证书号第2023180号

北京市海淀区地方税务局 印花税代扣专用章 国家知识产权局

发明专利证书

发明名称：一种Tat蛋白及其制备方法和应用

发明人：张辉;耿冠男

专利号：ZL 2014 1 0259996.8

专利申请日：2014年06月12日

专利权人：中山大学

授权公告日：2016年04月13日

本发明经过本局依照中华人民共和国专利法进行审查，决定授予专利权，颁发本证书并在专利登记簿上予以登记。专利权自授权公告之日起生效。

本专利的专利权期限为二十年，自申请日起算。专利权人应当依照专利法及其实施细则规定缴纳年费。本专利的年费应当在每年06月12日前缴纳。未按照规定缴纳年费的，专利权自应当缴纳年费期满之日起终止。

专利证书记载专利权登记时的法律状况。专利权的转移、质押、无效、终止、恢复和专利权人的姓名或名称、国籍、地址变更等事项记载在专利登记簿上。

局长
申长雨

中华人民共和国国家知识产权局

2016年04月13日

The United States of America

The Director of the United States Patent and Trademark Office

Has received an application for a patent for a new and useful invention. The title and description of the invention are enclosed. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law.

Therefore, this

United States Patent

Grants to the person(s) having title to this patent the right to exclude others from making, using, offering for sale, or selling the invention throughout the United States of America or importing the invention into the United States of America, and if the invention is a process, of the right to exclude others from using, offering for sale or selling throughout the United States of America, or importing into the United States of America, products made by that process, for the term set forth in 35 U.S.C. 154(a)(2) or (c)(1), subject to the payment of maintenance fees as provided by 35 U.S.C. 41(b). See the Maintenance Fee Notice on the inside of the cover.

Joseph Matal

Performing the Functions and Duties of the

Under Secretary of Commerce for Intellectual Property and

Director of the United States Patent and Trademark Office

MAINTENANCE FEE NOTICE

If the application for this patent was filed on or after December 12, 1980, maintenance fees are due three years and six months, seven years and six months, and eleven years and six months after the date of this grant, or within a grace period of six months thereafter upon payment of a surcharge as provided by law. The amount, number and timing of the maintenance fees required may be changed by law or regulation. Unless payment of the applicable maintenance fee is received in the United States Patent and Trademark Office on or before the date the fee is due or within a grace period of six months thereafter, the patent will expire as of the end of such grace period.

PATENT TERM NOTICE

If the application for this patent was filed on or after June 8, 1995, the term of this patent begins on the date on which this patent issues and ends twenty years from the filing date of the application or, if the application contains a specific reference to an earlier filed application or applications under 35 U.S.C. 120, 121, 365(c), or 386(c), twenty years from the filing date of the earliest such application ("the twenty-year term"), subject to the payment of maintenance fees as provided by 35 U.S.C. 41(b), and any extension as provided by 35 U.S.C. 154(b) or 156 or any disclaimer under 35 U.S.C. 253.

If this application was filed prior to June 8, 1995, the term of this patent begins on the date on which this patent issues and ends on the later of seventeen years from the date of the grant of this patent or the twenty-year term set forth above for patents resulting from applications filed on or after June 8, 1995, subject to the payment of maintenance fees as provided by 35 U.S.C. 41(b) and any extension as provided by 35 U.S.C. 156 or any disclaimer under 35 U.S.C. 253.

US009790255B2

(12) **United States Patent**
Zhang et al.

(10) **Patent No.: US 9,790,255 B2**
(45) **Date of Patent: Oct. 17, 2017**

(54) **TRANSACTIVATOR OF TRANSCRIPTION (TAT) PROTEINS AND PREPARATION METHOD**

(71) Applicant: **Sun Yat-Sen University**, Guangzhou (CN)

(72) Inventors: **Hui Zhang**, Guangzhou (CN); **Guannan Geng**, Guangzhou (CN)

(73) Assignee: **Sun Yat-Sen University** (CN)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **15/113,013**

(22) PCT Filed: **Jun. 11, 2014**

(86) PCT No.: **PCT/CN2014/079675**

§ 371 (c)(1),
(2) Date: **Jul. 20, 2016**

(87) PCT Pub. No.: **WO2015/188334**

PCT Pub. Date: **Dec. 17, 2015**

(65) **Prior Publication Data**

US 2017/0044215 A1 Feb. 16, 2017

(51) **Int. Cl.**
C07K 14/005 (2006.01)
A61K 38/00 (2006.01)

(52) **U.S. Cl.**
CPC ***C07K 14/005*** (2013.01); *A61K 38/00* (2013.01); *C12N 2740/16322* (2013.01)

(58) **Field of Classification Search**
CPC C07K 14/005; C07K 14/16; A61K 38/16; A61K 38/00; C12N 2740/16322
See application file for complete search history.

(56) **References Cited**

FOREIGN PATENT DOCUMENTS

CN 102206255 A 10/2011
CN 103073625 A 5/2013

OTHER PUBLICATIONS

International Search Report for International Patent Application No. PCT/CN2014/079615 dated Dec. 12, 2014.

Primary Examiner — Robert Landsman
(74) *Attorney, Agent, or Firm* — Lerner, David, Littenberg, Krumholz & Mentlik, LLP

(57) **ABSTRACT**

Disclosed is a Tat protein, the amino acid sequence of which is shown as SEQ NO: 1, SEQ NO: 2, SEQ NO: 3 and SEQ NO: 4. The Tat protein of the present invention has been studied and developed and can be a Latent infection of HIV-1 activating potential drug.

