EXHIBIT G

Docket 100/164-U.S.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

DAVID V. GOEDDEL ET AL.

Serial No. 06/483,052

Filed: April 7, 1983

For: HUMAN TISSUE PLASMINOGEN ACTIVATOR

Group Art Unit: 127

Examiner: J. Huleatt

A M E N D M E N T

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

Sir:

This is in response to the Official Action mailed 22 April 1985. The request for extension of time submitted herewith extends the time for response three months to 22 October 1985. Please amend this application in the following respects:

In the Specification

On page 1, line 29, between "quantity" and "material" insert --of--.

On pages 4 and 17, relocate the paragraph bridging lines 17 and 23 on page 4 to the location between lines 23 and 25 on page 17.

On page 5, line 8, replace "remainder" with --remaining--.

On page 5, replace line 22 with --In practice, through the use of recombinant DNA technology, one can express--.

B1

On page 7, change line 29 to read as follows: --Figure 1 shows a 10% sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS PAGE) of 35_S-methionine labelled proteins--

R2

On page 7, line 30, replace "extracted" with --secreted--.

On page 8, line 1, before "colonies" insert --bacterial--.

On page 8, line 15, before "cells" insert --E. coli--.

On page 8, line 24, cancel "transformed".

On page 14, line 6, replace "pBR 322" with --pBR322--.

On page 14, line 25, correct the spelling of "prokaryotes".

On page 16, line 20, correct the spelling of "provided".

On page 17, line 9, correct the spelling of "necessary".

on page 17, line 10, replace "resistant" with --deficient--.

On page 17, line 12, cancel the second occurrence of "are".

On page 17, line 18, before "trp" insert --lac and--.

On page 17, line 27, replace "wall" with --membrane--.

On page 19, line 4, after "ampicillin" insert --or tetracycline--.

On page 20, line 29, after "DNA" insert --encoding t-PA--.

On page 21, line 13, after "pg" insert --per cell per day--.

On page 21, lines 15 and 16, after "units" insert --per cell per day--.

On page 22, line 28, replace "acrylamide gel" with --PAGE--.

On page 23, line 7, after "the" and before "electrophoresis" insert --gel--.

On page 27, line 21, replace "for 24°" with --at 24°--.

On page 28, line 26, change "(51)", to --(54)--.

On page 32, line 23, after "infra.", replace "Clone 25E10" with --The cDNA insert of clone 25E10 (plasmid pPA25E10)--.

On page 35, line 13, cancel "the cDNA clone".

On page 35, line 14, replace "RSA" with --Rsa--.

On page 35, line 29, replace "clone" with --cDNA of --.

On page 35, line 35, replace "clone p25E10" with --plasmid pPA25E10--.

On page 37, line 15, change "asn_{184}" (second appearance) to --asn_{218}--.

On page 38, line 23, replace "shown" with a comma.

On page 44, line 15, after "1983," insert

B3 --corresponding to European Patent Application Publ. No. 117,060,--.

On page 44, lines 19 and 23, replace "pΔRIPA°" with --pt-PAtrp12--.

On page 44, line 32, after "1981," insert

B4 --corresponding to European Patent Application Publ. No. 73,656,--.

On page 45, lines 29 and 31, change "tPA" to --t-PA--.

On page 46, line 4, replace "pEdPAER400" with --pETPAER400--.

On page 46, line 27, replace "D.1.K.1.b" with --E.1.K.1.b--.

On page 47, line 23, cancel "a" and after "plasmid" insert --pETPFR was constructed--.

On page 47, line 24, cancel ", pETPFR, was constructed".

On page 47, line 25, replace "C.1.A." with --E.2.A.--.

On page 47, line 27, after "100/92" insert --, U.S. Serial No. 459,152, filed 19 January 1983, now U.S. Patent No. 4,713,339 issued December 15, 1987, corresponding to European Patent Application Publ. No. 117,058, incorporated herein by reference, --.

B5

On page 48, line 10, replace "best" with --most strongly--.

On page 48, line 12, replace "D.2.C." with --E.1.K.1.b.--.

On page 55, line 3, in reference 35, replace "(198)" with --(1980)--.

In Figure 11, upper left corner, replace "pΔR1PA°" with --pt-PAtrp12--.

In the Claims

B6

1. (amended). Recombinant human [Human] tissue plasminogen activator essentially free of other proteins of human origin.

B7

3. (amended). Human tissue plasminogen activator as produced by recombinant host cells.

4. (amended). Biologically active human [Human] tissue plasminogen activator in essentially pure form unaccompanied by protein with which it is ordinarily associated.

5. (amended). Human tissue plasminogen activator according to Claims 1-4 containing a sequence of a polypeptide [extending from] preceding the N-terminus of the ordinarily first amino acid of said human tissue plasminogen activator.

