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[Continued on next page]

(54) Title: TREATMENT OF SQUAMOUS CELL CARCINOMA WITH HSP27 ANTISENSE OLIGONUCLEOTIDES AND RADIOTHERAPY

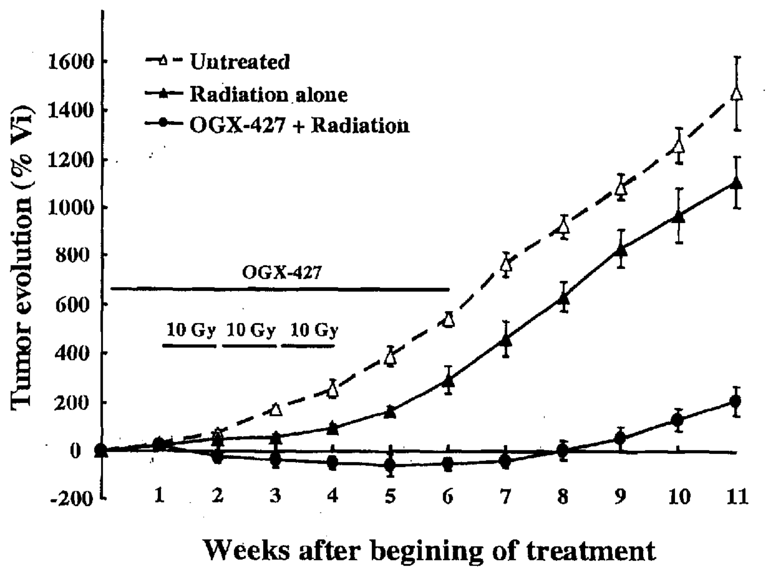

Fig. 3

(57) Abstract: Squamous cell carcinomas, such as squamous head and neck cancer, are treated with a combination of radio-therapy and a therapeutic agent that reduces the amount of hsp27 in the squamous cancer cells. In specific embodiments, the therapeutic agent that reduces the amount of hsp27 is an antisense oligonucleotide therapeutic agent.

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Treatment of Squamous Cell Carcinoma

This application claims the priority benefit of US Provisional Application No. 60/893,086, filed March 5, 2007, which application is incorporated herein by reference for all purposes.

Background of the Invention

This application relates to the treatment of squamous cell carcinomas using a combination of radiation and an agent that inhibits the level of heat shock protein 27 (hsp27) expression.

Squamous cell carcinoma is a cancer that begins in squamous cells -- thin, flat cells that look under the microscope like fish scales. Squamous cells are found in the tissue that forms the surface of the skin, the lining of hollow organs of the body, and the passages of the respiratory and digestive tracts. Squamous cell carcinomas may arise in any of these tissues. Thus, some skin cancer, head and neck cancer, lung cancer, mouth cancer, breast, esophageal cancer, and cervical cancer are cancers of squamous cell origin. Although it is rare, primary squamous cell cancer of the prostate may also occur. Treatment for squamous cell cancer may involve treatment with radiation with or without surgical removal of the tumor mass.

Hsp27 is a known inhibitor of apoptotic cell death in various types of cancers, including some squamous cell carcinomas and can act as a means for protecting cells against chemotherapy agents. Huot et al. (1991) Cancer Res. 51: 5245-5252; Oesterreich et al. (1993) The small heat shock protein hsp27 is correlated with growth and drug resistance in human breast cancer cell lines. Cancer Res. 53: 4443-4448; Garrido et al. (1997) Cancer Res. 57: 2661-2667; Yonekura et al., (2003) Cell Death and Differentiation 10, 313–322. On the other hand, it has been reported that there is no correlation between overexpression of hsp27 expression and resistance of squamous cell head and neck cancer to radiotherapy, even though there is a correlation between hsp27 over expression and heat and drug resistance. Fortin et al., (2000) Int. J. Radiation Oncology Biol. Phys. 46: 1259-1266.

US Patent No. 7,101,991, which is incorporated herein by reference, discloses oligonucleotide therapeutic agents that target hsp27. Reduction in hsp27 expression was shown to reduce progression of non-squamous prostatic tumor cells to androgen independence, and also to enhance to sensitivity of such prostate tumor cells to chemotherapy.

Summary of the Invention

Notwithstanding the absence of a correlation between hsp27 over-expression and resistance to radiotherapy, it has now been surprisingly found that reduction of hsp27 expression results in increased radio-sensitivity in squamous cell carcinoma. Thus, the present invention provides a method for treatment of squamous cell carcinoma comprising treating a patient diagnosed with a squamous cell carcinoma with a combination of radio-therapy and a therapeutic agent that reduces the amount of hsp27 in the squamous cancer cells. In specific embodiments, the therapeutic agent that reduces the amount of hsp27 is an oligonucleotide therapeutic.

Brief Description of the Drawings

Fig. 1 shows tumor evolution in untreated mice (NT) and in mice treated with the mismatch control oligonucleotide (MS), the hsp27 antisense alone (OGX), radiation alone, 10Gy (IR), the mismatch control oligonucleotide and radiation 10Gy (MS+IR) and the hsp 27 antisense and radiation 10Gy (OGX + IR).

Fig. 2 shows weight variation in the mice of Fig. 1.

Fig. 3 shows tumor evolution as the percentage of initial tumor volume at the beginning of treatment (Vi) when mice are treated with OGX-427 and radiation at a level of 30Gy.

Fig. 4 shows gluathione levels in mice treated with OGX-427 and radiation at a level of 30Gy.

Figs. 5A and 5B show the host toxicity profile of radiation therapy, as monitored by body weight measurements in tumor-bearing mice and survival percentage, respectively.

Detailed Description of the Invention

The present invention relates to the use of therapeutic agents that reduce the amount of active hsp27 in squamous cell cancer cells in combination with radio-therapy to provide enhanced therapeutic efficacy to the radio-therapy.