3 Claims, 6 Drawing Sheets

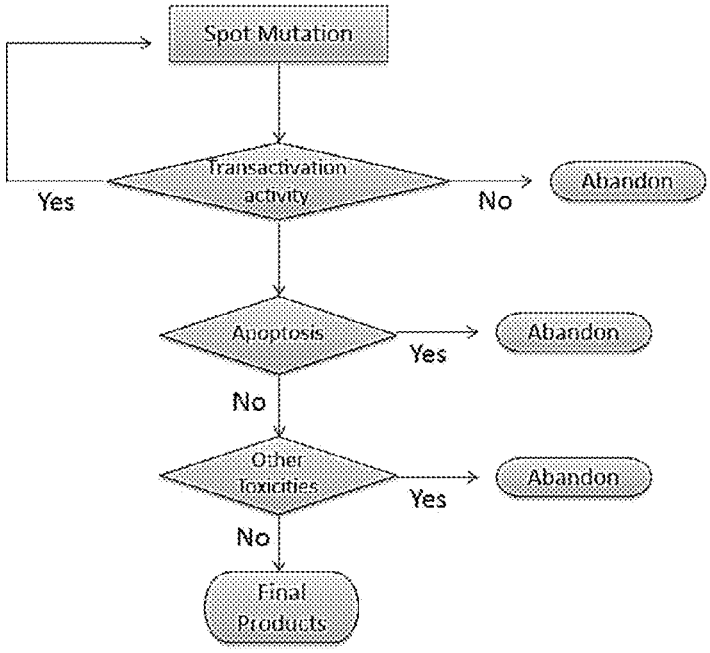


Figure 1

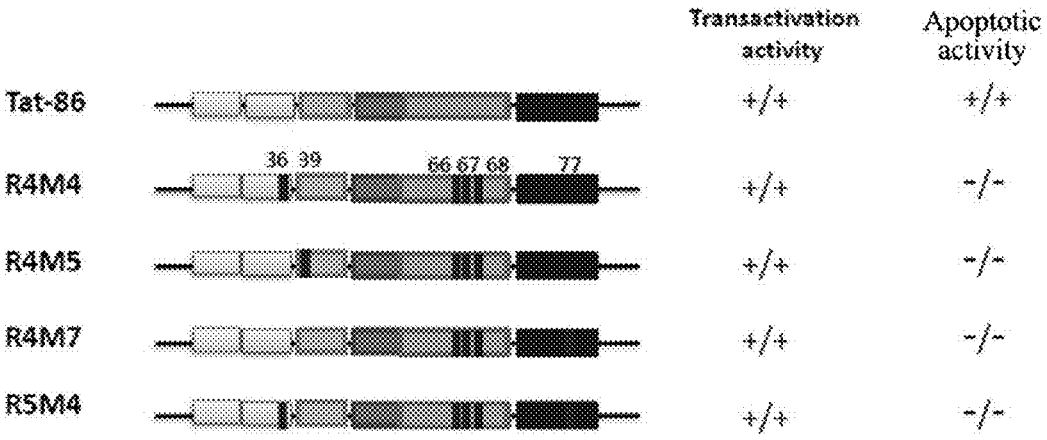


Figure 2

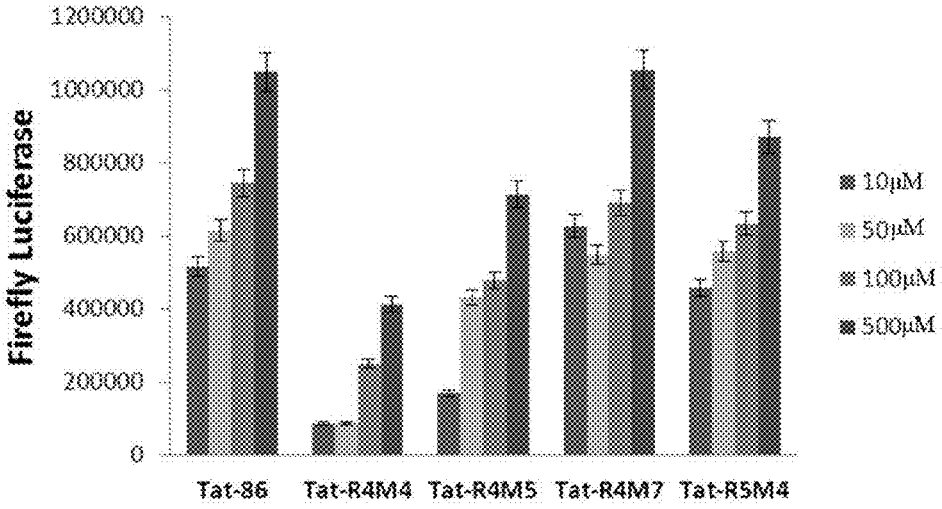


Figure 3