Please add the following claims:

B8

-- 16. A cell culture according to Claim 9, obtained by transforming a mammalian cell line.

17. A process according to Claims 14 or 15 wherein the host cell is a mammalian cell line. --.

Remarks

An Abstract of the Disclosure is enclosed with this response.

A Notice of Art cited by Applicant PTO-1449, is also enclosed. This form lists those references which have been cited in search reports obtained in various foreign counterparts of the present application. The submission of PTO-1449 does not constitute an assertion that a complete or fastidious search has been made. The more germane of these references deal with the purification to a degree from natural sources of plasminogen activator compounds or plasminogen activator activity. One reference which is considered relevant is European Patent Application Publn. No. 41766 which was published on 16 December 1981. That patent application is based upon the research underlying Reference R cited by the Examiner, namely, Rijken and Collen which appeared in the Journal of Biological Chemistry 256, 7035 (1981). Discussion of the patentability of the present invention over that disclosure follows. The remaining references on the enclosed list have either been cited by the U.S. Examiner or are believed to be no more relevant than those already of record and under which rejection has been made. Copies of these references are enclosed for convenience.

Formal drawings also accompany this response. It is noted that Figure 11 has been amended in these formal drawings in

line with the amendment given above to Figure 11, whose purpose is to correct the informal drawings in order to conform with the formal set.

Applicants and their attorney have critically reviewed the specification and claims. The amendments to the specification are for the purpose of correcting certain minor errors, improving textual quality, and correcting certain other inaccuracies which were inadvertently incorporated into the text when the application was prepared for filing. For the most part, these amendments are merely editorial in nature and require no extensive discussion. For example, on page 17, the addition of a reference to the lac promoter conforms with Figure 6. On page 19, line 4, the addition of tetracycline resistance as a selection marker conforms with Figure 9. The addition of "per cell per day" on page 21 merely continues recitation of these limitations from the first disclosure on line 12. The amendment on page 32 clarifies how the plasmid pPA25E10 (see line 31 on page 32) was prepared by using the cDNA found in a positive colony which was identified as 25E10 - see Figure 3. The amendment correcting the glycosylation site on page 37 line 15 is supported by Figure 12 where the four potential sites are indicated by the Z-shaped lines extending from the four ASN amino acids and by Figure 5b. Similarly, the amendments on pages 46, 47 and 48 correctly reference the assay procedure for the detection of the tissue plasminogen activator, and the correct vector construction example. There is no Section D.1.K.1.b,

C.1.A or D.2.C. in the specification.

That plasmid pt-PAtrp12 was intended instead of plasmid pΔRIPA° on page 44, lines 19 and 23, and in Figure 11, can be verified from the text describing the various DNA fragments removed from the three vectors, in conjunction with Figures 5, 6 and 9.

Plasmid pt-PAtrp12 contains the entire DNA sequence encoding t-PA, but lacks the leader sequence, as can be seen from Figure 9. Since the insertion of the DNA sequence encoding the leader could not conveniently be accomplished by direct insertion into pt-PAtrp12, the required vector was constructed from three fragments obtained from plasmids containing overlapping DNA sequences.

Example E.2 describes the preparation of full-length tissue plasminogen activator from transfected CHO cell culture. The vector thus contained the entire coding sequence for the mature molecule and its leader sequence, enabling the production of the mature molecule by secretion, during which the leader amino acid sequence is cleaved away. In the construction of this vector, three fragments supplied the requisite DNA.

The fragment generated from plasmid pPA17 contains 5' untranslated sequences and the coding region for the 35 amino acid leader and first six amino acids of the mature tissue

plasminogen activator molecule, namely, the "5' terminal t-PA sequence" as set forth on page 37, lines 2 to 5 and on line 22 on page 44 of the specification. The fragment terminates at its subcut Pst I site which is the first nucleotide (A) of the codon encoding the amino acid arg at the 7-position of the sequence for tissue plasminogen activator. This fragment contains approximately 200 base pairs, as disclosed in the specification on page 44, line 21 and as also can be seen from Figure 5A. Plasmid pPA17, therefore, supplied the DNA encoding the leader and the first six amino acids of t-PA.

The approximately 1645 base pair DNA fragment obtained from plasmid pPA25E10 by digestion with Nar I and Bgl II contains the DNA coding sequence of t-PA extending from the codon encoding amino acid 111, plus one nucleotide (C) thus forming the Nar I site, through part of the 3' untranslated region. Thus, pPA25E10 supplied the DNA encoding amino acids 111 through 527 of tissue plasminogen activator. Figure 9 of the specification illustrates this.

