

The opinion in support of the decision being entered today is not binding precedent of the Board.

Paper No. 199

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P.O. Box 1450
Alexandria, VA 22313-1450
Tel: 571-272-9797
Fax: 571-273-0042

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

JAY M. SHORT
Junior Party
(U.S. Patent 6,479,258)

v.

JUHA PUNNONEN, WILLEM P. C. STEMMER,
ROBERT G. WHALEN and RUSSELL HOWARD
Senior Party
(Application 09/724,869)

Patent Interference No. 105,188

Before: SCHAFFER, GRON and TORCZON, Administrative Patent Judges.
GRON, Administrative Patent Judge.¹

¹ Administrative Patent Judge Spiegel is presently unavailable. A reconstituted panel of Judges Schaffer, Gron and Torczon has been designated to consider the present request for rehearing. See In re Bose Corp., 772 F.2d 866, 869, 227 USPQ 1, 3-4 (Fed. Cir. 1985) (permissible exercise of discretion).

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MEMORANDUM IN SUPPORT OF RECOMMENDATION

Short requested rehearing (Paper No. 188) of Short Preliminary Motion 10, on June 24, 2004 (Paper No. 60). The motion was denied by Decision-Motion-Bd.R. 125(a) of the Board of Patent Appeals and Interferences (Board), mailed November 25, 5 2005 (Paper No. 181). Short's motion asked for judgment that Claim 47 of Senior Party Punnonen's Application 09/724,869, filed November 28, 2000² (Punnonen Exhibit 1007 (Exh. 1007)), is unpatentable under 35 U.S.C. § 103 in view of the combined 10 prior art teachings of Freeman PCT (WO 95/03408, published February 2, 1995) (Exh. 2040) and Short PCT (WO 91/16427, published October 31, 1991) (Exh. 2050). In discussing the teachings of the Freeman and Short PCTs, Short's motion set the state of the art and level of skill in the art on February 11, 15 1998, by reference to the Freeman and Short PCTs and Peach, et al. (Peach), "Both Extracellular Immunoglobulin-like Domains of CD80 Contain Residues Critical for Binding T Cell Surface Receptors CTLA-4 and CD28," J. Biol. Chem., Vol. 270, No. 36, pp. 21181-21187 (1995) (Exh. 2051); Stemmer, Willem P. C.

² Punnonen claims benefit of the February 10, 1999, filing date of U.S. Application 09/248,716, and the February 11, 1998, filing date of U.S. Provisional Application 60/074,294, under 35 U.S.C. § 120 for the full scope of the subject matter defined by Claim 47.

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(Stemmer), "Searching Sequence Space," Biotechnology, Vol. 13, pp. 549-553 (June 1995) (Exh. 2052); Patten, et al. (Patten), "Applications of DNA Shuffling to Pharmaceuticals and Vaccines," Current Opinion in Biotechnology, Vol. 8, pp. 724-733 (1997) (Exh. 2053); and Hoogenboom, et al. (Hoogenboom), "Multi-subunit proteins on the surface of filamentous phage: methodologies for displaying antibody (Fab) heavy and light chains," Nuc. Acid. Res., Vol. 19, No. 15, pp. 4133-4137 (1991) (Exh. 2054) (Paper No. 60, pp. 5-13). Punnonen filed a response to Short's request for rehearing on January 25, 2006 (Paper No. 191).

Short's request for rehearing (Paper No. 188) alleged that our decision overlooked and misapprehended teachings of the Freeman and Short PCTs, the state of the art, the knowledge of persons skilled in the art, and the full scope of subject matter defined by Claim 47 of Punnonen's Application 09/724,869 as of its February 11, 1998, filing date. While Short's request is no longer before us, we have reviewed the patentability of Punnonen's Claim 47 under 35 U.S.C. § 103 in view of the combined teachings of the prior art of record.

On reconsideration of the prior art of record, we conclude that Claim 47 of Punnonen's Application 09/724,869, filed November 28, 2000, prima facie would have been obvious under 35 U.S.C. § 103 to persons having ordinary skill in the art in

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view of the combined teachings of the Freeman PCT, the Short PCT, and Stemmer. Accordingly, on remand of Punnonen's Application 09/724,869, we recommend that further action be taken consistent with our conclusion.

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Discussion

1. Claim 47 of Punnonen's Application 09/724,869

Punnonen's Claim 47 (Paper No. 181, p. 18) is reproduced below:

10

Claim 47

A method for obtaining an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, or encodes a polypeptide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, the method comprising:

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a) creating a library of recombinant polynucleotides;
and

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b) screening the library to identify an optimized recombinant polynucleotide that has, or encodes a polypeptide that has, a modulatory effect on the immune response induced by a vector;

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wherein the optimized recombinant polynucleotide or the polypeptide encoded by the recombinant polynucleotide exhibits an enhanced ability to modulate an immune response compared to a polynucleotide from which the library was created; wherein said optimized modulatory effect on an immune response is induced by a genetic vaccine vector, wherein the optimized recombinant polynucleotide encodes a co-stimulator selected from B7-1 (CD80) or B7-2 (CD86) and the screening step involves selecting variants with altered activity through CD28 or CTLA-4, and whereby optimization is achieved by recursive sequence recombination.