As used in the specification and claims of this application, the term "active hsp27" refers to hsp27 which is active as a chaperone to stabilize protein structure at times of stress and in particular to inhibit the activity of caspase-3, a mediator of apoptosis. Reduction in levels of active hsp27 can be achieved by reducing the total amount of hsp27, either by restricting production of hsp27 or by degrading hsp27 at a rate faster than it is being produced, or by converting hsp27 to an inactive form, for example by sequestering hsp27 in an inactive complex such as with an anti-hsp27 antibody. Anti-hsp27 antibodies are known, for example from Tezel and Wax, *J. Neuroscience* 10:3553-3562 (2000).

The phrase "in combination with radio-therapy" refers to administration of the therapeutic agent that reduces hsp27 at a time before, during or after radiotherapy, provided that the time of administration is sufficiently close to the time of radiotherapy that the reduction in hsp27 overlaps in time with the cytotoxic/apoptotic effects of the radio-therapy.

The sequence of human hsp27 mRNA is known, for example from NCBI Accession Numbers AB020027, X54079, NM_006308, NM_001540 and NM_001541. The cDNA sequence (Seq. ID No. 91) forms the basis for the development of antisense oligonucleotides and RNAi nucleotide inhibitors. Suitable sequences for antisense, and for RNAi are those that target bases in the regions from nucleotides 131-161, 241-261, 361-371, 551-580, 661-681 and 744-764 in Seq. ID No. 91. In order to target bases within these regions, an antisense or RNAi molecule must have sequence specificity with a region that includes at least one of the listed bases, preferably at least 10 of the listed bases. Suitable antisense oligonucleotides have a length of from 12 to 35 oligonucleotides and have sequence specificity to the hsp27 mRNA sequence. Specific suitable antisense sequences are listed with DNA based only in Seq. ID Nos. 1 - 90. Modifications to include RNA bases in place of the corresponding DNA base may be made.

RNA interference or "RNAi" is a term initially coined by Fire and co-workers to describe the observation that double-stranded RNA (dsRNA) can block gene expression when it is introduced into worms (Fire et al. (1998) Nature 391, 806-811, incorporated herein by reference). dsRNA directs gene-specific, post-transcriptional silencing in many organisms, including vertebrates, and has provided a new tool for studying gene function. RNAi involves mRNA degradation, but many of the biochemical mechanisms underlying this interference are unknown. The use of RNAi has been further described in Carthew et al. (2001) Current Opinions in Cell Biology 13, 244-248, and Elbashir et al. (2001) Nature 411, 494-498, both of which are incorporated herein by reference. The RNAi molecules of the invention are double-stranded or single-stranded RNA of from about 21 to about 23 nucleotides which mediate RNA inhibition. That is, the isolated RNAi of the present invention mediate degradation of mRNA of the hsp27 gene. Specific sequences suitable for use in RNAi inhibitors of hsp27 are listed as Seq. ID Nos. 82-90.

The terms RNA, RNA molecule(s), RNA segment(s) and RNA fragment(s) may be used interchangeably to refer to RNA that mediates RNA interference. These terms include double-stranded RNA, single-stranded RNA, isolated RNA (partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA), as well as altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of the RNA or internally (at one or more nucleotides of the RNA). Nucleotides in the RNA molecules of the present invention can also comprise non-standard nucleotides, including non-naturally occurring nucleotides or deoxyribonucleotides. Collectively, all such altered RNAi compounds are referred to as analogs or analogs of naturally-occurring RNA. RNA of the present invention need only be sufficiently similar to natural RNA that it has the ability to mediate RNAi. As used herein the phrase "mediate RNAi" refers to and indicates the ability to distinguish which mRNA are to be affected by the RNAi machinery or process. RNA that mediates RNAi interacts with the RNAi machinery such that it directs the machinery to degrade particular mRNAs or to otherwise reduce the expression of the target protein. In one embodiment, the present invention relates to RNA molecules that direct cleavage of specific mRNA to which their sequence corresponds. It is not necessary that there be perfect correspondence of the sequences, but the correspondence must be sufficient to enable the RNA to direct RNAi inhibition by cleavage or lack of expression of the target mRNA.

Exemplary compositions useful in the invention are antisense hsp27 oligonucleotides or RNAi nucleotide inhibitors as described in US Patent No. 7,101,991. The invention further relates to the use of these compositions in the treatment of squamous prostate cancer and other squamous cell cancers that express hsp27 in elevated amounts.

The oligonucleotides employed as antisense or RNAi molecules may be modified to increase the stability of the oligonucleotides *in vivo*. For example, the oligonucleotides may be employed as phosphorothioate derivatives (replacement of a non-bridging phosphoryl oxygen atoms with a sulfur atom) which have increased resistance to nuclease digestion. MOE modification is also effective.

Administration of antisense oligonucleotides can be carried out using the various mechanisms known in the art, including naked administration and administration in pharmaceutically acceptable lipid carriers. For example, lipid carriers for antisense delivery are disclosed in US Patents No. 5,855,911 and 5,417,978 which are incorporated herein by reference. In general, the antisense is administered by intravenous, intraperitoneal, subcutaneous or oral routes, or direct local tumor injection.

The amount of antisense oligonucleotide or other therapeutic administered is one effective to reduce the amount of active hsp 27. It will be appreciated that this amount will vary both with the effectiveness of the antisense oligonucleotides or other therapeutic agent employed, and with the nature of any carrier used. The determination of appropriate amounts for any given composition is within the skill in the art, through standard series of tests designed to assess appropriate therapeutic levels. By way of specific non-limiting example, the 4-12-4 2'-MOE gapmer of Seq ID No. 92 discussed in the examples below is being tested in phase I human clinical trials in combination with docetaxel therapy using weekly injected doses of 200mg, 400mg, 600mg, 800mg or 1000mg.