The DNA encoding amino acids 7 through 110 was supplied by the remaining fragment. By calculation and reference to Figures 5A and 9, the total number of base pairs for this region is exactly 310, the number referred to in line 24 of page 44 of the specification.

Plasmid pΔRIPA° contains DNA encoding only from amino

acid 69 of t-PA onwards. This can be seen from Figure 6 and the specification on pages 33, lines 9 and 10, and 34, lines 8 to 12. Therefore, this plasmid could not be the source for the region of DNA encoding amino acids 7 through 110. Only plasmid pt-PAtrp12 could be the requisite source.

Plasmid pt-PAtrp12 contains the full length tissue plasminogen activator cDNA. The 310 base pair fragment generated by the Pst I-Nar I digestion called for in the specification on page 44, line 23, encodes amino acids 7 through 110. The Nar I site representing amino acid 110 (gly) is clearly shown in Figure 9. Although the Pst I site at the DNA encoding amino acids 6 and 7 is not explicitly designated in Figures 5A and 9, it is present, as shown below:

```
 5   6   7   8
ile cys arg asp
ATC TGC AGA GAT
TAG|ACG TCT CTA
  Pst I
```

Because the pΔRIPA° plasmid could not supply the DNA encoding amino acids 7 through 110, it is plain that what was intended on page 44, lines 19 and 23 was a reference to pt-PAtrp12 which contains, between the specified restriction sites, the required DNA fragment encoding these amino acids. It,

and not DNA from pΔRIPA°, when ligated with the 1645 base pair fragment from pPA25E10, encoding amino acids 111 to 527, and with the approximately 200 base pair fragment from plasmid pPA17, encoding the leader sequence and first six amino acids of the mature tissue plasminogen activator molecule, provided the entire coding sequence for full length tissue plasminogen activator. The designation of plasmid pΔRIPA° on page 44 was an error that was carried over into Figure 11.

To the extent that the foregoing amendments may not be considered merely editorial in nature, it is submitted that any added or corrected information was inherent in the specification as filed and does not constitute new matter. It is respectfully requested that these amendments be entered for the purpose of clarifying and correcting the specification.

The amendments to the claims have been made for the purpose of further clarification of that which Applicants regard as their invention. The amendments to Claims 1 and 3 to 5 are offered in the belief that the Examiner should and will reconsider and withdraw the restriction requirement. However, notwithstanding the fate of the restriction requirement, it is respectfully requested that these claim amendments be entered for the above stated purpose.

The amendment to Claim 1 clearly specifies that the human tissue plasminogen activator of the present invention is a

recombinant human tissue plasminogen activator as distinguished from that protein obtained in the prior art from natural source. Claim 4 further distinguishes Applicants' product by the phrase "unaccompanied by protein with which it is ordinarily associated", thus providing verbal distinctions corresponding to actual differences from the material of the prior art. "Biologically active" is added so as to make explicit the very result that Applicants observed upon isolation and testing of the human tissue plasminogen activator of the present invention, viz., activity as a fibrinolytic agent.

Support for the claim amendments is replete in the specification both explicitly and implicitly. For example, the term "recombinant human tissue plasminogen activator" can be found in the paragraph bridging lines 8 to 14 on page 13, and the language of amended Claim 4 finds support in the paragraph bridging lines 11 to 14 on page 11 as well as in the results of the bioassay procedure given in Tables 1 and 2 on page 43 of the specification. Support for new Claims 16 and 17 can be found in Section B of the specification bridging pages 13 to 17, and in particular in the paragraph bridging pages 15 and 16, and in the specifically exemplified use of a Chinese hamster ovary (CHO) cell line on page 46 of the specification.

Referring now specifically to the Official Action mailed 22 April 1985 (Paper no. 13), the first matter concerns the propriety of the restriction requirement made under 35 U.S.C.

112. The restriction requirement established two groups of claims: Group I, Claims 1 to 5 and 11 to 13; Group II, Claims 6 to 10, 14 and 15. (It is noted that the Official Action obviously intended to refer to claims 6 to 10 along with 14 and 15 for the claim Group II.). This requirement is respectfully traversed. However, in order to be responsive, Applicants confirm the provisional election of Group II.

The claims of the present application cover different aspects of a single inventive concept. If a first patent were to issue on the process claims of Group II, then under 35 U.S.C. 121, no double patenting issue would arise on a patent issuing later on the remaining subject matter. However, a later patent directed to the products of the claims of Group I could effectively bar the practice of the processes of the claims of Group II, even though the patent claiming such processes had already expired. It is submitted that this situation makes untenable a holding of divisible subject matter. The Examiner's assertion in support of the restriction requirement to the contrary notwithstanding, there is not, in the prior art, an alternative process for producing products having the degree of purity of those claimed in the present invention.