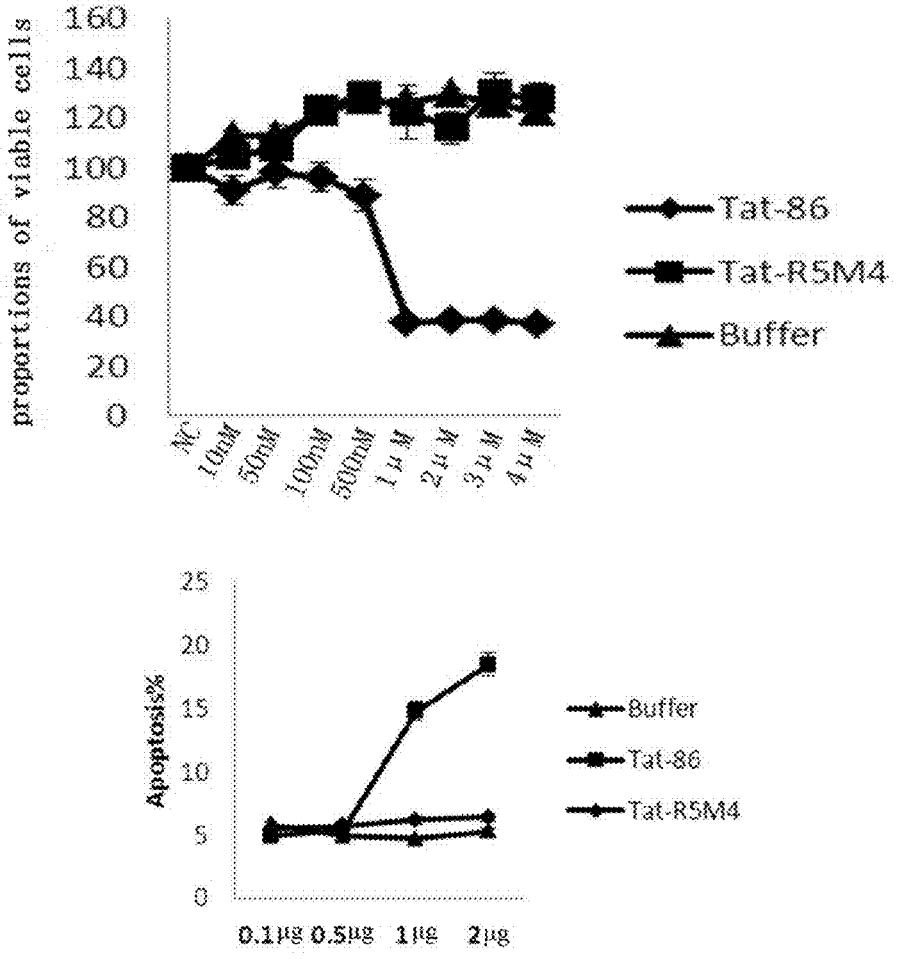


Figure 4

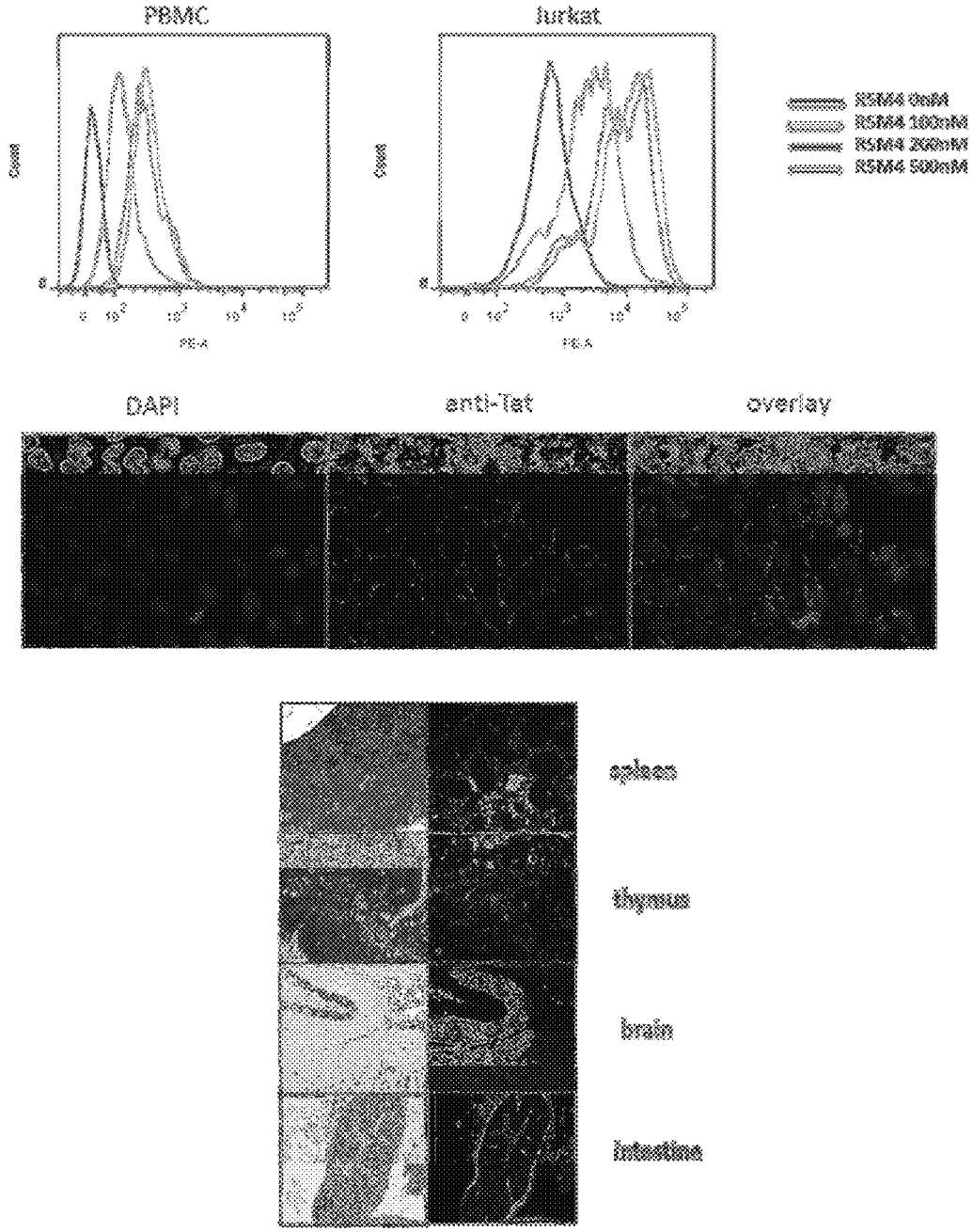

PBMC
Jurkat
RSM4 0nM
RSM4 100nM
RSM4 200nM
RSM4 500nM
DAPI
anti-Tat
overlay
spleen
thymus
brain
intestine

