EXHIBIT J

2E -104 GP 1814

PATENT

Attorney Docket No.: A54515-17/WHD/RMS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Examiner: D. Jacobson
Goeddel et al.	Group Art Unit: 1814
Serial No. 08/487,456	
Filed: 6 June 1995	
For: Human Tissue Plasminogen Activator	

CERTIFICATE OF MAILING

I hereby certify that this correspondence, including listed enclosures, is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, DC 20231 on 3 July 1996.

Signed: Maria Ciganovich
Maria Ciganovich

AMENDMENT

Assistant Commissioner of Patents
Washington, DC 20231

Sir:

This amendment is in response to the Office Action dated 5 January 1996 (Paper No. 4) received in the above-identified application.

A petition for a three month extension of time and the required fee is enclosed, making this a timely response. Consideration of the following

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remarks is respectfully requested. Please amend the above-identified application as follows:

In the Claims:

Please delete Claim 1 **without prejudice or disclaimer.**

Please add the following claims:

2. ~~Tissue~~ plasminogen activator as produced by recombinant expression of DNA encoding said tissue plasminogen activator in transformed host cells.

3. ~~Tissue~~ plasminogen activator according to claim ~~2~~ wherein said transformed host cells are mammalian host cells.

4. ~~Tissue~~ plasminogen activator according to claim ~~2~~ wherein said transformed host cells are bacterial host cells.

5. ~~Tissue~~ plasminogen activator according to claim ~~3~~ wherein said mammalian host cells are Chinese hamster ovary (CHO) cells.

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6. ~~Tissue~~ plasminogen activator according to claim ~~4~~ wherein said bacterial host cells are *E. coli* cells.

7. A composition comprising a therapeutically effective amount of tissue plasminogen activator according to claim ~~2~~ in admixture with a pharmaceutically acceptable carrier.

8. A composition comprising a therapeutically effective amount of tissue plasminogen activator according to claim ~~5~~ in admixture with a pharmaceutically acceptable carrier.

9. A composition comprising a therapeutically effective amount of tissue plasminogen activator according to claim ~~6~~ in admixture with a pharmaceutically acceptable carrier.

10. ~~Tissue~~ plasminogen activator according to claim ~~2, 5~~ or ~~6~~ having the amino acid sequence 1 to 527 set forth in Figures 5A, 5B and 5C hereof.

11. ~~Tissue~~ plasminogen activator according to claim ~~2, 5~~ or ~~6~~ having the amino acid sequence 69 to 527 set forth in Figures 5A, 5B and 5C hereof.--

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REMARKS

Claims 2-11 are pending, with the present submission.

The new claims clarify claim language to tissue plasminogen activators and pharmaceutical compositions incorporating such materials. The claims direct focus on the enabled prepartion of such tissue plasminogen activators in specific mammalian (CHO) and bacterial (E. coli) host cells and further embody the exemplified preparation of the full-length (527 amino acids) and amino acid delection derivative (69 to 527 amino acids) species.

Claim 1 is rejected under 35 U.S.C. §102(a) as being anticipated by Rijken et al.

Rijken et al. described isolation of human native tPA from human melanoma cells.

The current claims are directed to tPA as produced by recombinant expression of DNA in transformed host cells. As acknowledged by the Examiner, Rijken does not disclose recombinantly produced tPA.

The applicants submit that Rijken et al. does not anticipate the claims as amended due to the glycosylation differences between the protein isolated by Rijken et al. from a melanoma line and proteins made in other cells. The applicants have submitted voluminous arguments to this effect during the prosecution of the parent cases and these arguments are summarized herein.

First of all, the applicants submit the declaration of Dr. Michael Spellman, (enclosed herein as Exhibit A) originally cited in U.S.S.N. 07/012,694, which clearly shows that the t-PA produced by Rijken et al. (referred to in the Spellman declaration as the Collen et al. material, Collen being the senior author) has different glycosylation than the material produced in CHO cells and specifically claimed in claims 5 and 8. Furthermore, the glycosylated tPA produced by Rijken et al. is clearly different from the unglycosylated tPA produced in bacteria such as *E. coli*.

Dr. Spellman goes on to state that the work of Kagawa et al., (cited as Exhibit C of his declaration and attached herein as Exhibit B), shows that it is likely that glycosylation of proteins occurs in a host cell-specific manner and only partly under the influence of the protein sequence. Therefore, Dr. Spellman concludes,

> [I]t could be supposed that recombinant human tissue plasminogen activator produced in a recombinantly harnessed cell line will produce recombinant human tissue plasminogen activator having a carbohydrate structure different from that of native human tissue plasminogen activator of Collen et al. (see paragraph 18).

