Title: ANTISENSE INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN (IGFBP)-2-OLIGODEOXYNUCLEOTIDES FOR PROSTATE AND OTHER ENDOCRINE TUMOR THERAPY

Abstract: Compositions and a method are provided for the treatment of prostate and other endocrine tumors in mammals, including humans, by administration of an antisense oligodeoxynucleotide (ODN) which is complementary to a portion of the gene encoding IGFBP-2. Using the human prostate cancer LNCaP tumor model in vitro and in vivo, the administration of such an ODN was shown to reduce proliferation of tumor cells, and also to delay the progression to androgen independence. Thus, treatment of prostate and other hormone-regulated cancer in mammals, including humans, and delay of the progression of prostate tumors to androgen independence is accomplished by administering to the mammal a therapeutically effective amount of an antisense oligodeoxynucleotide which is complementary to a portion of the nucleic acid sequence encoding IGFBP-2 and which reduces the amount of IGFBP-2 in the treated cells.
Antisense Insulin-Like Growth Factor Binding Protein (IGFBP)-2 Oligodeoxynucleotides for Prostate and other Endocrine Tumor Therapy

DESCRIPTION

Field of the Invention:
The present invention relates generally to antisense oligonucleotide therapy for cancer. More specifically, prostate and breast cancer are targeted.

Background of the Invention:
This application relates to the treatment of prostate tumors making use of an antisense oligonucleotide that has a sequence complementary to the sequence encoding insulin-like growth factor binding protein (IGFBP)-2.

Prostate cancer is the most common cancer that affects men, and the second leading cause of cancer death in men in the Western world. Because prostate cancer is an androgen-sensitive tumor, androgen withdrawal, for example via castration, is utilized in some therapeutic regimens for patients with advanced prostate cancer. Androgen withdrawal leads to extensive apoptosis in the prostate tumor, and hence to a regression of the disease. However, castration-induced apoptosis is not complete, and a progression of surviving tumor cells to androgen-independence ultimately occurs. This progression is the main obstacle to improving survival and quality of life, and efforts have therefore been made to target androgen-independent cells. These efforts have focused on non-hormonal therapies targeted against androgen-independent tumor cells; however, no non-hormonal agent has improved survival thus far (Oh et al., *J. Urol* 160: 1220-1229 (1998)). Alternative approaches are therefore indicated. Recent studies in our laboratory suggest that increased levels of IGFBP-5 (Miyake et al, *Endocrinology* 141:2257-2265, (2000)) and IGFBP-2 after androgen ablation enhance IGF-1 mitogenesis and cell survival, thereby accelerating progression to androgen ablation.

Insulin-like growth factor (IGF)-I and IGF-II are potent mitogens for many normal and malignant cells. Accumulating evidence suggests that IGFs play an important role in the pathophysiology of prostatic disease and breast cancer (Boudon et al., *J. Clin. Endocrin. Metab.* 81: 612-617 (1996); Angelloz-Nicoud et al., *Endocrinology* 136: 5485-
5492 (1995); Nickerson et al., *Endocrinology* 139: 807-810 (1998); Figueroa et al., *J. Urol.* 159: 1379-1383 (1998)).

The biological response to IGF’s is regulated by various factors, including IGFBPs. To date, six IGFBPs have been identified whose function is believed to involve modulation of the biological actions of the IGFs through high affinity interactions (Rajaram et al. *Endocrin. Rev.* 18: 801-813 (1997)). However, some evidence suggests biological activity for IGFBPs that are independent of IGFs (Andress et al., *J. Biol. Chem.* 267: 22467-22472 (1992); Oh et al., *J. Biol. Chem.* 268: 14964-14971 (1993)), and both stimulatory and inhibitory effects of IGFBPs on cell proliferation have been reported under various experimental conditions (Andress et al., *supra*; Elgin et al., *Proc. Nat’l. Acad. Sci. (USA)*, 84: 3254-3258 (1987); Huynh et al., *J. Biol. Chem.* 271: 1016-1021 (1996); Damon et al., *Endocrinology* 139: 3456-3464 (1998)). Thus, the precise function of IGFBPs remains controversial. Because of this, while the reported results implicate IGF in prostate cancer, they do not clearly suggest a therapeutic approach based upon this involvement.

