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(71) Applicant *(for all designated States except US):* TANOX BIOSYSTEMS, INC. [US/US]; 10301 Stella Link, Houston, TX 77025 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)* : CHANG, Tse, Wen [US/US]; 3323 Robinhood, Houston, TX 77003 (US).

(74) Agent: MIRABEL, Eric, P.; Tanox Biosystems, Inc., 10301 Stella Link, Houston, TX 77025 (US).

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(54) Title: ANTI-SENSE OLIGONUCLEOTIDES FOR ISOTYPE-SPECIFIC SUPPRESSION OF IMMUNOGLOBULIN PRODUCTION

(57) Abstract

The invention includes oligonucleotides which are complementary to the 3′-end untranslated regions or to at least some of the splicing recognition region of an mRNA transcript precursor for a particular immunoglobulin heavy chain. More particularly, the oligonucleotides are complementary to at least a continuous 12 nucleotide segment of the sequence upstream to the 3′ splice junction up to and including the branch point A residue. Sequences of some of the oligonucleotides of the invention are the underlined portions of SEQ ID NOS: 3-33. These oligonucleotides will hybridize with the splicing recognition region and prevent or inhibit maturation of the mRNA. This will prevent production of mRNA encoding a complete immunoglobulin, so that it will not be translated into a functional immunoglobulin. The invention also pertains to the preparation of these anti-sense oligonucleotides for specifically suppressing IgG and/or IgM production in patients suffering from autoimmune diseases and for specifically suppressing IgE in patients suffering from allergic diseases manifested by immediate-type hypersensitivities.

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ANTI-SENSE OLIGONUCLEOTIDES FOR ISOTYPE-SPECIFIC SUPPRESSION OF IMMUNOGLOBULIN PRODUCTION

Background of the Invention

5 In man and many mammals, five classes of immunoglobulins, namely IgD, IgM, IgG, IgA, and IgE, are synthesized. An antibody monomer contains two identical light chains of one of the two isotypes, κ and λ, and two identical heavy chains of one of the five isotypes, δ, μ, γ, α, and ϵ. Some of these isotypes have subclasses. For example, in man, γ has $\gamma 1$, $\gamma 2$, $\gamma 3$, and $\gamma 4$ subclasses.

10 Antibodies have enormously diverse binding specificity characterized by their individually unique binding sites. Each heavy chain or light chain has its own unique variable region segment, as well as constant region segments which define the isotype and subclass. A mature, immunocompetent, resting B cell has a rearranged genes encoding for the heavy and light chain variable regions and, 15 therefore, a defined antigen specificity. However, while undergoing activation and clonal expansion process, a B cell can further mature and differentiate into a cell expressing one or more immunoglobulins of the five classes, all of which bear the same variable regions and antigen specificity.

The five classes of antibodies, which could probably bear the same 20 variable regions and the same antigen specificities, have different immune functions and are differently involved in the pathogenesis of immune system-related disease. IgM exists as a pentamer and is multivalent, and fixes complement effectively; IgG generally is most effective in mediating antibody-

dependent cellular cytotoxicity and crosses the placenta to the fetus; IgA is most important in mucosal surface; IgE is dominant in sensitizing mast cells and basophils in pharmacological mediator release; IgD is on the B cell surface and is probably crucial in the B cell activation process.

5 Among the different classes of antibodies, those of IgG isotype are most conspicuously involved as autoantibodies in autoimmune diseases, such as in rheumatoid arthritis, system lupus erythematosus, Graves' disease, Hashimoto's thyroiditis, myasthenia gravis, pemphigus vulgaris, and idiopathic thrombocytopenia purpura. IgE is mainly responsible for immediate type 10 hypersensitivities manifesting allergic rhinitis, extrinsic asthma, drug and food allergies, and atopic dermatitis.

For treating certain diseases, such as one of the autoimmune or allergic diseases, it is desirable to suppress the antibodies causing the disease. Since IgG is responsible for many autoimmune diseases, and IgE is mainly responsible for 15 immediate-hypersensitivities, it is desirable that the antibody suppression be isotype-specific. By doing so, the adverse effects to the immune system will be minimized. A number of experimental approaches for studying isotype-specific suppression have been proposed. One approach involves inhibiting the lymphokines involved in isotype-specific switching, *e.g.* experiments have been 20 done with an anti-interleukin 4 monoclonal antibody in an attempt to inhibit IgE synthesis. Another approach is to use monoclonal antibodies specific for membrane-bound immunoglobulin of specific isotypes to target B cells producing

the specific isotypes. The present invention employs anti-sense oligonucleotides for targeting the mRNA precursors of the specific isotypes and even of particular subclasses of isotypes.

Anti-sense technology is based on the principle that a properly designed
5 oligonucleotide or analogue capable of binding to a key segment of an RNA or a DNA can enter a cell, binding to the crucial element of the mRNA, or its precursor RNA, or DNA, and effectively inhibit the function of the RNA or DNA species. In mammals, DNA generally exist as double helices of two complementary strands, complexing with nuclear proteins such as histones,
10 forming extremely complex but orderly structural units that compact together to form a chromosome. The DNA strand is a genetically inherited sequence of millions of four deoxyribonucleotides distinguished by their base side chains, adenine ("A"), thymine ("T"), cytosine ("C"), and guanine ("G"). The DNA strand is functionally a linear stretch of thousand of genes, some of which encode
15 structural polypeptides and some of which carry regulatory information or functions.

Messenger RNA is a transcribed product of a segment, generally a gene, in DNA. Messenger RNA is a sequence of ribonucleotides with A, C, G, and uracil ("U") bases. In mammals, RNA is generally single stranded and hence is
20 complementary to a segment of only one of the two double-stranded DNA. A naturally occurring mRNA and its complementary DNA strand is a "sense" strand. An artificially designed segment, either synthesized *in vitro* or *in vivo*,

is an "anti-sense" strand.