Moreover, a search of the subject matter encompassing all of the claims would not represent an undue burden on the Patent and Trademark Office. In fact, a complete search sufficient to constitute a full examination of either of the two

groups as defined in the Official Action would necessarily extend to all of the claimed subject matter.

It is therefore respectfully requested that the restriction requirement be reconsidered and withdrawn.

The objection to the specification under 35 U.S.C. 112 is based upon an assertion that certain starting plasmids must be deposited in order for Applicant to have provided an adequate disclosure. The underlying assertion, it is respectfully submitted, is incorrect and therefore the objection is respectfully traversed. For each of the plasmids referred to, an adequate disclosure detailing preparation is provided. The Examiner's attention is respectfully invited to the specification on pages 34 to 37 where such disclosure for plasmid pPA17 is provided, to page 38 where such disclosure for plasmid pLeIFAtrp103 is provided (Reference 53; which provides a description of its preparation; see also page 26, lines 29 to 31), page 33 where such disclosure for preparing pdeltaR1SRC is provided (This plasmid designation is synonymous with pΔRIexsrc, as can be seen by comparing the text on page 33, lines 18 to 32 with the extreme right portion of Figure 6.) and finally to page 32 where such disclosure for the preparation of plasmid pPA25E10 is provided. (The amendments to page 32 may help to clarify this for the Examiner.)

In view of this complete disclosure that enables

construction of the plasmids, and further, in view of the disclosure of the complete DNA sequence encoding t-PA, which enables the construction of any of the foregoing plasmids by known techniques, Applicants maintain that a deposit is unnecessary. Indeed, a number of U.S. patents have issued in this field with very similar descriptions for the preparation of various plasmids, evidencing that the Patent Office does issue patents in this field without a requirement of deposit for recombinant organisms or recombinant plasmids where their production is otherwise enabled.

Applicants submit that the specification of the present application adequately enables, thus making deposit of plasmids unnecessary, given the detailed steps of how to prepare them. In view of these facts it is respectfully requested that this objection be reconsidered and withdrawn.

The claims are rejected under 35 U.S.C. 103 as "being unpatentable over Rijken et al. (R) or Rijken et al. (S) in view of Bollen et al. and Hung et al." This rejection is traversed for the following reasons:

The claims under rejection are directed to the various means for preparing human tissue plasminogen activator via recombinant DNA technology. Applicants were the first to have accomplished and enabled others to accomplish the identification and isolation of a DNA sequence encoding human tissue plasminogen

activator. This is therefore claimed in Claim 6. This DNA sequence was first employed by Applicants to provide replicable expression vectors, producers in suitable host cells of the tissue plasminogen activator via expression thereof. Claim 7 covers such expression vectors. Claim 8 claims two specific expression vectors. Claims 9 and 10 and new Claim 16 relate to transformation of a host cell, such as a microorganism (e.g. E. coli) or a cell culture (e.g. CHO line) with expression vectors thus creating micro factories for producing the human tissue plasminogen activator product. Claims 14 and 15 cover this entire sequence of steps by way of process claims.

Prior to the present invention, recombinant DNA technology had not been successfully employed to produce human tissue plasminogen activator, nor was there any disclosure sufficient to enable anyone to do so. Applicants were the first to produce, and teach the world how to produce, a biologically functional human tissue plasminogen activator using recombinant technology.

None of the cited references, save U.S. Patent 4,370,417 (Hung et al.), contains any relevant disclosure of the use of recombinant DNA technology. Hence, they are not directly applicable as references to the "recombinant claims" elected. Moreover, to the extent that these references might provide a disclosure of isolation from a natural source of a plasminogen activator or of plasminogen activator activity, even the product

claims to this application that relate to recombinant tissue plasminogen activator, distinguish over what these references disclose.

Although Hung et al. profess to disclose the use of recombinant DNA technology to produce a plasminogen activator, the disclosure of Hung et al. is incapable of enabling the production of human tissue plasminogen activator. The terminology of Hung et al. notwithstanding, that patent disclosure relates only to urokinase. Urokinase is a protein which has been reported to have plasminogen activator-like activity, but it is clearly a distinct compound, not tissue plasminogen activator. The tissue plasminogen activator protein of the present invention is structually, chemically and immunologically distinct from urokinase, and does not cross-react with antibodies raised against urokinase. Further, urokinase has little or no binding affinity for fibrin, a characteristic feature of the human tissue plasminogen activator of the present invention and one which makes tissue plasminogen activator a highly superior product in clinical use.