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2. Freeman PCT

First, we revisit the Freeman PCT. On consideration of its complete disclosure, we fail to see how its recognition that a “soluble B7-2 extracellular region fragment or derivative that binds CTLA4Ig (or CTLA-4 or CD28 or CD28Ig)” need not “necessarily [be] . . . capable of providing the second co-stimulation signal required to induce T cells to proliferate and become functional . . .” (Paper 181, pg. 133, ll. 11-19)” establishes that the Freeman PCT does not describe “obtaining an optimized polynucleotide encoding a B7-1 or B7-2 co-stimulator variant having an enhanced ability to modulate an immune response induced by a genetic vaccine vector and having altered activity through CD28 or CTLA-4 as compared to a predecessor B7-1 or B7-2 molecule, e.g. wild-type B7-1 or B7-2 . . .” (Paper 181, pg. 129, ll. 2-7, and pg. 142, ll. 2-9)”.

The Freeman PCT (Exh. 2040) explicitly states (Exh. 2040, p. 2, ll. 20-33):

This invention pertains to isolated nucleic acids encoding novel molecules which costimulate T cell activation. Preferred costimulatory molecules include antigens on the surface of B lymphocytes, professional antigen presenting cells . . . which present antigen to immune cells, and which bind either CTLA4, CD28, both CTLA4 and CD28 or other known or as yet undefined receptors on immune cells. Such costimulatory molecules are referred to herein as CTLA4/CD28 binding counter receptors or B lymphocyte antigens, and are capable of providing costimulation to activated T cells to thereby induce T cell

5 proliferation and/or cytokine secretion. Preferred B lymphocyte antigens include B7-2 and B7-3 and soluble fragments or derivatives thereof which bind CTLA4 and/or CD28 and have the ability to inhibit or induce costimulation of immune cells. In one embodiment, an isolated nucleic acid which encodes a peptide having the activity of the human B7-2 B lymphocyte antigen is provided. . . .

10 In describing further aspects of the invention disclosed, the Freeman PCT teaches (Exh. 2040, p. 6, ll. 10-19, emphasis added):

15 Macrophages that express a peptide having the activity of a B lymphocyte antigen, such as the B7-2 antigen, can be used as antigen presenting cells, which, when pulsed with an appropriate pathogen-related antigen or tumor antigen, enhance T cell activation and immune stimulation.

20 Mammalian cells can be transfected with a suitable expression vector containing a nucleic acid encoding a peptide having the activity of a novel B lymphocyte antigen, such as the B7-2 antigen, ex vivo and then introduced into the host mammal, or alternatively, cells can be transfected with the gene in vivo via gene therapy techniques. For example, the nucleic acid encoding peptide having B7-2 activity can be transfected alone, or in combination with nucleic acids encoding other costimulatory molecules.

25 On the other hand, the Freeman PCT acknowledges in the Summary of the Invention another aspect of its invention which relates to proteins which block or fail to deliver a co-stimulatory signal (Exh. 2040, p. 6, l. 24, to p. 13):

30 The invention also provides methods for inducing both general immunosuppression and antigen-specific tolerance in a subject by, for example, blocking the functional interaction of the novel B lymphocyte antigens of the invention, e.g. B7-2 and B7-3, to their natural ligand(s) on T cells or other immune system cells, to thereby block co-stimulation through the receptor-ligand pair. In one
35 embodiment, inhibitory molecules that can be used to block the interaction of the natural human B7-2 antigen to its

5 natural ligands (e.g., CTLA4 and CD28) include a soluble peptide having B7-2 binding activity but lacking the ability to costimulate immune cells, antibodies that block the binding of B7-2 to its ligands and fail to deliver a co-stimulatory signal (so called "blocking antibodies", such as blocking anti-B7-2 antibodies), B7-2-Ig fusion proteins, which can be produced in accordance with the teachings of the present invention, as well as soluble forms of B7-2 receptors, such as CTLA4Ig or CD28Ig. . . .

10 Another aspect of the invention features methods for upregulating immune responses by delivery of a costimulatory signal to T cells through use of a stimulatory form of B7-2 antigen, which include soluble, multivalent forms of B7-2 protein, such as a peptide having B7-2 activity and B7-2 fusion proteins. Delivery of a stimulatory form of B7-2 in conjunction with antigen may be useful prophylactically to enhance the efficiency of vaccination against a variety of pathogens and may also be useful therapeutically to upregulate an immune response against a particular pathogen during an infection or against a tumor in a tumor-bearing host.