Figure 5

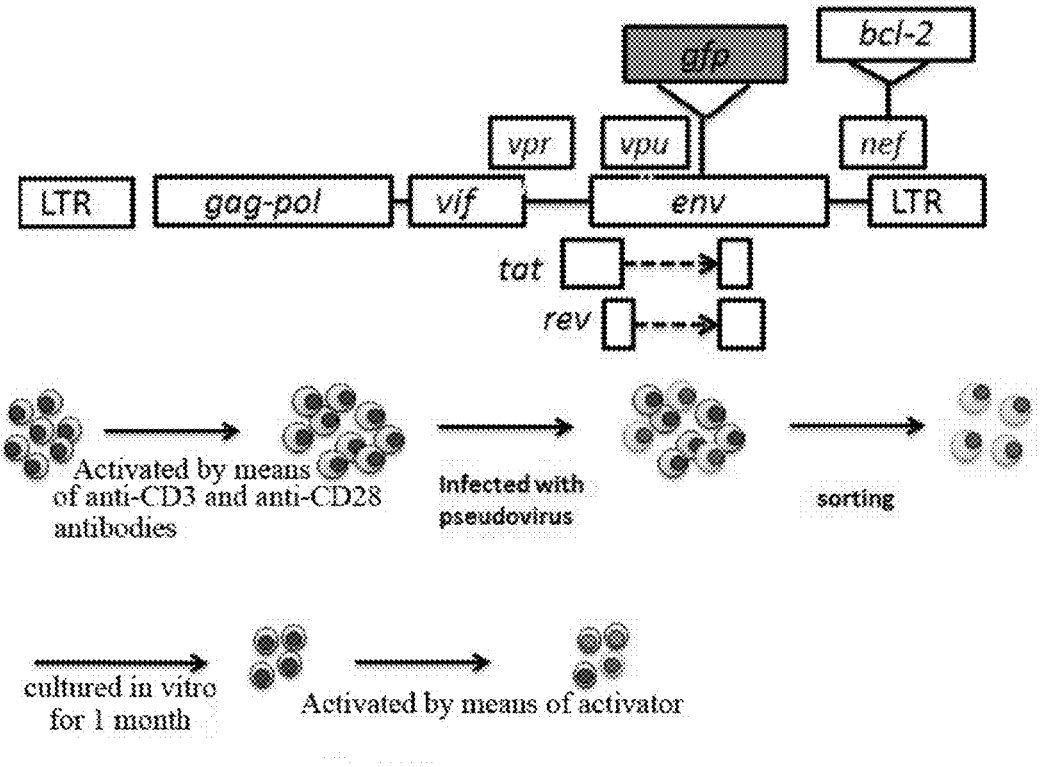


Figure 6

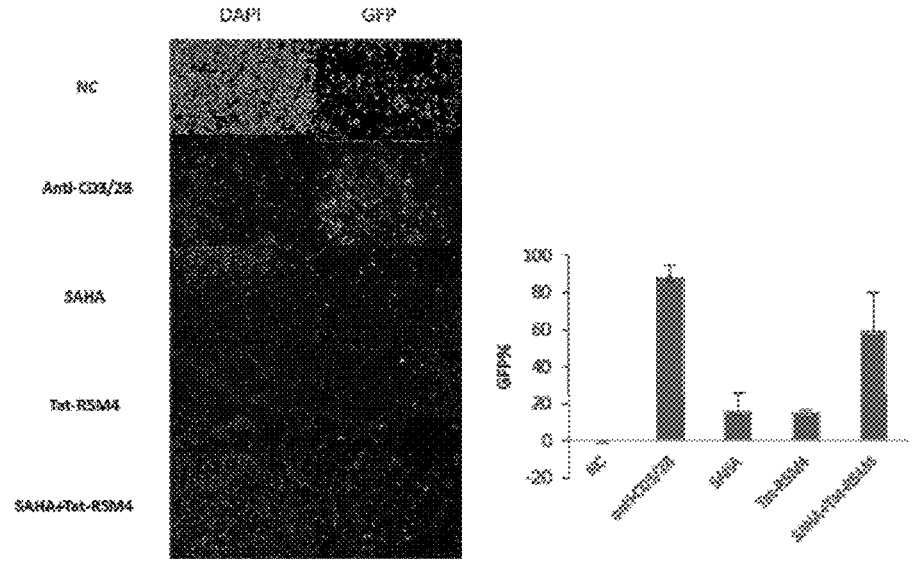


Figure 7

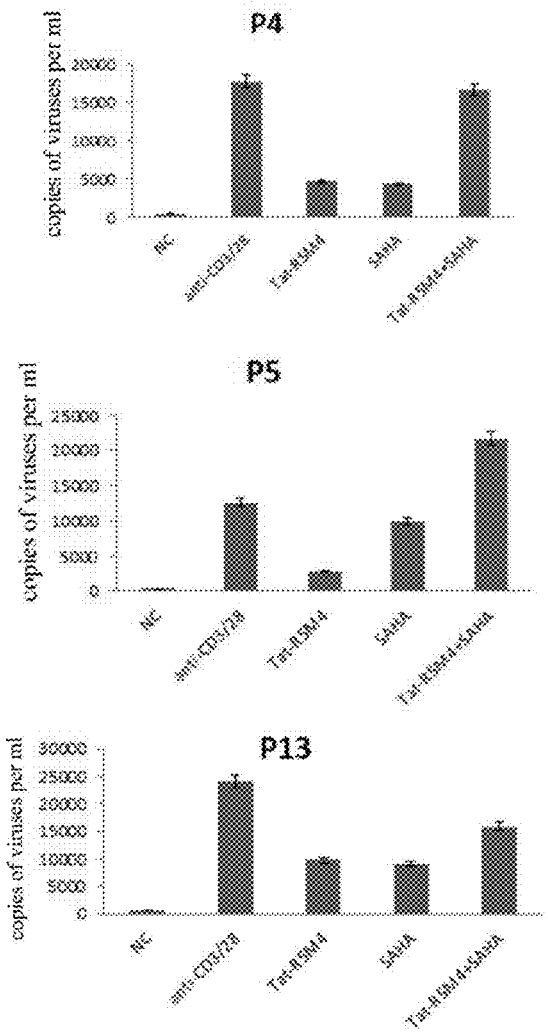
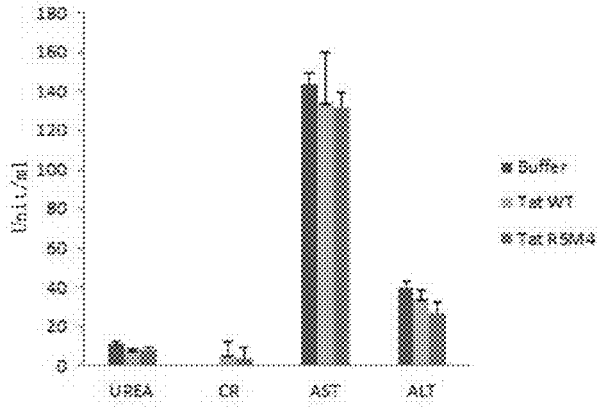

P4
copies of viruses per ml
NC
anti-CD3/28
Tat-RSM4
SAHA
Tat-RSM4+SAHA
P5
copies of viruses per ml
NC
anti-CD3/28
Tat-RSM4
SAHA
Tat-RSM4+SAHA
P13
copies of viruses per ml
NC
anti-CD3/28
Tat-RSM4
SAHA
Tat-RSM4+SAHA