At the time this invention was made, it was unknown (a) what effect glycosylation differences would have on the biological activity of a protein, and (b) whether the cell type used for expression of the protein would effect the glycosylation pattern. Taken together, this shows that the Rijken et al.

reference does not anticipate the present claims which are directed to recombinantly produced tPA.

It would not have been predictable whether glycosylation differences would, in fact, produce intact, functionally and biologically active glycoprotein. On this point, even later published papers reiterate this uncertainty. For example, three back-to-back papers published in 1989 show both uncertainty.

In the first paper, Parekh et al., (enclosed herein as Exhibit C), the authors report on differences found in the glycosylation structure between tissue plasminogen activator form a human colon fibroblast cell strain and from a Bowes melanoma cell line. The last two paragraphs of that article emphasize that even as of 1989 there was substantial uncertainty on what effects, if any, differences in carbohydrate structure impose upon a given glycoprotein, that are products of different cell lines. The unanswerable questions posed create the unpredictability that forms the basis of patentability of the claims:

> [D]oes each cell within the population express all glycoforms, a unique subset, or just one? Should the N-glycosylation of one polypeptide change under external influences, is there a similar and concomitant change in the N-glycosylation of all the other glycoproteins being expressed by the cellular population in question? If not, how is such a change avoided? Why do such changes in the N-glycosylation of a polypeptide not lead to immune rejection?

> [T]hese results have significant implications for the genetic engineering of mammalian glycoproteins, including t-PA. Expression of the desired polypeptide in recombinant form in a cell type in which it is not normally expressed would probably lead to the production of a non-physiological set of glycoforms . . . [T]hese glycoforms may have unusual additional properties arising from novel N-glycosylation. These may include new circulatory properties, changes in tropism and immunogenecity (page 7661, emphasis added).

In the second paper by Wittwer et al. (enclosed herein as Exhibit D), the authors report that both "qualitative and quantitative differences in t-PA N-glycosylation influence its in vitro enzymatic activities" (page 7663, left column, lines 9 to 11).

These authors thus emphasize the unpredictability as to what effects, if any, changes in glycosylation may have on the biological profile of a given glycoprotein (see the last sentence of the article).

In the last article on page 7670 by Parekh et al. (enclosed herein as Exhibit E), these researchers relate additional interesting comments that touch poignantly on the question of the patentability of the claims:

> Expression of a polypeptide in a cell type other than that in which it is normally expressed might be expected to lead to a N-glycosylation pattern different from that of the native form. Altered N-glycosylation could disturb functions normally influenced by oligosaccharides, as well as conferring new ones, or even render the recombinant glycoprotein immunogenic by creating novel epitopes or raising the levels of ones that were previously subimmunogenic (page 7670, right column, lines 12-19, emphasis added).

Again, this indicates that even in 1989, the scientific community continued to speculate as to what the resultant properties of a glycoprotein would be, if the N-glycosylation patterns would be altered, once again suggesting that the state of glycosylation of a given glycoprotein could not be predicted, or if it were, the biological profile whether viewed in terms of rank biological activity or immunogenicity, is not predictable or reasonably foreseeable to one skilled in the art.

Thus, at the time this invention was made, it could not have been predicted with reasonable certainty that the recombinant t-PA products having glycosylation structure different from that disclosed by the prior art, would be useful in the manner that they have proved to be, namely, in therapeutic application in a safe manner to human beings.

In conclusion, the protein purified by Rijken et al. from a native source does not anticipate the present claims directed to tPA recombinantly produced in a transformed host cell, as the glycosylation patterns of the tPA have been shown to be different when produced in different cell types (i.e. the Spellman declaration, the two Parekh et al. references, and the Wittwer et al. reference). Accordingly, the rejection under 35 U.S.C. §102(a) should be withdrawn.

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Claim 1 is provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims in U.S.S.N. 08/264,134.

To the extent this rejection may be applicable at all to new claims 2 to 11 herein, the applicants respectfully request this matter to be held in abeyance until an indication of otherwise allowable subject matter.

It is the position of the applicants that the claims are now in condition for allowance and an early notification of such is solicited.

Respectfully submitted,

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