The chemical basis of the complementarity between the two strands in a double helix or between an "anti-sense" segment and a sense segment is that A or T (or U in the case of RNA) and G and C, of two neighboring nucleotides in
5 the two strands form stable hydrogen bonds. Both DNA and RNA strands have directions, with their ends being referred as 5' and 3' ends, pertaining to the hydroxy linkages on the ribosyl monosaccharide backbone of the nucleotides.

During the transcription process (the production of RNA), a set of regulatory proteins and enzymes separate the sense strand of DNA from its
10 complementary strand at the control region of a gene and allow RNA polymerase to assemble an RNA molecule according to the DNA sequence in that gene. The newly synthesized RNA precursor is processed by another set of proteins and enzymes to form a mature mRNA species, which is then transported from the nucleus to the cytosol. In the translation process, a set of regulatory proteins and
15 enzymes bind to the control segment near the 5' end of the mRNA and read the information along the mRNA sequence (from the 5' end to the 3' end) to assemble amino acids to form a unique polypeptide chain.

In applying the "anti-sense" concept, oligonucleotides for inhibiting the production of crucial viral proteins or products or oncogenic proteins (potentially
20 for use in infectious diseases and cancer) can be placed into one of two categories in terms of target specificity. Cohen, J.S. Trends Pharm. Sci. 10:435 (1989); LeDoan, T. et al. Bull. Cancer 76:849 (1989); Takayama, K.M. and Inouye, M.

Crit. Rev. Biochem. Mol. Biol. 25:155 (1990); Rothenberg, M. et al. J. Natl. Cancer Inst. 81:1539 (1989); Eguchi, Y. et al. Ann. Rev. Biochem. 60:631 (1991). The first group target the control region of mRNA or its precursor RNA and interfere the proper expression of the messenger species. The anti-sense
5 compounds can interfere with proper maturation of the precursor RNA transcripts and prevent them from becoming functional mRNA. They can also affect the translation process by interfering with the regulatory components of the gene. They can also affect the stability of the mRNA, making it more susceptible to RNase. The other group of anti-sense compounds target the control region of a
10 gene and hence inhibit the transcription process. In the latter cases, the oligonucleotide binds to one strand of the DNA as the two strands of DNA open up, and forms an apparent "triplex" structure.

In the past few years, the development of anti-sense technology for pharmaceutical application has made substantial progress because of the successful
15 development of methods of synthesizing analogues of oligonucleotides. The oligonucleotides made from the native nucleotides are highly charged and penetrate the cellular plasma membrane very poorly. They are also very sensitive to nuclease digestion in the blood. The modified oligonucleotides include phosphorothioate oligomers, methylphosphonate oligomers, α-anomeric
20 oligomers, and others. These oligonucleotides hybridize with the complementary RNA or DNA segments equally well. However, since they are less hydrophilic and more lipophilic, they can get into cells more effectively. They are also much

more resistant to nuclease cleavage than the native oligonucleotides.

As for the identification of segments in a precursor RNA or in a mature mRNA species for designing anti-sense compounds, there are a number of potential regions that are more likely to contain "effective" target segments.
5 Based on experience with many mRNA and precursor RNA targets, the most probable regions are the 5' regulatory regions, the splicing junctions, and the 3'-end untranslated regions that control mRNA stability. Even segments of the coding regions can provide targets for anti-sense compounds. One widely used process would be to synthesize several oligonucleotides of 10 to 20 nucleotides
10 in length covering portions of the regions mentioned above, and to test the effects on inhibiting the expression of the encoded protein. When a oligonucleotide shows significant inhibition, additional oligonucleotides with slight variations in length and in the exact positions in the region would also be synthesized and tested. The ones with optimal effect would be selected.

15 Generally speaking, in mammals, a DNA gene consists of not only segments encoding peptide sequences (exons), but also dispersing segments of non-coding DNA (introns). During transcription, the RNA which is initially transcribed corresponds to the entire sequence, including the regulatory regions at the 5' end, the exons, and the introns. This RNA precursor is processed in a
20 sequence of steps, in which the introns are excised and the exons are spliced together to form a mature mRNA, which contains a contiguous coding sequence of a polypeptide, along with regulatory elements flanking the 5' and 3' ends.

The mechanism of mRNA splicing is complex. It may involve U1- and U2- containing small RNA-protein complexes (U1, U2 snRNPs). The U1 RNA has a sequence at its 5' end that is precisely complementary to the nine-nucleotide consensus for the 5' splice site of an exon. Binding of U1-snRNP to the 5' splice site results in a cut at the 5' splice junction. The cut occurs through ATP hydrolysis accompanied by formation of an unusual 2'-5' phosphodiester bond between the 5'-terminal G residue of the intron and a site about 30 nucleotides upstream from the 3' end of the intron. This creates an intermediate RNA lariat structure in which an A residue invariably forms the branch point.

During the splicing reaction, U2 snRNPs associate with the intron branch point, while U1 snRNPs interact with the 5' splice site and some other component binds to the 3' splice junction in the pre-mRNAs. All these bindings seem to assemble along an ordered pathway. Signals crucial for the splicing of mammalian pre-mRNAs reside directly at the splice junction. Analysis of the intron sequences of mammalian genes showed that exon-intron boundaries in pre-mRNAs are highly conserved. The conserved sequences are generally regarded as mRNA splice sites, and are shown in SEQ ID NO:1 and SEQ ID NO:2.

