Exhibit 22

PATENT

11/B
B. White
12-30-94

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Kozal *et al.*

Serial No.: 07/883,327 Group Art Unit: 1807

Filed: May 14, 1992 Examiner: A. Marschel

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

Attorney Docket No.: 7627-002-999

RESPONSE UNDER 37 C.F.R. §1.115

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Responsive to the Office Action of October 5, 1994, please consider the following amendments and remarks.

IN THE SPECIFICATION

Please amend the Specification as follows:

On Page 1, Line 7 insert the following paragraph:

--This invention was made with Government support under contracts AI27762-04 & AI27666-07 awarded by the National Institutes of Health. The Government has certain rights in this invention.--

STAN 006370

EXPRESS MAIL CERTIFICATION

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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.

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IN THE CLAIMS

Amend Claims 24 and 29 as follows:

24. A method of evaluating the effectiveness of antiretroviral therapy of [a patient] an HIV-infected patient who is being treated with an antiretroviral agent comprising:

(i) collecting a plasma sample from [an HIV-infected patient who is being treated with an antiretroviral agent] the patient; and

(ii) determining whether the plasma sample comprises nucleic acid encoding HIV RT having a mutation at codon 215,

in which the presence of the mutation correlates positively with future immunologic decline of the patient within a six to twelve month period.

29. A method of evaluating the effectiveness of antiretroviral therapy of [a patient] an HIV-infected patient who is being treated with an antiretroviral agent comprising:

(i) collecting PBMC from [an HIV-infected patient who is being treated with an antiretroviral agent] the patient; and

(ii) determining whether the PBMC comprise proviral HIV DNA which comprises a mutation at codon 215,

in which the presence of the mutation correlates positively with future immunologic decline of the patient within a 4-11 month period.

Cancel Claims 1-23 without prejudice, in compliance with the Examiner's requirement for restriction.

REMARKS

The Amendments

Claims 24 and 29 have been amended by moving the phrase "an HIV-infected patient who is being treated with an antiretroviral agent" from step (i) to the preamble. This minor amendment indicates that the method is directed to evaluating therapy of an ***HIV-infected*** patient. Claims 24 and 29 have been further amended by inserting "future" before "immunologic decline." This amendment is support by the specification at, *e.g.*, page 14, lines 16-20, which states that codon 215 mutations "precede immunologic decline."

No new matter is introduced by the amendments, and their entry is respectfully requested.

Section 112, first paragraph

Claims 24-33 have been rejected under §112, first paragraph, on the grounds that the disclosure is enabling only for claims limited to HIV mutation analysis, whereas the preambles are more broadly directed to retroviral therapy. This rejection has been obviated by amendment of the preamble of independent claims 24 and 29 to recite that the claimed method is directed to antiretroviral therapy "of an HIV-infected patient." The term "antiretroviral therapy" itself is correct and has been retained because some common antiretroviral agents exhibit activity against retroviruses other than HIV; therefore the term is proper to refer to the

group of agents used in this therapy. Since the preambles now recite the limitation that therapy is of an HIV-infected patient, the claims cannot be construed as broader than the enablement of the present disclosure.

The Examiner is respectfully requested to withdraw this rejection.

Section 112, second paragraph

Claims 23-33 have been rejection as indefinite for reciting "antiretroviral" therapy. This rejection has been obviated by the amendment described and explained under the reply to the §112, first paragraph, rejection *supra*.

The claims have been further rejected as indefinite for not reciting that the mutation predicts *future* immunologic decline. This rejection has been obviated by insertion in Claims 24 and 39 of the word "future" before "immunologic decline."

The Examiner is respectfully requested to withdraw the rejections under §112, second paragraph.

Section 102(a)

Claims 24, 25, 29 and 30 have been rejected under §102(a) as anticipated by Richman *et al*. This rejection is respectfully traversed.

Richman *et al*. teach that mutations at four codons (215, 70, 67, and 219) correlate with ZDV chemotherapy and resistance. They state that "a mutation may first appear at any codon" of the four (*see* page 1077, first column, last full paragraph). After one year of therapy, 63% of specimens had a

mutation at codon 215. However, a total of 105% (33% + 59% + 13%) had a mutation at one of the three other codons (the total is greater than 100% because some specimens contained multiple mutations; *see* page 1077, second column, first paragraph). These data do not support an inference that the codon 215 mutation is by itself predictive of future immunologic decline, as is presently claimed. Indeed, the data in Table 1 indicate that isolates with codon 215 mutations exhibit a broad range of drug resistance values; many of these values are within the normal (*i.e.*, drug-susceptible) range. Figure 5 also indicates that most drug-resistant isolates have multiple mutations at codons 70, 215, and 219. These results demonstrate that a codon 215 mutation is not sufficient *per se* to cause drug resistance.

