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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: VIRAL ENVELOPE MEDIATED FUSION ASSAY

(57) Abstract: This invention provides a method of screening a sample for susceptibility to a viral entry inhibitor. The invention further provides a method for monitoring a patient for sensitivity to a viral entry inhibitor and a method of determining an inhibitory concentration of a viral entry inhibitor to inhibit virus infection in a patient. A method of screening for viral entry inhibitors is also provided.



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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/18556

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : G01N 33/567, 33/569, 33/53, 33/573; C12N 5/00, 5/08, 5/10, 5/22
US CL : 435/7.2, 7.21, 7.24, 7.93, 7.4, 69.1, 440, 325, 339, 366, 367, 373

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.2, 7.21, 7.24, 7.93, 7.4, 69.1, 440, 325, 339, 366, 367, 373

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LI, B. the synthetic peptide WKYVM attenuates the fusion of the chemokine receptor CCR5 and CXCR4 through activation of formyl peptide receptor-like 1. BLOOD, May 2001, Vol. 97, No. 10, pages 2941-2947, see pages 2941-2943, Fig. 7 and 8 on page 2946.	1-6, 8-13
X	DEY, B. Multiple Antiviral Activities of Cyanovirin-N: Blocking of Human	1-6, 8-10, 12-13
---	Immunodeficiency Virus Type 1 gp120 Interaction with CD4 and Cpreceptor and	-----
Y	Inhibition of Diverse Enveloped Viruses. J. Virol. May 2000, Vol. 74, No. 10, Pges 4562-4569, see pages 4563.	11
X	NUSSBAAUM, O. Fusogenic Mechanisms of Enveloped-Virus Glycoproteins Analyzed by a Novel Recombinant Vaccinia Virus-Based Assay Quantitatiing Cell Fusion-	1-6, 8-10, 12-13
---	DependentReporter Gene Activation. J. Virol. Semptember 1994, Vol. 68, No. 9, pages 5411-5422, see entire document.	-----
Y		11
X	TAKASHIMA, K. Inhibition Effectes of Small-Molecule CCR5 Antagonists on Human	1-6, 8-10, 12-13
---	Immunodeficiency Virus Type 1 Envelope-Mediated membrane Fusion and Viral	-----
Y	Replication,. J. Virol. December 2001, Vol. 45, No. 12, pages 3538-3543, see pages 3539-3540.	11

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

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

"E" earlier application or patent 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 when the document is taken alone

"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

Date of the actual completion of the international search

13 November 2003 (13.11.2003)

Date of mailing of the international search report

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Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

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

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
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, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ 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 claims Nos.: 1-6, 8-13 in the scope of receptor CXCR4 and beta-galactosidase

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

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BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-6, 8-13, drawn to a method for screening a sample for sensitivity to viral entry inhibitor, wherein the effector is a cell.

Group II, claim(s) 1, 7-13, drawn to a method for screening a sample for sensitivity to viral entry inhibitor, wherein the effector is a pseudovirus.

Group III, claim(s) 14-24, drawn to a method for monitoring a patient for sensitivity to a viral entry inhibitor.

Group IV, claim(s) 25-27, drawn to a method for identifying viral inhibitors.

Group V, claim(s) 28-31, drawn to a method for determining an inhibitory concentration of a viral entry inhibitor.

Group VI, claim(s) 32-40, drawn to a method for screening a sample for sensitivity to a viral entry inhibitor.

Group VII, claim(s) 41-45, drawn to a method for identifying viral entry inhibitor.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows:

Species I of receptor: 1). CXCR4, 2). CCR5, 3). CCR2b and 4). CCR3.

Species II of reporter: a). beta-galactosidase, b). luciferase, c). chloramphenicol acetyltransferase, d). alkaline phosphatase, e). green fluorescent protein and f). beta lactamase.

The claims are deemed to correspond to the species listed above in the following manner:

Claims 11, 23, 27, 38 and 44 are corresponding for the Species I of receptor.

Claims 13, 24 and 40 are corresponding for the Species II of reporter.

The following claim(s) are generic: claims 11, 23, 27, 38, 44 are generic for Species I and claims 13, 24 and 40 are generic for species II.

The inventions listed as Groups I to do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technique feature of current a system used for screening an inhibitor of viral entry is known in the art as evidenced by (Dey et al. J. Virol. 2000, Vol. 74, No. 10, pages 4562-4569, see entire document, especially see section of MATERIALS AND METHODS on pages 4562-4564). The system comprises co-culturing an effector cell line with another target cell line in the presence of a possible viral entry inhibitor, wherein the effector cells express a viral envelope protein element and a transcription factor and the target cells express a virus receptor and comprise an nucleic acid encoding an reporter gene sequence operably linked with a promoter that is susceptible to be activated in response to the transcription factor activation expressed by the effector cells. Upon the target cells fuse with the effector cells, the transcription factor expressed by the effector cells acts on the promoter carried by the target cells and consequently activate the reporter gene expression.

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The species listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: different viral receptors have different structures and function, e.g. the receptor CXCR4 mediates the T-tropism HIV entry the T cells, whereas the CCR5 mediates M-tropism HIV entry the monocytes. Moreover, different reporter gene encoding different reporter that word in different mechanism. Therefore, the difference of species cannot link all inventions under a same technical feature.

Continuation of B. FIELDS SEARCHED Item 3:

MEDLINE, STN APLUS

search terms: HIV, fusion assay, Hela cells, tat transfect,