Referring to SEQ ID NO:1, the nine nucleotide consensus sequence for the 5' junctions extends three residues upstream and six residues downstream from the splice point, which is between the 3rd and 4th G-base nucleotides. The C at the 5' end of SEQ ID NO:1 can also be an A, and the 6th A from the 5' end can be a G.

Referring to SEQ ID NO:2, the 3' splice junction includes a pyrimidine-rich region of variable length (but always greater than ten nucleotides). In SEQ ID NO:2, the C can be a T. This pyrimidine-rich region is followed by a short consensus sequence extending only three residues upstream and one residue
5 downstream from the splice point, which is between the two Gs at the 3' end.

It is noted that at both the 3' and 5' splice junctions, the conserved sequences extend further into the intron than into the exon, thereby placing only minimal constraints on the sequence of the mature mRNA.

The sequence of the introns upstream of the 3' splice junction in the CH1
10 region and the 5' end of the exons for the constant regions of the human immunoglobulins $\gamma1$, $\gamma2$, $\gamma3$, $\gamma4$, δ, $\alpha1$, $\alpha2$, ϵ and μ are shown respectively in SEQ ID NOS: 3, 4, 5, 6, 7, 8, 9, 10 and 11.

An examination of SEQ ID NOS: 3, 4, 5, 6, 7, 8, 9, 10 and 11 shows that the AG dinucleotide occurs invariantly at the 3' intron-exon junction, and the
15 branch point A residue occurs either 32 residues upstream (in the case of the IgGs, IgD, and IgE) or 35 residues upstream (in the case of the IgAs) or 34 residues upstream (for IgM) from the 3' splice junction. Between these invariant residues lie the nucleotide sequences which are considerably non-homologous among immunoglobulins of different isotypes. Since the intron sequences
20 between the 5' splice junction and the branch point are not needed for splicing reactions, oligonucleotide sequences which would successfully interfere with splicing would be complementary to the region about 30 nucleotides upstream of

the 3' splice junction.

In the germ-line organization, the variable (V) gene family of 50 or more genes and the cluster of heavy chain constant region genes are in separate chromosomes. During the somatic maturation process of B cells, a V gene is rearranged with the constant region gene cluster at a certain pre-B cell stage. Hinds, K.R. and Litman, G.W. Nature 320:546 (1986); Blackwell, T.K. and Alt, F.W. Ann. Rev. Genet. 23:605 (1989). The heavy chain cluster in man is organized in the sequence of μ, δ, $\gamma3$, $\gamma1$, $\alpha1$, $\gamma2$, $\gamma4$, ϵ and $\alpha2$. Flanagon, J.G. and Rabbitts, T.H. Nature 300:709 (1982). In a further maturation process, the fused VDJ segment may be switched to one of the γ's or α, or ϵ, this switching being regulated by certain T cell factors and some other yet-to-be characterized factors. Thus, for a B cell expressing a particular isotype and subclass, the VDJ segment is adjacent to the particular heavy chain constant region gene segment. For example, for a B cell expressing IgG_1, the VDJ segment is thought to be adjacent to the constant region gene of $\gamma1$.

In the genomic organization, the peptide sequence coding segments are encoded by exons which are separated by introns. Generally speaking, for a heavy chain immunoglobulin gene, the leader sequence serves as secretion signal at the endoplasmic reticulum. The fused VDJ segment, each of the constant region domains (including CH1, CH2, and CH3, and for μ and ϵ, CH4), and the membrane anchor peptide segments, all are encoded for by discrete exons separated by introns. For δ chain, the hinge region of the immunoglobulin is

encoded by a separate exon. The control regions for transcription and translation are at the 5' end flanking the exon of the leader sequence. The transcription termination sequence is at the 3' end flanking the membrane exons.

During the transcription process of an immunoglobulin heavy chain, an
5 RNA transcript complementary to the entire length of the genomic structure from the 5' end control regions to the 3' end transcription termination signal sequence is made. This RNA precursor is then processed through a sequence of splicing steps, in which the introns are excised and the peptide-coding exons are spliced together to form mature mRNA. In this RNA splicing process, the 3' end of an
10 exon is spliced to the 5' end of the next exon in sequence. For example, the 3' end of the fused VDJ segment is spliced to the 5' end of the CH1 exon.

As discussed above, the same VDJ may be rearranged to the constant region genes of various heavy chain isotypes during B cell maturation. In one embodiment of the present invention, the splicing between the VDJ exon and an
15 isotype specific CH1 exon is inhibited by anti-sense oligonucleotides that bind to the splicing recognition sequence 5' to the CH1 exon or that bind to the splicing junctional segment of CH1. The invention is explained further below.

Summary of the Invention

The invention includes oligonucleotides which are complementary to the
20 splicing recognition region of an mRNA transcript precursor for a particular immunoglobulin heavy chain, in one or more of the CH regions of the immunoglobulin gene. More particularly, the oligonucleotides are complementary

to a portion of the sequence upstream of the 3' splice junction and towards the branch point A residue, or to a portion of the sequence upstream and downstream of the 3' splice junction. The oligonucleotides generally should be complementary to at least a continuous 12 nucleotide segment of these sequences
5 in order to bind stably. Longer segments may be used, if desired.

The oligonucleotides of the invention would be complementary to some or all of the underlined portions of SEQ ID NOS:3-33, and/or to some of these portions as well as some of the portions downstream of the 3' splice junction and in the exon region (shown in bold face type). The underlined portions represent
10 the conserved portions of the oligonucleotides. In these sequences the exons are shown in bold face type, and the introns in normal type. The 3' splice point is the point of transition between these regions. The branch point A residue is also in bold face type.