Richman *et al.* further teach that multiple codon mutations *in addition* to the above four mutations may be required to cause drug resistance, even when resistance is determined by an *in vitro* assay rather than by clinical indicia. For example, patient A027 had a mutation at codon 215, but did not change from drug sensitive to drug resistant until some two months thereafter. The authors suggest that "additional mutations, not at one of the four codons, also contribute to reduced susceptibility," (*see* page 1077, last paragraph). This observation does *not* suggest that the codon 215 mutation itself is predictive of future drug resistance, since it does not indicate that the necessary additional mutations are likely to occur.

Furthermore, even if Richman *et al.* had suggested that the codon 215 mutation is predictive of future drug

resistance, this would not anticipate the *claimed* invention. The claims specify the prediction of future ***immunologic decline***; this limitation requires that immune system status or condition becomes impaired or lessened. By contrast, in the report of Richman *et al.*, drug resistance is determined by an *in vitro* plaque assay using the HeLa laboratory cell line. The *in vitro* HeLa cell assay clearly is not a measurement of the patient's immunologic condition.

Nor had the HeLa cell assay been taught by the prior art to correlate with the condition of the patient's immune system. The HeLa cell assay for drug resistance was described initially in Larder *et al.* (1989) Science 243: 1731-1734, a copy of which is enclosed as Exhibit A. Larder *et al.* state:

> The critical issue is whether the development of a less sensitive virus phenotype results in clinical resistance to drug therapy. ... [I]t will be difficult to correlate emergence of resistant variants with changes in clinical status or other markers.

Id. at 1733, first paragraph. Since the claims recite the prediction of ***immunologic decline*** rather than merely development of drug resistance, the publication of Richman *et al.* cannot be an anticipation of the claimed invention, under even the most generous interpretation of its teachings.

Finally, Richman *et al.* expressly disclaim the use of mutation analysis for prediction of a patient's future condition, as is recited in the claims. The authors state that "genotypic analysis will not provide precise susceptibility information in any individual patient. Susceptibilities may vary widely, even within the same genotypic combination at the four codons," (page 1081, second

paragraph). They further conclude that "the many complexities of zidovudine resistance render the assay of limited use for application to individual patients;" (abstract, page 1075, last sentence).

In view of these differences between Richman *et al.* and the claimed invention, the Examiner is respectfully requested to withdraw the rejection under §102(a).

Section 103

The Examiner has rejected Claims 24-32 as obvious over the combination of Japour *et al.* and Mullis *et al.* This rejection is respectfully traversed.

First, the Examiner is invited to note that Japour *et al.* had actual knowledge of the polymerase chain reaction (PCR) technique for which the publication of Mullis *et al.* was cited. Japour *et al.* discuss PCR amplification as a technique for recovering a reverse transcriptase (RT) DNA fragment from HIV; *See* p. 3093, column 1, last paragraph. Japour *et al.* used the PCR amplified product to determine the DNA sequence of RT from zidovudine (ZDV or AZT)-treated patients. Therefore the cited publication of Mullis *et al.* adds nothing to Japour *et al.* The Examiner need not speculate as to what inferences or conclusions one skilled in the art might draw from combining the teachings of Japour *et al.* and Mullis *et al.*; Japour *et al.* state what those skilled in the art actually *did* conclude from such teachings.

In posing this rejection, the Examiner has mischaracterized the invention presently claimed. Independent Claims 24 and 29 do recite the step of determining whether an

HIV-infected patient's sample comprises a mutation at codon 215 of RT. However, these claims do *not* recite that the codon 215 mutation correlates with development of resistance to antiretroviral therapy. Instead, the claims recite that "the presence of the mutation correlates positively with future immunologic decline of the patient" within a 6-12 or 4-11 month period, respectively. Immunologic decline is a clinically meaningful phenomenon, as indicated, *e.g.*, by the decline in patient $CD4^+$ cell counts. By contrast, ZDV resistance is a phenomenon determined in the cited publications by *in vitro* assays, such as plaque reduction in cultured HeLa cells or enzymatic RT activity. However, as already described with respect to the §102(a) rejection, *in vitro* drug resistance had not been shown prior to the present invention to correlate with future immunologic decline. Indeed, Richman *et al.*, cited *supra* by the Examiner, state in the second sentence of their publication that "the clinical importance of drug-resistant HIV has not been defined...".