20 In the Detailed Description of the Invention, the Freeman PCT teaches:

25 Costimulatory molecules within the scope of the invention are referred herein as CTLA4/CD28 ligands (counter-receptors) or B lymphocyte antigens. Novel B lymphocyte antigens which provide costimulation to activated T cells to thereby induce T cell proliferation and/or cytokine secretion include the B7-2 (human and murine) and the B7-3 antigens described and characterized herein.

30 (Exh. 2040, p. 10, ll. 6-10);

35 [O]ne aspect of this invention pertains to isolated nucleic acids comprising a nucleotide sequence encoding a novel costimulatory molecule, such as the B lymphocyte antigen B7-2, fragments of such nucleic acids, or equivalents thereof. . . . The term equivalent is intended to include nucleotide sequences encoding functionally equivalent B lymphocyte antigens or functionally equivalent peptides having an activity of a novel B lymphocyte antigen, i.e.,

5 the ability to bind to the natural ligand(s) of the B lymphocyte antigen on immune cells, such as CTLA4 and/or CD28 on T cells, and inhibit (e.g., block) or stimulate (e.g., enhance) immune cell costimulation. Such nucleic acids are considered equivalents of the human B7-2 nucleotide sequence provided in Figure 8 (SEQ ID NO:1) and murine B7-2 nucleotide sequence provided in Figure 14 (SEQ ID NO:22) and are within the scope of this invention.

10 (Exh. 2040, p. 10, ll. 24-35);

15 In one embodiment, the nucleic acid is a cDNA encoding a peptide having an activity of the B7-2 B lymphocyte antigen. Preferably, the nucleic acid is a cDNA molecule consisting of a least a portion of a nucleotide sequence encoding human B7-2

(Exh. 2040, p. 10, l. 36, to p. 11, l. 1);

20 A "fragment" of a nucleic acid encoding a novel B lymphocyte antigen is defined as a nucleotide sequence having fewer nucleotides than the nucleotide sequence encoding the entire amino acid sequence of the B lymphocyte antigen and which encodes a peptide having an activity of the B lymphocyte antigen (i.e., the ability to bind to the natural ligand(s) of the B lymphocyte antigen on immune cells, such as CTLA4 and/or CD28 on T cells and either stimulate or inhibit immune cell costimulation). Thus, a peptide having B7-2 activity binds CTLA4 and/or CD28 and stimulates or inhibits a T cell mediated immune response, as evidenced by, for example, cytokine production and/or T cell proliferation by T cells that have received a primary activation signal. In one embodiment, the nucleic acid fragment encodes a peptide of the B7-2 antigen which retains the ability of the antigen to bind CTLA4 and/or CD28 and deliver a costimulatory signal to T lymphocytes. In another embodiment, the nucleic acid fragment encodes a peptide including an extracellular portion of the human B7-2 antigen (e.g., approximately amino acid residues 24-245 of the sequence provided in Figure 8 (SEQ ID NO:2)) which can be used to bind CTLA4 and/or CD28 and, in monovalent form, inhibit costimulation, or in multivalent form, induce or enhance costimulation.

45 (Exh. 2040, p. 12, ll. 18-33);

5 This invention further pertains to expression vectors containing a nucleic acid encoding at least one peptide having the activity of a novel B lymphocyte antigen, as described herein, operably linked to at least one regulatory sequence.

(Exh. 2040, p. 14, ll. 7-9);

10 The invention also features methods of producing peptides having an activity of a novel B lymphocyte antigen. For example, a host cell transfected with a nucleic acid vector directing expression of a nucleotide sequence encoding a peptide having activity of the B7-2 protein can be cultured in a medium under appropriate conditions to
15 allow expression of the peptide to occur.

(Exh. 2040, p. 14. ll. 29-33);

20 Transfected cells which express peptides having activity of one or more B lymphocyte antigens (e.g., B7-2, B7-3) on the surface of the cell are also within the scope of this invention.

(Exh. 2040, p. 15, ll. 29-31); and

25 [A] peptide having an activity of the B7-2 protein is defined herein as a peptide having the ability to bind to the natural ligand(s) of the B7-2 protein on immune cells, such as CTLA4 and/or CD28 on T cells and either stimulate or
30 inhibit cell costimulation. Thus, a peptide having B7-2 activity binds CTLA4 and/or CD28 and stimulates or inhibits a T cell mediated immune response (as evidenced by, for example, cytokine production and/or proliferation by T cells that have received a primary activation signal).
35 Alternatively, a peptide having both B7-2 binding activity and the ability to deliver a costimulatory signal to T cells is used to stimulate or enhance T cell proliferation and/or cytokine secretion in a subject.

40 (Exh. 2040, p. 16, ll. 22-30; emphasis added).

