# Exhibit 25

#17

Express Mail No.: EM 061 036 1764S

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Merigan et al.

Application No.: 08/470,885 Group Art Unit: 1809

Filed: June 6, 1995 Examiner: Marschel, A.

For: POLYMERASE CHAIN REACTION ASSAYS FOR MONITORING ANTIVIRAL THERAPY AND MAKING THERAPEUTIC DECISIONS IN THE TREATMENT OF ACQUIRED IMMUNODEFICIENCY SYNDROME

Attorney Docket No.: 7627-009

DECLARATION OF DR. MICHAEL J. KOZAL
UNDER 37 C.F.R. § 1.132



Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, MICHAEL J. KOZAL, do declare that:

1. I currently hold the position of Assistant Professor of Infectious Diseases, Department of Internal Medicine at the University of Iowa College of Medicine, Iowa City, Iowa, where I have been employed since 1995. I received the degree of Bachelor of Science in Chemistry in December of 1983 from Creighton University, Omaha, Nebraska, and the degree of Doctor of Medicine (M.D.) in 1988 from the University of Nebraska College of Medicine, Omaha, Nebraska.

My technical experience can be summarized as follows:

Postgraduate and Professional Experience

| | |
|---|---|
| 1995-present | Assistant Professor<br>Infectious Diseases<br>Department of Internal Medicine<br>University of Iowa College of Medicine<br>Iowa City, Iowa |
| Sept. 1996-present | Medical Director<br>Iowa City VA, HIV/ID Clinic<br>Iowa City, Iowa |
| 1994-1995 | Clinical Instructor<br>Division of Infectious Diseases<br>Department of Medicine<br>Stanford University School of Medicine<br>Stanford, California |
| 1994-1995 | Head of Infectious Diseases<br>Department of Molecular Biology<br>AFFYMETRIX-DNA CHIP COMPANY<br>Santa Clara, California |
| 1991-1994 | Fellow<br>Infectious Disease<br>Stanford University<br>Stanford, California |
| 1988-1991 | Resident<br>University of Nebraska Medical Center<br>Omaha, Nebraska |

Clinical Teaching Activities

| | |
|---|---|
| 1997 | Patient Consult Service<br>University of Iowa Hospitals and Clinics (UIHC)<br>General Medical Clinical Resident<br>UIHC<br>Outpatient Virology Clinic, UIHC<br>Outpatient Infectious Disease Clinic VA |
| 1995-present | Inpatient Infectious Disease Wards, IUHC<br>Outpatient Infectious Disease Clinic<br>UIHC<br>Outpatient Medicine Clinic UIHC<br>Outpatient Infectious Disease Clinic VA |
| 1991-1994 | Stanford University Hospitals and Clinics |

Other Activities

1996 AIDS Conference, Co-Director
University of Iowa
Iowa City, Iowa

1997 AIDS Conference, Co-Director
University of Iowa
Iowa City, Iowa

Prevention and Medical Management of HIV Infection in Women and Infants
University of Iowa
Iowa City, Iowa

University of Minnesota AIDS Conference: HIV Drug Resistance
Minneapolis, Minnesota

Infectious Disease Grand Rounds: HIV Drug Resistance
Saint Paul Ramsey Hospital
St. Paul, Minnesota

AIDS Conference: HIV Drug Resistance
University of Texas Southwestern
Dallas, Texas

Quad Cities Regional Virology AIDS Conference: Gene Amplification Technology in AIDS
Davenport, Iowa

Kansas City Area AIDS Conference: HIV Drug Resistance
Kansas City, Missouri

Honors

| | |
|---|---|
| 1997-2001 | VA Career Development Award |
| 1997-1999 | Pfizer Scholar Award |
| 1997 | American Federation For Medical Research Early Career Development Award |
| June 1996 | Obermann Fellow for Advanced Studies |
| 1991-1994 | National Research Service Award International Conference on Antimicrobial Agents in Chemotherapy (ICAAC) Grant for Fellows |

2. I have had extensive clinical, research and teaching experience in the area of infectious diseases,

including the study of HIV infection and the diagnosis and treatment of AIDS. In this connection, I am currently the Medical Director of the Iowa City VA, HIV/Infectious Disease Clinic in Iowa City, Iowa and I have been diagnosing and treating AIDS patients since my residency at the University of Nebraska Medical Center in 1990. My clinical experience includes numerous clinical trials involving the therapeutic evaluation of HIV-infected patients, particularly with regard to drug resistance and/or drug efficacy. My research experience includes the study of HIV disease pathogenesis and in particular, the pathogenesis of drug resistance.

3. I am familiar with the contents of the above-identified patent application, United States Serial No. 08/470,885. I have also reviewed the following documents: (1) the first Office Action dated May 31, 1996 in this case; (2) the outstanding Advisory Action dated September 3, 1997; (3) copies of the prior art references which have been cited against this application under 35 U.S.C. § 103 in the Actions referred to above; and (4) a copy of the Submission Under 37 C.F.R. § 1.129(a) to which this Declaration will be attached. Copies of these documents were provided to me by the attorneys who are prosecuting this application for Applicants.

