were considered to be a collection
of 50 or more cells and were counted on a per dish basis.
15   Survival was determined from the number of cells per dish,
normalized by unirradiated controls treated with the same
concentration of taxol or DMSO.

Cells were irradiated with 6 Gy of ^{137} gamma rays at 0, 8,
20   and 24 hr after addition of 10 nM taxol. At each of the
time points noted above, cells were additionally prepared
for flow cytometry as follows: the medium was aspirated,
cells washed with HBS, then trypsinized and washed in normal
medium. Cells were rewashed and then resuspended in cold
25   methanol and refrigerated. Cells were resuspended in 50
μg/ml propidium iodide prior to flow cytometry using a
Coulter dual laser EPICS 752. Flow studies measured the
number of cells versus DNA content and allowed for the
determination of the fraction of cells in each phase of the
30   cell cycle. Cells were also studied (a) after exposure to
a range of concentrations and (b) as a function of length of
exposure to 10 nM taxol.

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Results and Discussions

The cytotoxic effect on the human astrocytoma cell line G18
of a range of concentrations of taxol are shown in Figure 1.
5   At 1 nM, and for a 24-hour exposure, there is no significant
difference from the control, while after a 100 nM treatment,
about 0.1% of cells retain clonogenic potential relative to
controls.

10   The morphological consequences of 24-hour taxol exposures on
these astrocytoma cells are shown in Figure 2. Cells were
photographed 24 hours after taxol addition and even at 1 nM
(Fig. 2, b), there is a clear change in cellular morphology,
with cells being unable to spread. They have lost polarity,
15   yet show little evidence of nuclear anomalies. At 10 nM
(Fig. 2, c), there are clear changes in nuclear morphology,
with many cells showing nuclei fragmented into numerous
micronuclei.   These may represent abortive attempts at
division, and hence, cells will accumulate with a G2/M DNA
20   content. This situation is dramatically enhanced following
a 100 nM treatment (Fig. 2, d), where there are clearly
considerably fewer cells, an overall flattening of cells,
and with the vast majority of cells showing a
multimicronucleate phenotype.
25   .

Overall, this microtubule stabilizing agent has a dose
dependent effect on cellular behavior, consistent initially
with a breakdown of cytoskeletal function and hence cellular
shape and mobility, and then accumulation of cells in late
30   G2/M. The inability of cells to erect functioning spindles
then results in either the reformation of nuclear membranes
around groups of chromosomes (multi-micronucleation), or
else, the nuclear membrane breaks down only partially and
then reforms in clusters.   The results would then be

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expected to be a time- and concentration-dependent increase
in the proportion of cells with a G2 DNA content and
accumulation in the G2/M phases of the cell cycle. This was
confirmed in the astrocytoma cell line by flow cytometric
5   analysis of cellular DNA contents. The fraction of cells at
different stages of the cell cycle following treatment with
10 nM taxol is shown in Table 1.

Table 1

10   FLOW CYTOMETRY OF HUMAN ASTROCYTOMA CELLS
             INCUBATED WITH 10 nM TAXOL AT VARIOUS TIME INTERVALS

Treatment	Time (hrs.)	G1(%[*])	S(%)	G2/M(%)
Control	0	58.1	25.3	16.6
	24	56.6	23.9	19.5
10 nM taxol	2	54.1	27.7	18.2
	8	16.6	32.4	51.0
	24	0.0	3.5	96.5

20

[*]% = Percentage of cells in different stages of the cell
cycle.

After two hours of treatment with 10 nM taxol, there is no
25   significant difference from the control distributions.
However, at eight hours there is a clear change in the
proportion of cells in each of the phases, with a diminution
of cells in G1 phase and an augmentation of cells in G2/M
(to approximately 50%). By 24 hours, cellular DNA contents
30   are consistent with the vast majority of cells (96.5%)
having progressed through the cell cycle and accumulated
with G2/M DNA contents. That is, cells cannot complete a
successful mitosis. The flow cytometric data and cellular
observations complement each other and indicate that even
35   following taxol removal, the micronucleated state of cells
is incompatible with continued proliferative potential.