Figure 8


U/L
UREA
CR
AST
ALT
Buffer
Tat WT
Tat RSM4

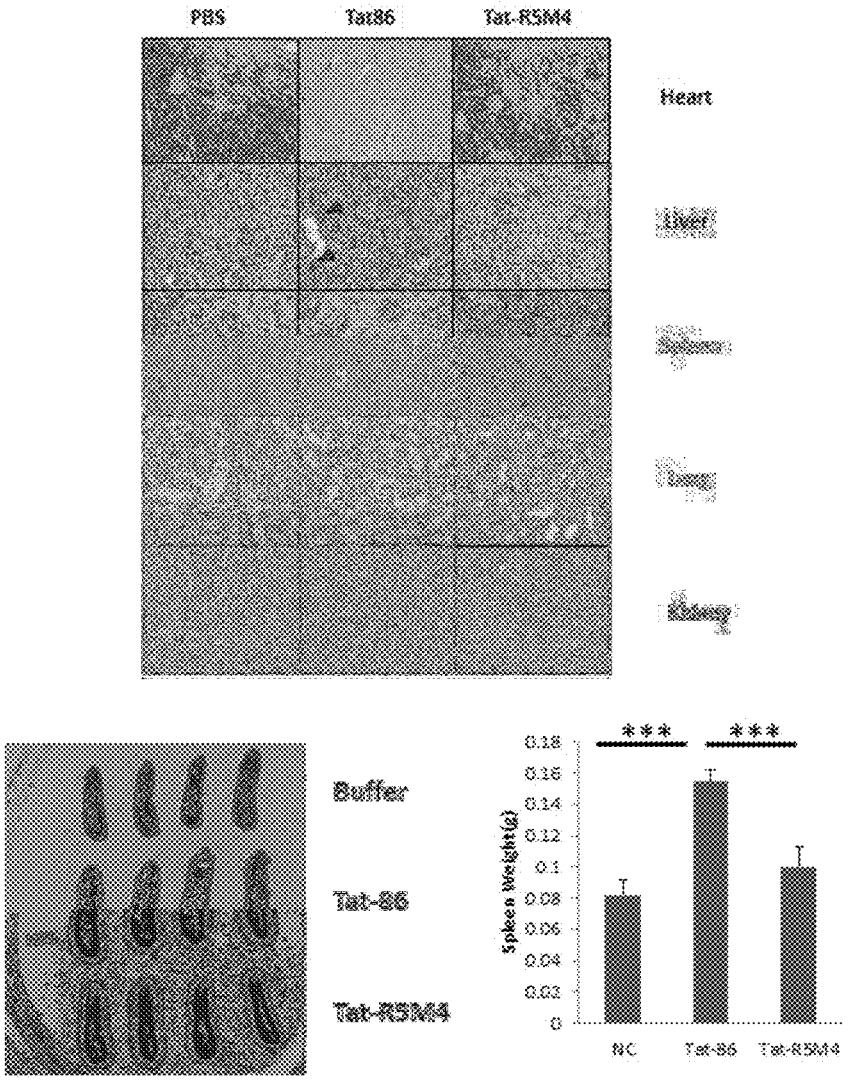

PBS
Tat86
Tat-RSM4
Heart
Liver
Buffer
Tat-86
Tat-RSM4
***
***
Spleen Weight(g)
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
NC
Tat-86
Tat-RSM4

Figure 9

TRANSACTIVATOR OF TRANSCRIPTION (TAT) PROTEINS AND PREPARATION METHOD

CROSS REFERENCE TO RELATED APPLICATIONS

The present application is a national phase entry under 35 U.S.C. §371 of International Application No. PCT/CN2014/079675, filed Jun. 11, 2014, published as International Publication No. WO 2015/188334 A1, which is hereby incorporated herein by reference.

TECHNICAL FIELD

The present invention relates to an antiviral compound, and more particularly, to an improved Tat protein and preparation method and use thereof.

BACKGROUND

Highly active antiretroviral therapy (HAART) can effectively control the amount of viruses in patients to an undetectable extent, but it is hard to be remove precursor viruses of latently infected HIV-1 (human immunodeficiency virus type 1) after integrated into a genome of a host to form a reservoir pool. Long-term medication is necessary for patients to suppress a viral replication, and a rebound of the viral replication may be caused once withdrawal of drugs. How to clear latent infection of HIV-1 has become a bottleneck problem for completely curing AIDS.

Cytokines such as interleukin-2 (IL-2) and anti-CD3 etc. have been used for activating latent infection of HIV-1, which activate the cells in an overall level, leading to huge poisonous side effects on an organism. A plurality of histone deacetylase inhibitors (HDACi) are also latent activators studied more currently. On one hand, abnormal expressions of other genes are easily caused due to activation of HDACi to genes being broad-spectrum effects; on the other hand, HDACi having better effects in vitro such as valproc acid (VPA) and Vorinostat (ie. Suberoylanilide hydroxamic acid, SAHA), and being not good in clinical manifestations, cannot be put into practical use. Therefore, finding out new, strong in specificity, highly effective and safe latent activators is an urgent mission.

HIV-1 Tat is a specific trans-activation factor of HIV-1, which specifically binds to TAR of HIV-1 5' LTR, raising several hundreds of times the transcription of HIV-1 mRNA. TAT protein is also a critical factor in the latent infection of HIV-1. This protein with cell-penetrating peptides has been proven to have a function of efficiently penetrating cytomembrane; and has been designed as a vaccine for HIV-1 used in clinical experiment, which is relatively safe for human body. However, Tat protein has been proven to have a function of inducing apoptosis, and may affect functions of immunocyte.