These oligonucleotides will hybridize with the splicing recognition region
15 and prevent or inhibit maturation of the mRNA. This will prevent production of mRNA encoding a complete immunoglobulin, so that it will not be translated into a functional immunoglobulin.

The oligonucleotides can be tested for activity *in vitro* on B cell lines or peripheral blood mononuclear cells (PBMCs), to determine their ability to inhibit
20 the production of antibodies of a particular class of interest. When an oligonucleotide demonstrates significant activity, additional oligonucleotides of varying lengths covering the target segment can be synthesized and tested, with

those of optimal length and sequence being selected as product candidates.

The present disclosure focuses on the splice junctions of the various immunoglobulin domains as the target segments for designing anti-sense compounds. The 3'-end untranslated regions of each antibody isotype, which are known to regulate the stability of the mRNA species, are also attractive targets for regulation with anti-sense compounds. A systematic approach for identifying effective target segments for anti-sense compounds is to synthesize over-lapping oligonucleotides of about 15 nucleotide long, covering the entire length of the 3'-end untranslated region. These oligonucleotides may overlap with adjacent oligonucleotides by up to 5 nucleotides on each end. Since oligonucleotide synthesis with synthesizers is a very established procedure, to make overlapping oligonucleotides covering a gene region of 1000 to 2000 nucleotide long is feasible. These oligonucleotides can then be tested *in vitro* against B cell lines or PBMCs, to determine their ability to inhibit the production of a particular class of antibodies. Those which inhibit production to the greatest extent would be selected.

In applying the oligonucleotides of the invention for *in vivo* therapy, use of one of the oligonucleotides at a time achieves isotype-specific suppression of immunoglobulins, in that only one immunoglobulin isotype is suppressed. This is advantageous because one can effect treatment while selectively deleting only one isotype of humoral immunity. Alternatively, several oligonucleotides of different specificities could be used to cause a generalized humoral

immunosuppression.

This selective suppression of immunoglobulin isotypes is suitable for treating autoimmune diseases and allergies. The immunoglobulins IgG and IgM have been associated with autoimmune diseases, including especially rheumatoid arthritis. Suppressing IgG and/or IgM production would be useful in treating rheumatoid arthritis, and probably also in treating other autoimmune diseases including systemic lupus erythematosus, scleroderma, and myasthenia gravis.

Allergies of the immediate-type hypersensitivity class are all mediated by IgE. Suppression of IgE by using the appropriate oligonucleotide of the invention would inhibit or prevent allergic reactions.

Another use for the oligonucleotides of the invention is in production of monoclonal antibodies. The oligonucleotides can be used to selectively inhibit production of various immunoglobulin isotypes, either by B cells extracted from the immunized animal prior to fusion with an immortalized cell line, or by the hybridomas after the fusion. This allows production of immunoglobulins of a particular desired isotype, or the prevention of production of immunoglobulins of a particular isotype. As a result, no monoclonal antibodies of these isotypes would be produced.

Detailed Description of the Preferred Embodiments

The invention pertains to use of anti-sense compounds to achieve isotype-specific suppression for treating a number of clinical conditions. Because the 5' regulatory regions and the variable domains of the immunoglobulins are highly

variable and are not unique to particular isotypes, these regions are not suitable targets for anti-sense compounds.

The most suitable targets in the immunoglobulin mRNA or the precursor RNA are at the splice junctions and in the 3'-end untranslated regions. The
5 coding regions or other areas of the untranslated regions may also provide targets for anti-sense intervention. These other regions are much longer and may require larger numbers of oligonucleotides to determine the precise target areas.

As an example of how to identify a target segment and how to design anti-sense compounds to that segment, one can consider the 3'-end untranslated region
10 of human ϵ chain. The region SEQ ID NO:34 between the stop codon TGA at the end of the 4th constant domain and the poly A signal is 126 nucleotides long. Seno M. et al. *Nucl. Acids Res.* **11**:719-727 (1983). A set of 23 complementary oligonucleotides of 15 nucleotides in length, overlapping by 5 nucleotides, are synthesized. These oligonucleotides, at concentrations ranging from 0.1 to 200
15 μg/ml are tested for their effect on the synthesis of IgE by treating SKO-007 cells (ATCC, Rockville, Maryland). These IgE-expressing cells are incubated with the potential inhibitors for 1 to 3 days and the IgE secreted into the medium is assayed by a standard IgE ELISA, such as that described in Sun L. et al. *J. Immunol.* **146**:199-205 (1991). The tests may also be done with PBMCs which
20 are isolated from donors with high serum IgE (atopic individuals).

When an oligonucleotide, or an adjacent oligonucleotide, causes inhibition of IgE synthesis by the SKO-007 cells, the susceptible region is then identified.

An additional set of oligonucleotides, ranging from 10 to 12 nucleotides in length which overlap by only one or two nucleotides and covering the identified region, are then synthesized and tested for effect on IgE production. By doing so, the segment with the optimal sequence and length can be identified. Analogues of 5 oligonucleotides with enhanced lipophilicity and resistance to RNase can also be synthesized and tested. In this way, the optimal oligonucleotides can be identified.

Similar systematic approaches can be undertaken to find anti-sense compounds for suppression of other regions of the ϵ gene and other 10 immunoglobulin isotypes. A similar approach may also be used for designing anti-sense compounds to target the 3'-end untranslated regions of the four subclasses of γ chains. The region between the ATG stop codon and AATAA signal is 100 nucleotides long for $\gamma 1$ (SEQ ID NO:35), and is 101 nucleotides long for $\gamma 2$, $\gamma 3$, and $\gamma 4$, (SEQ ID NOS: 36, 37 and 38, respectively). *See* Huck 15 S. et al. *Nucl. Acids Res.* **14**:1779-1789 (1986) . The regions have only a few nucleotide differences among the four subclasses. A set of 18 oligonucleotides can be synthesized for each subclass, with most of these oligonucleotides shared among the subclasses. These oligonucleotides can then be tested for ability to inhibit cell lines secreting the specific subclass of IgG.