While recognizing that taxol alone is a potent cytotoxic

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agent, the time and concentration dependence in the proportion of cells in the late G2/M phase of the cell cycle is exploitable by the use of ionizing irradiations. The rationale for combining ionizing radiation and taxol therapeutically is that cells in G2 or M are relatively more radiosensitive than cells in other phases of the cell cycle. Clonogenicity results for 24-hour treatments with different concentrations of taxol alone, radiation alone and combined treatments of taxol and radiation at twenty four hours are shown in Figure 3A. Control plating efficiencies during the course of these experiments ranged from 53-72%. It can readily be seen that taxol alone is dose-dependently cytotoxic, with less than 0.1% clonogenic capacity at 100 nM, about 5% at 10 nM and about 90% at 1 nM. Constant treatment with taxol (i.e., for the 10 day cell incubation period) again resulted in about 90% clonogenicity at 1 nM but less than 0.01% clonogenicity at 10 nM, indicating a time- and concentration-dependent cytotoxicity.

The relative increase in radiosensitivity is demonstrated by the change in the shape of the survival curves at higher taxol concentrations. This change is more clearly demonstrated in Figure 3B, where the survival curves are normalized by the plating efficiencies of cells treated with the same taxol concentration and without radiation, i.e., survival for taxol alone is defined as 100% for each of these curves. The relative decrease in survival for a given radiation dose is apparent. There was an 87% relative survival after 2 Gy alone, which is compatible with that expected from this comparatively radio-resistant astrocytoma cell line. In the presence of 10 nM taxol, relative survival declined to 61%, and as radiation doses increase, there is an enhanced decrease in the surviving fraction for the combined treatment, reaching a factor of 54 at a dose of

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8 Gy (5%) versus 8 Gy plus 10 nM taxol (0.093%, data not shown in the Figure).

Combined treatment studies indicate that radiation is
5   relatively more cytotoxic as the concentration of taxol is
increased (see Figure 3). The enhancement of the response
to radiation by taxol, based on the cell cycle principles
discussed above, is seen in the concentration range where
kinetic and cell cycle effects were observed. For purposes
10   of the future use of the combination of these two
modalities, their interaction can be viewed in two ways. At
the higher taxol concentration of 10 nM, the drug itself
demonstrates a significant degree of cytotoxicity. In this
case, the interaction could be viewed as an interaction
15   between a chemotherapeutic agent and radiation.   For
concentrations in the 1 nM range, there is no significant
cell death without the radiation, but there is the
suggestion of increased cytotoxicity for a given dose of
radiation. Taxol would be classified as a radiosensitizer
20   when employed at this concentration (15). The sensitizer
enhancement ratio (SER) at 10% survival for 10 nM taxol was
approximately 1.8 and for 1 nM taxol the SER was
approximately 1.2.

25   Experimental data supporting the proposed mechanism of
interaction between taxol and radiation are shown in Figure
4. The longer cells are exposed to 10 nM taxol , the more
cells accumulate in the G2 or M phases of the cell cycle
(8), as shown in Figure 4C and Table 1. Plating efficiency
30   decreases as the incubation time with 10 nM taxol increases
(see Figure 4A). As the number of cells in G2 or M
increases, the relative sensitivity of the cell population
to radiation also increases. Figure 4B shows the time-
dependent increase in cell sensitivity to radiation, which

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is similar to the time-dependent sensitivity to cytotoxicity
(see Figure 4A). It is important to note that survival as
shown in Figure 4B is expressed relative to unirradiated
controls exposed to taxol for the same length of time (i.e.,
5   the data presented in Figure 4A). Thus, these data
represent the relative increase in the interaction between
taxol and irradiation. The interaction between taxol and
radiation therefore depends on the cell cycle distribution
of cells and is not a result of direct interaction between
10   the two modalities.

Clearly then, a 24-hour treatment with 10 nM taxol results
in a dramatic enhancement in the fraction of cells becoming
clonogenically incompetent. As is obvious from the flow
15   cytometric analysis of DNA contents (see Table 1), the
majority of cells irradiated following the 24-hour taxol
treatment are accumulated in the G2/M cycle phases. The
enhanced level of cell kill is then consistent (at least in
part) with the greater radiosensitivity of G2/M cells. To
20   demonstrate that this effect depends on the distribution of
cells in the cell cycle, cells were irradiated with 6 Gy
after 10 nM taxol treatment for 2, 8 and 24 hours. No
significant difference was seen between 6 Gy and taxol at 2
and 8 hours (9-14% relative survival). However, the results
25   of Figure 3 were confined to the 24 hour time point, namely
14% relative clonogenicity after 6 Gy and 0.14% relative
cell clonogenicity for the 24-hour taxol-treated cells
irradiated with 6 Gy. Note that the results obtained at 8
hours were for cells where there is a significant depletion
30   of G1 cells and enhancement of G2 cells (see Table 1), but
at this time, accumulations and/or cellular modifications
are inadequate to demonstrate differences in radiation
sensitivity.