SUMMARY OF THE INVENTION

One of the purposes of the present invention is to find out a new anti-HIV approach.

Firstly, provided is a use of a Tat protein in preparing anti-HIV drugs, and TAT-86 is used in the present invention to demonstrate the effect.

More further provided is a use of an attenuated Tat protein in preparing anti-HIV drugs, an amino acid sequence of the attenuated Tat protein is shown as: SEQ NO: 1, SEQ NO: 2, SEQ NO: 3, or SEQ NO: 4.

More further provided is an attenuated Tat protein, an amino acid sequence of which is shown as: SEQ NO: 1 (R4M4), SEQ NO: 2 (R4M5), SEQ NO: 3 (R4M7), or SEQ NO: 4 (R5M4).

Further provided is a method for preparing the above-described attenuated Tat protein, characterized in that, firstly selecting sites for site-directed mutagenesis, performing site-directed mutagensis on these sites in genes of Tat protein, finally obtaining trans-activation functions of a large number of the remaining Tat, and removing apoptotic and other active attenuated proteins therein.

The invention has the following advantages:

1. The purpose of the present invention is to provide several attenuated HIV-1 Tat proteins for activating latent infection of HIV-1.

2. The present invention provides a thought of using a trans-activation protein Tat of HIV-1 itself as a means for activating latent infection, and makes the Tat protein more reliable in safety by modifying the Tat proteins through accumulative mutagenesis.

3. The present invention provides four kinds of attenuated Tat proteins, which can specifically activate latent infection of HIV-1 at a cellular level.

4. The present invention provides a manner for constructing an improved model of latent infection in vitro of HIV-1.

5. The present invention provides a new method for activating latent infection of HIV-1 using a combination of the attenuated Tat protein with SAHA.

6. The attenuated Tat proteins provided by the present invention have no effect on the function of an immunocyte.

7. It is found in the present invention, that the Tat proteins having superior trans-activation activities can effectively raise a transcription of HIV-1 mRNA.

8. The Tat proteins have good membrane-penetrating activities, and can effectively enter cells and various tissues to function.

DESCRIPTION OF FIGURES

FIG. **1**: A principle for modifying and screening HIV-1 Tat proteins.

FIG. **2**: A diagram of pattern for four kinds of attenuated Tat.

FIG. **3**: The four kinds of attenuated Tat can activate a promoter of HIV-1 in Tzm-b1.