4. It has been explained to me by the attorneys for Applicants that the Examiner takes the position that the methods for evaluating the effectiveness of anti-HIV therapy as claimed in the above-identified patent application are rendered obvious by the cited references under § 103 of the patent laws. It has further been explained to me that, under § 103, the inquiry is whether the cited references would have

suggested to one of ordinary skill in the art at the time this invention was made (which would be May of 1992) that the claimed methods should be carried out and, in carrying out those methods, one of skill in the art would have had a reasonable expectation of success. I have reviewed the cited references as well as the present patent application and, as one of ordinary skill in the art, I can state that the cited references would not have suggested the claimed methods of evaluating HIV therapy nor would one of ordinary skill in the art have had a reasonable expectation of success in carrying out those methods.

First, I agree with the argument contained in Applicants' attached Submission that the cited Cantin reference relates to a defective PCR experiment, wherein both the control OMP-C sequence as well as the active OMP-A agent show substantially the same drastic reduction of HIV sequences. Because the PCR experiment of Cantin contains this failed control, no correlation can be made between drug efficacy and a reduction in HIV sequences based on this experiment. In fact, because the experiment is inconclusive, one of skill in the art would not be likely to use the PCR data of Cantin for any valid comparison.

Furthermore, the Cantin reference merely contains one in vitro PCR experiment relating to the possible effect of OMP-A on HIV; one of ordinary skill in the art would not extrapolate from this one experiment, even if it were not defective, to the use of PCR in a clinical context for the evaluation of HIV therapy in patients nor does the Cantin

reference suggest that PCR be used to evaluate the efficacy of drug treatment in a clinical setting.

Finally, I note that Cantin does not suggest any of the specific parameters of the claimed invention, e.g., the use of plasma as the source of HIV sequences via PCR, the use of about 30 cycles of amplification or any of the specific correlations used in the claimed invention to determine efficacy, for example, that an RNA copy number of less than 500 per 200 μl of plasma correlates with efficacy.

The cited Hart reference also does not suggest these specific parameters of the invention. The Hart reference deals only with the detection of HIV RNA sequences in HIV-infected cells. While Hart vaguely mentions that PCR might be useful, in the future, for monitoring therapy, there is no suggestion in Hart as to how such monitoring would be accomplished, e.g., what are the parameters for PCR amplification or what are the criteria or correlations by which efficacy will be determined.

Furthermore, Hart uses cells as the source of the HIV RNA detected by PCR; however, this source of HIV RNA is not one that would have been favored by those of skill in the art at the time this invention was made for any clinical evaluation of anti-HIV therapy. This is so because HIV-infected cells contain not only full-length virus but various mRNA transcripts, e.g., encoding HIV viral regulatory proteins. Thus, the amount of HIV RNA present in infected cells may not reflect the true extent of HIV viral infection in the body. In addition, the HIV virus found in infected cells is generally older virus and may not be representative

of the virus strain that is currently active in the patient. Thus, the disclosure of Hart as to the detection by PCR of HIV RNA from cellular sources would not have been found to be applicable to the clinical context by those of ordinary skill in the art at the time this invention was made.

Using plasma as the source of HIV RNA would be more appropriate in a clinical context, as presently claimed; however, the cited Hart reference does not indicate how one would use plasma as a source of HIV RNA for the detection of HIV via PCR, much less how one would use plasma as a source of HIV RNA in PCR for the evaluation of drug efficacy. As Applicants point out in the accompanying Submission, while Hart does mention some unpublished experiments purporting to use serum samples for the detection of HIV RNA via PCR, there is no disclosure in Hart as to how those experiments were performed or whether in fact they were even successful. This is not a trivial point because at the time this invention was made, it was known that the detection of HIV RNA from plasma via PCR was problematic (see cited Ottmann reference, p. 274). Thus, the mere mention that PCR could be performed using plasma (or serum) for the detection of HIV RNA would not have suggested to one of ordinary skill in the art that (a) this could be readily and successfully accomplished or (b) how it could be accomplished.

I note that the Examiner cites the Ottmann reference as teaching the equivalence of using plasma or serum samples versus cells for detecting HIV RNA using PCR. However, Ottmann, like Hart, is only detecting HIV RNA; there is no suggestion in Ottmann that PCR on plasma samples can be used

for any clinical evaluation of AIDS treatment. In fact, as the Examiner correctly notes, Ottmann's disclosure is limited to detection. This is because Ottmann used plasma as the source of its HIV RNA and HIV RNA in plasma is hard to isolate in sufficient amounts for PCR (as noted by Ottmann); thus, the only way Ottmann could even achieve detection of the HIV RNA was by using a very high number of amplification cycles. However, such a high cycle number would not be applicable to a clinical evaluation of drug efficacy, as recognized by the Examiner.

