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Published
With international search report.
With amended claims.

(54) Title: TREATMENT OF AIDS-TYPE DISEASE

(57) Abstract

Treatment of patients suffering from AIDS-type disease with a colony stimulating factor alone or together with erythropoietin, and/or an anti-viral agent and/or IL-2.

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TREATMENT OF AIDS-TYPE DISEASE

Acquired Immune Deficiency Syndrome, commonly known as AIDS, is a generally lethal disease of increasing notoriety. A less malignant form called AIDS-Related Complex (referred to as ARC) is also increasing in prevalence. For purposes of this invention these and related maladies may be hereinafter referred to generically as AIDS-type disease.

AIDS-type disease affects humans and other primates and is characterized by a general loss of the host immune response to invading pathogens. Another characteristic is pancytopenia, i.e. a marked depression in the hematological profile of the host. The etiologic agent is believed to be viral, and more specifically, retroviruses of the HTLV/LAV-type, now known as the human immunodeficiency virus (HIV). Related viruses with similar pathogenicity are found in monkeys and other simians. Despite various reports of successful treatments with diverse agents, there is still much need for effective therapy of AIDS-type disease.

According to my invention one or more primate colony stimulating factors are used to treat patients suffering from AIDS-type disease. The beneficial effects of this treatment can be measured by improved hematological profile (e.g. the reduction of cytopenia) and restoration of immune function.

Colony stimulating factors (CSFs) are a recognized class of proteins whose natural counterparts are produced in very low concentrations in the body. They stimulate the growth and development of various bone marrow progenitor cells into mature cells such as granulocytes, macrophages, megakaryocytes, erythrocytes, lymphocytes and mast cells. They obtain their name from the in vitro assay which measures the stimulation of colony formation by bone

marrow cells plated in semi-solid culture media. These factors induce the formation of such colonies. Stated another way, CSFs are factors required for survival, proliferation and differentiation of the myeloid, lymphoid and erythroid progenitors.

A leading and preferred example of such a colony stimulating factor for use in this invention is GM-CSF, also known as granulocyte-macrophage colony stimulating factor. It is described in detail in Wong et al, Science, Vol. 228, pp. 810-815 (May 17, 1985) and references cited therein. Wong et al. also teach its production via recombinant DNA techniques. While GM-CSF had been recognized as exhibiting significant in vitro activity on the various hematopoietic progenitor cells, its use in treatment of clinical conditions has been conjectural.

Another example of such colony stimulating factor is primate G-CSF, also known as beta-CSF or granulocyte-CSF. This factor produces colonies which contain primarly granulocytes and has been recently cloned. See Nagata et al., Nature, 319:415-418 (1986) and Souza et al. Science, 232:61-65, (1986).

Another colony stimulating factor for use in the treatment of this invention is primate IL-3, also known as pluripotent or multi-CSF. This CSF acts in an earlier stage in hematopoiesis. It has been described in Yang et al., Cell, 42:3-10, (1986).

Still another CSF within the scope of this invention is M-CSF, also known as CSF-1 or CSF-69. It produces colonies which contain primarily macrophages. It has been described in Kawasaki et al., Science, 230:291-296 (1985) and publications by E. R. Stanley. Its production by recombinant DNA techniques is described in U.S. application Serial No. 860,377. A truncated version is described in PCT/US86/00238.

Yet a further example of a CSF of this invention is CSF-309. This CSF acts in the early stages of hematopoiesis.

It is described in U.S. CIP application Serial No. 885,905 and Hirano et al, Nature, 324:73-76 (November 6, 1986).

The CSFs can be systemically administered either intravenously or subcutaneously. One preferred form is via a subcutaneous implant, e.g., an osmotic continuous infusion pump. Numerous said implants are known in the art. The dosage regimen will be determined by the attending physician considering the condition of the patient, the severity of any infection and other clinical factors. Generally, the regimen as a continuous infusion should be in the range of 1 - 500 units per kilogram of body weight per minute. A preferred dose is in the range of 5 to 50 units per kilogram of body weight per minute. Progress can be monitored by periodic assessment of the hematological profile, e.g. hematocrit, CBC, reticulocyte count, platelet count and the like.

In another aspect of this invention, a primate patient having a depressed hematological profile is treated by co-administering effective amounts of a primate CSF and primate erythropoietin. The relative amount of erythropoietin should be within the range of 1-100 units per kilogram of body weight per minute. I contemplate that these two materials act synergistically to reduce both pancytopenia and anemia.