The present invention utilizes antisense oligodeoxynucleotides (ODNs) targeted to IGFBP-2 as a treatment for prostate and other endocrine cancers. Antisense ODNs are stretches of single-stranded DNA that are complementary to mRNA regions of a target gene, and thereby effectively inhibit gene expression by forming RNA/DNA duplexes (Figueroa et al., *J. Urol.*, 159: 1379-1383 (1998)). Phosphorothiate ODNs are stabilized to resist nuclease digestion by substituting one of the nonbridging phosphoryl oxygens of DNA with a sulfur. Recently, several antisense ODNs specifically targeted against genes involved in neoplastic progression have been evaluated both *in vitro* and *in vivo*, and demonstrated the efficacy of antisense strategy as potential therapeutic agents (Monia et al., *Nature Med.* 2: 668-675 (1996); Cucco et al., *Cancer Res.* 56: 4332-4337 (1996); Ziegler et al., *J. Natl. Cancer Inst.* 89: 1027-1036 (1997); Jansen et al., *Nature Med.* 4: 232-234 (1998)).
Summary of the Invention:

In accordance with the invention, compositions and a method are provided for the treatment of prostate and other endocrine tumors in mammals, including humans, by administration of an antisense oligodeoxynucleotide (ODN) which is complementary to a portion of the gene encoding IGFBP-2. Using the androgen-sensitive human prostate cancer LNCaP and androgen-dependent murine Shionogi tumor model in vitro and in vivo, the administration of such an ODN was shown to reduce proliferation of tumor cells, and also to delay the progression to androgen independence. Thus, treatment of prostate cancer and other hormone-regulated cancers in mammals, including humans, and delay of the progression of prostate tumors to androgen independence is accomplished by administering to the mammal a therapeutically effective amount of an antisense oligodeoxynucleotide which is complementary to a portion of the nucleic acid sequence encoding IGFBP-2 and which results in a reduction of IGFBP-2 levels in the target cancer cells.

Brief Description of the Figures:

Fig. 1 shows densitometry traces for Northern analysis demonstrating increased IGFBP-2 mRNA levels in LNCaP cells after castration and during androgen-independent progression;

Fig. 2 depicts in vitro levels of viable human prostate LNCaP cells showing dose-dependent decreases in cell number after antisense IGFBP-2 treatment;

Figs. 3a and 3b show that treatment of LNCaP-tumor bearing mice after castration with IGFBP-2 ASO’s reduces tumor growth rates and rises in serum PSA and delays time to androgen independent progression; and

Fig. 4 shows that treatment of human LNCaP tumor cells with IGFBP-2 ASO’s resulted in greater than 50% growth inhibition in a time- and dose-dependent manner.
Detailed Description of the Invention:

The present invention provides a method for delaying the progression of prostatic tumor cells to androgen independence, a therapeutic method for the treatment of individuals, including humans, suffering from hormone-regulated cancer such as prostate or breast cancer, and therapeutic agents effective for use in such methods. In addition, the compositions of the invention can be used to inhibit or delay the growth and metastatic progression of such cancers. The therapeutic method of the invention will most commonly be used in the treatment of individuals with advanced prostate cancer, but may also be used in conjunction with hormonal therapies of other endocrine malignancies, such as breast cancer.

In accordance with the first embodiment of the invention, the progression of androgen-sensitive prostatic cancer cells to androgen independence can be delayed by reducing the amount of IGFBP-2 in the cells. Experiments were performed in vitro and in vivo in the androgen-sensitive human prostate cancer LNCaP and androgen-dependent murine Shionogi tumor models. The Shionogi tumor model is a xenograft of an androgen dependent mouse mammary carcinoma that grows subcutaneously in male syngeneic hosts. Shionogi tumor cells are highly tumorigenic and locally invasive. The cells have been shown to respond to androgen withdrawal in a manner which mimics the observed behavior of prostatic tumor cells, and have been accepted as a valid model for prostate cancer in humans (Bruchovsky et al., Cancer Res. 50: 2275-2282 (1990); Rennie et al., Cancer Res. 48: 6309-6312 (1988); Bruchovsky et al., Cell 13: 272-280 (1978); Gleave et al., in Genitourinary Oncology, pp. 367-378, Lange et al. eds., Lippencott (1997); Gleave et al., J. Urol. 157: 1727-1730 (1997); Bruchovsky et al., The Prostate 6: 13-21 (1996)).