Thus, the disclosure of Ottmann does not supplement the disclosure of Hart (or Cantin) in any way that would suggest the methods of this invention for the evaluation of anti-HIV therapy. Ottmann's PCR methodology using HIV RNA in plasma samples is clearly restricted to high cycle numbers for detection, while Hart does not even indicate how plasma HIV RNA can be used for detection via PCR. Thus, the combination of cited references -- Cantin, Hart, Ottmann and Murakawa[1] -- at best suggests the use of PCR for the detection of HIV sequences in cells or in plasma but using high cycle numbers. None of these references, alone or in combination, suggests or provides a reasonable expectation of success in carrying out the claimed methods of evaluating anti-HIV drug therapy or even that such methods could be developed.

5. It should be understood that, at the time this invention was made, nobody knew that PCR would be useful for

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[1] Murakawa is merely cited for its disclosure that HIV sequences were available for the synthesis of primers for use in PCR.

the clinical evaluation of anti-HIV therapy. As the cited references demonstrate, all that was known was that PCR could be used to detect HIV sequences, either in cells or, under certain conditions, in plasma. However, it was not known or suggested that PCR would allow the quantitation of cell-free plasma HIV RNA for the evaluation of drug efficacy. Moreover, it was not known or suggested that cell-free plasma HIV RNA, e.g., in the form of RNA copy number, could be used as a marker of circulating HIV viral load to assess anti-HIV treatment or clinical outcome. Applicants made these discoveries as set forth in the present patent application, which discoveries led to the methods claimed in this application. The combination of references cited against the claimed methods would not have suggested the claimed invention to one of ordinary skill in the art and, as one of skill in the art, I can attest to the fact that the disclosures of these references would not have suggested the claimed invention to me.

Moreover, it should be understood that, at the time this invention was made, it was not known how many PCR amplification cycles should be used to quantitate HIV RNA in plasma samples to assess anti-HIV therapy. As noted above, the isolation of cell-free plasma HIV RNA was difficult and Ottmann had only been able to detect plasma HIV RNA using very high cycle numbers which would not be effective for quantitation purposes. In the present invention, Applicants determined that about 30 cycles of PCR was necessary; substantially more cycles would result in a loss of quantitation and substantially less would result in a loss of

sensitivity, i.e., detectability. Thus, while the Examiner states that typical PCR amplification involves 30 or fewer cycles (Advisory Action, p. 3), the fact is that no one knew how many cycles would be necessary for the quantitation of plasma HIV RNA for the evaluation of anti-HIV therapy. It was Applicants in the present application that disclosed the appropriate number of cycles to be used according to the claimed methods.

6. I declare further that all statements made in this Declaration are of my own knowledge and are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 12/10/97

MICHAEL J. KOZAL

Cp 1807

PATENT

UNITED STATES PATENT AND TRADEMARK OFFICE

#108 12-11-96 TGray

Application of: Kozal *et al.*

Serial No.: 08/470,885 Group Art Unit: 1807

Filed: June 6, 1995 Examiner: Marschel, A.

For: POLYMERASE CHAIN REACTION ASSAYS FOR MONITORING ANTIVIRAL THERAPY AND MAKING THERAPEUTIC DECISIONS IN THE TREATMENT OF ACQUIRED IMMUNODEFICIENCY SYNDROME

Attorney Docket No.: 7627-009-999



1806

**RESPONSE AND AMENDMENT UNDER 37 C.F.R. § 1.115**

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In response to the outstanding Office Action dated May 31, 1996 (Paper No. 4), and pursuant to the provisions of Rule 115 of the Rules of Practice, please consider the following amendments and remarks. The Applicant submits herewith (1) a Petition to Extend Time, accompanied by the appropriate fee, (2) a Petition by the Inventors under 37 C.F.R. §1.132; (3) a Petition under 37 C.F.R. § 1.48(b) to remove Dr. Michael Kozal as an inventor on this application, accompanied by the appropriate fee; and (4) a Supplemental Information Disclosure Statement, accompanied by the appropriate fee.

EXPRESS MAIL CERTIFICATION

"Express Mail" label No. TB 668 686 073 US Date of Deposit October 30, 1996

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Jennifer Mann

(Type or print name of person mailing paper or fee)

(Signature of person mailing paper or fee)

PEMP-61674.1

**IN THE SPECIFICATION:**

Please amend the specification as follows:

On page 1, line 7, insert the following paragraph:

-- This application is a divisional application of United States Application Serial No. 07/883,327, filed May 14, 1992, now abandoned which is incorporated herein by reference in its entirety. This invention was made with Government support under contracts AI27762-04 and ~~AI27666~~ AI27766-07 awarded by the National Institutes of Health. The Government has certain rights in this invention. --

On page 3, line 31, delete "condon" and substitute therefor --codon--.

On page 10, line 6; page 11, lines 1 and 14; and page 31, line 19, after "(5'-TTCCCATTAGTCCTATT-3')", insert the phrase --(SEQ ID NO:1)--.

On page 10, line 15; page 11, lines 2 and 15; and page 31, line 20, after "3')", insert the phrase --(SEQ ID NO:2)--.

On page 11, line 21, after "(5'-GGATGGAAAGGATCACC-3')", insert the phrase --(SEQ ID NO:3)--.