The cited publication of Japour *et al.* neither teaches nor suggests that the presence of a codon 215 mutation correlates with future immunologic decline within 6-12 or 4-11 months. Japour *et al.* studied ZDV resistance of viral strains isolated from five patients, using an *in vitro* assay for drug resistance. The assay is based on RNA-RNA hybridization for detection and quantitation of viral replication in cultured peripheral blood mononuclear cells (PBMC) infected with virus obtained from the patients. The cases are described on pages 3093-3094 and in Table 1 of Japour *et al.* Of the two more drug-resistant HIV isolates, EP113089 (Case 1) was obtained

from a patient who, "shortly thereafter, developed an opportunistic infection and expired." This description does not imply that "immunologic decline" occurred subsequent to the mutation at codon 215, since the patient apparently was in immunologically poor condition for at least six months prior to their detecting the mutation, as indicated by the earlier development of recurrent fevers. Therefore, this sample does not suggest that the presence of mutation may be predictive of future immunologic decline. Furthermore, the EP113089 HIV isolate exhibited 18 nucleotide mutations resulting in eight amino acid changes; *see* page 3093, second column, fourth paragraph. These data do not imply any special predictive significance for the codon 215 mutation.

The other highly resistant HIV isolate, B101990 (Case 2), was obtained from a patient who remained stable after five months. Two other patients (Cases 4 and 5) whose samples yielded HIV isolates of intermediate drug resistance, remained "stable" or "well" after 8 months on the study. Japour *et al.* did not report whether any of these subjects exhibited a codon 215 mutation.

In characterizing the contemporaneous knowledge in the art, Japour *et al.* state:

> Unfortunately, no objective clinical manifestations or surrogate laboratory markers predict the emergence of ***clinically relevant resistant HIV*** isolates.

Id. at 3092, column 1, last sentence (emphasis added). This statement supports the conclusion that the art prior to Japour *et al.* does not teach or enable a method of evaluating therapy using detection of the codon 215 mutation as a laboratory

marker to predict the emergence even of clinically relevant HIV drug-resistant strains. *A fortiori*, the codon 215 mutation could not have been known to predict the future immunologic decline of the patient, as is claimed.

Moreover, the teachings of Japour *et al.* do not supply these deficiencies. Japour *et al.* describe the significance of their reported RNA-RNA hybridization assay in this manner:

> [T]his assay may provide an objective quantifiable method to establish the clinical significance of HIV resistance...

Id. at 3092, column 2, last paragraph before "Materials and Methods." Thus they themselves did not conclude that the "clinical significance of HIV resistance" had been established by their work, as published in their cited article. Their RNA-RNA hybridization method was shown to provide an alternative to the assay of Larder *et al.* for *in vitro* measurement of drug resistance, but their results do not even purport to show that the presence of the codon 215 mutation predicts future immunologic decline.

Japour *et al.* mention that "clinical deterioration was seen in one of two patients with high-level ZDV resistance." However, as already explained, the clinically deteriorated patient was apparently in poor condition at least six months prior to the time that codon 215 mutation and ZDV resistance were determined. The presence of the 215 mutation in this patient could not be interpreted, and was not suggested, as ***predicting future*** immunologic decline.

Furthermore, even if the codon 215 mutation had been shown to occur prior to clinical decline, the poor condition of the one drug-resistant patient is countered by the stable

condition of the other drug-resistant patient. One skilled in the art could not reasonably conclude from these data that the presence of ZDV resistance predicts future immunologic decline, since at least one of the two drug-resistant patients showed no future decline.

Finally, the results of Japour *et al.* fail to show that the presence of a codon 215 mutation predicts even ZDV resistance, much less immunologic decline. Japour *et al.* note (page 3095, last paragraph) that the 215 mutation, which they found in a single sample, was also among the four mutations reported by Larder and Kemp. However, the single sample of Japour *et al.* also contained three other nonconservative mutations and four conservative mutations. The authors conclude:

> Since the observations of both groups are based on a small number of clinical ZDV-resistant isolates that have been fully sequenced, more resistant isolates must be identified and analyzed before genotypic mutations associated with ZDV resistance can be conclusively confirmed.

Id. at 3096. This statement is especially notable because it disclaims an established correlation between *any* mutations and *present* ZDV resistance. Clearly, from these results the presence of a codon 215 mutation cannot be extrapolated to prediction of *future* ZDV resistance, and still less to prediction of future immunologic decline.

Japour *et al.* explain the significance of their results by stating:

> This method [of RNA-RNA hybridization] should also be useful for extensive testing of isolates from patients.. and permit the correlation of *in vitro* HIV resistance with clinical outcome.

Id. Thus they implicitly acknowledge that they had not yet established a correlation between HIV drug-resistance and clinical outcome.

In summary, Japour *et al.* do not teach that the codon 215 mutation predicts future *in vitro* drug-resistance, and furthermore do not teach that either the codon 215 mutation or *in vitro* drug resistance correlates with future immunologic decline.

In view of these deficiencies of Japour *et al.* in teaching or suggesting the present by claimed invention, the Examiner is respectfully requested to withdraw the rejection under §103.

CONCLUSION

The present amendments and remarks are believed to place the application in condition for allowance. Early notice of acceptance is earnestly solicited.

Respectfully submitted,

Dated: December 8, 1994

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Enclosure

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