Thus, androgen withdrawal precipitates apoptosis and tumor regression in a highly reproducible manner. Further, changes in expression and peptides such as TRPM-2 and Bcl-2 in human prostate cancer following castration and during progression to androgen-independence are similar to those observed in Shionogi tumor cells. Because of these similarities, the Shionogi tumor model mimics human prostate cancer and provides a very useful model for the evaluation of the ability of compounds to delay the onset of androgen-independence. Despite complete tumor regression after castration, rapidly growing androgen-independent Shionogi tumors invariably recur after one month, which
provides a reliable end point to evaluate agents which can delay the progression to androgen-independence.

In the study leading to the present invention, we initially characterized the changes of IGFBP expression in the Shionogi tumor model after castration and during AI progression. Northern blot analyses were used to characterize changes in IGFBP mRNA expression in AD intact tumors before castration, regressing tumors 4 and 7 days after castration, and AI recurrent tumors 28 days after castration. Various patterns of changes in IGFBP-2, -3, -4, and -5 mRNA expression were observed. IGFBP-1 and IGFBP-6 mRNAs are undetectable in the Shionogi tumor model.

Northern blotting was used to characterize changes in IGFBP-2 mRNA expression in AD intact LNCaP tumors before castration and at various time points after castration. As shown in Fig. 1, IGFBP-2 expression increased gradually beginning 14 days after castration, and by 28 days after castration was > 2-fold compared to levels before castration (two-sided \( p < 0.05 \), student’s \( t \)). Increased IGFBP-2 levels after castration was also identified using LNCaP and human prostate cancer tumor tissue microarrays. Mean IGFBP-2 staining intensity increased from +1 in AD tumors (n = 20 spots) before castration to +2.3 in AI tumors (n = 40 spots, 28 and 35 days after castration). The mean intensities of other groups were +1 for 3 days, +1.2 for 5, 7 and 10 days, +1.4 for 14 days, and +1.6 for 21 days after castration. Immunohistochemical staining results generally corresponded with results from Northern blotting.

In accordance with the present invention, antisense ODN’s which are complementary to the sequence encoding IGFBP-2 are administered. When the subject is human, the sequence administered is based on human IGFBP-2. Specific antisense ODN are listed in Table 2 and are identified as Seq. ID Nos. 1-56. Seq. ID. No. 1 which includes the translation initiation site was the most active of those tested, and was used in the majority of the experiments reported herein. The ODNs employed may be modified to increase the stability of the ODN in vivo. For example, the ODNs may be employed as phosphorothioate derivatives (replacement of a non-bridging phosphorl oxygen atom with a sulfur atom) which have increased resistance to nuclease digestion. Increased ODN stability can also be achieved using molecules with 2-methoxyethyl substituted backbones.
Administration of antisense ODNs can be carried out using the various mechanisms known in the art, including naked administration and administration in pharmaceutically acceptable carriers. For example, lipid carriers for antisense delivery are described in US Patents Nos. 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.

The amount of antisense ODN administered is one effective to reduce the levels of IGFBP-2 in prostatic cells or other hormone-regulated tumor cells. In the context of the present invention, applicants do not intend to be bound by any specific mechanism by which this reduction may occur, although it is noted that the reduction may occur as a result of reduced expression of IGFBP-2 if the antisense molecule interferes with translation of the mRNA, or via an RNase mediated mechanism. Furthermore, it will be appreciated that the appropriate therapeutic amount will vary both with the effectiveness of the specific antisense ODN 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.

The method for treating prostate cancer in accordance with the invention may further include administration of chemotherapy agents and/or additional antisense ODNs directed at different targets. For example, conventional chemotherapy agents such as taxol (paclitaxel or docetaxel) and mitoxantrone may be used. Similarly, combinations of antisense IGFBP-2 ODN with other antisense sequences such as antisense Bcl-2 ODN, TRPM-2 ODN, or IGFBP-5 ODN may be used.
Examples:

The invention will now be further described with reference to the following, non-limiting examples.