Erythropoietin is a natural protein factor which increases the differentiation and proliferation of erythrocytes. Human erythropoietin, along with its method of production via recombinant DNA techniques, is described in Jacobs et.al., Nature, Vol. 313, pp. 806-810 (February 28, 1985) and references cited therein.

In a further embodiment of this invention, an immunocomprised primate patient is treated by co-administering a primate CSF and primate IL-2. The relative amount of IL-2 should be within the range of 50-500 units per kilogram of body weight per minute. I contemplate that these two materials will synergistically act to improve the patient's

immune status. The relative ratios reflect the normal therapeutic amounts of each protein given separately. In a still further embodiment, erythropoietin is also co-administered in the above described amount with the CSF and IL-2 to further aid the immuno-compromised patient who is pancytopenic or anemic.

In another embodiment of the invention the CSF is co-administered with an anti-viral agent to a primate patient suffering from AIDS. A preferred anti-viral agent for use in conjuction with this invention is 3'-azido-3'deoxythymidine commonly referred to as AZT. A highly preferred combination is GM-CSF with AZT. The amount of AZT in this co-administra-tion can be greater than the amount when AZT is the sole administered agent, thus permitting a higher therapeutic ratio.

The relative ratios and total amounts of CSF, anti-viral agent, erythropoietin and/or IL-2 will be determined by the attending physician considering the condition of the patient, his hematological profile, the severity of any infection and other clinical factors. When systemically administered, the proteins for use in this invention are in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability and the like, are within the skill of the art.

The following illustrate treatments according to this invention utilizing GM-CSF.

EXAMPLE I

The patient was a severely debilitated, pancytopenic rhesus monkey naturally infected with the type D retrovirus (an AIDS-type disease). This animal received an aqueous solution of highly purified, pyrogen free recombinant human GM-CSF prepared as described in Wong et. al, supra. It was subcutaneously administered by means of a continuous osmotic infusion pump (Model 2ML1 produced by Alza Corpor-

ation, Palo Alto, CA.) at a rate of 500 units per kilogram of body weight per minute for a period of seven days.

The patient's hematological progress is charted in Figure 1. After an initial lag of three days, the monkey's WBC began to rise significantly. From an initial leukocyte count of 1600/ul (98% lymphocytes, 2% monocytes), the white blood cell count was elevated to 23,900 on day 8 (52% polys, 2% bands, 42% lymphocytes, and 4% monocytes). Upon termination of the treatment on day 8 the white blood cell count peaked at 43,700 on day 10 and then began to decline returning to 1900 by day 19. Figure 2 monitors the reticulocyte count, an important barometer of the patient's erythroid response.

The reticulocyte count revealed a dramatic increase upon infusion with this protein. This subsequently led to a significant increase in red blood cell count.

This illustrates the significant improvement in hematological profile obtainable by systemically treating a patient suffering an AIDS-type disease with a CSF.

EXAMPLE 2

The patient was a 35 year old human male having Kaposi's sarcoma and diagnosed as having AIDS in accordance with the criteria established by the United States Center for Disease Control. In a controlled study at the New England Deaconess Hospital, Boston, MA, he was continuously infused intravenously with recombinant human GM-CSF having a specific activity of about 4×10^6 units/mg. at the rate of 3 units per kilogram of body weight per minute. This patient responded favorably to the infusion as measured by the white blood cell count increasing from 2,800 four days prior to the start of the study to 14,900 after receiving the aforesaid infusion for two weeks. The increase in total peripheral white blood cell count included absolute increases in neutrophils, banded neutrophils, and eosinophils. The patient expressed a subjective feeling of

betterment and evinced a reduction in the size of Kaposi's sarcoma. Upon termination of the infusion two days later, the white blood cell count decreased to 4,000. While receiving the infusion the patient demonstrated no adverse effects to the administration of the protein.

EXAMPLE 3

In another 39 year old male AIDS patient who received a dose of 1 ug/kg per day of recombinant human GM-CSF as a continuous infusion, similar results were observed. This patient also responded favorably to the infusion, with an increase in the total white blood cell count of from 2,200 on the day of admission to 9,900 two weeks after the start of therapy. The increase in total peripheral white blood cell count included absolute increases in all subtypes of such cells including lymphocytes.

Other CSF's such as M-CSF, IL-3, G-CSF, and CSF-309 can be substituted for GM-CSF for an analogous treatment to that described above. Similarly, analogs or so-called second generation CSFs may be analogously employed.

WHAT IS CLAIMED IS:

1. A method of treating a patient suffering AIDS-type disease comprising administering thereto an effective amount of colony stimulating factor.