On page 11, line 22, after "(3'-TGGTGTGGTCTGTTTTTTGTA-5')", insert the phrase --(SEQ ID NO:4)--.

On page 11, line 24, after "AAGTGTGGTCTGTTTTTTGTA-5')", insert the phrase --(SEQ ID NO:5)--.

After page 41, insert the Sequence Listing as submitted in parent application 07/883,327, as pages 42 and 43.

Please renumber original specification pages 42 through 48 as pages 44 through 50.

**IN THE CLAIMS:**

In Claims 1, 6, 7, 8, 9, 14 and 19, line 2 of each, replace the term "antiretroviral" with -- anti-HIV --.

In Claims 1, 6, 7 and 8, on both line 9 and line 11 of each, replace the term "HIV sequence" with -- HIV-encoding nucleic acid --.

In Claim 9, line 11, after "500", insert the term -- per 200 μl of plasma--.

In Claim 14, line 11, after "200", insert the term -- per 200 μl of plasma--.

In Claims 3, 11, 16 and 21, line 2 of each, after "3')", insert the phrase --(SEQ ID NO:2)--.

In Claims 4, 12, 17 and 22, line 2 of each, after "3')", insert the phrase --(SEQ ID NO:1)--.

In Claims 5, 13, 18 and 23, line 2 of each, replace the word "zidvudine" with the word --zidovudine--.

REMARKS

Claims 1, 3-9, 11-14, 16-19 and 21-23 have been amended in order to correct several informalities and to more particularly point out that which Applicants regard as the claimed invention. These amendments are fully supported in the application as filed. For example, the amendments to Claims 9 and 14 are supported by the specification at, *inter alia*, page 22, lines 13-14.

Also submitted with this response is a petition under 37 C.F.R. § 1.48(b) to remove Dr. Michael Kozal as an inventor on the instant application. Dr. Kozal is not an inventor on the currently pending claims, and this petition is being diligently filed. Applicants respectfully request the granting of this petition and removal of Dr. Michael Kozal as a named inventor in the instant application.

In the outstanding Office Action, Claims 1-23 were rejected under 35 U.S.C. § 112, second paragraph, § 102(a), and § 103.

**1. The Invention.**

In patients suffering from HIV infection, opportunistic infections arising as a result of a compromised immune system can be rapidly fatal. It is therefore extremely important to strive to avoid deterioration of the immune system in these patients.

The present invention is directed to methods of monitoring, via polymerase chain reaction (PCR), the clinical progression of human immunodeficiency virus (HIV) infection and its response to antiviral therapy. It is based, in part, on the discovery that plasma HIV RNA copy number, as measured using PCR, may be used as a sensitive marker of the

circulating HIV viral load to assess the therapeutic effect of antiretroviral compounds. An increase in plasma HIV RNA copy number was found to correlate with disease progression and refractoriness to treatment, while successful antiretroviral therapy was found to correlate with a decline in plasma HIV RNA copy number.

The present invention offers the advantage of being more sensitive in measuring HIV virus than standard methods which measure plasma p24 antigen or infectious virus detectable by culture techniques. Additionally, because the present invention enables the early prediction of immunological decline, it allows alteration of a patient's therapeutic regimen so as to avoid opportunistic infections, and therefore may be used to promote survival and improve the quality of life of HIV-infected patients.

**2. The Rejections Under 35 U.S.C. § 112, Second Paragraph, Should Be Withdrawn.**

The Examiner asserted that Claims 1-23 were indefinite under 35 U.S.C. § 112, second paragraph, in the recitation of "antiretroviral therapy" in the preamble. The Examiner states that it is unclear whether only HIV or all retroviral therapy is being evaluated. To facilitate prosecution, Applicants have amended Claims 1, 6-9, and 14 by replacing the term "antiretroviral therapy" with the term --anti-HIV therapy--. In light of this amendment, Applicants respectfully request withdrawal of the rejections under 35 U.S.C. § 112, second paragraph.

**3. Holodniy *et al.* Is Not Prior Art To The Claimed Invention.**

Claims 1, 2, 5, 7, 8, 14, 15, 18-20 and 23 are rejected under 35 U.S.C. § 102(a) as anticipated by Holodniy *et al.* ("Holodniy"), whereas Claims 6, 9, 10 and 13 are rejected under 35 U.S.C. § 103 as obvious over Holodniy.

Applicants contend that the Holodniy reference is not available as prior art. To support their contention, Applicants provide the accompanying Declaration by the