20 The anti-sense compounds of the invention also target the segments at the splicing junctions, or at the segments known to be involved in splicing. These oligonucleotides are complementary to at least about a continuous 12 nucleotide

segment of the underlined portions of the sequences of SEQ ID NOS:3-33. The sequences represented by SEQ ID NOS:3-11 are explained above. SEQ ID NOS:12, 13, 14 and 15 represent, respectively, the sequences of the intron and exon near the 3' splice sites of the CH2 regions of $\gamma1$, $\gamma2$, $\gamma3$, $\gamma4$. SEQ ID
5 NOS:16, 17, 18 and 19 represent, respectively, the sequences of the intron and exon near the 3' splice sites of the CH3 regions of $\gamma1$, $\gamma2$, $\gamma3$, $\gamma4$. SEQ ID NOS:20 and 21 represent, respectively, the sequences of the intron and exon near the 3' splice sites of the CH2 and CH3 regions of δ. SEQ ID NOS:22 and 23 represent, respectively, the sequences of the intron and exon near the 3' splice
10 sites of the hinge 1 and hinge 2 regions of δ. SEQ ID NOS:24 and 25 represent, respectively, the sequences of the intron and exon near the 3' splice sites of CH2 and CH3 of $\alpha1$. SEQ ID NOS:26 and 27 represent, respectively, the sequences of the intron and exon near the 3' splice sites of CH2 and CH3 of $\alpha2$. SEQ ID NOS:28, 29 and 30 represent, respectively, the sequences of the intron and the
15 exon near the 3' splice sites of CH2, CH3 and CH4 of ϵ. SEQ ID NO: 31, 32, 33 represent the sequence of the intron and exon near the 3' splice sites of CH2, CH3, and CH4 for μ. The exons are shown in bold face type, and the introns in normal type. The 3' splice point is the point of transition between these regions. The branch point A residue is also in bold face type.

20 In general, the preferred length for the oligomers of the invention is 15 nucleotides, although they can be as short as about 12 nucleotides. As noted above, the oligomers or the invention hybridize with the segment of the

nucleotide upstream of the 3' splice junction (underlined in the sequence listing portion), and/or with a portion of this segment as well as a continuous portion of the segment downstream of the 3' splice junction (shown in bold letters in the sequence listing). The oligonucleotides of the invention can therefore either be RNA (having a U instead of a T base nucleotide as in DNA) or DNA.

The oligonucleotides of the invention can include the entire 32 or 35 nucleotide portion upstream from the 3' splice point which they hybridize with, and/or they can even include a portion downstream of the 3' splice point. However, they can also be shorter, to a length of 12 nucleotides or even less. The shorter oligonucleotides, again, can include just the portion upstream of the 3' splice point, or some of the upstream portion and some of the downstream (exon) portion. The disadvantage of segments much shorter than 12 nucleotides is that they may not hybridize in a stable fashion. The disadvantage of segments much longer than 15 nucleotides is the added cost of synthesizing longer oligonucleotides, and the fact that they may not readily permeate the cellular membranes so as to get into the cytoplasm.

All of the oligonucleotides of the invention can be synthesized with techniques which are well known in the art, such as in an Applied Biosystems 380A synthesizer.

As noted above, the oligonucleotides of the invention are useful in treating autoimmune diseases, allergies, and in causing humoral immunosuppression. For treatment of autoimmune diseases, oligonucleotides complementary to the

appropriate portions of SEQ ID NOS:3, 4, 5, 6, 11, 12, 13, 14, 15, 16, 17, 18, 19, 30, 31, 32, and 33 are preferred, as they will cause suppression of IgG and IgM production. For IgG, it is noted that there is also a hinge region gene between the sequence encoding the CH1 region (SEQ ID NOS:3, 4, 5, 6) and the 5 sequence encoding the CH2 region (SEQ ID NOS:12, 13, 14, 15). An oligonucleotide complementary to the portion of the hinge region sequence upstream of the hinge region 3' splice junction, or a portion upstream and a continuous downstream portion, could also be used as an oligonucleotide of the invention.

10 For allergy treatment, one would use oligonucleotides complementary to the appropriate portion of SEQ ID NOS:10, 27, 28 and 29, as they would cause suppression of IgE production. For inducing humoral immunosuppression, one would use oligonucleotides complementary to the appropriate portion of SEQ ID NOS:3-33, as they would suppress production of all immunoglobulins. As noted 15 above, the oligonucleotides administered can be DNA or RNA, as long as they are complementary to the target segments, and hybridize with them.

The oligonucleotides of the invention can be used *in vitro* and *in vivo* in non-modified form. In the alternative, in order to increase their hydrophobicity and make them more lipophilic, they can be chemically modified, for example, 20 by changing them to phosphorothioate, methylphosponate, or α-anomeric oligonucleotides. The manner of making these modifications is well-known in the art.

To use the oligonucleotides of the invention *in vivo*, one would administer a large excess of the oligonucleotides to the patient. They could be administered intravenously, or possibly even orally. The dosage could be readily calculated. Depending on which isotype one wishes to suppress, one would calculate the total 5 number of B cells producing that isotype, and then administer enough of the oligonucleotide to attach to the appropriate gene segment in each B cell.