We find that the Freeman PCT alone describes procedures for obtaining polynucleotides encoding active B7-2 co-stimulator

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variants having an enhanced ability to modulate an immune response induced by a genetic vaccine vector and having altered activity through CTLA4 and/or CD28 as compared to a predecessor B7-2 molecule, e.g., natural or wild-type B7-2. The new polynucleotides obtained in accordance with the procedures described in the Freeman PCT may encode proteins which upregulate immune responses by either inhibiting delivery of a costimulatory signal to T cells or enhancing delivery of a costimulatory signal to T cells. The altered B7-2 antigens include soluble, multivalent forms of B7-2. While the reference teaches that enhanced delivery of stimulatory B7-2 may be useful to improve the efficiency of vaccination against a variety of pathogens, it also teaches that delivery of nonstimulatory B7-2 may be useful to construct B7-2 "knock out" animals. In either case, the procedures described by the Freeman PCT are designed to produce polynucleotides that encode peptides having an altered modulatory effect on an immune response as compared to the response prior to alteration.

Whether or not each of the methods expressly described by the Freeman PCT optimizes the modulatory effect of the encoded protein is indeterminable from the teaching of the Freeman PCT. We find, however, that all the methods described in the Freeman PCT were designed to improve the modulatory effect of

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the encoded protein on an immune response as compared to the response prior to alteration. The procedures described in the Freeman PCT for obtaining new polynucleotides comprise the steps of: a) creating a library comprising recombinant polynucleotides, and b) screening the library to identify polynucleotides which encode B7-1 and/or B7-2 variants having altered modulatory effects on an immune response, whether the effects are enhanced immune responses or inhibited immune responses to a vaccine vector. For a description of construction of a cDNA library, see the Freeman PCT at page 18, l. 27, to page 19, l. 20 (II. Isolation of mRNA and Construction of cDNA Library); page 70, l. 11, to page 72, l. 4 (Example 4: Cloning, Sequencing and Expression of the B7-2 Antigen, A. Construction of cDNA Library); and page 78, l. 17, page 80, l. 6 (Example 6, Cloning and Sequencing of the Murine B7-2 Antigen, A. Construction of cDNA Library). On consideration of more detailed instructions in the Freeman PCT, we also find that the inventive procedures described therein include recursive screenings for DNA encoding altered, active B7-2 antigens (Exh. 2040, III. Transfection of Host Cells and Screening for Novel B Lymphocyte Activation Antigens, p. 19, l. 23, to p. 20, l. 6; emphasis added):

The thus prepared cDNA library is then used to clone the gene of interest by expression cloning techniques.

. . . .

5 According to one embodiment, plasmid DNA is introduced into a simian COS cell line . . . by known methods of transfection . . . and allowed to replicate and express cDNA inserts. The transfectants expressing B7-1 antigen are depleted with an anti-B7-1 monoclonal antibody . . . and anti-murine IgG and IgM coated immunomagnetic beads. Transfectants expressing human B7-2 antigen can be positively selected by reacting the transfectants with the fusion proteins CTLA4Ig and CD28Ig, followed by panning with anti-human Ig antibody coated plates. Although human CTLA4Ig and CD28Ig fusion proteins were used in the examples described herein, given the cross-species reactivity between B7-1 and, for example murine B7-1, it can be expected that other fusion proteins reactive with another cross-reactive species could be used. After panning, episomal DNA is recovered from the panned cells and transformed into a competent bacterial host Plasmid DNA is subsequently reintroduced into COS cells and the cycle of expression and panning repeated at least two times. After the final cycle, plasmid DNA is prepared from individual colonies, transfected into COS cells and analyzed for expression of novel B lymphocyte antigens by indirect immunofluorescence with, for example, CTLA4Ig and CD28Ig.

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25 Example 4 (Exh. 2040, B. Cloning Procedure, p. 72, ll. 6-32) and Example 6 (Exh. 2040, B. Cloning Procedure, p. 80, ll. 8-34) explain how the recursive screening steps of the procedures described in the Freeman PCT select polynucleotide sequences which encode novel human or murine B7-2 variants having markedly enhanced or inhibited immune responses to a vaccine vector.

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35 Focusing on the detailed teachings of the Freeman PCT, we find that one method described in this primary reference for obtaining an immunomodulatory polynucleotide that encodes a B7-2 antigen which has an altered modulatory effect on an immune response induced by a genetic vaccine vector as compared to the

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response to the unaltered antigen comprises: a) creating a library of DNA encoding a natural B7-2 antigen and DNA altered to encode a B7-2 variant having reduced activity or enhanced inactivity; b) inducing homologous recombination between the
5 natural DNA and the altered DNA comprising a DNA library; and
c) screening all new DNA resulting from homologous recombination of natural DNA and altered DNA comprising the DNA library for expression of B7-2 variants which reduce the animal's immune response to a vaccine as compared to its natural immune response.