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Conclusions

The results obtained with cycling, aerated, radio-resistant
brain tumor cells indicate that significant advantage may
5   derive from appropriate time-concentration dependent
interactions in combined modality protocols. A role for
radiation therapy and taxol, particularly in those tumors
where taxol alone shows some promise, is thereby worthy of
consideration. The combination may also be useful in the
10   treatment of refractory brain tumors, since normal tissue is
quiescent. This difference in cellular behavior should
result in a therapeutic gain for combined treatment.

The potential for the combination of taxol and radiation
15   being clinically useful is based on a number of factors.
The experiments presented here were performed at clinically
achievable drug concentrations (10 nM or less). Clinical
trials have shown that drug concentrations of 200 nM can be
maintained for greater than thirty hours using a 6 hour
20   infusion of 230 mg per m^2 taxol (12). Peak concentrations
of roughly 4000 nM were achieved. Regimens of this type
have been used with acceptable levels of taxol-related
toxicity.

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What is claimed is :

1.   A method of increasing the sensitivity of cells to the
       cytotoxic effects of ionizing radiation which comprises
5   first incubating the cells with a spindle poison in a
       suitable carrier at a concentration effective to
       inhibit the cells from progressing through the G2 or M
       phases of the cell cycle for an amount of time
       effective to inhibit division of the cells and then
10   administering an effective cytotoxic dose of ionizing
       radiation to the cells.

2.   The method of claim 1, wherein the cells are tumor
       cells.

15
3.   The method of claim 2, wherein the tumor cells are
       brain tumor cells.

4.   The method of claim 3, wherein the brain tumor cells
20   are   astrocytoma,   glioblastoma   multiforme   or
       medulloblastoma cells.

5.   The method of claim 2, wherein the tumor cells are
       ovarian tumor cells.

25
6.   The method of claim 5, wherein the ovarian tumor cells
       are epithelial, sex-chord stromal, lipid, germ or
       gonadoblastoma cells.

30   7.   The method of claim 2, wherein the tumor cells are lung
       tumor cells.

8.   The method of claim 7, wherein the lung tumor cells are
       adenocarcinoma, large cell, small cell or squamous cell

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tumor cells.

9. The method of claim 2, wherein the tumor cells are
    breast tumor cells.

10. The method of claim 9, wherein the breast tumor cells
    are invasive duct carcinoma, medullary carcinoma or
    mucinous carcinoma cells.

11. The method of claim 2, wherein the tumor cells are
    melanoma cells.

12. The method of claim 1, wherein the spindle poison is a
    taxol-related compound.

13. The method of claim 12, wherein the taxol-related
    compound is taxol.

14. The method of claim 13, wherein taxol is a natural
    product of the yew <u>Taxis</u> Sp. L.

15. The method of claim 1, wherein the suitable carrier is
    an aqueous solution.

16. The method of claim 15, wherein the aqueous saline
    solution comprises dimethyl sulfoxide.

17. The method of claim 1, wherein the effective inhibiting
    concentration of the spindle poison is a concentration
    from about 1 nM to about 50 μM.

18. The method of claim 1, wherein the effective inhibiting
    amount of time amount is an amount from about 6 hours
    to about 24 hours.

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19. The method of claim 18, wherein the effective inhibiting amount of time is an amount from about 8 hours to about 20 hours.

5   20. The method of claim 19, wherein the effective inhibiting amount of time is an amount about 18 hours.

21. The method of claim 1, wherein an effective cytotoxic dose of ionizing radiation is administered to the cells

10   by exposing the cells to a beam of radiation.

22. The method of claim 21, wherein the beam of radiation is produced by an irradiator.

15   23. The method of claim 22, wherein the irradiator comprises the radioisotope Cesium-137.

24. The method of claim 22, wherein the irradiator comprises the radioisotope Iridium-192.

20

25. The method of claim 1, wherein an effective cytotoxic dose of ionizing radiation is administered to the cells by contacting the cells with an aqueous solution containing a radioisotope.