More importantly, the Hung et al. disclosure is not enabling as to any plasminogen activator. The Examiner's attention is respectfully directed to the four affidavits filed concurrently with this amendment in the names of Diane Pennica, Margaret Winkler, Herbert Heyneker and Ellson Chen. These affidavits singly and collectively conclusively demonstrate that

the Hung et al. disclosure is inoperative even with respect to a method for producing the immunologically distinct urokinase protein. Applicants obtained a culture of the Hung et al. E. coli X1776 containing the purported "expression vector" pABB26. This culture, which is alleged to produce "urokinase", was deposited by Hung et al. in the ARS culture collection, U.S. Department of Agriculture, Peoria, Illinois under accession number B12122. Applicants and their colleagues undertook experiments to determine whether, in fact, the culture produced urokinase or any plasminogen activator activity. The four affidavits referred to summarize the results of these experiments.

The affidavit by Diane Pennica illustrates that the DNA insert of the Hung et al. patent has no restriction enzyme analysis pattern similar to the pattern displayed by either the authentic human tissue plasminogen activator gene or the urokinase gene. The affidavit by Margaret Winkler demonstrates that, in her hands, the material obtainable from the Hung et al. "expression vector" by following the disclosed procedure does not exhibit plasminogen activator activity, in constradistinction to the assertion made in Hung et al. The affidavit by Herbert Heyneker demonstrates that the DNA insert of the Hung et al. patent did not hybridize with probes prepared to detect DNA encoding either authentic human tissue plasminogen activator or urokinase. Finally, the Ellson Chen affidavit conclusively establishes that there is no identity or even similarity by way

of homology of the Hung et al. DNA insert to either authentic human tissue plasminogen activator DNA or to human urokinase DNA. Indeed, the sequence analysis of the Hung et al. DNA insert indicates that there is no open reading frame in any of the six possible. This means that there are frequent stop translation codons scattered throughout the DNA insert so any translation that were to occur would quickly terminate. It is interesting to note that the sequence of the DNA insert of the Hung et al. patent was also compared with a computer databank containing virtually all of the reported DNA sequences to date, and there was no homology to any of these. What sort of product, if any, the DNA insert might encode remains a mystery.

For all of the the above reasons, Hung et al. must be eliminated as a basis for the rejection.

This leaves the three references to Rijken et al. (R), Rijken et al. (S) and Bollen et al. (T) for consideration. However, as noted above none of these references provides even a hint of relevant recombinant DNA technology that could affect the instant claims. The Examiner's assertion that it "would be obvious to utilize the methods of Hung et al....to produce a human tPA made by recombinant techniques" falls as Hung et al. is merely an erroneous prophetic disclosure.

The Bollen et al. reference teaches far less than the Examiner implies that it does. Contrary to the assertion that

this reference teaches the isolation of mRNA for urokinase, a fair reading discloses that all that was taught was the separation of a melange of mRNAs from other RNA present in human kidney or human fetal kidney cells. In vitro translation of the mRNA mixture produced a mixture of proteins, some of which behaved like urokinase in the assay system employed and were immunoprecipitated with what were obviously not very specific antibodies to urokinase. (See, for example, the last column of Table 1 which shows that significant amounts of immunoprecipitable material were obtained even in the control using globin mRNA.) Even the authors refer to the allegedly active material as "putative urokinase" or "urokinase-like material", indicating considerable uncertainty as to the true identity of the material. Be that as it may, the authors give no clue as to how the desired urokinase mRNA could be separated from the mixture or how to use the mixture to prepare the cDNA that would have to be inserted into an appropriate vector before urokinase could be produced by recombinant techniques. The most that Bollen et al. reveal is that human kidney cells contain mRNA that can be translated in vitro to produce a urokinase-like material, hardly a disclosure that would render the present invention obvious.

The addition of Hung et al. to Bollen et al. provides no more support to the rejection on obviousness than does a rubber crutch to a man with a bad leg. As was previously discussed and as demonstrated by the accompanying affidavits, the Hung et al.

reference is utterly incapable of enabling the production of any plasminogen activator. Combining one reference that gives no clue as to how one would obtain the requisite DNA with another reference that demonstrably fails to enable the expression of DNA encoding any plasminogen activator does not qualify as a patentability defeating combination.

Given the knowledge of the existence of a tissue plasminogen activator, it might be argued that it may have been obvious to try to produce it by recombinant techniques. But it is now well established that obviousness to try is not the standard of 35 U.S.C. 103. See for example In re Goodwin, 198 U.S.P.Q. 1 (CCPA 1978) and In re Yates, 211 U.S.P.Q. 1149 (CCPA 1981). In this respect, the Bollen et al. reference therefore is of no consequence, just as the Hung et al. patent is of no consequence, being directed at best to an immunologically distinct protein the preparation of which cannot be repeated by following its teachings.