2. A method according to claim 2 wherein the colony stimulating factor is administered subcutaneously.

3. A method according to claim 2 wherein the colony stimulating factor is administered intravenously.

4. A method according to claim 1 wherein the colony stimulating factor is administered in an amount within the range of 1 to 500 units per kilogram of body weight per minute.

5. A method according to claim 4 wherein the amount is within the range of 5 to 50 units per kilogram of body weight per minute.

6. A method according to claim 1 wherein exogeneous erythropoietin is co-administered to said patient.

7. A method of treating a patient exhibiting a depressed hematological profile comprising systemically co-administering synergistically effective amounts of a colony stimulating factor and erythropoietin.

8. A method according to claim 7 wherein said patient exhibits pancytopenia.

9. A method according to claim 7 wherein said patient exhibits anemia.

10. A method of treating an immuno-compromised patient comprising systemically co-administering effective amounts of both a colony stimulating factor and IL-2.

11. A method according to claim 1 wherein anti-viral agent is co-administrered to said patient.

12. A method according to claim 11 wherein said anti-viral agent is AZT.

13. A method according to any one of claims 1-12 wherein said colony stimulating factor is GM-CSF.

14. A method according to any one of claims 1-12 wherein said colony stimulating factor is M-CSF.

15. A method according to any one of claims 1-12 wherein said colony stimulating factor is IL-3.

16. A method according to any one of claims 1-12 wherein said colony stimulating factor is G-CSF.

17. A method according to any one of claims 1-12 wherein said colony stimulating factor is CSF-309.

AMENDED CLAIMS

[received by the International Bureau on 27 March 1987 (27.03.87);
original claims 1–17 replaced by new claims 1–19 (2 pages)]

1. A method of treating a patient suffering from AIDS-type disease comprising administering thereto an effective amount of colony stimulating factor.

2. A method according to claim 1 wherein the colony stimulating factor is administered subcutaneously.

3. A method according to claim 1 wherein the colony stimulating factor is administered intravenously.

4. A method according to claim 1 wherein the colony stimulating factor is administered in an amount within the range of 1 to 500 units per kilogram of body weight per minute.

5. A method according to claim 4 wherein the amount is within the range of 5 to 50 units per kilogram of body weight per minute.

6. A method according to claim 1 wherein exogeneous erythropoietin is co-administered to said patient.

7. A method of treating a patient exhibiting a depressed hematological profile comprising systemically co-administering synergistically effective amounts of a colony stimulating factor and erythropoietin.

8. A method according to claim 7 wherein said patient exhibits pancytopenia.

9. A method of treating a patient infected with human immunodeficiency virus (HIV) comprising administering thereto an effective amount of colony stimulating factor.

10. A method of treating an immuno-compromised patient comprising systemically co-administering effective amounts of both a colony stimulating factor and IL-2.