25

26. The method of claim 25, wherein the radioisotope is Phosphorous-32.

27. The method of claim 21 or 25, wherein the effective

30   cytotoxic dose of ionizing radiation is a dose from about 1 Gy to about 10 Gy.

28. The method of claim 27, wherein the effective cytotoxic dose of ionizing radiation is a dose from about 2 Gy to

about 8 Gy.

29. A method of treating a cancer patient which comprises
first administering to the patient a spindle poison in
a suitable carrier in an amount effective to inhibit
tumor cells in the patient from progressing through the
G2 or M phases of the cell cycle for an amount of time
effective to inhibit division of the tumor cells and
then administering an effective cytotoxic dose of
ionizing radiation to the patient.

30. The method of claim 29, wherein the administration of
the spindle poison comprises intravenous injection.

31. The method of claim 29, wherein the patient is a human
patient.

32. The method of claim 29, wherein the spindle poison is
a taxol-related compound.

33. The method of claim 32, wherein the taxol-related
compound is taxol.

34. The method of claim 33, wherein taxol is a natural
product of the yew _Taxus_ Sp. L.

35. The method of claim 29, wherein the suitable carrier is
an aqueous infusion solution.

36. The method of claim 35, wherein the aqueous infusion
solution comprises 5% dextrose injection USP.

37. The method of claim 29, wherein the amount of the
spindle poison effective to inhibit the progression of

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cells through the G2 or M phases of the cell cycle is an amount sufficient to establish a concentration of the spindle poison in the blood of the patient effective to inhibit the cells from progressing through
5   the G2 or M phases of the cell cycle.

38.   The method of claim 37, wherein the effective inhibiting concentration of the spindle-blocking agent is a concentration from about 10 nM to 100 nM.
10

39.   The method of claim 37, wherein the effective inhibiting concentration of the spindle poison in the blood of the patient is maintained by readministering the agent to the patient at an interval of time after
15   the preceding administration.

40.   The method of claim 39, wherein the interval of time comprises an interval of about one week.

20   41.   The method of claim 29, wherein the effective inhibiting amount of time is an amount greater than about 24 hours.

42.   The method of claim 29, wherein an effective cytotoxic
25   dose of ionizing radiation is administered by exposing the patient to a beam of radiation produced by a linear accelerator.

43.   The method of claim 42, wherein the effective cytotoxic
30   dose of ionizing radiation is a dose from about 4,000 cGy to about 8,000 cGy.

44.   The method of claim 43, wherein the effective cytotoxic dose of ionizing radiation is a dose from about 6,500

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cGy to about 7,500 cGy.

45. The method of claim 42, wherein the effective cytotoxic dose of ionizing radiation is administered in a schedule of increments.

46. The method of claim 45, wherein the schedule of increments comprises a dose of ionizing radiation of about 200 cGy per day for five days per week for a period from about six weeks to about eight weeks.

47. The method of claim 29, wherein an effective cytotoxic dose of ionizing radiation is administered by implanting a radioisotope in tumors in the body of the patient for an amount of time sufficient to expose the tumor to the effective cytotoxic dose.

48. The method of claim 47, wherein the effective cytotoxic dose of ionizing radiation is a dose from about 8,000 cGy to about 20,000 cGy.

49. The method of claim 48, wherein the effective cytotoxic dose of ionizing radiation is a dose about 16,000 cGy.

50. The method of claim 47, wherein the radioisotope is Iridium-192.

51. The method of claim 47, wherein the radioisotope is Iodine-125.

52. The method of claim 47, further comprising exposing the patient to a beam of radiation.

53. The method of claim 52, wherein the beam of radiation

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is produced by a linear accelerator.

54.   The method of claim 52, wherein the effective cytotoxic dose of ionizing radiation comprises a dose of ionizing radiation from about 4,000 cGy to about 6,000 cGy administered by the beam of radiation and a dose of ionizing radiation from about 2,000 cGy to about 4,000 cGy administered by the radioisotope implant.

55.   The method of claim 29, wherein the effective cytotoxic dose of ionizing radiation is administered by applying radiosurgery to the patient.

56.   The method of claim 55, wherein the effective cytotoxic dose of ionizing radiation is a dose from about 1,200 cGy to about 2,800 cGy.