This leaves for consideration the two Rijken et al. references. Again, since these references in no way suggest a product prepared by recombinant means, the provisionally elected claims are patentable over these references, directed as these claims are to recombinant DNA, expression vectors and cultures. However, even the non-recombinant composition of matter claims of the present invention are considered to be patentable over these references for the following reasons:

Dealing first with Reference S, it is plain from the article itself that the body of knowledge that had existed prior to the Reference S method was voluminous and related to attempts to isolate a plasminogen activator. A large number of plasminogen activators characterized by many different molecular weights had been detected. However, this body of knowledge related primarily to disclosure of detection techniques, not isolation and purification of a plasminogen activator.

The work reported in Reference S went beyond the earlier work in that it represented an attempt to purify a plasminogen activator, i.e., that normally present in human uterine tissue. However, there is no evidence in Reference S that this was done for purposes other than merely increasing the available scientific knowledge of the characteristics of one plasminogen activator from among the many the existence of which had already been suggested and detected or that the material obtained was of sufficient purity to warrant clinical utilization.

Reference R reports the purification of human tissue plasminogen activator to a degree not achieved by Reference S or other prior art. This higher degree of purity made possible the preliminary evaluation of the thrombolytic properties of the purified tissue plasminogen activator.

However, the distinction that the claims of the present invention provide over Reference R is that the present invention

enables the first preparation of human tissue plasminogen activator via recombinant means, thus enabling for the first time the preparation of material which is substantially free of any proteins with which it is ordinarily associated in its native state. Thus, while Reference R produced a much purer human tissue plasminogen activator composition than Reference S, one suitable for pharmaceutical testing, it was derived from a native source, and because of limitations inherent in protein purification methods, was apparently accompanied by some amount of contaminants with which it is ordinarily associated. The present invention made possible for the first time a distinct departure from this prior art by enabling the production of a human tissue plasminogen activator which, because if does not entail the use of human tissues, is substantially pure with respect to unrelated proteins of human origin with which the natural material would ordinarily be associated.

This invention clearly provides material of a purity unattainable by the prior art and represents an invention which was neither anticipated nor could be considered obvious from any of the cited art.

In this regard, attention is specifically directed to the concurrently filed affidavit of Stuart Builder that demonstrates that the protein preparation of Reference R is different from the preparation obtained via the means disclosed in the present application in that it is associated with some

unidentified protein that is not found in preparations obtained in accordance with the present invention.

The Examiner's attention is directed to the recent decision of the Court of Appeals for the Federal Circuit, In re Marosi, 218 U.S.P.Q. 289 (Federal Circuit, 1983), where the court held that the claim language "essentially free of alkali metal" defined a patentable invention over prior art which had larger amounts of alkali metal. In the present application, the prior art preparation contains at least one protein that is not found in the composition provided via the recombinant DNA technology of the present invention, as demonstrated by the Builder affidavit. The claim language in the present application distinguishes from and is patentable over the prior art under the holding of In re Marosi.

The desirability of producing human tissue plasminogen activator does not ensure its achievability or enable one to predict with a reasonable degree of confidence that it can be achieved by "a person having ordinary skill in the art". The fact is that there is nothing in the art of record or in the state of the art at the time the present invention was made that would have taught anyone how to prepare recombinant tissue plasminogen activator except in the most general terms that would constitute the definition of an objective rather than the means for attaining that objective.

The principal flaw in the Examiner's rejection, even as applied to the non-elected claims, is that there is no basis in the art of record for predicting with reasonable certainty that human tissue plasminogen activator could be expressed in a recombinant system, that it would be compatible with recombinant host cells, or that bioactive tissue plasminogen activator of a degree of purity enabled by the present invention could be produced by any practical means.

It would have been appreciated by those skilled in the art at the time this invention was made that the expression of human tissue plasminogen activator in transformed cells would be fraught with many potential difficulties. The art of recombinant DNA technology appears to be deceptively straightforward but is inherently unpredictable. A case in point is the Hung et al. patent, which appears to be based only on predictability and is clearly a prophetic disclosure but is, in fact, inoperative.

One of the reasons for not being able to reasonably predict the ability of a recombinant cell to successfully produce by expression a heterologous protein concerns the fate of foreign DNA in a host cell system. For example, it is not predictable that mRNA, if produced at all from such DNA, will be stable or that it will be accurately translated into a full-length protein. Even if it is, one cannot be certain that the protein will not be degraded by enzymes, either within the cell or extracellularly, or that the recombinant cell will properly fold the molecule

conformationally so that it will exhibit its desired biological activity. The human tissue plasminogen activator of the present invention contains some 527 amino acids, with many potential cleavage sites and some essential conformational requirements for biological activity. Thus, it would certainly have been unpredictable before the fact that one could obtain by recombinant DNA technology a biologically active protein such as the one forming the basis of the present invention. At the same time, it is clear that only recombinant DNA technology can assure that a human tissue plasminogen activator absolutely free of unrelated proteins of human origin can be obtained.