The RNAi molecules of the invention are used in therapy to treat patients, including human patients, that have cancers or other diseases of a type where a therapeutic benefit is obtained by the inhibition of expression of the targeted protein. siRNA molecules of the invention are administered to patients orally, by one or more daily injections (intravenous, subcutaneous, intravesical, or intrathecal) or by continuous intravenous or intrathecal administration for one or more treatment cycles to reach plasma and tissue concentrations suitable for the regulation of the targeted mRNA and protein.

The ability of therapeutic agents that reduce the level of hsp27 to enhance the radio-sensitivity of squamous cell carcinoma was determined in experiments using a radio-resistant human cell line derived from a head and neck squamous cell carcinoma as described in the example below. In these examples, the therapeutic agent is a 4-12-4 2'-MOE gapmer oligonucleotide with phosphorothiolated internucleotide linkages which can be represented as 5'-GGGAMeCGMeCGGMeCGMeCTMeCGGMeUMeCAMeU-3', (Seq. ID No. 92) where G, A, MeC, and T represent the nucleosides 2'-deoxyguanosine, 2'-deoxyadenosine, 2'-deoxy-5-methylcytidine, and 2'-deoxythymidine (DNA nomenclature). The underlined nucleosides (G, A, MeC, and MeU) denote 2'-O-methoxyethyl (2'-MOE) modifications of the nucleosides (RNA nomenclature for guanosine, adenosine, 5-methylcytidine and 5-methyluridine). The internucleotide linkages are phosphothioate diester (sodium salts). This compound is also known by the name OGX-427 and CAS Registry No. 915443-09-3. This oligonucleotide was observed to reduce the amount of hsp27 protein in the cells after treatment with combined oligonucleotide and radiation therapy by over 68% as compared to treatment with radiation and a mismatch oligonucleotide control sequence.

Fig. 1 shows tumor evolution in untreated mice (NT) and in mice treated with the mismatch control oligonucleotide (MS), the hsp27 antisense alone (OGX), radiation alone (IR), the mismatch control oligonucleotide and radiation (MS+IR) and the hsp 27 antisense and radiation (OGX + IR). As is readily apparent, the combination of hsp 27 antisense and radiation resulted in a dramatic change in the rate of tumor evolution.

No significant additional tissue damage or toxicity (as reflected by weight loss) was observed in mice treated with the antisense oligonucleotide, with or without radiation, as compared to mismatch oligonucleotide treatments. Fig. 2 shows weight variation for the same mice as Fig. 1. This figure shows that minimal variation in weight occurred for the effective treatment regime.

EXAMPLES

Example 1

A) Protocol

- Tumor Cell Line

Radioresistant SQ20B human cell line, derived from a head and neck squamous cell carcinoma (HNSCC), established in John Little's laboratory (Department of Cancer Biology, Harvard School of Public Health, Boston, USA) (Gery B et al., (1996). Int J Radiat Oncol Biol Phys 34: 1061-1071.). These cells are characterized by a high level of Hsp27 protein expression (1.53µg/mg total protein). Cells were grown in DMEM-Glutamax I™ medium supplemented with 10% fetal calf serum (Rodriguez-Lafrasse C et al., (2001). Biochem 357:407-416).

- Treatment of cells with antisense oligonucleotide

SQ20B cells were plated at the density of 24000 cells per cm^2 and treated the day after for 1 or 2 days with OGX-427 (OncoGenex Technologies, Inc., Vancouver, Canada) or mismatch control (MS) oligonucleotides at a concentration of 200 nM after a preincubation for 20 minutes with 3.5 mg/ml of OligofectAMINE™ (Invitrogen-Life Technologies, Inc) in serum-free OPTI-MEM™ (Invitrogen). Four hours after the beginning of the incubation, the medium was replaced with standard culture medium.

- Western blot analysis

Hsp27 protein levels were assessed by Western Blot analysis with a mouse monoclonal anti-Hsp27 (Stressgen). GAPDH was used as a loading control. For densitometric analysis, scanned autoradiographs were quantified using 1Dscan EX3.1 software.

- Clonogenic assay

Clonogenic survival of SQ20B treated cells was assessed by a standard colony-formation assay as described by Alphonse et al. (2004) Oncogene. 23(15):2703-15. Three hours after treatment, cells were harvested and seeded in 25 cm^2 culture flasks at densities of 16 to 120 cells/cm^2. Cells were then irradiated with a Clinac 600C™ X-ray irradiator, at doses varying between 0.5 and 8 Gy. The survival variables A and B were plotted according to the linear quadratic equation ($SF = \exp[- A X \ D - B X \ D^2]$), where D represents the dose of irradiation. The survival fraction at 2 Gy (SF2) was determined as an index of radiosensitivity.

B) Results

- Dose-dependant inhibition of Hsp27 expression by OGX-427

SQ20B treated cells were collected at different times after transfection and the decrease of Hsp27 protein expression was measured by Western Blot analysis. OGX-427 treatment dose-dependently inhibited Hsp27 expression, up to 70% for 200 nM treatment and 90% for 2x200 nM at 24 hours post-transfection. MS control oligonucleotide did not modify Hsp27 expression.

- OGX-427 increases clonogenic cell death

To determine whether the attenuation of Hsp27 protein expression sensitizes SQ20B cells to irradiation, survival curves were established after treatment. A significant increase of clonogenic cell death was observed, the survival fraction at 2 Gy (SF2) shifted from 0.72 to 0.49 in SQ20B treated with 2x200 nM OGX-427 (SF2=0.71 in SQ20B MS cells).

<u>Example 2</u>

We next evaluated the effect of OGX-427 treatment in combination to irradiation on SQ20B heterotopic xenograft tumors.