57.   A method of increasing the sensitivity of cells to the cytotoxic effects of bleomycin which comprises first incubating the cells with a spindle-poison in a suitable carrier at a concentration effective to inhibit the progression of the cells through the G2 or M phases of the cell cycle for an amount of time effective to inhibit cell division and then exposing the cells to an effective cytotoxic concentration of bliomycin.

58.   A kit useful for treating a cancer patient which comprises:
(i) a spindle poison in an amount sufficient to establish a concentration of the poison in the body of the patient effective to inhibit the progression of tumor cells through the G2 or M phases of the cell cycle; and

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(ii)an effective cytotoxic dose of an agent selected
from the group consisting of a radioisotope in aqueous
solution, an antibody-conjugated radioisotope or
bleomycin.

5

59.   The kit of claim 58, further comprising instructions
for administering the spindle poison and cytotoxic
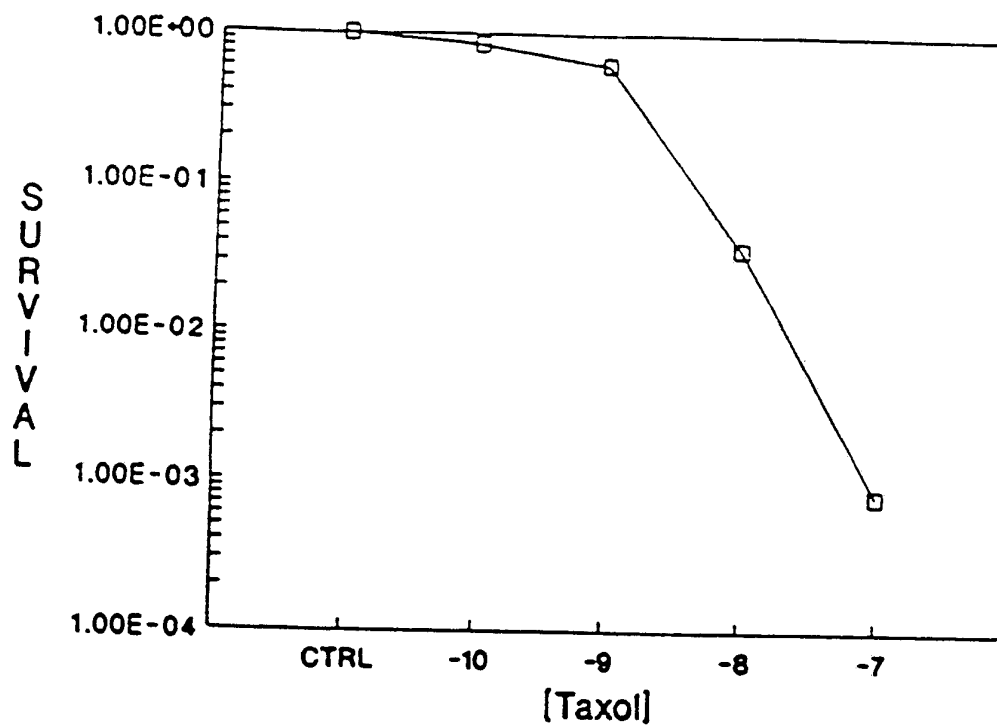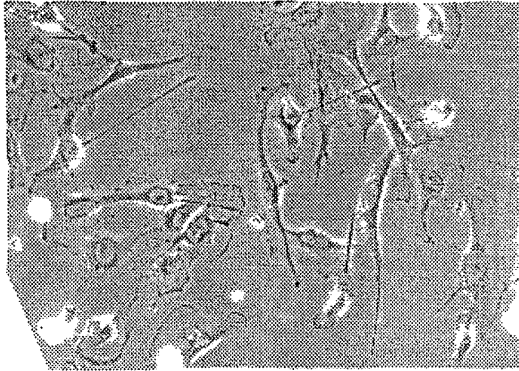agent.
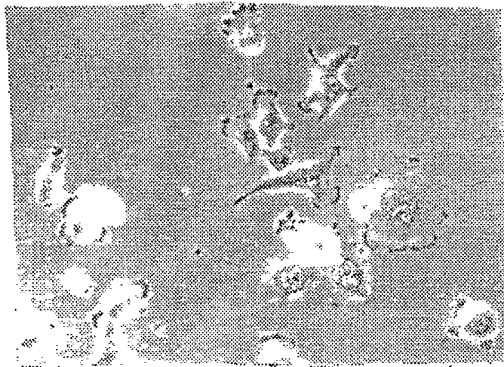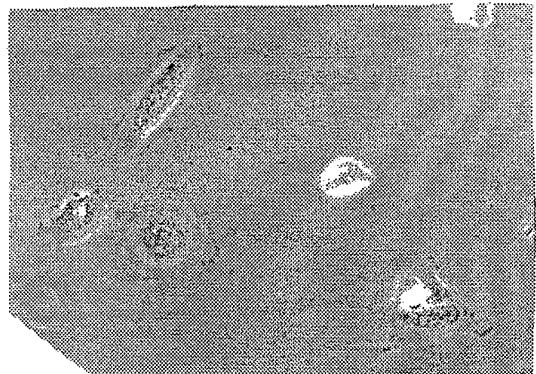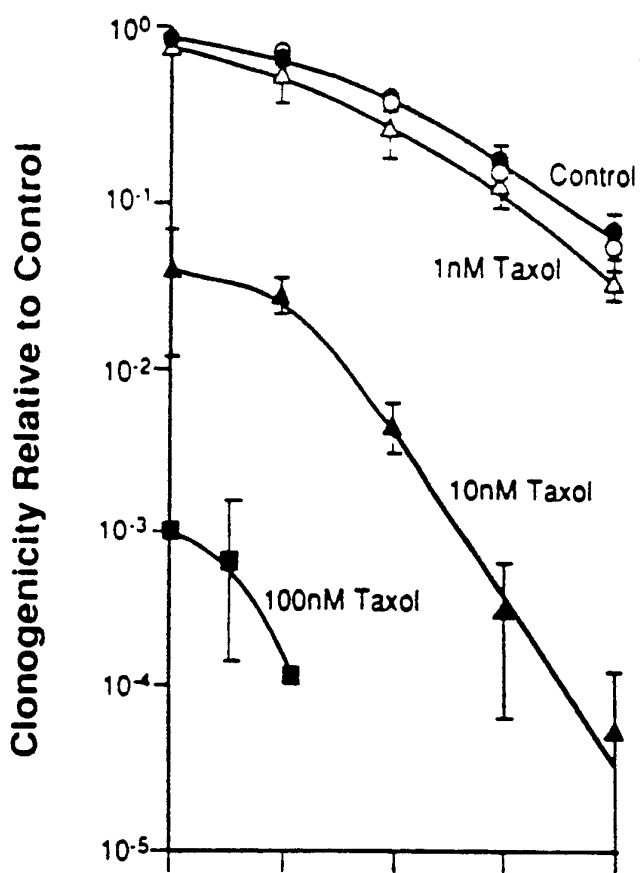
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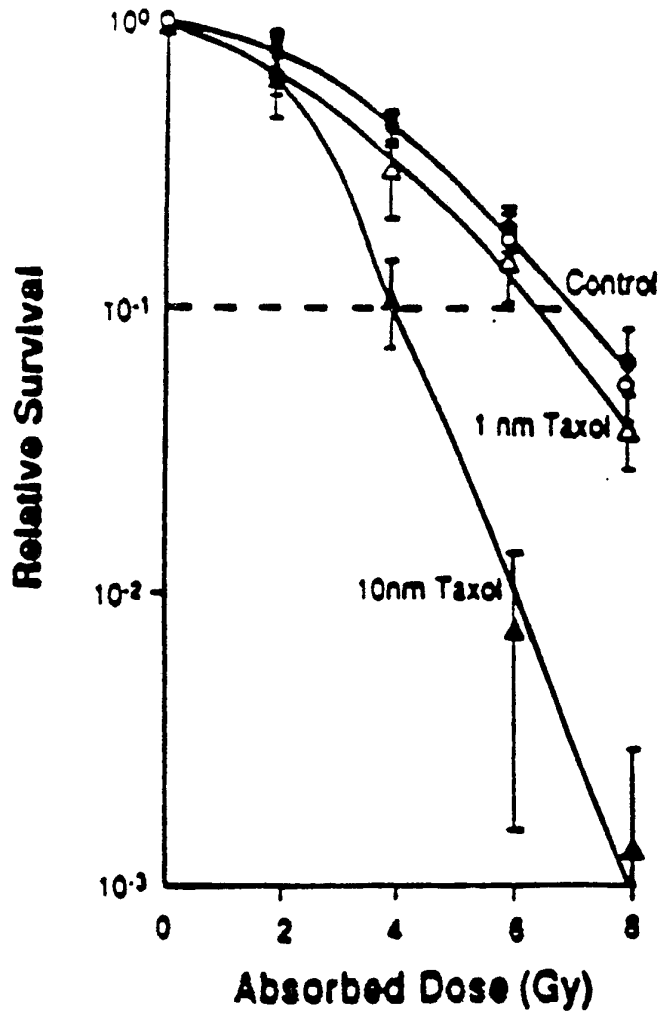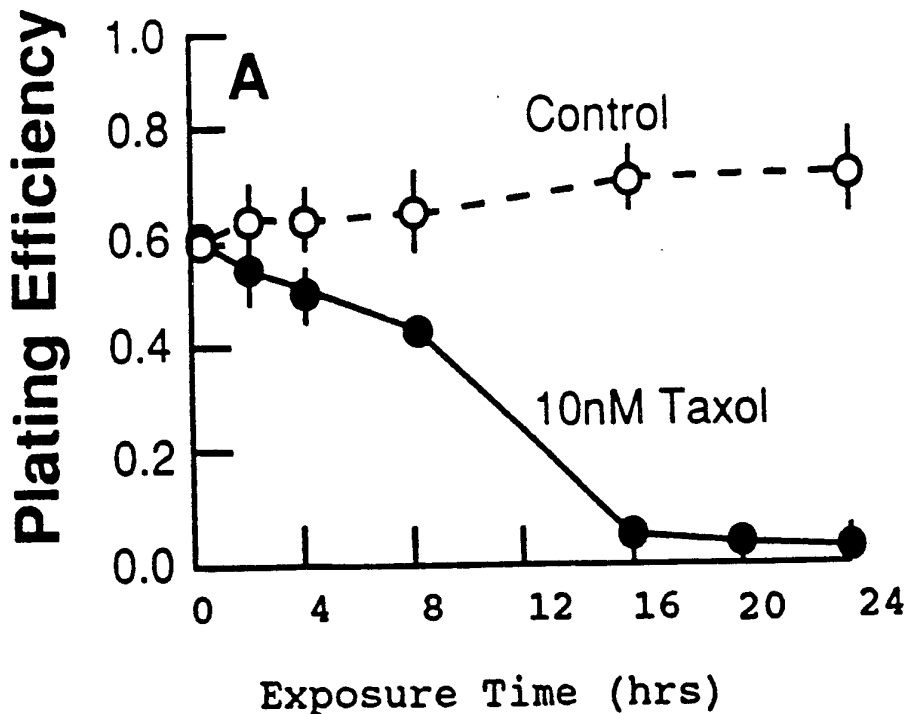