10 See the following excerpts from the Freeman PCT (Exh. 2040, p. 22, l. 17, to p. 23, l. 17; emphasis added):

15 A transgene is a DNA which is integrated into the genome of a cell from which a transgenic animal develops. In one embodiment, murine B7-2 cDNA or an appropriate sequence thereof can be used to generate transgenic animals that contain cells which express B7-2 protein. Methods for generating transgenic animals, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009.
20 Typically, particular cells would be targeted for B7-2 transgene incorporation with tissue specific enhancers, which could result in T cell costimulation and enhanced T cell proliferation and autoimmunity. Transgenic animals that include a copy of a B7-2 transgene introduced into the
25 germ line of the animal at an embryonic stage can be used to examine the effect of increased B7 expression. Such animals can be used to examine the effect of increased B7 expression. Such animals can be used as tester animals for reagents thought to confer protection from, for example, autoimmune disease. In accordance with this facet of the
30 invention, an animal is treated with the reagent and a reduced incidence of the disease, compared to untreated animals bearing the transgene, would indicate a potential therapeutic intervention for the disease.

5 Alternatively, the non-human homologues of B7-2 can be used to construct a B7-2 "knock out" animal which has a
10 defective or altered B7-2 gene as a result of homologous recombination between the endogenous B7-2 gene and altered B7-2 genomic DNA introduced into an embryonic cell of the animal. For example, murine B7-2 cDNA can be used to clone genomic B7-2 in accordance with established techniques. A portion of the genomic B7-2 DNA (e.g., such as an exon which encodes an extracellular domain) can be deleted or replaced by another gene, such as a gene encoding a selectable marker which can be used to monitor integration. Typically, several kilobases of unaltered flanking DNA (both at the 5' and 3' ends) are included in the vector (see e.g., Thomas, K.R. and Capecchi, M. R. (1987) Cell 51:503 for a description of homologous recombination vectors). The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the induced DNA has homologously recombined with the endogenous DNA are selected (see e.g., Li, E. et al. (1992) Cell 69:915). The selected cells are then injected into a blastocyst of an animal (e.g., mouse) to form aggregation chimeras (see e.g., Bradley, A. in Teratocarcinomas and Embryonic Stem Cells: A Practical Approach, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). The chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term to create a "knock out" animal. Progeny harbouring the homologously recombined DNA in their germ cells can be identified by standard techniques and used to breed animals in which all cells of the animal contain the homologously recombined DNA. Knockout animals can be characterized for their ability to accept grafts, reject tumors and defend against infectious diseases and can be used in the study of basic immunobiology.

35 We have reviewed the findings at page 142, first paragraph, of our prior decision in light of the reference's teaching as a whole (Paper No. 181). We now find that the Freeman PCT prima facie describes a method for obtaining an optimized polynucleotide encoding a B7-2 co-stimulator variant having an enhanced ability to modulate an immune response induced by a

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genetic vaccine vector and having altered activity through CTLA4 or CD28 as compared to a predecessor B7-2 molecule, e.g., wild-type B7-2. However, the reference also teaches that optimization may be achieved by recursive activity whereby (Exh. 2040, pp. 19-20, III. Transfection of Host Cells and Screening for Novel B Lymphocyte Activation Antigens) a prepared cDNA library is used to clone the B7-2 gene of interest, plasmid DNA is introduced into a simian COS cell line by known methods of transfection and allowed to replicate and express the cDNA inserts, transfectants expressing human B7-2 antigen are selected by reacting the transfectants with the fusion proteins CTLA4Ig, CD28Ig and other cross-reactive proteins and panning, episomal DNA is recovered from the panned cells and transformed into E. coli, plasmid DNA is reintroduced into COS cells and the expression and panning cycle is repeated at least two times, and the COS cells of the final repeat are analyzed for expression of novel B lymphocyte antigens using CTLA4Ig and CD28Ig.

Moreover, we find explicit teaching in the Freeman PCT that optimization may be achieved by homologous recombination of DNA comprising a library of DNA including endogenous and altered B7-2 DNA (Exh. 2040, p. 22, l. 17, to p. 23, l. 17). Accordingly, while our previous finding that Short failed to establish that the Freeman PCT explicitly describes recursive sequence

recombination appears to have been correct, we did not consider the more general teachings in the Freeman PCT to obtain an immunomodulatory polynucleotide having an optimized modulatory effect on an immune response as compared to the response prior to optimization by methods whereby optimization is achieved by homologous sequence recombination or selecting and recycling DNA exhibiting altered antigenic activity.

3. Short PCT

We now revisit the Short PCT. We consider the teaching of the Short PCT anew in light of our finding herein that the teaching of the Freeman PCT is much more pertinent to the subject matter defined by Punnonen's Claim 47 and much less deficient than we previously had found.