To use the oligonucleotides of the invention *in vitro*, *i.e.*, in production of monoclonal antibodies, one would administer enough of the oligonucleotides to suppress the B cells which produce the isotype(s) which one desires to 10 suppress. It would be preferable to do this before fusion with the immortalized cell line, which is done to produce hybridomas. However, it could also be done after the fusion.

It should be understood that the foregoing terms and expressions are descriptive only and not limiting, and that the invention is defined only in the 15 claims which follow, and includes all equivalents of the subject matter of those claims.

SEQUENCE LISTING

(1) General Information:
(i) Applicant: Chang, Tse Wen
(ii) Title of Invention: ANTI-SENSE OLIGONUCLEOTIDES FOR ISOTYPE-
5 SPECIFIC SUPPRESSION OF IMMUNOGLOBULIN PRODUCTION
(iii) Number of Sequences: 38
(iv) Correspondence Address:
(A) Addressee: Tanox Biosystems, Inc.
(B) Street: 10301 Stella Link Rd.
10 (C) City: Houston
(D) State: Texas
(E) Country: USA
(F) Zip: 77025
(v) Computer Readable Form:
15 (A) Medium Type: Diskette, 3.5 inch
(B) Computer: IBM PS/2
(C) Operating System: DOS 3.30
(D) Software: Wordperfect 5.1
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(viii) Attorney/Agent Information:
(A) Name: Mirabel, Eric P.
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(C) Reference/Docket Number: TNX91-6-PCT
30 (ix) Telecommunication Information:
(A) Telephone: (713) 664-2288
(B) Telefax: (713) 664-8914
(2) Information for SEQ ID NO:1:
(i) Sequence Characteristics:
35 (A) Length: 9 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:1
40 CAGGTAAGT 9
(2) Information for SEQ ID NO:2:
(i) Sequence Characteristics:
(A) Length: 4 nucleotides
(B) Type: nucleic acid

(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:2
CAGG 4
5 (2) Information for SEQ ID NO:3:
(i) Sequence Characteristics:
(A) Length: 55 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double-stranded
10 (D) Topology: linear
(xi) Sequence Description: SEQ ID NO:3
CACACCGCGG TCACATGGCA CCACCTCTCT TGCAGCCTCC 40
ACCAAGGGCC CATCG 55
(2) Information for SEQ ID NO:4:
15 (i) Sequence Characteristics:
(A) Length: 55 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
20 (xi) Sequence Description: SEQ ID NO:4
CACACCGCGG TCACATGGCA CCACCTCTCT TGCAGCCTCC 40
ACCAAGGGCC CATCG 55
(2) Information for SEQ ID NO:5:
(i) Sequence Characteristics:
25 (A) Length: 55 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:5
30 CACACCGCAG TCACATGGCG CCATCTCTCT TGCAGCTTCC 40
ACCAAGGGCC CATCG 55
(2) Information for SEQ ID NO:6:
(i) Sequence Characteristics:
(A) Length: 55 nucleotides
35 (B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:6
CACACCGGGG TCACATGGCA CCACCTCTCT TGCAGCTTCC 40
40 ACCAAGGGCC CATCC 55
(2) Information for SEQ ID NO:7:
(i) Sequence Characteristics:
(A) Length: 55 nucleotides
(B) Type: nucleic acid

(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:7
TCCAGCCTCA GACTCCCACT GTGTCTGTCT TCCAGCACCC 40
ACCAAGGCTC CGGAT 55
(2) Information for SEQ ID NO:8:
(i) Sequence Characteristics:
(A) Length: 55 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:8
AGGGCCGCGT CCTCACAGTG CATTCTGTGT TCCAGCATCC 40
CCGACCAGCC CCAAG 55
(2) Information for SEQ ID NO:9:
(i) Sequence Characteristics:
(A) Length: 55 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:9
AGGGCCGCGT CCTCACAGCG CATTCTGTGT TCCAGCATCC 40
CCGACCAGCC CCAAG 55
(2) Information for SEQ ID NO:10:
(i) Sequence Characteristics:
(A) Length: 54 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:10
CTGAGGCTGG CACTGACTAG GTTCTGTCCT CACAGCCTCC 40
ACACAGAGCC CATC 54
(2) Information for SEQ ID NO:11:
(i) Sequence Characteristics:
(A) Length: 56 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:11
TCACCCTCCC TTTCTCTTTT GTCTGCGGGT CCTCAGGGAG 40
TGCATCCGCC CCAACC 56
(2) Information for SEQ ID NO:12:
(i) Sequence Characteristics:
(A) Length: 55 nucleotides

(B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:12
GGGTGCTGAC ACGTCCACCT CCATCTCTTC CTCAGCACCT 40
GAACTCCTGG GGGAC 55
(2) Information for SEQ ID NO:13:
(i) Sequence Characteristics:
(A) Length: 52 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:13
GGGTGCTGAC ACATCTGCCT CCATCTCTTC CTCAGCACCA 40
CCTGTGGCGG AC 52
(2) Information for SEQ ID NO:14:
(i) Sequence Characteristics:
(A) Length: 55 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:14
GGGTGCTGAC ACATCTGCCT CCATCTCTTC CTCAGCACCT 40
GAACTCCTGG GAGAC 55
(2) Information for SEQ ID NO:15:
(i) Sequence Characteristics:
(A) Length: 55 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:15
GGGTGCTGAC ACATCTGCCT CCATCTCTTC CTCAGCACCT 40
GAGTTCCTGG GGGAC 55
(2) Information for SEQ ID NO:16:
(i) Sequence Characteristics:
(A) Length: 54 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:16
GAGAGTGACC GCTGTACCAA CCTCTGTCCT ACAGGGCAGC 40
CCCGAGAACC ACAG 54
(2) Information for SEQ ID NO:17:
(i) Sequence Characteristics:

(A) Length: 55 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
5 (xi) Sequence Description: SEQ ID NO:17
GGGAGTGACC GCTGTGCCAA CCTCTGTCCC TACAGGGCAG 40
CCCCGAGAAC CACAG 55
(2) Information for SEQ ID NO:18:
(i) Sequence Characteristics:
10 (A) Length: 55 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:18
15 GGGAGTGACC GCTGTGCCAA CCTCTGTCCC TACAGGACAG 40
CCCCGAGAAC CACAG 55
(2) Information for SEQ ID NO:19:
(i) Sequence Characteristics:
(A) Length: 55 nucleotides
20 (B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:19
GGGAGTGACC GCTGTGCCAA CCTCTGTCCC TACAGGGCAG 40
25 CCCCGAGAAC CACAG 55
(2) Information for SEQ ID NO:20:
(i) Sequence Characteristics:
(A) Length: 55 nucleotides
(B) Type: nucleic acid
30 (C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:20
CGGCCACAGC TGCTCTCGTT TGCTCTCCCC TGCAGAGTGT 40
CCGAGCCACA CCCAG 55
35 (2) Information for SEQ ID NO:21:
(i) Sequence Characteristics:
(A) Length: 55 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double-stranded
40 (D) Topology: linear
(xi) Sequence Description: SEQ ID NO:21
GGCCAGAGCC TGACAGCCCC CCCACCTCCC CGCAGCTGCG 40
CAGGCACCCG TCAAG 55
(2) Information for SEQ ID NO:22:

(i) Sequence Characteristics:
(A) Length: 55 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double-stranded
5 (D) Topology: linear
(xi) Sequence Description: SEQ ID NO:22
CCTAAGCCTC ACCTGCACTT TTCCTTGGAT TTCAGAGTCT 40
CCAAAGGCAC AGGCC 55
(2) Information for SEQ ID NO:23:
10 (i) Sequence Characteristics:
(A) Length: 55 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
15 (xi) Sequence Description: SEQ ID NO:23
AACATGTATA ATTTTTGTCA ATTAAAAATT TTTAGGAAGA 40
GGAGGAGAAG AGAAG 55
(2) Information for SEQ ID NO:24:
(i) Sequence Characteristics:
20 (A) Length: 55 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:24
25 TGTGCTGACC AGCTCAGGCC ATCTCTCCAC TCCAGTTCCC 40
TCAACTCCAC CTACC 55
(2) Information for SEQ ID NO:25:
(i) Sequence Characteristics:
(A) Length: 55 nucleotides
30 (B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:25
TGCCCAGCCC CCTGACCTGG CTCTCTACCC TCCAGGAAAC 40
35 ACATTCCGGC CCGAG 52
(2) Information for SEQ ID NO:26:
(i) Sequence Characteristics:
(A) Length: 55 nucleotides
(B) Type: nucleic acid
40 (C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:26
CGTTCTGACC AGCTCAGGCC ATCTCTCCAC TCCAGTCCCC 40
CACCTCCCCA TGCTG 55

(2) Information for SEQ ID NO:27:
(i) Sequence Characteristics:
(A) Length: 55 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:27
TGCCCAGCCC CCTGACCTGG CTCTCTACCC TCCAGGAAAC 40
ACATTCCGGC CCGAG 55

(2) Information for SEQ ID NO:28:
(i) Sequence Characteristics:
(A) Length: 55 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:28
GCCAACAACC CTCATGACCA CCAGCTCACC CCCAGTCTGC 40
TCCAGGGACT TCACC 55

(2) Information for SEQ ID NO:29:
(i) Sequence Characteristics:
(A) Length: 55 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:29
GGGTGGGCCT CACACAGCCC TCCGGTGTAC CACAGATTCC 40
AACCCGAGAG GGGTG 55

(2) Information for SEQ ID NO:30:
(i) Sequence Characteristics:
(A) Length: 55 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:30
AGCGGGGCCC GGGCTGACCC CACGTCTGGC CACAGGCCCG 40
CGTGCTGCCC CGGAA 55

(2) Information for SEQ ID NO:31:
(i) Sequence Characteristics:
(A) Length: 58 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:31
AGTGGCGGGC ACCGGGCTGA CACGTGTCCT CACTGCAGTG 40

ATTGCCGAGC TGCCTCCC 58
(2) Information for SEQ ID NO:32:
(i) Sequence Characteristics:
(A) Length: 57 nucleotides
5 (B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:32
ACCTGCCCCA CCTCTGACTC CCTTCTCTTG ACTCCAGATC 40
10 AAGACACAGC CATCCGG 57
(2) Information for SEQ ID NO:33:
(i) Sequence Characteristics:
(A) Length: 56 nucleotides
(B) Type: nucleic acid
15 (C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:33
CCACTGCCCC GCCCTCACCA CCATCTCTGT TCACAGGGGT 40
GGCCCTGCAC AGGCCC 56
20 (2) Information for SEQ ID NO:34:
(i) Sequence Characteristics:
(A) Length: 126 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double-stranded
25 (D) Topology: linear
(xi) Sequence Description: SEQ ID NO:34
CGTACTCCTG CCTCCCTCCC TCCCAGGGCT CCATCCAGCT 40
GTGCAGTGGG GAGGACTGGC CAGACCTTCT GTCCACTGTT 80
GCAATGACCC AGGAAGCTAC CCCCAATAAA CTGTGCCTGC 120
30 TCAGAA 126
(2) Information for SEQ ID NO:35:
(i) Sequence Characteristics:
(A) Length: 100 nucleotides
(B) Type: nucleic acid
35 (C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:35
GTGCGACGGC CGGCAAGCCC CGCTCCCCGG GCTCTCGCGG 40
TCGCACGAGG ATGCTTGGCA CGTACCCCCT GTACATACTT 80
40 CCCGGGCGCC CAGCATGGAA 100
(2) Information for SEQ ID NO:36:
(i) Sequence Characteristics:
(A) Length: 101 nucleotides
(B) Type: nucleic acid