A) Protocol

Approximately 3 x 10^6 SQ20B cells diluted in 0.2 ml PBS were subcutaneously inoculated in the right flank region of 4 weeks old female athymic nude mice (SWISS nu/nu) (Charles River, France) via a 23-gauge needle, under ketamine and xylazine anesthesia. When tumors reached 400 to 500 mm3, usually 4 to 5 weeks after injection, mice were randomly divided into six treatment groups:

1- Untreated (10 mice)

2- Radiation alone (10 mice),

3- MS (10 mice),

4- OGX-427 (10 mice),

5- MS + radiation (10 mice),

6- OGX-427 + radiation (11 mice).

ASO-Hsp27 (OGX-427) and MS at doses of 10 mg/kg (diluted in PBS) were administered by intraperitoneal injection (3, 4, 5, 6 groups). Mice were treated for six weeks. In the first week, oligonucleotides were injected during 5 consecutive days (from Wednesday to Sunday). The 2, 5, 6 groups of mice were irradiated at a dose of 2 Gy/day (1 Gy from each side of the tumor) for 5 consecutive days (from Monday to Friday) combined to 3 injections of oligonucleotides for the 5,6 groups (on Monday, Wednesday and Friday, after irradiation). The last four weeks of treatment, 3 injections/week were performed (on Monday, Wednesday and Friday) on 3, 4, 5, 6 groups. Before irradiation on the Clinac X-ray irradiator (Radiotherapy Department of Lyon-Sud Hospital Center), mice were anaesthetized with ketamine (120 mg/kg) and xylazine (10 mg/kg). Tumors were measured once weekly by use of calipers and their volumes were calculated by the formula: 0.5236 a x b2, where a and b are long and short diameters respectively.

The delivered amount of OGX-427 was selected in reference to the works of Rocchi P et al 2004. Cancer Res. 64: 6595-6602, Rocchi P et al 2005. Cancer Res. 65:11083-11093, Yip KW et al 2005. Clin Cancer Res. 11:8131-8144. The radiation dose of 2 Gy per day was chosen as being the therapeutic dose given daily to a patient.

Tumor growth, toxicity (weight) and mortality were followed along the treatment. Apoptosis and Hsp27 protein expression were analysed on tumors taken after irradiation (at the end of the second week) and at the end of the treatment. Table 1 shows the number of mice sacrificed at each step, and mice death.

Apoptosis was detected using TUNEL staining (Promega), and Hsp27 protein level was determined by Western Blot analysis.

B) Results

- Effects of OGX-427 combined to irradiation on SQ20B tumor growth

Tumor evolution was represented by the percentage of initial tumor volume at the beginning of treatment. Table 2 shows the number of mice used for measurement of tumor volume in each week of the experiment. The results of these measurements are summarized in Fig. 1.

Table 1

	After irradiation (end 2nd week)	End of treatment (end 6th week)	Mortality 17 weeks following treatment
Untreated (10 mice)	4	2	4*
Radiation alone (10 mice)	3	2	1" 4*
MS (10 mice)	4	2	4*
OGX-427 (10 mice)	3	2	5*
MS + radiation (10 mice)	3	2	1", 4*
OGX-427+radiation(11 mice)	3	2	1", 3*

* Natural death (during treatment)

" Death due to anaesthesia

Table 2

	Week	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**11**
Mice*									
NT		10	10 (4)	6	5	5	5 (2)	3	1
MS		10	10 (4)	6	5	5	5 (2)	3	1
OGX		9	9 (3)	6	6	6	6 (2)	4	3
IR		10	9 (3)	6	6	6	6 (2)	4	4
MS+IR		9	8 (3)	5	4	4	4 (2)	2	2
OGX+IR		11	10 (3)	7	7	6	6 (2)	3	3

Table 2 shows number of mice on which the measurement of tumor volume was taken. (number) of sacrificed mice at the end of week 2 and 6.

In vivo, OGX-427 monotherapy had no effect on tumor evolution (same slope as non-treated and MS mice). Irradiation alone and MS + irradiation induced a tumor regression on the second and third week of treatment. From the fourth week, the curve of tumor evolution paralleled that of non-treated mice. Combining OGX-427 treatment with radiotherapy inhibited tumor growth very significantly. At the end of treatment (week 6), the tumor volume began to increase. Apoptosis was evaluated on tumor taken at the end of the second week of treatment by the TUNEL immuno-staining. Apoptosis was significantly increased in tumors from mice treated by OGX-427 + IR, as compared to MS or MS+IR.

To determine the level of Hsp27 protein decrease induced by OGX-427, Western blots were performed on tumors taken at the end of the second week of treatment. Hsp27 was significantly decreased in OGX-treated tumors, up to 65% compared to MS-treated tumors.

No significant tissue damage, toxicities (weight loss) were observed on mice treated with OGX-427 combined or not with irradiation, compared to MS-treated groups as reflected in Fig. 2. Two mice which received the combined treatment (OGX + IR) were still alive at week 17, while all the mice from the other groups (untreated, IR, MS, MS+IR and OGX mice) died on weeks 13-14.

<u>Example 3</u>

In the sutdy reported in Example 2,, we observed the sensitization of OGX-427 treatment on HNSCC xenograft tumors irradiated with a total dose of 10 Gy. In order to confirm the effect of OGX-427 combined to irradiation, a second *in vivo* study was performed. Mice received the same OGX-427 treatment but were irradiated for a total dose of 30 Gy. In the absence of significant difference in the evolution of tumors from mice treated with MS-control alone or associated with irradiation observed in Example 2, this study was only conducted in three groups of mice: Untreated, Radiation 30Gy, and OGX-427 + 30 Gy.

A) Experimental protocol

Female athymic nude mice (Charles River, France) were injected s.c with 3 x 106 of SQ20B cells in the right flank region via a 23-gauge needle under ketamine/xylazine anesthesia. When tumors reached a volume of 300 to 400 mm3, usually 5 to 6 weeks after injection, mice were randomly divided into three treatment groups (16 mice/group): Untreated, Radiation 30Gy, OGX-427 + radiation 30Gy. Mice received the same OGX-427 (10 mg/kg for six weeks) treatment as in the first study. Tumors were irradiated for a total dose of 30 Gy delivered at a dose of 2 Gy/day (5 days/week) (weeks 2, 3, 4) (dose rate of 2 Gy/min). Irradiations were performed with a Saturne 42 irradiator, under ketamine/xylazine anesthesia.