The Short PCT teaches (Exh. 2050, p. 33, l. 35, to p. 35, l. 17; emphasis added):

The present invention also provides a novel method for screening variants of a parental clone or clones. If the parental clone or clones contain two nucleotide sequences that, when expressed together, create a phenotype, then such nucleotide sequences can be altered to create populations of variants of such nucleotide sequences. If the two variant populations are coexpressed in a random fashion (that is with no correlation between the specific alterations made in the two different nucleotide sequences), then a combinatorial collection of such nucleotide sequence variants has been created. Such combinatorial collections may be screened for the presence of phenotypes that are unlike the parental clone or clones. Generally, the method combines the following elements:

1. Replicating a clone containing a nucleotide sequence under conditions that allow mutations to occur.
2. Replicating a second clone containing a second nucleotide sequence under conditions that allow mutations to occur.
3. Randomly combining and co-expressing the two mutated populations of nucleotide sequences.
4. Screening clones containing combinations of the mutated nucleotide sequences for phenotypes that were not present in either parent clone.

Alternatively, the methods combine the following elements:

1. Replicating at least portions of two nucleotide sequences contained within a single clone under conditions that allow mutations to occur in either nucleotide sequence.
2. Allowing recombination events between the two nucleotide sequence populations to reassociate mutant nucleotide sequences to form new pairs of the two sequences that were not paired in the original mutated, replicated population.
3. Screening clones containing combinations of nucleotide sequences for phenotypes that were not present in the parent clone or in the mutant replicas of the parent clone.

For example, assume a parent clone containing two nucleotide sequences A and B is replicated under mutating conditions such that variant clones are formed:

Parent: A/B
Variant 1: A1/B
Variant 2: A/B1
Variant 3: A2/B1
Variant 4: A/B2
Variant 5: A3/B

However, within this mutated population, the combinations A1/B2, A2/B, A2/B2, A3/B1, and A3/B2, do not occur. If the mutant population (including non-mutated parent clones) is allowed to recombine sequences A and B and their variants, then combinations such as A1/B2, A2/B etc. can be created. Such new combinations may express a desired phenotype that was not present in the parental or variant population.

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We find that the hereinabove transcribed paragraphs of the Short PCT describe methods for obtaining polynucleotides having an optimized phenotype comprising: a) creating a library of recombinant polynucleotides; and b) screening the library to
5 identify those polynucleotides having a variant phenotype, whereby optimization is achieved by recursive sequence recombination. The Short PCT adds (Exh. 2050, p. 40, ll. 5-18):

10 In some cases, it is desirable to bias the repertoire for a preselected activity, such as by using as a source of nucleic acid cells (source cells) from vertebrates in any one of various stages of age, health and immune response. For example, repeated immunization of a healthy animal prior to collecting rearranged B cells results in obtaining a
15 repertoire enriched for genetic material producing a ligand binding polypeptide of high affinity.

Accordingly, the foundation for our previous finding that "the Short PCT fails to remedy the deficiencies of the Freeman PCT [because] . . . Short has not shown that the Short PCT
20 describes using recursive sequence recombination to create recombinant polynucleotide libraries as required by Punnonnen claim 47" (Paper No. 181, p. 142, third full paragraph) did not take into account the full scope and content of the teachings of the Freeman PCT.

25 4. Stemmer

We find that, as of the date Punnonnen's involved application was first filed, recursive sequence recombination was a procedure

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well-known in the pertinent art for creating polynucleotide variants encoding polypeptides eliciting an optimized immune response in an expression host as compared to the immune response elicited by the polypeptides encoded by the library of polynucleotides from which the novel polynucleotide variants were recombined. This finding is consistent with the prior art teaching of the publication of Punnonen's co-inventor Stemmer of record. Willem P. C. Stemmer (Stemmer), "Searching Sequence Space," Biotechnology, Vol. 13, pp. 549-553 (June 1995)

(Exh. 2052), teaches that recursive sequence recombination is a procedure well-known for rapidly creating the best polynucleotide sequences for a specific task (Paper No. 60, p. 6, para. 12).

The 1995 Stemmer publication teaches:

Experiments conducted to date suggest that benchtop in vitro evolution techniques are capable of creating libraries as large as molecules. Researchers can mimic natural evolution by searching these libraries for the best candidates for a specific task: Repeated round[s] . . . of selection and amplification of candidates has already been shown to produce novel molecules capable of binding with equal or higher affinity than their natural counterpart.

(Exh. 2052, p. 549, col. 1, third para.);

The problem with point mutagenesis strategies can be traced to the fact that they introduce random "noise" into a message at every cycle. If the noise level is too high -relative to the library size and the selection stringency- the message will gradually become nonsensical. This problem has generated a great deal of speculation about how to create "biased" libraries that attempt to use similar, rather than random, amino acid substitutions. The optimum

level of random point mutations introduced at each cycle would be much lower if existing, already proven, point mutations or combinations could be permuted by recombination.

5 (Exh. 2052, p. 550, col. 2, paragraph bridging cols. 1-2);

10 Since recombination can occur even with "naked" DNA, "it would seem that it would be easy to employ recombination techniques in order to evolve complex sequences-or even whole genomes-at the benchtop. But in reality this has not been the case. To the surprise of most researchers, recombination methods for mutagenesis, selection, and replication have been extremely difficult to develop in the laboratory.