Example 1

Three oligonucleotides were prepared with the sequences given in Seq. ID. Nos. 1-3. Seq. ID No. 1 spans the translation initiation site of the IGFBP-2 mRNA starting with base number 64. Seq. ID Nos. 2 and 3 correspond to bases 131-151 and 630-650 respectively. Two base IGFBP-2 mismatch oligonucleotides (Seq. ID Nos. 57-59) were also prepared as controls.

Initial screening on these three oligonucleotides was done using LNCaP cells. Lipofectin, a cationic lipid (Life Technologies Inc. Gaithersburg, MD) was used to increase uptake of the oligonucleotides into the cells. LNCaP cells were treated with one of the three oligonucleotides (Seq. ID. Nos. 1-3), 1000 nM, or the corresponding mismatch control (Seq. ID Nos. 57-59). Total RNA was extracted and analyzed by Northern Blot analysis for levels of IGFBP-2 encoding RNA. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) was used as a control. The probes used had the sequences given by Seq. ID. Nos. 60-63. The RNA blots were hybridized with human IGFBP_2 probe labeled with $[^32P]dCTP$ by random primer labeling. Washing and densitometric analysis was carried out. After detecting the IGFBP-2 encoding RNA, the membranes were re-probed using human G3PDH probes to verify integrity.

Seq. ID No. 1 was most effective, causing up to 80-90% reduction in IGFBP-2 mRNA levels. Seq ID. Nos. 2 and 3 were also effective, albeit less so, causing a decrease of about 50%.

Example 2

The LNCaP model is an androgen-sensitive, PSA-secreting, human prostate cancer cell line that can be induced to form tumors in athymic mice under a variety of conditions. Like in human prostate cancer, serum PSA levels in this model are regulated by androgen and are directly proportional to tumor volume. After castration, serum and tumor-cell PSA levels decrease up to 80% and remain suppressed for 3-4 weeks. Beginning 4 weeks after castration, however, PSA production gradually increases above pre-castrate levels in the absence of testicular androgens, heralding the onset of androgen-
independent progression. The pattern of changes in gene expression after castration in the LNCaP model is similar to that in the Shionogi system, with increased expression of Bcl-2, TRPM-2, and IGFBP-2 following castration of mice bearing LNCaP tumors. It is important to stress that many of the changes in gene expression in the LNCaP and Shionogi models also occur in human prostate cancer (e.g., Bcl-2, clusterin, IGFBP’s, PSA, Bcl-xL), which validates their use as models of the human disease for functional genomics and preclinical proof of principle experiments. In the study leading to the present invention, we initially characterized changes of IGFBP expression in the LNCaP tumor model after castration and during AI progression. Northern blot analyses showed that IGFBP-2 levels increased up to 2-3 fold in androgen-independent tumors compared to androgen dependent tumors prior to castration, suggesting IGFBP-2 increases may be associated with the development of the androgen-independent phenotype (Fig. 1).

Example 3

The Shionogi tumor model mimics human prostate cancer and provides a very useful model for the evaluation of the ability of compounds to delay the onset of androgen-independence. Despite complete tumor regression after castration, rapidly growing androgen-independent Shionogi tumors invariably recur after one month, which provides a reliable end point to evaluate agents which can delay the progression to androgen-independence. In the study leading to the present invention, we initially characterized changes of IGFBP expression in the Shionogi tumor model after castration and during AI progression. Northern blot analyses were used to characterize changes in IGFBP mRNA expression in AD intact tumors before castration, regressing tumors 4 and 7 days after castration, and AI recurrent tumors 28 days after castration. IGFBP-2 levels increased up to 2-3 fold in androgen-independent tumors compared to androgen dependent tumors prior to castration, suggesting IGFBP-2 increases may be associated with the development of the androgen-independent phenotype.

Example 4

Treatment of human LNCaP cells with IGFBP-2 ASO (Seq. ID. No. 1) resulted in dose-dependent and sequence-specific downregulation of IGFBP-2 mRNA and protein levels. IGFBP-2 levels were decreased by 90% after treatment with 500 nM IGFBP-2
ASO (Seq. ID. No. 1). Cell viability also decreased in a dose-dependent manner. (Fig. 2).