Mice were monitored by general clinical observation as well as by body weight and tumor growth. Assessment of tumor volume was followed up weekly over 11 weeks from the beginning of the treatment and was calculated according to the formula: 0.5236 (L x W2), where (L) and (W) are the length and width diameters, respectively. Histological analysis and quantifications of biochemical markers were performed on tumors taken after irradiation and at the end of treatment.

B) Results

- OGX-427 combined to 30 Gy significantly inhibits SQ20B tumor growth

Tumor evolution was represented by the percentage of initial tumor volume at the beginning of treatment (Vi) and the results are summarized in Fig. 3. Combining OGX-427 treatment with 30 Gy radiation significantly increased the inhibition of tumor growth. At the end of treatment, a respective 720 and 500% reduction of mean tumor volume was measured compared to the non-treated and only irradiated mice groups.

- Combined treatment of OGX-427 and radiation enhances apoptosis and decreases cell proliferation

Immunohistochemical analyses showed higher levels of apoptosis (TUNEL staining) and a decrease of tumor cell proliferation (Ki-67 staining) in tumors from mice treated with OGX-427 plus radiation. An amplification of the level of apoptosis by increasing the dose of radiation without changing OGX-427 treatment was also observed. In the same way, a greater decrease in cell proliferation was observed in tumors treated with OGX-427 combined with 30 Gy irradiation (Ki-67 positive cells).

The increase of apoptosis was associated with an attenuation of the antioxidant defense capacity of tumor cells. OGX-427 combined with 30 Gy irradiation decreases significantly the intratumoral glutathione levels. As reflected in Fig. 4, at the end of treatment (week 6), glutathione level decreased respectively of 68 and 59% compared to non-treated and irradiated mice groups.

- Combined treatment had no significant impact on host toxicity

OGX-427 did not significantly alter the host toxicity profile of radiation therapy, as monitored by body weight measurements in tumor-bearing mice (See. Fig. 5A and B). No significant tissue damage (liver, brain) was observed in mice receiving combined treatment. The absence of toxicity was also confirmed by the increased survival in the group of mice treated with OGX-427 plus radiation as compared with control mice.

These results confirm those obtained in the study of Example 2. Increasing dose of tumor radiation (30 Gy) in combination with OGX-427 treatment significantly amplifies the inhibition of tumor growth, the increase of apoptosis and the decrease of tumor cell proliferation. In addition, we observed a significant decrease of the intratumoral level of glutathione, an antioxidant protector.

CLAIMS

1. A method for treatment of squamous cell carcinoma comprising treating a patient diagnosed with a squamous cell carcinoma with a combination of radio-therapy and a therapeutic agent that reduces the amount of hsp27 in the squamous cancer cells.

2. The method of claim 1, wherein the therapeutic agent that reduces the amount of hsp27 is an oligonucleotide therapeutic.

3. The method of claim 2, wherein the oligonucleotide is an antisense oligonucleotide.

4. The method of claim 3, wherein the oligonucleotide comprises Seq. ID No. 82.

5. The method of claim 4, wherein the oligonucleotide is a 4-12-4 2'-MOE gapmer oligonucleotide with phosphorothiolated internucleotide linkages which can be represented as 5'-<u>GGGA</u>MeCGMeCGGMeCGMeCTMeCGG<u>MeUMeCAMeU</u>-3', (Seq. ID No. 92)
where G, A, MeC, and T represent the nucleosides 2'-deoxyguanosine, 2'-deoxyadenosine, 2'-deoxy-5-methylcytidine, and 2'-deoxythymidine, the underlined nucleosides denote 2'-O-methoxyethyl (2'-MOE) modifications of the nucleosides, and the internucleotide linkages are phosphothioate diester, sodium salts.

6. The method of any one of claims 1 to 5, in which the squamous cell carcinoma is head and neck cancer.

7. Use of a composition that reduces the amount of hsp27 in the formulation of a therapeutic for combination therapy with radiation in the treatment of sqaumous cell carcinomas.

8. Use of claim 7, wherein the therapeutic agent that reduces the amount of hsp27 is an oligonucleotide therapeutic.

9. Use of claim 8, wherein the oligonucleotide is an antisense oligonucleotide.

10. Use of claim 9, wherein the oligonucleotide comprises Seq. ID No. 82.

11. Use of claim 10, wherein the oligonucleotide is a 4-12-4 2'-MOE gapmer oligonucleotide with phosphorothiolated internucleotide linkages which can be represented as 5'-GGGAMeCGMeCGGMeCGMeCTMeCGGMeUMeCAMeU-3', (seq. ID No. 92) where G, A, MeC, and T represent the nucleosides 2'-deoxyguanosine, 2'-deoxyadenosine, 2'-deoxy-5-methylcytidine, and 2'-deoxythymidine, the underlined nucleosides denote 2'-O-methoxyethyl (2'-MOE) modifications of the nucleosides, and the internucleotide linkages are phosphothioate diester, sodium salts.