Inventors under 37 C.F.R. § 132, signed by Dr. Thomas Merigan, Dr. David Katzenstein and Dr. Mark Holodniy. In this declaration, the inventors verify that the conception of so much of the subject matter of Holodniy as relates to the pending claims was a portion of their own work, and that the contribution of co-author Dr. Dennis M. Israelski to said subject matter was not inventive and largely consisted of providing access to several of his patients from whom samples were obtained for performance of the experiments reported in the paper. In view of this Declaration by the Inventors, Holodniy is not prior art under Section 102. In particular, Holodniy is not prior art under Section 102(a) because it is a description of the inventors' own work. *In re Katz,* 215 USPQ 14, 17 (CCPA 1982); MPEP § 715.01(c); *Reading & Bates Construction v. Baker Energy Resources,* 223 USPQ 1168, 1171-1172 (Fed.Cir. 1984) (*quoting In re Fout,* 213 USPQ 532 (CCPA 1982)) ("Absent a statutory bar under 3 U.S.C. §§ 102(b), (c), or (d), an applicants' own invention cannot be prior art to him.") Because Holodniy is not prior art under Section 102, it is likewise not available to show obviousness. *In re Bass,* 177 USPQ 178, 189 (CCPA 1973) (Rich, J., concurring) (only acts and references encompassed by subsections (a), (b), (e), and (g) of section 102 are the prior art under section 103); *Symbol Technologies v. Opticon, Inc.,* 17 USPQ2d 1737, 1740 (S.D.N.Y. 1990), *aff'd,* 19 USPQ2d 1241 (Fed. Cir. 1991) ("For prior technology to be considered 'prior art' in an obviousness determination it must: (a) come within one or more of the pertinent subdivisions of 35 U.S.C. § 102 ... "); *Fout, supra.*

In view of this declaration, the Holodniy reference is not properly available as prior art under 35 U.S.C. §§ 102(a) and 103. Accordingly the rejections under Sections 102 and 103 in view of Holodniy are improper and should be withdrawn.

**4. Claims 1-23 Are Patentable Over Cantin or Hart, Taken In View Of Ottmann, And Further In View Of Murakawa.**

The Examiner asserts that Claims 1-23 are obvious over Cantin *et al.* ("Cantin") or Hart *et al.* ("Hart"), taken in

view of Ottmann *et al.* ("Ottmann"), and further in view of Murakawa *et al.* ("Murakawa"). Cantin is cited for the use of PCR to detect HIV RNA synthesis in isolated cells. The Examiner argues that this suggests the use of PCR for evaluation of therapy against HIV infection. The Examiner asserts that Hart similarly discloses PCR detection in patient samples, and further asserts that Hart utilizes serum samples for detection by PCR. Ottmann allegedly discloses that detection of HIV by PCR is equivalently detectable in both serum and plasma. Finally, the Examiner alleges that Murakawa discloses the HIV viral nucleic acid sequence which is missing from the Cantin and Hart references. Applicants respectfully traverse this rejection.

To establish a *prima facie* case of obviousness, three basic criteria must be met. The Examiner must present prior art references which, when combined, teach or suggest all the claim limitations. Additionally, there must be some suggestion or motivation found within the cited references to combine them. Finally, there must be a reasonable expectation of success. The teachings or suggestion to make the claimed combination and the expectation of success must both be found in the prior art and not in Applicants' disclosure. Applicants respectfully submit that the Examiner has failed to meet all three of these criteria.

Cantin or Hart, taken in view of Ottmann, and further in view of Murakawa do not teach or suggest all the limitations of the instantly claimed invention. The invention, as claimed, specifies a method of evaluating the effectiveness of anti-HIV therapy by obtaining a plasma sample from an HIV-infected patient, performing a PCR reaction of about 30 cycles on that sample, and testing for the presence of HIV sequence in the product of the PCR reaction. None of the references cited by the Examiner specifies that the PCR reaction be performed on a serum sample for about 30 cycles. Hart and Cantin, which only perform PCR on nucleic acid isolated from infected cells, describe PCR reactions of 35 and 20 (6 reverse transcriptase cycles followed by 14 DNA polymerase cycles), respectively. Ottmann, the only reference which describes the

results of PCR experiments on serum samples, teaches a PCR reaction of 40 cycles. Indeed, as described in the specification at page 28, lines 21 through 31, the ability to show the quantitative changes demonstrated in the instant application with 30 cycles of PCR is lost at the high PCR cycle numbers used by Ottmann. Therefore, not only do the cited references fail to provide all of the limitations of the claimed invention, the Ottmann reference teaches away from the presently claimed invention.

Nor do the cited references, taken together, suggest or motivate the presently claimed invention. Cantin describes the use of a PCR reaction to assay the production of HIV RNA in vitro isolated from cultured cells after OMP treatment with anti-sense and sense (control) constructs. There is no suggestion to perform PCR analysis on patient serum samples generated in vivo. Cantin also presents data on the development of multinucleated cells (a CPE assay) in response to the same treatment (see Cantin, Figure 1 and Table I). Inhibition of syncytial giant cell formation (CPE) is presented as a measure of antiviral potency (Cantin, col. 4, lines 39-40). While the anti-sense OMP construct, OMP-A, was effective in inhibiting CPE, the control OMP-C sense construct produced no inhibition of CPE (Cantin, col. 4, lines 24-25, and Table I).

In contrast, Cantin's PCR results presented in Figure 2 of Cantin show that the control OMP-C sense construct drastically reduced the PCR signal from HIV RNA. *See* figure 2, compare lanes E (control OMP-C sense construct) and F (OMP-A antisense construct) with lanes C and D (positive control HIV infected cells with no OMP treatment). Thus, not only does Cantin not suggest PCR analysis on patient serum samples to assess anti-HIV therapy, the data presented in Cantin shows that PCR analysis of cellular RNA from cultured HIV-infected cells does not correlate with their phenotypic assay of antiviral potency (CPE).