(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:36
GTGCCACGGC CGGCAAGCCC CCGCTCCCCA GGCTCTCGGG 40
5 GTCGCGTGAG GATGCTTGGC ACGTACCCCG TGTACATACT 80
TCCCAGGCAC CCAGCATGGA A 101
(2) Information for SEQ ID NO:37:
(i) Sequence Characteristics:
(A) Length: 101 nucleotides
10 (B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:37
GTGCGACAGC CGGCAAGCCC CCGCTCCCCG GGCTCTCGGG 40
15 GTCGCGCGAG GATGCTTGGC ACGTACCCCG TGTACATACT 80
TCCCGGGCAC CCAGCATGGA A 101
(2) Information for SEQ ID NO:38:
(i) Sequence Characteristics:
(A) Length: 101 nucleotides
20 (B) Type: nucleic acid
(C) Strandedness: double-stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:38
GTGCCAGGGC CGGCAAGCCC CCGCTCCCCG GGCTCTCGGG 40
25 GTCGCGCGAG GATGCTTGGC ACGTACCCCG TCTACATACT 80
TCCCAGGCAC CCAGCATGGA A 101

What Is Claimed Is:

1. An oligonucleotide complementary to the splicing recognition region of an mRNA transcript precursor for a particular immunoglobulin heavy chain.

2. The oligonucleotide of claim 1 which is either RNA or DNA.

5 3. The oligonucleotide of claim 1 complementary to at least a continuous 12 nucleotide sequence of the region extending about 30 nucleotides upstream to a CH exon or about 30 nucleotides upstream to the intron-exon junctional segment of a CH or to some of the sequence upstream of the CH exon or the intron-exon junctional segment and some of the continuous sequence downstream thereof.

10 4. The oligonucleotide of claim 1 in which the CH exon is CH1.

5. The oligonucleotide of claim 1 in which the immunoglobulin heavy chain is ϵ, α or μ.

6. The oligonucleotide of claim 1 wherein the oligonucleotide is a phosphorothioate, methylphosponate, an α-anomeric oligomer, or another type of

15 hydrophobized oligomer.

7. An oligonucleotide of claim 4, which is complementary to at least a continuous 12 nucleotide sequence of the underlined portions of one of SEQ ID NOS:3-11, or to some of the underlined portions and a continuous segment of the bold face portions.

20 8. An oligonucleotide of claim 5, which is complementary to at least a continuous 12 nucleotide sequence of the underlined portions of one of SEQ ID NOS:8, 9, 10, 11, 24, 25, 26, 27, 28, 29, 30, 31, 32, and 33, or to some of the

underlined portions and to a continuous segment of the bold face portions.

9. A composition for causing suppression of IgG production, comprising oligonucleotides complementary to at least a continuous 12 nucleotide sequence of the underlined portion of SEQ ID NOS: 3, 4, 5, 6, 12, 13, 14, 15, 16, 17, 18
5 and 19, or to some of the underlined portions and to a continuous segment of the bold face portions.

10. A composition for causing suppression of IgM production, comprising oligonucleotides complementary to at least a continuous 12 nucleotide sequence of the underlined portion of SEQ ID NOS:11, 30, 31, 32, 33, or to some of the
10 underlined portions and to a continuous segment of the bold face portions.

11. A composition for causing suppression of IgE production, comprising oligonucleotides complementary to at least a continuous 12 nucleotide sequence of the underlined portion of SEQ ID NOS:10, 27, 28 and 29, or to some of the underlined portions and to a continuous segment of the bold face portions.

15 12. A method of suppressing production of a particular immunoglobulin isotype comprising administering an oligonucleotide of claim 1.

13. The method of claim 12 comprising administering one or more of the oligonucleotides of claims 8, 9, 10, or 11.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/10024

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07H 21/00; A61K 45/00
US CL :536/27; 514/44; 435/6, 91, 172.3
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. : 536/27; 514/44; 435/6, 91, 172.3

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)

APS, Dialog: Biosis, Medline, CAS, Biotechnology Abstracts
search terms: Antisense and (Splic?(w) site or region)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Molecular Biology, Vol. 24, no. 1, Part 2, August 1990 (Translation of January - February, 1990), Vlasov et al., "Inhibition of translation of immunoglobulin mRNA in vitro by means of an alkylating derivative of an oligonucleotide", pages 151-155, see entire document.	1-13
Y	P.N.A.S. USA, vol. 85, August 1988, Goodchild et al., "Inhibition of human immunodeficiency virus replication by antisense oligonucleotides", pages 5507-5511, see entire document.	1-13
Y	EMBO Journal, Vol. 7, no. 8, 1988, Munroe, S.H., "Antisense RNA inhibits splicing of pre-mRNA in vitro", pages 2523-2532, see entire document.	1-13
X Y	Journal of Allergy & Clinical Immunology, vol. 17, no 1, Part 2, Abstracts, #414, Hall et al., "Specific inhibition of IgE antibody production in vitro by an antisense oligonucleotide", page 243, abstract only.	1-13 1-13
Y	"E.A. Kabat et al., "Sequences of protein of immunological interest", Fourth edition, published 1987, cover sheet only.	1-13

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

* Special categories of cited documents:

"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

"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
14 January 1993	05 FEB 1993
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer JOHN L. LEGUYADER Telephone No. (703) 308-0196