**Example 5**

Treatment of human LNCaP tumor cells with IGFBP-2 ASO (Seq. ID. No. 1) decreased target mRNA and protein levels greater than 90% and resulted in greater than 50% growth inhibition in a time- and dose-dependent manner (Figure 2).

**Example 6**

Systemic administration of IGFBP-2 ASO (Seq. ID. No. 1) in mice bearing human LNCaP prostate tumors after castration significantly delayed the growth of AI recurrent tumors and time to sacrifice. LNCaP tumor growth and rises in serum PSA were both significantly delayed in mice treated with IGFBP-2 ASO’s compared to controls treated with mismatch ASO’s (Figs. 3a and 3b). These findings provide the first evidence that upregulation of IGFBP-2 after castration enhances the mitogenic activity of IGF-I, and illustrates a potential use for IGFBP-2 ASO therapy for prostate cancer.

**Example 7**

Treatment of human LNCaP tumor cells with IGFBP-2 ASO (Seq. ID. No. 1) decreased target mRNA and protein levels greater than 90% and resulted in greater than 50% growth inhibition in a time- and dose-dependent manner, an effect that could not be reversed by exogenous IGF-I (Fig. 4). IGFBP-2 ASO (Seq. ID. No. 1) plus IGF-I antibody treatment had additional inhibitory effect on LNCaP tumor cell growth in vitro.

**Example 8**

To examine the effects on cell cycle regulation of decreases in IGFBP-2 levels by IGFBP-2 ASO treatment, changes in cyclin D1 levels were evaluated in LNCaP cells after treatment with IGFBP-2 ASO (Seq. ID. No. 1). Western analysis demonstrated a greater than 50% decrease in cyclin D1 after IGFBP-2 ASO (Seq. ID. No. 1) treatment, illustrating that decreases in IGFBP-2 by ASO treatment inhibits IGF-I signaling and results in cell cycle arrest. Apoptosis induction after IGFBP ASO treatment was also shown by LNCaP cell cycle analysis by flow cytometry after treatment with IGFBP-2
ASO. LNCaP cells were treated daily with IGFBP-2 ASO or mismatch control oligonucleotide with or without 1 nM DHT (dihydrotestosterone) for 2 days. Table 1 shows cell populations in each phase (Sub G1-G0, G1-G0, S, and G2+M) in % for the various treatments. Each datum represents the mean value of triplicate experiments. After IGFBP-2 ASO treatment, the percent of cells in Sub G1-G0 increased 3-fold (p < 0.05), while percent of cells in G2+M decreased by 50%.

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Example 9
Metastatic prostate and breast cancer frequently invade bony tissue. Treatment of such metastases is very difficult, and progression of the cancer into the bone generally indicates a poor prognosis for long term survival. Thus, it would be desirable to have a methodology for inhibiting or delaying this progression. It was logical to assume that since IGF-I and IGFBP-2 are important factors for growth of IGF-I sensitive cancer, including in particular prostate and breast cancer, that the presence of high levels of IGFBP-2 in bone could be an important mechanism for promoting the growth and progression of metastatic lesions. Accordingly, Western analysis was performed on samples of primary human bone tissue cultures. This experiment confirmed the presence
of high levels of IGFBP-2 in bone. Inhibition of these levels using antisense IGFBP-2 ODN in accordance with the invention should provide an effective therapy for inhibiting or delaying the progression of metastatic lesions in the bone.
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- 15 -

CLAIMS


2. The composition of claim 1, wherein the antisense oligonucleotide is complementary to a region of IGFBP-2 mRNA spanning the translation initiation or termination site.

3. The composition of claim 1, wherein the antisense oligonucleotide comprises a series of contiguous bases as set forth in any one of Seq. ID’s No. 1 through 56, inclusive.

4. The composition of claim 2 or 3, wherein the antisense oligonucleotide has a length of from 15 to 30 nucleotides.

5. The composition of claim 1, wherein the antisense oligonucleotide consists of a series of contiguous bases as set forth in Seq. ID No. 1.