11. A method according to claim 1 or 9 wherein an anti-viral agent is co-administered to said patient.

12. A method according to claim 11 wherein said anti-viral agent is AZT.

13. A method according to any one of claims 1-12 wherein said colony stimulating factor is GM-CSF.

14. A method according to any one of claims 1-12 wherein said colony stimulating factor is M-CSF.

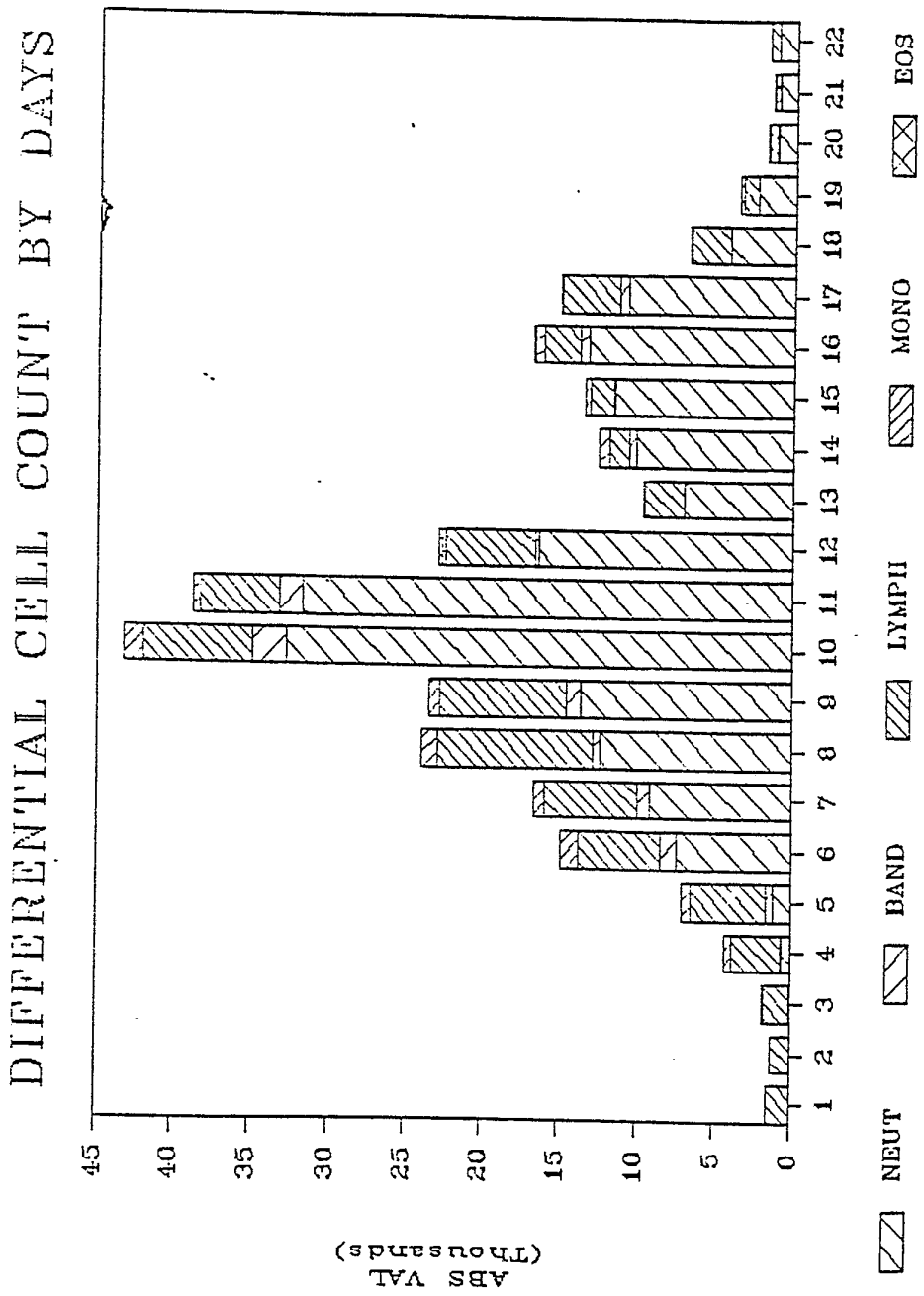
15. A method according to any one of claims 1-12 wherein said colony stimulating factor is IL-3.