FIG. **4**: The modification of Tat-R5M4 reduces cytotoxicity and apoptotic activity.

FIG. **5**: Tat-R5M4 can effectively penetrate membranes and enter cells and various tissues.

FIG. **6**: Construction of a model for latent infection in vitro of HIV-1.

FIG. **7**: Tat-R5M4 can effectively activate a model for latent infection in vitro of HIV-1.

FIG. **8**: Tat-R5M4 can effectively activate $CD4^+$T cells in latent infection state from peripheral blood of clinical patients.

FIG. **9**: Experiment of acute toxicity and detection of immunogenicity.

MODE OF CARRYING OUT THE INVENTION

The present invention has been described in further detail below in combination with the drawings and the specific embodiments. The reagents, equipment and methods used in the present invention are conventional and commercially

available reagents and equipment, and conventionally used methods in this technical field, unless otherwise specified.

Embodiment 1: Modification and Detection for Activity of Tat

Over the past decades, the structure and the function of HIV-1 Tat have been studied in great detail. In the present invention, we remove apoptotic activity by accumulating mutagenesis and modification, and retain most of transactivation-active Tat proteins. Since the study for the first three structure domains (amino acids 1-59) of Tat proteins has been very detailed, we initially select amino acids 60-72 studied less to perform point mutagenesis, connect the mutated genes with an eukaryotic expression vector to transfect into Tzm-b1 cells, and examine the activity of luciferase afterwards, wherein only M36, M39, M51, M66, M67, M68, M69, and M77 remain 80% or more of the trans-activation activity. Then, we combine and superpose these mutagenesises, finally obtaining four candidate proteins after six rounds of mutagenesis: R4M4, R4M5, R4M7, and R5M4, wherein R5M4 accumulates at most five point mutagenesises.

Wherein, R4M4 in site-directed mutagenesis has replaced valine at the 36th position with alanine, glutamine at the 66th position with alanine, valine at the 67th position with alanine, and serine at the 68th position with alanine.

Wherein, R4M5 in site-directed mutagenesis has replaced isoleucine at the 39th position with alanine, glutamine at the 66th position with alanine, valine at the 67th position with alanine, and serine at the 68th position with alanine.

Wherein, R4M7 in site-directed mutagenesis has replaced glutamine at the 66th position with alanine, valine at the 67th position with alanine, serine at the 68th position with alanine, and serine at the 77th position with alanine.

Wherein, R5M4 in site-directed mutagenesis has replaced valine at the 36th position with alanine, glutamine at the 66th position with alanine, valine at the 67th position with alanine, serine at the 68th position with alanine, and serine at the 77th position with alanine.

R4M4, R4M5, R4M7 and R5M4 are cloned into a prokaryotic expression vector to perform expression and purification, TZM-b1 cells are treated with the obtained proteins, and the activity of Firefly Luciferase is examined after 48 hours.

This experiment proves that the modified R4M4, R4M5, R4M7, and R5M4 have good transactivation activity in Tzm-b1.

Embodiment 2: Detection for Cytotoxicity and Apoptosis Activity

10 nM, 50 nM, 100 nM, 500 nM, 1 μM, 2 μM, 3 μM and 4 μM of Tat-86 or Tat-R5M4 are co-cultured with TZM-b1 cells, and viabilities of the cells are examined by means of MTS method after 48 hours. 0.1 μg, 0.5 μg, 1 μg, and 2 μg of Tat-86 (subjected to prokaryotic expression according to a conventional technique, cited references: Expression of full length Tat in *E. coli* and its purification) and Tat-R5M4 are co-incubated with Jurkat cells respectively, and double stained by means of Annexin-V-FITC and PE after 48 hours, and apoptosis ratio is examined by flow cytometry.

The present experiment proves that Tat-R5M4 obviously reduces cytotoxicity and an ability inducing apoptosis.

Embodiment 3: Detecting Membrane-Penetrating Activity of Tat-R5M4

To verify whether the membrane-penetrating activity of Tat-R5M4 is affected after mutagenesis, the purified Tat-R5M4 proteins are labeled with NHS-Rhodamine, and a state of Tat entering cells is examined by flow cytometry 6 hours after respectively treating PBMC and Jurkat cells with the labeled proteins. Tat-R5M4 has good membrane-penetrating activity. To detect the distribution situation of Tat-R5M4 in cytoplasms after entering into cells, Tzm-b1 cells are treated with the purified Tat-R5M4 proteins, and then subjected to a detection of immunofluorescence with Tat-R5M4 antibody, and it is found that Tat-R5M4 is distributed mostly in cytoplasm after entering cells, with only a small amount of proteins entering nucleus. To detect the distribution situation of Tat-R5M4 proteins in mice, Tat-R5M4 labeled with NHS-rhodamine is injected into mice through caudal vein, slices prepared from the tissues of spleen, thymus, brain, and intestine etc. are examined after 6 hours, and it is found that Tat-R5M4 is obviously distributed in these tissues.

The present experiment confirms that the modified proteins completely maintain membrane-penetrating characteristics thereof, and can efficiently enter cells and tissues to function.

Embodiment 4: Construction of Latent Infection Model in Vitro of HIV-1

The activated primary $CD4^+$T cells are infected with pseudoviruses having bcl-2 gene, with a positive rate generally between 5% and 10%. After 3 days of infection, the GFP-positive cells are sorted out by flow sorting, then continue to be cultured in PRM1640 medium added with IL-2, and simultaneously added with CD3 and CD28 antibodies for activating again. After one week, PRM1640 medium without any cell factors is used instead, and after culturing for 3-4 weeks, the cells gradually come into resting state with removal of IL-2. These cells in resting state may be used for detection of latent activator.