12. Use of any one of claims 7 to 11, in which the squamous cell carcinoma is head and neck cancer.

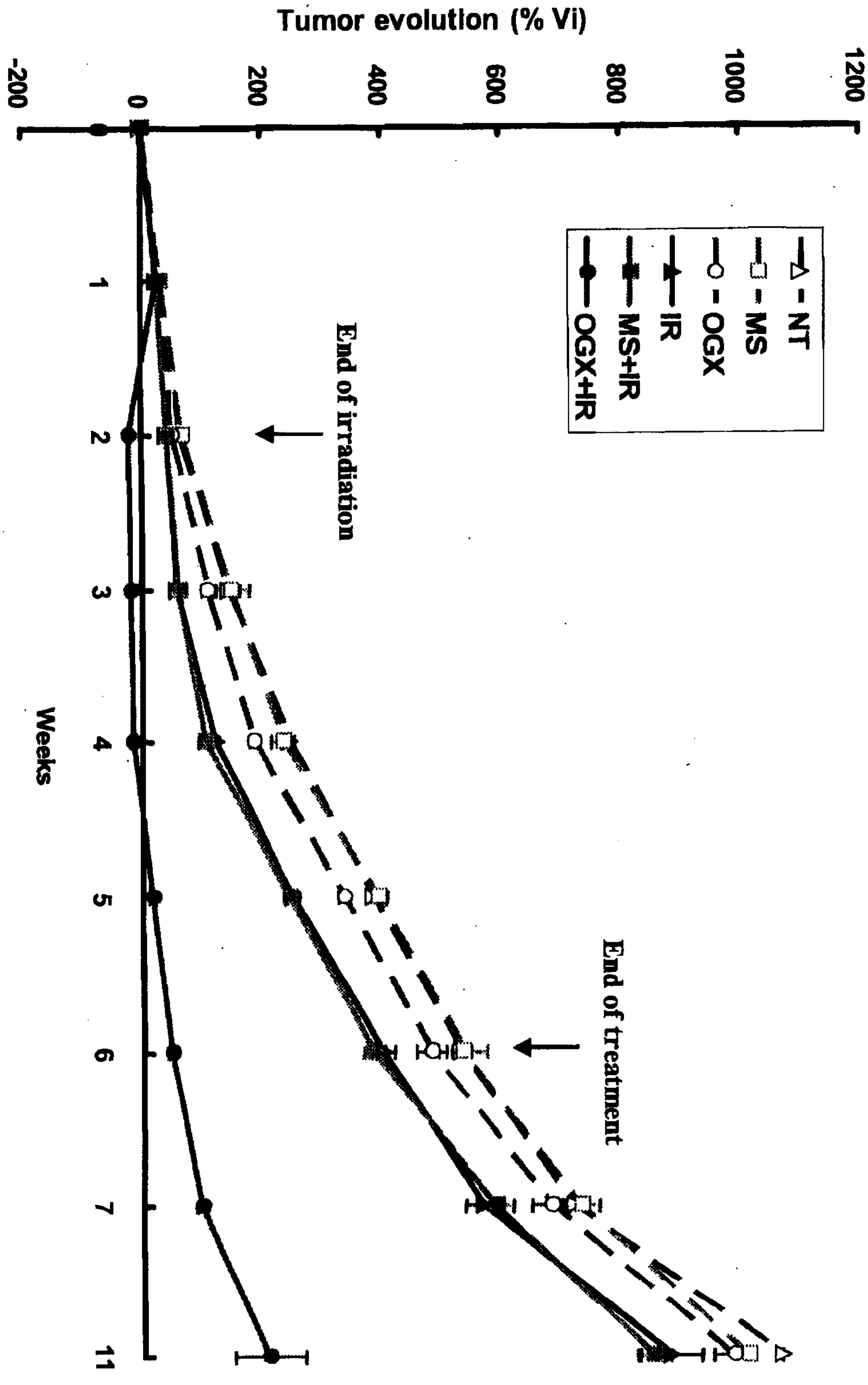
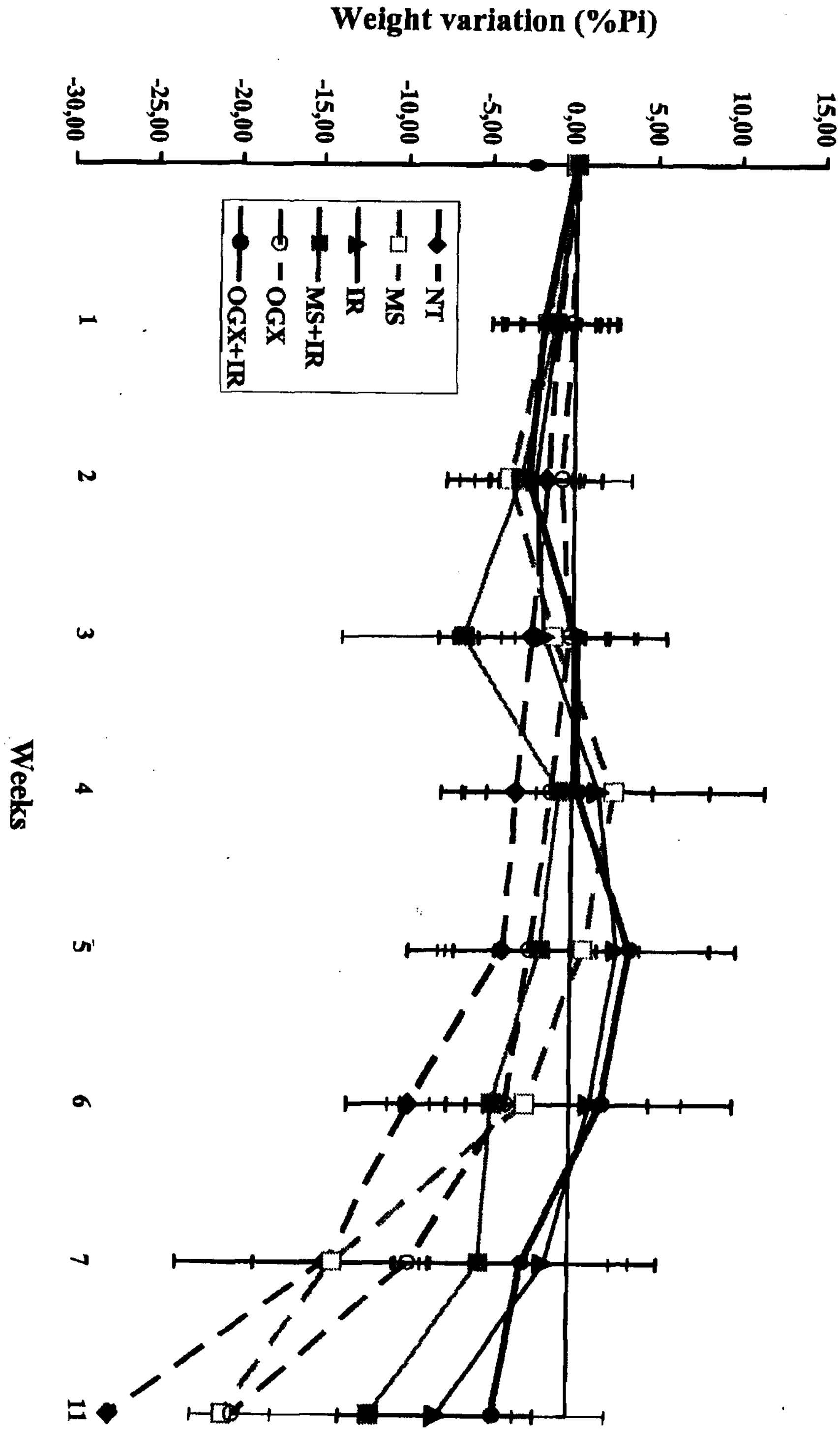