In the absence of reasonable predictability in this field, as discussed above, the art of record becomes at most a mere invitation to experiment. This is not an acceptable obviousness standard according to judicial interpretation of 35 U.S.C. 103, as noted above.

The unpredictability in this field is underscored by the difficulties the Applicants had to overcome before they could achieve success in producing human tissue plasminogen activator via recombinant DNA technology. Numerous endeavors to obtain a full length cDNA copy of tissue plasminogen activator messenger RNA (mRNA) by the techniques usually employed resulted only in a cDNA fragment that was still lacking the sequences encoding the N-terminal amino acids. Albeit a biologically active product was encoded by the cDNA obtained, it was recognized that it was not

the full length t-PA protein.

In order to overcome this difficulty, a new approach was devised, one that involved the synthesis of numerous DNA oligonucleotide primers and probes and required the isolation of the gene encoding tissue plasminogen activator from the human chromosome. This work is described beginning on page 34 (Example E.1.H.) of the specification. A synthetic oligonucleotide 5' TTCTGAGCACAGGGCG 3' was used for priming the preparation of cDNA from a gel fractionated pool of mRNA's containing t-PA mRNA. The oligonucleotide that was successfully employed was chosen from the nucleotide sequence near the very end of the cDNA cloned in example E.1.F. (pPA25E10; page 31 of specification). Numerous other primers from regions contained in the clone of E.1.F. (not detailed in the specification) were apparently unable to produce cDNA longer than that described in E.1.F. Use of the synthetic oligonucleotide described above (see Example E.1.H.) allowed the synthesis of cDNA encoding the N-terminal region of t-PA beyond this point but then a new source of hybridization probe had to be obtained to identify the clone that contained the full length cDNA. As shown in the specification, in order to confirm that the resultant cDNA was indeed DNA from the N-terminus of t-PA and not some other DNA which fortuitously hybridized with the probe used in the priming, a genomic DNA probe had to be obtained and used. First it had to be determined that t-PA was encoded by a single unique gene and not a family of genes, in the human chromosome. That gene had to be cloned, analyzed by DNA

sequencing, and a portion from the N-terminal part had to be obtained and used as a probe. Applicants could not be sure that the resultant cDNA obtained by using the oligonucleotide primer indeed encoded the entire remainder of the N-terminus of t-PA, particularly in view of the previous numerous failures to obtain full length DNA. Fortunately, as can be seen from the specification (see E.1.H.), the strategy employed did lead to a successful synthesis of the N-terminal portion of t-PA cDNA gene and the production of full length t-PA itself.

Even with the approach that ultimately proved successful, an enormous effort was still required. In all, a total of over 20,000 recombinant clones were produced with a number of primers and tested with various probes. (These numerous failed experiments are not detailed in the specification.) As can be seen from the specification, approximately 1,500 transformants were obtained with the primer described in E.1.H. Of these clones, only 18 contained a t-PA DNA sequence hybridized with the probe obtained from a human genomic DNA library. (See specification page 36, line 32.) Only seven of these hybridized with the oligonucleotide used as the primer, and one clone (pPA17) was shown to contain the correct 5' N-terminal region of tissue plasminogen activator, a signal leader sequence and an 84 bp 5' untranslated region. Only by combining the two clones, pPA25E10 and pPA17 could the entire

cDNA gene coding for the t-PA gene be constructed. This work which resulted in obtaining full length cDNA encoding t-PA is detailed on pages 34-37 of the specification.

The results of human clinical trials for human tissue plasminogen activator reflect enormous success in arresting heart attacks in progress, underscoring the inference that the lives of a number of heart attack victims have been saved, and has prompted such headlines as:

"New Heart Attack Drug Acclaimed".*

This success was made possible by the invention of the present Applicants.

The significance of the contribution made by the Applicants of the present invention is best judged not by a hindsight reconstruction of the prior art, but by the contemporaneous judgement of those skilled in the relevant art. Dr. Diane Pennica, one of the coinventors of the present invention, presented a paper disclosing the work at an international scientific meeting. At the end of her presentation, she received a standing ovation from those present. Clearly, they didn't believe that the accomplishment was obvious.

*The Miami News, 12 November 1984.

For all of the foregoing reasons, it is respectfully submitted that the present invention represents a significant and patentable advance in the art and that the present application is allowable. An early notice to that effect is respectfully requested.

Respectfully submitted,

GENENTECH, INC.

By
Walter H. Dreger
Reg. No. 24,190

October 21, 1985
460 Point San Bruno Boulevard
South San Francisco, California 94080
(415) 952-1000; X6764

L007.48b

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231 on 21 Oct. 85
(Date)
Walter H. Dreger
Typed name of person signing, assignee or Registered Representative
Signature
21 Oct. 85
Date of Signature

Docket 100/164-U.S.