Similarly Hart, who again only describes results of PCR analysis on cellular nucleic acid (from cultured H9 cells and PBMCs) also found no correlation between an indicator of AIDS

patients' prognosis (low T-helper cell count) and their PCR results (*see* Hart, page 598, last partial paragraph). Contrary to the Examiner's assertion, Hart does not utilize serum samples for their PCR detection, although they mention in passing that another group "are using PCR technology to detect HIV RNA of whole virus in serum samples." (Hart, page 598). Instead, Hart only utilizes serum samples for a serum antigen assay, and Hart discloses that HIV antigen was only detected in serum from 6 (29%) of the 21 seropositive subjects (*see* Hart, the section entitled "Patients and Methods"; the sentence bridging pages 597 and 598; and Table I). Therefore, Hart, like Cantin, teaches that their PCR results on cellular nucleic acids do not correlate with other prognostic markers.

Nor do Ottmann and Murakawa cure the deficiencies of Cantin and Hart. As discussed in the instant specification at page 28, Ottmann detected HIV RNA in plasma from 24 out of 25 patients who were receiving AZT. This contrast between Ottmann and the presently claimed invention is attributable to methodological differences. These differences, in part, prevented Ottmann from being able to show the quantitative results exhibited by the claimed invention (*see* specification page 28). Murakawa, like Cantin and Hart, analyzes RNA isolated from cells. Although this reference was cited as allegedly disclosing the entire HIV viral nucleic acid, Murakawa only presents a figurative drawing of the HIV genome. Murakawa in fact discloses only various synthetic primers to the HIV 3' ORF, but cites to other references well known in the art which disclose the nucleic acids of HIVs. Additionally, Fitzgibbon *et al.*, cited by the Examiner as cumulative to the above references, only describes PCR analysis of cellular DNA from an AIDS patient, and therefore does not disclose or suggest the instantly claimed invention.

Therefore, taken as a whole, the references cited by the Examiner do not suggest or motivate PCR analysis, using about 30 cycles of PCR, of HIV nucleic acid in a serum sample from a patient in order to predict therapeutic effectiveness of antiretroviral therapy and/or absolute CD4 count. Instead, the references cited, as a whole, teach away from the claimed

invention, and do not indicate to one of skill in the art any reasonable probability of success in using PCR as claimed in the instant methods of the invention. In light of the above remarks, Applicants respectfully submit that the rejection under 35 U.S.C. § 103 in view of Cantin, Hart, Ottmann, and Murakawa is improper and should be withdrawn.

CONCLUSION

Entry of the foregoing remarks into the file of the above specified application is respectfully requested. Applicant believes that each ground for rejection and objection has been successfully overcome or obviated and that the claims are in condition for allowance. Withdrawal of all the Examiner's rejections and objections is requested and an early allowance is earnestly sought. If any issues remain in connection herewith, the Examiner is respectfully invited to telephone the undersigned to discuss the same.

Respectfully submitted,

Date October 30, 1996

SaraLynn Mandel 31,853
SaraLynn Mandel (Reg. No. )
for Laura A. Coruzzi
Reg. No. 30,742

**PENNIE & EDMONDS**
1155 Avenue of the Americas
New York, N.Y. 10036-2711
(415) 854-3660

Enclosures

Sheet 1 of 1

| LIST OF REFERENCES CITED BY APPLICANT *(Use several sheets if necessary)* | ATTY. DOCKET NO: 7627-009-999 | SERIAL NO: 08/470,885 |
|---|---|---|
| | APPLICANT: Kozal, *et al.* | |
| | FILING DATE: June 6, 1995 | GROUP: 1809 |

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U.S. PATENT DOCUMENTS

| *EXAMINER INITIAL | | DOCUMENT NUMBER | DATE | NAME | CLASS | SUBCLASS | FILING DATE IF APPROPRIATE |
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| | | | | | | | |

FOREIGN PATENT DOCUMENTS

| | | DOCUMENT NUMBER | DATE | COUNTRY | CLASS | SUBCLASS | TRANSLATION YES | TRANSLATION NO |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |

OTHER REFERENCES *(Including Author, Title, Date, Pertinent Pages, Etc.)*

| | | |
|---|---|---|
| AM | BA | ~~Holodniy~~ Holodniy, *et al.*, "Quantitation of HIV-1 RNA in the Serum of ARC and Aids Patients Using the Polymerase Chain Reaction (PCR)", Abstract presented at the Sixth International Conference on AIDS, San Francisco, California, June 22, 1990. |
| AM | BB | ~~Holodniy~~ Holodniy, *et al.*, "Quantitation of HIV-RNA in Serum and Correlation with Disease Status Using the Polymerase Chain Reaction (PCR)", *Journal of Cellular Biochemistry*, Supplement 14D, 1990, Abstract of UCLA Symposia on Molecular and Cellular Biology, March 11-April 6, 1990. |
| AM | BC | Winters, *et al.*, "Rapid Detection of HIV Infectivity and Measurement of Antiviral Activity Using the Polymerase Chain Reaction (PCR)", *Journal of Cellular Biochemistry*, Supplement 15E, 1991, Abstract of Keystone Symposia on Molecular and Cellular Biology, March 8-March 26, 1991. |
| | BD | ~~Holodniy, *et al.*, "Reduction in Plasma HIV-RNA Copy Number following Dideoxynucleoside Therapy as determined by the Polymerase Chain Reaction", Abstract presented at the 31st Interscience Conference on Antimicrobial Agents and Chemotherapy, McCormick Place, Chicago, Illinois, September 29 October 2, 1991.~~ |
| | | |

EXAMINER: Azdin Marashy — DATE CONSIDERED: 3/3/97

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

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#13/C (AE) 8/27/97

Express Mail No.: EM 202 007 917 US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Kozal *et al.*

Serial No.: 08/470,885 Group Art Unit: 1807

Filed: June 6, 1995 Examiner: Marschel, A.



For: POLYMERASE CHAIN REACTION ASSAYS FOR MONITORING ANTIVIRAL THERAPY AND MAKING THERAPEUTIC DECISIONS IN THE TREATMENT OF ACQUIRED IMMUNODEFICIENCY SYNDROME

Attorney Docket No.: 7627-009-999

**RESPONSE UNDER 37 C.F.R. § 1.116**

OK TO ENTER AM, 8-29-97

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In response to the outstanding Office Action dated March 3, 1997 (Paper No. 11), and pursuant to the provisions of Rule 116 of the Rules of Practice, please consider the following remarks. Applicants submit herewith a Petition to Extend Time, accompanied by the appropriate fee.

**IN THE SPECIFICATION:**

Please amend the specification as follows:

On page 1, line 7, after "Application Serial No. 07/883,327, filed May 14, 1992," please insert --now abandoned,--.

REMARKS

The specification has been amended to update the status of the parent application to which priority is claimed.

The Examiner notes that an abstract by Holodniy *et al.* (reference BD) was not contained in the supplemental IDS which he received. Applicants enclose herewith another copy of this reference, and request that it be made of record in the file history of the instant application.

In the outstanding Office Action, Claims 1-23 were rejected under 35 U.S.C. § 103.

**1. The Invention.**

In patients suffering from HIV infection, opportunistic infections arising as a result of a compromised immune system can be rapidly fatal. It is therefore extremely important to strive to avoid deterioration of the immune system in these patients.

The present invention is directed to methods of monitoring, via polymerase chain reaction (PCR), the clinical progression of human immunodeficiency virus (HIV) infection and its response to antiviral therapy. It is based, in part, on the discovery that plasma HIV RNA copy number, as measured using PCR, may be used as a sensitive marker of the circulating HIV viral load to assess the therapeutic effect of antiretroviral compounds. An increase in plasma HIV RNA copy number was found to correlate with disease progression and refractoriness to treatment, while successful antiretroviral therapy was found to correlate with a decline in plasma HIV RNA copy number.

The present invention offers the advantage of being more sensitive in measuring HIV virus than standard methods which measure plasma p24 antigen or infectious virus detectable by culture techniques. Additionally, because the present invention enables the early prediction of immunological decline, it allows alteration of a patient's therapeutic regimen so as to avoid opportunistic infections, and therefore may be used to promote survival and improve the quality of life of HIV-infected patients.

**2. Claims 1-23 Are Patentable Over Cantin or Hart, Taken In View Of Ottmann, And Further In View Of Murakawa.**

The Examiner asserts that Claims 1-23 are obvious over Cantin *et al.* ("Cantin") or Hart *et al.* ("Hart"), taken in view of Ottmann *et al.* ("Ottmann"), and further in view of Murakawa *et al.* ("Murakawa"). This rejection is respectfully traversed for the following reasons.

A reference should be considered as a whole, and portions arguing against or teaching away from the claimed invention must be considered. *Bausch & Lomb, Inc. v. Barnes-Hind/Hydrocurve, Inc.*, 230 U.S.P.Q. 416 (Fed. Cir. 1986). As the Court of Customs and Patent Appeals has held:

> It is impermissible within the framework of section 103 to pick and choose from any one reference only so much of it as will support a given position to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one skilled in the art.

*In re Wesslau*, 147 U.S.P.Q. 391, 393 (CCPA 1965). Applying this standard to the instant case, the references cited by the

Examiner do not teach or suggest the instantly claimed invention.

The presently claimed invention is drawn towards methods of evaluating the effectiveness of anti-HIV therapy by assaying for the presence or levels of HIV-encoding nucleic acid in plasma samples using about 30 cycles of PCR. In embodiments of the invention, the levels of HIV assayed in this manner are correlated to therapeutic effectiveness or to absolute $CD4^+$ cell counts. In sharp contrast, Cantin, Hart and Ottmann teach or suggest that there is no correlation between their PCR results and either anti-HIV effectiveness (Cantin and Ottmann) or $CD4^+$ cell counts (Hart).