Tumor evolution (% Vi)
-200
0
200
400
600
800
1000
1200
Weeks
1
2
3
4
5
6
7
11
NT
MS
OGX
IR
MS+IR
OGX+IR
End of irradiation
End of treatment

Fig. 1


Weight variation (%Pi)
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10,00
5,00
0,00
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-20,00
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-30,00
NT
MS
IR
MS+IR
OGX
OGX+IR
1
2
3
4
5
6
7
11
Weeks

Fig. 2

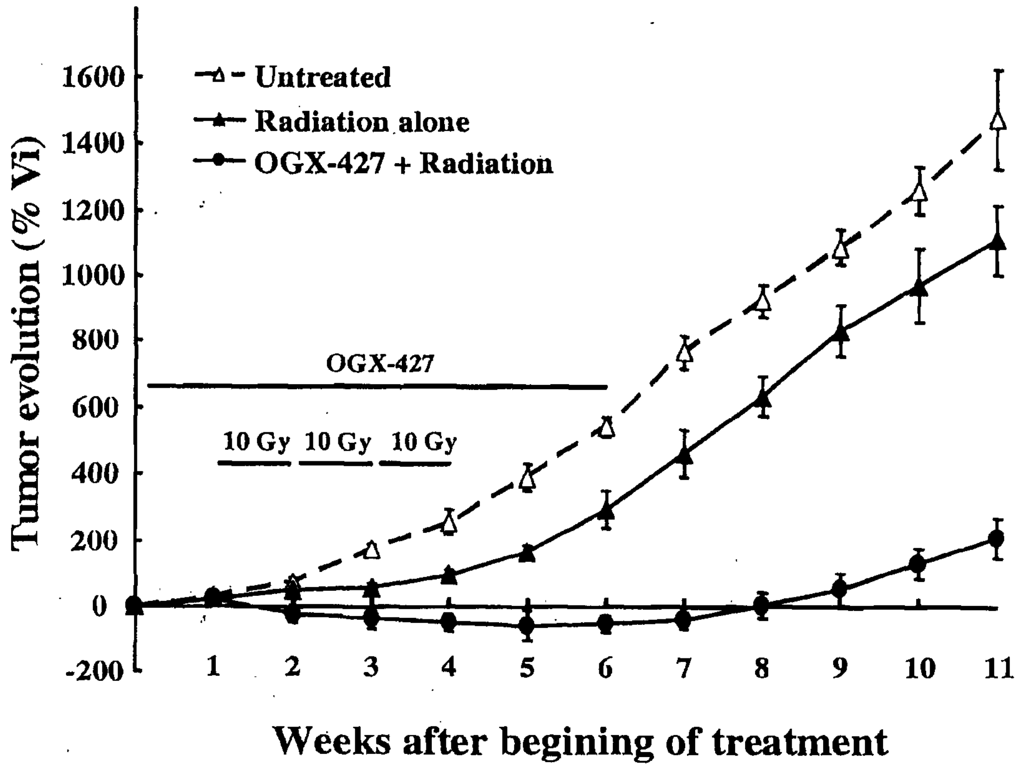
Untreated
Radiation alone
OGX-427 + Radiation
Tumor evolution (% Vi)
1600
1400
1200
1000
800
600
400
200
0
-200
OGX-427
10 Gy 10 Gy 10 Gy
1 2 3 4 5 6 7 8 9 10 11
Weeks after begining of treatment

Fig. 3

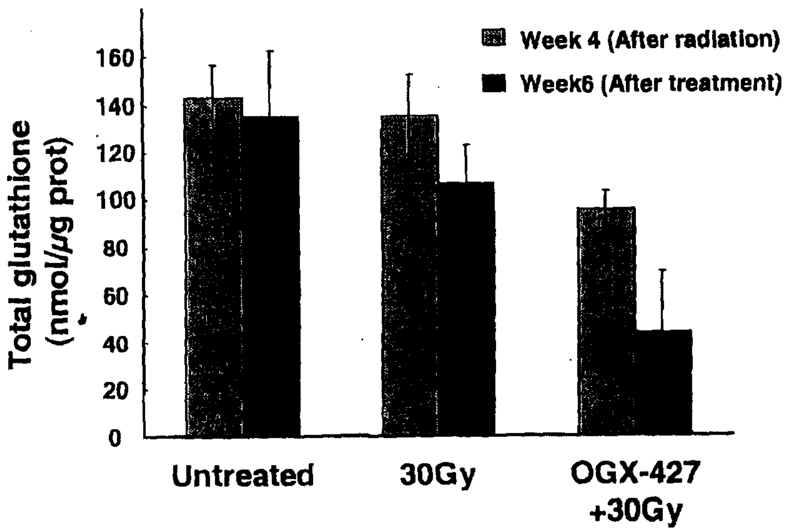
Week 4 (After radiation)
Week6 (After treatment)
Total glutathione (nmol/μg prot)
160
140
120
100
80
60
40
20
0
Untreated
30Gy
OGX-427 +30Gy