6. Use of a composition according to any claims 1-5 in formulating a pharmaceutical composition for delaying progression of hormone-regulated tumor cells to an hormone-independent state by treating hormone-sensitive tumor cells with an antisense oligonucleotide which inhibits expression of IGFBP-2 by the tumor cells.

7. The use of claim 6, wherein the tumor cells are prostatic tumor cells.

8. The use of claim 6, wherein the tumor cells are breast cancer cells.

9. Use of a composition according to any of claims 1-5 in formulating a pharmaceutical composition for treating a hormone-responsive cancer in an individual
suffering from hormone-responsive cancer, wherein the pharmaceutical composition is administered to the individual after initiation of hormone-withdrawal to induce apoptotic cell death of hormone-responsive cancer cells in the individual, and thereby delays the progression of hormone-responsive cancer cells to a hormone-independent state in the individual.

10. The use of claim 9, wherein the hormone-responsive cancer is prostate cancer.

11. Use of a composition in accordance with any of claims 1-5 in formulating a pharmaceutical composition for inhibiting or delaying metastatic bony progression of an IGF-I sensitive tumor in a mammal, including a human, by administration of the composition to the mammal in an amount effective to inhibit expression of IGFBP-2 by the hormone-responsive cancer cells, thereby inhibiting or delaying metastatic bony progression of the tumor.

12. The use of claim 11, wherein the IGF-I sensitive tumor is a prostate cancer.

13. A method for treating a hormone-responsive cancer in an individual suffering from hormone-responsive cancer, comprising administering to the individual a composition comprising an antisense oligonucleotide which inhibits expression of IGFBP-2 by hormone-regulated tumor cells.

14. The method of claim 13, wherein the composition is administered to the individual after initiation of hormone-withdrawal to induce apoptotic cell death of hormone-responsive cancer cells in the individual, and thereby delays the progression of hormone-responsive cancer cells to a hormone-independent state in the individual.

15. A method for delaying progression of hormone-regulated tumor cells to an hormone-independent state comprising the step of treating hormone-sensitive
tumor cells with an antisense oligonucleotide which inhibits expression of IGFBP-2 by the tumor cells.

16. A method for inhibiting or delaying metastatic bony progression of an IGF-I sensitive tumor in an individual suffering from the IGF-I sensitive tumor, comprising the step of administering to the individual a composition comprising an antisense oligonucleotide which inhibits expression of IGFBP-2 by the tumor cells in an amount effective to inhibit expression of IGFBP-2 by the IGF-I sensitive cancer cells, thereby inhibiting or delaying metastatic bony progression of the tumor.

17. The method of any of claims 13-16, wherein the individual is a human.

18. The method of any of claims 13-16 wherein the tumor cells are prostatic tumor cells.

19. The method of claim 18, wherein the individual is a human.

20. The method of any of claims 13-16, wherein the tumor cells are breast cancer cells.

21. The method of claim 20, wherein the individual is a human.
Figure 1
Figure 4

IGF-1  - + - - + - - + - - + - - + - - + - - +
Anti IGF-1 - - + - - + - - + - - + - - + - - +
AS IGFBP-2 - - + + + - - - - - + + + - - -
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<110> University of British Columbia
    Cleave, Martin
    Kiyama, Satoshi
    Nelson, Colleen
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<120> Antisense Insulin-Like Growth Factor Binding Protein (IGFBP)-2 Oligodeoxynucleotides for Prostate and Endocrine Tumor Therapy

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/28748

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C07H 21/04; C12Q 1/68; A61K 48/00; C12N 15/85
US CL. : 514/44; 436/6, 325, 366, 375; 536, 23.1, 24.5
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.: 514/44; 436/6, 325, 366, 375; 536, 23.1, 24.5

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)
West, Captus, Embase, SciSearch, and Medline

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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See patent family annex.

Date of the actual completion of the international search
15 October 2001 (15.10.2001)

Date of mailing of the international search report
12 FEB 2002

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703)305-3230

Authorized officer
Andrew Moore
Telephone No. 703-303-0196

Form PCT/ISA/210 (second sheet) (July 1998)
INTERNATIONAL SEARCH REPORT

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Remark on Protest □ The additional search fees were accompanied by the applicant’s protest. |
□ No protest accompanied the payment of additional search fees.