ABSTRACT OF DISCLOSURE

Human tissue plasminogen activator (t-PA) is produced in useful quantities using recombinant DNA techniques. The invention disclosed thus enables the production of t-PA free of contaminants with which it is ordinarily associated in its native cellular environment. Methods, expression vehicles and various host cells useful in its production are also disclosed.

INFORMATION DISCLOSURE CITATION

(Use several sheets if necessary)

MAIL ROOM OCT 24 28 1985 PAT. & TRADEMARK OFFICE

ATTY. DOCKET NO.	SERIAL NO.
100/164	06/483,052
APPLICANT: DAVID V. GOEDDEL ET AL.	
FILING DATE: April 7, 1983	GROUP: 127

U.S. PATENT DOCUMENTS

*EXAMINER INITIAL	DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
JAH	4,245,051	Jan. 13, 1981	Reich et al.	435	212	
JAH	4,317,882	March 2, 1982	Horiguchi et al.	435	212	
JAH	4,314,994	Feb. 9, 1982	d'Hinterland et al.	424	95	
JAH	3,904,480	Sept. 9, 1975	Hull et al.	435	212	

RECEIVED NOV 14 1985 GROUP 120

FOREIGN PATENT DOCUMENTS

	DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION YES	TRANSLATION NO
JAH	0 040 176	Dec. 16, 1981	EPC (Collen et al.)	435	212		
JAH	1 551 275	Aug. 30, 1979	UK (d'Hinterland et al.)	424	94.64		
JAH	1 492 959	Nov. 23, 1977	UK (d'Hinterland et al.)	424	94.64		
JAH	2 092 154	Aug. 11, 1982	UK (Sugimoto)	435	215		

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

JAH Hoylaerts et al., J.B.C. 257, No. 6, 2912-2919 (1982)

JAH Weimar et al., The Lancet 2, 1018-1020 (November 7, 1981)

JAH Widman et al., Nature 272, No. 5648, 549-550 (March 2, 1978)

EXAMINER	DATE CONSIDERED
Joseph A. Halluin	Jan. 15, 1986

*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.

Form PTO-FB-A820 (also form PTO-1449)

Patent and Trademark Office - U.S. DEPARTMENT of COMMERCE

FORM PTO-1083

Case Docket No. 100/164-U.S.

In re application of : DAVID V. GOEDDEL ET AL.
Serial No. 06/483,052
Filed: April 7, 1983
For: HUMAN TISSUE PLASMINOGEN ACTIVATOR

RECEIVED
NOV 14 1985
GROUP 120

THE COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

Sir:

Transmitted herewith is an amendment in the above-identified application.

☐ Small entity status of this application under 37 CFR 1.9 and 1.27 has been established by a verified statement previously submitted.

☐ A verified statement to establish small entity status under 37 CFR 1.9 and 1.27 is enclosed.

☐ No additional fee is required.

The fee has been calculated as shown below:

EXTENSION FEE FOR RESPONSE WITHIN THIRD MONTH

	(Col. 1) CLAIMS REMAINING AFTER AMENDMENT		(Col. 2) HIGHEST NO. PREVIOUSLY PAID FOR	(Col. 3) PRESENT EXTRA	SMALL ENTITY RATE	SMALL ENTITY ADDIT. FEE	OR	OTHER THAN A SMALL ENTITY RATE	OTHER THAN A SMALL ENTITY ADDIT. FEE
TOTAL	*	MINUS	**	=	x5 =	$		x10 =	$
INDEP.	*	MINUS	***	=	x15 =	$		x30 =	$
☐ FIRST PRESENTATION OF MULTIPLE DEP. CLAIM					+50 =	$		+100 =	$
					TOTAL ADDIT. FEE	$	OR	TOTAL	$

*If the entry in Col. 1 is less than the entry in Col. 2, write "0" in Col. 3.
**If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, write "20" in this space.
***If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, write "3" in this space.
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found from the equivalent box in Col. 1 of a prior amendment or the number of claims originally filed.

☒ Please charge my Deposit Account No. 07-0630 in the amount of $ 390.00. A duplicate copy of this sheet is attached.

☐ A check in the amount of $_____ is attached.

☒ The Commissioner is hereby authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 07-0630. A duplicate copy of this sheet is attached.

☒ Any filing fees under 37 CFR 1.16 for the presentation of extra claims.

☒ Any patent application processing fees under 37 CFR 1.17.

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231 on 21 Oct 85
Walter H. Dreger
Name of Applicant, Assignee or Registered Representative
Signature
21 Oct. 85
Date of Signature

Respectfully submitted,

Walter H. Dreger, Reg. No. 24,190