Fig. 4

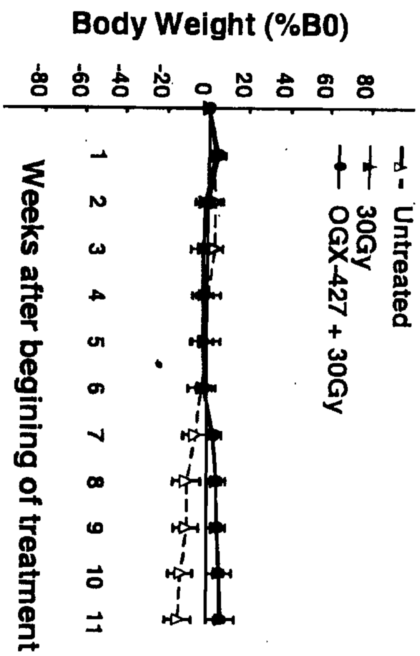
Body Weight (%B0)
80
60
40
20
0
-20
-40
-60
-80
Untreated
30Gy
OGX-427 + 30Gy
1 2 3 4 5 6 7 8 9 10 11
Weeks after begining of treatment

Fig. 5A

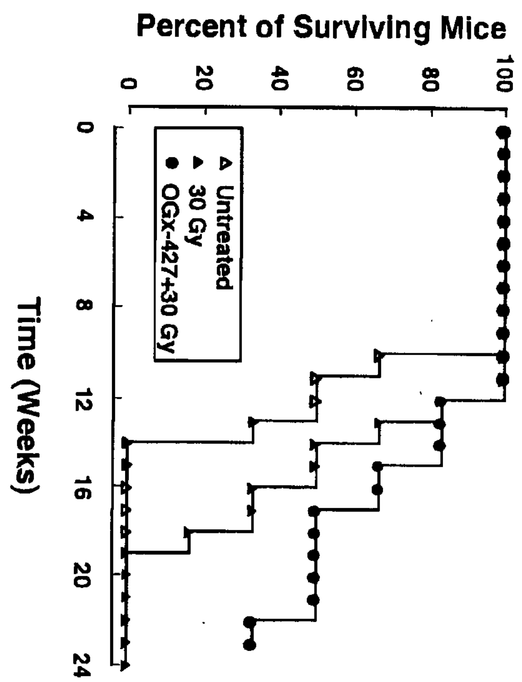
Percent of Surviving Mice
100
80
60
40
20
0
Untreated
30 Gy
OGX-427+30 Gy
0 4 8 12 16 20 24
Time (Weeks)

Fig. 5B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2008/000419

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***A61K 48/00*** (2006.01) , ***A61K 31/7088*** (2006.01) , ***A61K 31/712*** (2006.01) , ***A61K 31/7125*** (2006.01) , ***A61P 35/00*** (2006.01) , *A61N 5/10* (2006.01)
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)
IPC (2006.01): A61K 48/00, A61K 31/7088, A61K 31/712, A61K 31/7125, A61P 35/00 and A61N 5/10

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Databases: Delphion, Pubmeb, Scirius, Scopus, CAplus, Medline, Biosis, Canadian Patent Database, Geneseq
Keywords: heat shock, hsp27, radiotherapy, radi*, radiosensi*, squamous cell carcinoma, cancer

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TEIMOURIAN, S. et al. Down-regulation of Hsp27 radiosensitizes human prostate cancer cells. International Journal of Urology, September 2006, Vol. 13(9), pages 1221-1225, ISSN 0919-8172, eISSN 1442-2042. whole document	1-12
Y	MIYAZAKI, T. et al. Predictors of response to chemo-radiotherapy and radiotherapy for esophageal squamous cell carcinoma. Anticancer Research, Jul-Aug 2005, Vol. 25(4), pages 2749-55, ISSN 0250-7005. whole document	1-12

[X] Further documents are listed in the continuation of [ ] See patent family annex.

* Special categories of cited documents :
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"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
"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
"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
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 April 2008	21 May 2008 (21-05-2008)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Brad Temple 819- 934-7599

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the	Relevant to claim No.
P,X	ALOY, M.T. et al. Protective role of Hsp27 protein against gamma radiation-induced apoptosis and radiosensitization effects of Hsp27 gene silencing in different human tumor cells. International Journal of Radiation Oncology, February 2008, Electronic publication 5 November 2007, Vol 70(2), pages 543-53, ISSN 0360-3016. whole document	1-12
A	MESE, H. et al. Prognostic significance of heat shock protein 27 (HSP27) in patients with oral squamous cell carcinoma. Oncology reports, March-April 2002, Vol. 9(2), pages 341-344, ISSN 1021-335X, eISSN 1791-2431.	
A	LO, W.Y. et al. Identification of over-expressed proteins in oral squamous cell carcinoma (OSCC) patients by clinical proteomic analysis. Clinica Chimica Acta, February 2007, Electronic pubulication 30 June 2006, Vol. 376(1-2), pages 101-107, ISSN 0009-8981.	

Box No. II **Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. :
   because they relate to subject matter not required to be searched by this Authority, namely :

   Claims 1-6 are directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search. However, this Authority has carried out a search based on the alleged effects or purposes/uses of the product defined in claims 1-6.

2. [ ] Claim Nos. :
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. [ ] Claim Nos. :
   because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III **Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows :

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest [ ] The additional search fees were accompanied by the applicant’s protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.