Figure 1

FIGURE 2A
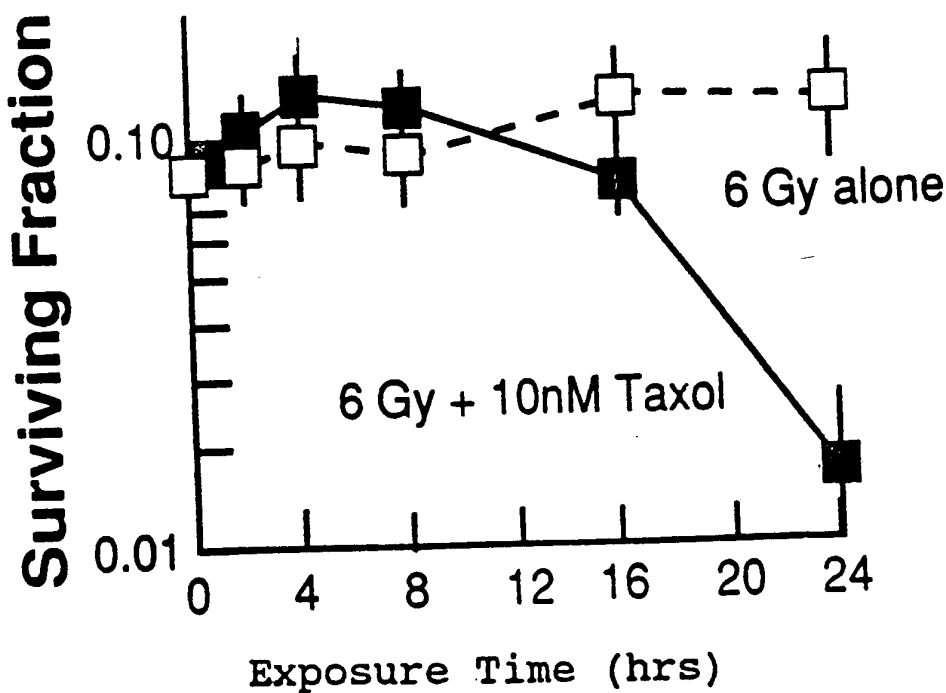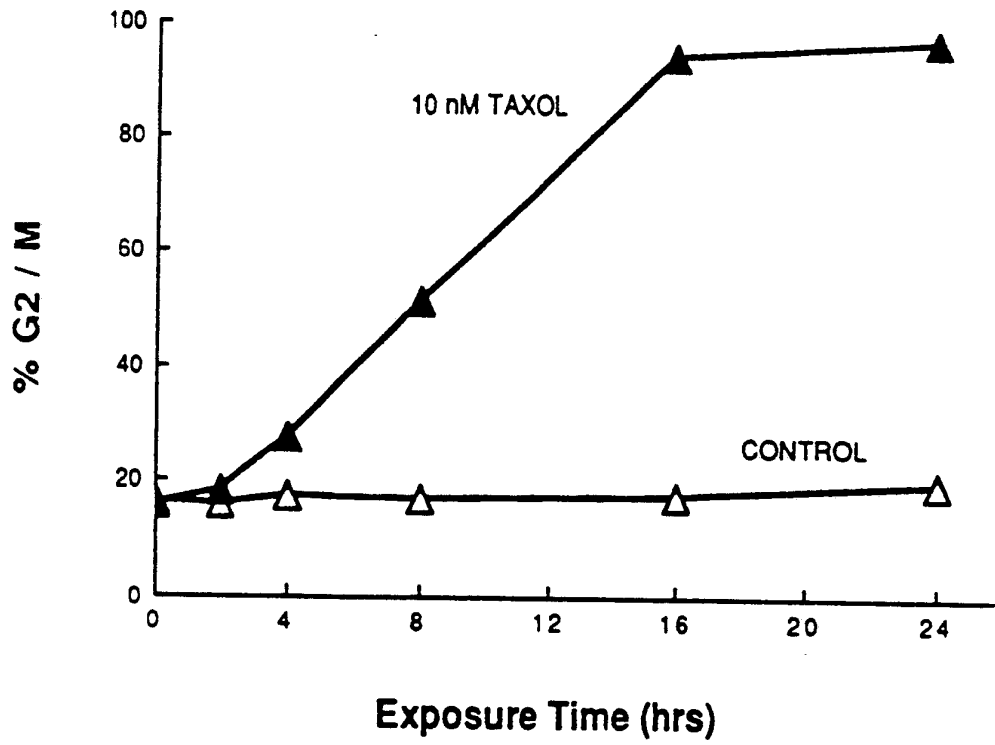
FIGURE 2B

FIGURE 2C

FIGURE 2D

FIGURE 3A

FIGURE 3B

FIGURE 4A

FIGURE 4B

FIGURE 4C

INTERNATIONAL SEARCH REPORT

A.   CLASSIFICATION OF SUBJECT MATTER

IPC(5)   :A61K 43/00, A01N 43/02
US CL :424/1.1, 514/449
According to International Patent Classification (IPC) or to both national classification and IPC

B.   FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :   424/1.1, 514/449

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN Express, files Reg, CA, Medline taxol and radiat?
APS Text + Dialog taxol and (radiat? or radiosens.)

C.   DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US,A, 4,814,470 (Colin et al.) 21 March 1989 See columns 1-3.	
A	US,A, 4,876,399 (Holton et al.) 24 October 1989 See columns 1 and 2.	
A	US,A, 4,960,790 (Stella et al.) 02 October 1990 See columns 1 and 2.	
A	US,A, 5,059,699 (Kingston et al.) 22 October 1991 See column 1.	
A,E	US,A, 5,157,049 (Haugwitz et al.) 20 October 1992 See columns 1 and 2.	

☐   Further documents are listed in the continuation of Box C.   ☐   See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
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