15 (Exh. 2052, pp. 550-551, bridging para.);

20 So far, a technique called sexual PCR, or DNA shuffling, comes closest to mimicking natural recombination by allowing the in vitro homologous recombination of DNA.

(Exh. 2052, p. 551, col. 1, first full para.);

25 This method offers practical and theoretical advantages over existing recursive mutagenesis methods, such as error-prone PCR or recursive oligonucleotide directed mutagenesis. By recombining point mutations and wild-type sequences, followed by selection for function,^[3] sexual PCR will rapidly fine tune the mutation load present in different parts of a protein. Over several cycles, those areas that tolerate a high mutation load accumulate more diversity than those areas that are less permissive to modification.

30 (Exh. 2052, p. 551, cols. 1-2, bridging para.);

35 Sexual PCR's advantages for searching sequence space can be further explained by understanding how it employs poolwise and pairwise recombination strategies. Recombination by sexual PCR is, in principal, poolwise-more than two parental sequences can contribute to form the

³ This resembles the procedure used in the Freeman PCT (Exh. 2040, pp. 22-23, bridging para.).

5 chimeric molecule. This is a very efficient method of combining useful mutations from many different parental sequences through multiple crossovers. Sexual PCR's high recombination rate takes advantage of this by recombining a large number of mutations in a minimum number of selection cycles.

(Exh. 2052, p. 551, col. 2, first full para.); and

10 The advantage of sexual PCR is that it uses a very low rate of random mutation The longer the sequence, the lower the rate needs to be. Recombination and selection quickly yield functional blocks containing combinations of compatible point mutations. At a larger scale, these
15 functional building blocks are permuted and the best combinations are selected. The low point mutation rate and the homology requirement for recombination make it a process that is mostly gentle and conservative, but also capable of
20 big sequence jumps.

(Exh. 2052, p. 551, col. 2, second to last full para.).

Regarding a promising alternative technique called exon shuffling, Stemmer states (Exh. 2052, p. 552, cols. 1-2, bridging
25 para.):

30 Exon shuffling may also be used to obtain random peptide libraries of very large effective sizes. Without homologous introns, the degree of homology between peptides selected from a random library would be insufficient for recombination. Recombination inside the exon is unlikely because of their random sequence origin and much shorter length. The recombined exons are cloned back into the phage, and a new library of 10^8 is obtained and
35 screened. This process is repeated until no further improvement occurs.

5. Prima facie obviousness

We have considered the combined teachings of the Freeman and Short PCTs in light of Stemmer's description of the knowledge and

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skill of a person having ordinary skill in the art at the
pertinent time. We conclude that the method defined by Claim 47
of Punnonnen's involved application prima facie would have been
obvious to persons having ordinary skill in the art as of the
5 effective filing date of Punnonnen's application.

Prior art disclosures should be considered for everything
they would have taught a person having ordinary skill in the art
when the issue of patentability under 35 U.S.C. § 103 in view of
that prior art is raised. "The description of . . . 'specific
10 preferences in connection with . . . [the] generic' is
determinative in an analysis of anticipation under 35 U.S.C.
§ 102. . . . But in a section 103 inquiry, 'the fact that a
specific [embodiment] is taught to be preferred is not
controlling, since all disclosures of the prior art, including
15 unpreferred embodiments, must be considered.' In re Lamberti,
545 F.2d 747, 750, 192 USPQ 278, 280 (CCPA 1976)." Merck & Co.
Inc. V. Biocraft Laboratories Inc., 874 F.2d 804, 807, 10 USPQ2d
1843, 1846 (Fed. Cir. 1989). In re Fracalossi, 681 F.2d 792, 794
n.1, 215 USPQ 569, 570 n.1 (CCPA 1982), teaches:

20 It is axiomatic that a reference must be considered in its
entirety, and it is well established that the disclosure of
a reference is not limited to specific working examples
contained therein. In re Lamberti, 545 F.2d 747, 750,
192 USPQ 278, 280 (CCPA 1976).
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In re Boe, 355 F.2d 961, 965, 148 USPQ 507, 510 (CCPA 1966),
instructs, "All of the disclosures in a reference must be
evaluated for what they fairly teach one of ordinary skill in the
art." Similarly, In re Bode, 550 F.2d 656, 661, 193 USPQ 12, 17
5 (CCPA 1977), directs, "A reference must be evaluated for all it
teaches and is not limited to its specific embodiments." "[A]
prior art reference is relevant for all that it teaches to those
of ordinary skill in the art." In re Fritch, 972 F.2d 1260,
1264, 23 USPQ2d 1780, 1782 (Fed. Cir. 1982). Summarizing, In re
10 Heck, 699 F.2d 1331, 1332-33, 216 USPQ 1038, 1039 (Fed. Cir.
1983), instructs:

As the Court of Customs and Patent Appeals said in a section
103 case (In re Lemelson, 397 F.2d 1006, 1009, 158 USPQ 275,
277 ([CCPA] 1968)):

15 "The use of patents as references is not limited to
what the patentees describe as their own inventions or
to the problems with which they are concerned. They
20 are part of the literature of the art, relevant for all
they contain."