16. A method according to any one of claims 1-12 wherein said colony stimulating factor is G-CSF.

17. A method according to any one of claims 1-12 wherein said colony stimulating factor is CSF-309.

18. A method according to claim 9 wherein an effective amount of IL-2 is co-administered to said patient.

19. A method according to claim 9 wherein erythropoietin is co-administered to said patient.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15
20
25
30
35
40
45
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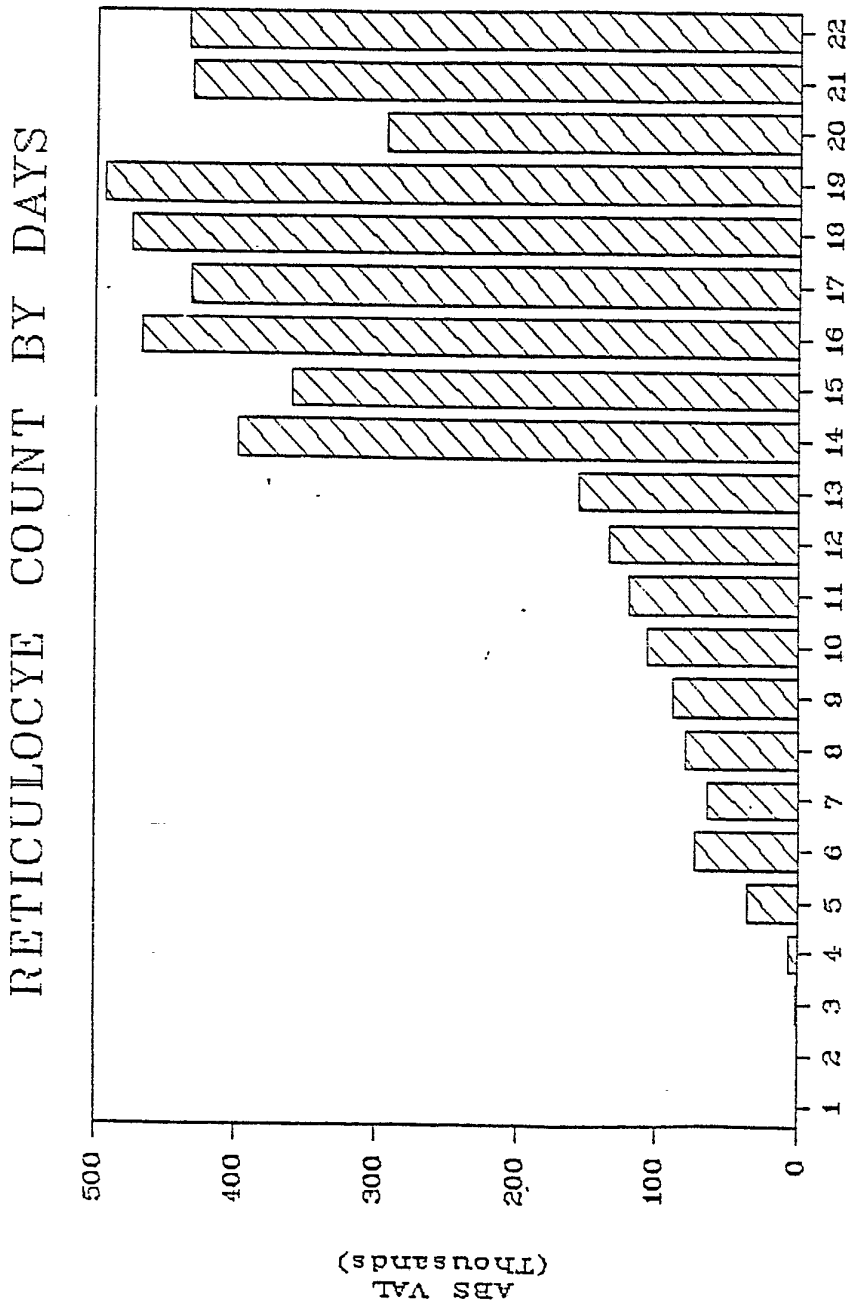
NEUT
BAND
LYMPH
MONO
EOS

Fig.1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ABS VAL
(Thousands)
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300
200
100
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

Fig.2

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 86/02575

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (4): A 61 K 37/02
U.S. Cl. 514/2,8

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	514/8,885,2

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

Databases: CASOnline file CA, file Reg and BIOSIS

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	US, A, 4,230,697 (NISHIDA et al), 28 October 1980, see abstract and columns 1-2.	1-10
Y	Chemical Abstracts, Volume 99, issued 1982 (Columbus, Ohio, USA), Kazuo Motoyoshi et al, "Phase I and early Phase II studies on human urinary colony stimulating factor", abstract No. 1046a, Jpn. J. Med. 1982, 21 (3), 187-91 (Eng), see entire document.	1-10

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [3]	Date of Mailing of this International Search Report [3]
6 January 1987	14 JAN 1987
International Searching Authority [1]	Signature of Authorized Officer [20]
ISA/US	Jackie Stone Jackie Stone

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	Biological Abstracts, Volume 79 (11), issued 1985 (Philadelphia, Pennsylvania, USA), P. Kern et al, "Preliminary Clinical Observations with recombinant Interleukin-2 in patients with acquired immune deficiency syndrome or persistent lymphadenopathy syndrome", Ref. No. 100547, BLUT 50(1): 1-6, 1985, see entire document.	9-10

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers....., because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
Y	ROOK et al, "Interleukin-2 enhances the natural killer cell activity of acquired immunodeficiency syndrome patients through a gamma-interferon-independent mechanism", J. Immunol., issued March 1985, volume 134 No. 3, see pages 1503, 1507.	10
Y	Biological Abstracts, Volume 78 (12), issued 1984 (Philadelphia, Pennsylvania, USA), Jerry L. Spivak et al, "Hematologic abnormalities in the acquired immune deficiency syndrome", Ref. No. 92073, Am. J. Med. 77(2): 224-228, 1984, see entire document.	1-10
Y	Chemical Abstracts, Volume 91, issued 1979 (Columbus, Ohio, USA), D. Metcalf et al, "Interactions between purified GM-CSF, purified erythropoietin and spleen conditioned medium on hemopoietic colony formation in vitro", Abstract No. 49943c, J. Cell Physiol., 1979, 99(2), 159-74 (Eng), see entire document.	5-8