Using a non-quantitative PCR assay, Ottmann found that HIV nucleic acid could be detected in serum or plasma regardless of antiretroviral treatment or disease status. In particular, Ottmann states in the abstract:

> PCR on HIV-1 genomic cDNA was positive in 38 out of the 40 plasma or serum samples (95%), **regardless of the clinical stage of the infection**: HIV-1 was detected in 14 of the 15 untreated subjects and in 24 of the 25 AZT-treated patients. . . . The results show that **this method is suitable for the detection of viral particles in plasma or serum from HIV-1-infected individuals irrespective of antiviral treatment.**

Ottmann, page 273, emphasis added. Therefore, in apposition to the presently claimed invention, Ottmann teaches that HIV nucleic acid will be detected in plasma or serum even if the infection is latent or whether the patient is undergoing effective or ineffective antiviral treatment.

Hart specifically states that there was no correlation between a low $CD4^+$ cell count and their PCR results on

peripheral blood mononuclear cells (PBMC). Applicants draw the Examiner's attention to the last partial paragraph, column 2, on page 598 of Hart, where it is stated:

> In our study, by Fisher's exact test, there was no statistical correlation between a low T-helper cell count . . . and the PCR results.

As the Examiner is aware, T-helper cells are $CD4^+$ cells. Accordingly, since Hart did not find any correlation between indicatives of patient prognosis and PCR results from PBMCs, Hart does not suggest the methods of the presently claimed invention which do in fact correlate plasma PCR results with therapeutic effectiveness and $CD4^+$ cell count. At best, Hart provides only an invitation to the artisan to engage in unlimited experimentation in order to disprove Hart's negative preliminary results.

Cantin used both an HIV-antisense nucleic acid (OMP-A) and a control sense nucleic acid (OMP-C) to treat HIV-infected cultured H-9 cells. The antisense OMP-A construct inhibited viral potency (as measured by a CPE assay), while the control OMP-C construct had no effect in this CPE assay. However, when PCR was used to examine HIV RNA levels in the cultured H-9 cells, both the antisense OMP-A and the sense OMP-C nucleic acids drastically decreased HIV RNA levels (see Figure 2, lanes e and f, as compared to no treatment in lanes c and d). Therefore, Cantin found no correlation between reduction in viral potency, as measured by CPE assay, and PCR analysis.

In response to the data in Cantin, described above, indicating that PCR was perhaps ineffective in predicting

inhibition of viral potency (therapeutic effectiveness), the Examiner states:

> It is acknowledged that conditions may be specially selected that interfere with an assay such as PCR, but someone of ordinary skill in the art would certainly avoid such interfering conditions given its disclosure in a reference. This does not, however, negate the functionality of assays performed so as to avoid interferences that are known in the prior art.

(Office Action, page 4). Applicants respectfully submit that one of skill in the art would not disregard the significance of controls in assessing his/her experimental results. In particular, one of skill in the art would not conclude, upon assessing the PCR results of Cantin, that the PCR results were functional in predicting therapeutic effectiveness but that the OMP-C control should be avoided or that the PCR should be modified. Rather, upon observing that the control sense construct OMP-C gave similar PCR results as the experimental antisense construct OMP-A, one of skill would judge that the reduction in PCR signal was more likely due to a non-specific effect from both the sense and anti-sense constructs. In other words, Cantin does not demonstrate a functional PCR assay for determining therapeutic effectiveness, because the results shown by Cantin demonstrate that the PCR results do not correlate with their CPE measure of therapeutic effectiveness. For the Examiner to suggest otherwise is improper reconstruction of Applicants' invention using Applicants' own disclosure.

Therefore, contrary to the assertion of the Examiner, the cited references do not demonstrate that "the presence of HIV

nucleic acids in various forms . . . are detectable and correlatable to therapy effectiveness evaluation." (Office Action mailed March 3, 1997, paper no. 11, page 4). Instead, the cited references Cantin, Hart and Ottmann show that PCR detection of HIV nucleic acids does not correlate to therapy effectiveness evaluation.

Taken as a whole, the references cited by the Examiner do not disclose or suggest the instantly claimed invention. Instead, the references cited, as a whole, teach away from the claimed invention, and do not indicate to one of skill in the art any reasonable probability of success in using PCR as claimed in the instant methods of the invention. In light of the above remarks, Applicants respectfully submit that the rejection under 35 U.S.C. § 103 in view of Cantin, Hart, Ottmann, and Murakawa is improper and should be withdrawn.

## CONCLUSION

Entry of the foregoing remarks into the file of the above specified application is respectfully requested. Applicants believe that each ground for rejection and objection has been successfully overcome or obviated and that the claims are in condition for allowance. Withdrawal of all the Examiner's rejections and objections is requested and an early allowance is earnestly sought. If any issues remain in connection

herewith, the Examiner is respectfully invited to telephone the undersigned to discuss the same.

Respectfully submitted,

Kathleen Fowler P-40,611

Date August 4, 1997

For Laura A. Coruzzi 30,742

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Enclosures