We previously focused on the preferred embodiments of both
the Freeman PCT and the Short PCT and their shortcomings relative
to the specific subject matter defined by Punnonen's Claim 47.
25 However, Stemmer's view of the state of the art (Exh. 2052) is
entitled to considerable weight because it is the unbiased view
of one of Punnonen's co-inventors and it was published long

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before the effective filing dates of both Punnonnen's involved application and Short's involved patent.

We conclude that the method defined by Claim 47 of Punnonnen's involved application prima facie would have been
5 obvious under 35 U.S.C. § 103 to a person having ordinary skill in the art on the effective filing date of Punnonnen's involved application in view of the combined teachings of the Freeman PCT, the Short PCT, and Stemmer. Our conclusion newly emphasizes Stemmer's view of the knowledge, skill and state of the art at
10 the pertinent time. We emphasize our view that the Freeman PCT describes, and/or reasonably would have suggested, all aspects of a method encompassed by Claim 47 of Punnonnen's involved application to persons having ordinary skill in the art but for the limitation "whereby optimization is achieved by recursive
15 sequence recombination" (Claim 47, Paper No. 181, p. 18). However, as we have indicated above, we find in the Freeman PCT suggestions to optimize the modulatory effect on an immune response sought by the methods taught in the Freeman PCT by use of mutation procedures, including homologous recombination and
20 point or sequence mutations, followed by screening, recycling and/or repeating the procedures for optimum results. In light of the knowledge and skill in the art at the pertinent time, as evident from the disclosures of the Short PCT and Stemmer, the

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use of recursive sequence recombination and/or other recursive
mutation techniques for creating novel DNA using a recombinant
DNA library would have been obvious to achieve optimum results.
Persons having ordinary skill in the art would have required no
5 more instruction and/or motivation than is provided by the prior
art relied upon to make and use the method of Punnonen's
Claim 47.

Where, as here, the general conditions of a claimed process
are disclosed in the prior art, it is not inventive to optimize
10 by routine experimentation. In re Aller, 220 F.2d 454, 456,
105 USPQ 233, 235 (CCPA 1955). Here, the evidence shows that
persons having ordinary skill in the art understood that optimum
results could be achieved by recursive homologous recombination
at the pertinent time without undue experimentation. Stemmer
15 acknowledges that it was within the ordinary skill of the artisan
to optimize the modulatory effect on an immune response of a
protein encoded by recombinant DNA by recursive sequence
recombination. Moreover, the obviousness of the method of
Claim 47 of Punnonen's involved application is kindled by
20 specific instructions in the Short PCT to repeat recombination
procedures to create a desired phenotype (Exh. 2050, p. 33,
l. 35, to p. 35, l. 17). The Short PCT directs persons skilled
in the art to utilize recursive sequence mutations, including

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recursive sequence recombinations, to produce novel DNA which encodes a polypeptide having desirably enhanced properties.

Accordingly, we conclude that the combined teachings of the Freeman PCT, the Short PCT, and Stemmer reasonably would have led persons having ordinary skill in the art to optimize the modulatory effect of B7-2 variants encoded by novel DNA produced by homologous recombination by: a) creating a library of recombinant DNA encoding B7-2, b) promoting sequence recombination in the recombinant DNA library, c) screening the library for novel recombinant DNA which exhibits an enhanced ability to modulate an immune response induced by a genetic vaccine vector, and optimizing the enhanced ability of the novel recombinant DNA to modulate the immune response by recursive DNA sequence recombination, and reasonably expect success.

Subject matter defined by Punnonen's Claim 47 appears to be unpatentable under 35 U.S.C. § 103 as having been prima facie obvious to a person having ordinary skill in the art in view of the combined teachings of the Freeman PCT, the Short PCT, and Stemmer. Therefore, we recommend that further examination and subsequent action relating to the patentability of the subject

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cc (via overnight mail):

Attorney for Short:

5 Jane M. Love Ph.D., Esq.
 Caren K. Khoo, Esq.
 WILMER CUTLER PICKERING HALE and DORR LLP.
 399 Park Avenue
10 New York, New York 10022
 Tel: (212) 937-7233
 Fax: (212) 937-7300

Attorney for Punnonen:

15 R. Danny Huntington, Esq.
 Sharon E. Crane, Ph.D., Esq.
 BINGHAM McCUTCHEN LLP.
 2020 K Street, NW
20 Washington, DC 20006-1806
 Tel: (202) 373-6000
 Fax: (202) 373-6367

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