Embodiment 5: Activation Role of Tat-R5M4 in $CD4^+$T Cells from Sources of Latent Infection Model in Vitro of HIV-1 and Clinical Patients

The ability of activating latent infection of Tat-R5M4 is examined by means of a system for latent infection constructed in vitro, CD3/CD28 and SAHA are used and treated as positive controls meanwhile, and the proportion of GFP-positive cells is observed by a fluorescence microscope after 3 days.

CD3/CD28 antibodies and SAHA are used as positive controls, and the isolated $CD4^+$T cells of clinical samples are treated by means of Tat-R5M4 or a combination of Tat-R5M4 with SAHA meanwhile. A supernatant is taken after 18 hours, and then RNA is extracted to examine the content of HIV-1 RNA in the supernatant.

The above experiment proves that Tat-R5M4 can effectively activate $CD4^+$T cells in latent state from sources of latent infection model in vitro of HIV-1 and clinical patients' samples, and can enhance trans-activation effects thereof in combination with SAHA meanwhile.

Embodiment 6: Acute Toxicity Experiment and Detection of Immunogenicity

To provide subsequent animal experiments with a certain theory support, the toxicity of Tat-R5M4 protein is further examined Firstly, acute toxicity experiments with Babl/c mice indicate that the mice still live well when a dose of tail vein injection reaches 40 mg/ml. Indicators of the mice such

as alanine aminotransferase or glutamic-pyruvic transaminase (ALT), aspartate aminotransferase or glutamic oxalacetic transaminase (AST), urea nitrogen (BREA) and CR (creatinine) etc. are examined to be all in normal levels. The pathological sections show that heart, liver, spleen, lungs and kidneys of the mice have all not shown obvious damage, except that a wild type Tat-86 protein causes infiltration of local inflammatory cells in liver, which suggests that Tat-R5M4 is safe with respect to experiments in mice. Meanwhile, obvious intumescence of spleen is found one week later from the tail vein injection of Tat-86, which indicates that Tat-86 may cause obvious immune response, but Tat-R5M4 would not cause a similar phenomenon. Then, the mice are immunized by hypodermic injection with Tat-86 and Tat-R5M4 respectively, and blood is taken upon amputation of tail in 7 days, 14 days, and 21 days respectively, to detect a concentration of Tat antibody, it is found that an amount of the antibody produced by a stimulation of Tat-R5M4 is obviously lower than that of Tat-86.

The present experiment demonstrates that the modified Tat-R5M4 is relatively safe for the mice, has significantly lower immunogenicity, and is more suitable for experiments in vivo than the wild type Tat-86.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 4

<210> SEQ ID NO 1
<211> LENGTH: 86
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Tat Protein

<400> SEQUENCE: 1

Met Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser
1 5 10 15

Gln Pro Lys Thr Ala Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Phe
20 25 30

His Cys Gln Ala Cys Phe Ile Thr Lys Ala Leu Gly Ile Ser Tyr Gly
35 40 45

Arg Lys Lys Arg Arg Gln Arg Arg Arg Pro Pro Gln Gly Ser Gln Thr
50 55 60

His Ala Ala Ala Leu Ser Lys Gln Pro Thr Ser Gln Ser Arg Gly Asp
65 70 75 80

Pro Thr Gly Pro Lys Glu
85

<210> SEQ ID NO 2
<211> LENGTH: 86
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Tat Protein

<400> SEQUENCE: 2

Met Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser
1 5 10 15

Gln Pro Lys Thr Ala Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Phe
20 25 30

His Cys Gln Val Cys Phe Ala Thr Lys Ala Leu Gly Ile Ser Tyr Gly
35 40 45

Arg Lys Lys Arg Arg Gln Arg Arg Arg Pro Pro Gln Gly Ser Gln Thr
50 55 60

His Ala Ala Ala Leu Ser Lys Gln Pro Thr Ser Gln Ser Arg Gly Asp
65 70 75 80

Pro Thr Gly Pro Lys Glu
85

<210> SEQ ID NO 3
<211> LENGTH: 86
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:
<223> OTHER INFORMATION: Tat Protein

<400> SEQUENCE: 3

Met Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser
1 5 10 15

Gln Pro Lys Thr Ala Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Phe
20 25 30

His Cys Gln Val Cys Phe Ile Thr Lys Ala Leu Gly Ile Ser Tyr Gly
35 40 45

Arg Lys Lys Arg Arg Gln Arg Arg Arg Pro Pro Gln Gly Ser Gln Thr
50 55 60

His Ala Ala Ala Leu Ser Lys Gln Pro Thr Ser Gln Ala Arg Gly Asp
65 70 75 80

Pro Thr Gly Pro Lys Glu
85

<210> SEQ ID NO 4
<211> LENGTH: 86
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Tat Protein

<400> SEQUENCE: 4

Met Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser
1 5 10 15

Gln Pro Lys Thr Ala Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Phe
20 25 30

His Cys Gln Ala Cys Phe Ile Thr Lys Ala Leu Gly Ile Ser Tyr Gly
35 40 45

Arg Lys Lys Arg Arg Gln Arg Arg Arg Pro Pro Gln Gly Ser Gln Thr
50 55 60

His Ala Ala Ala Leu Ser Lys Gln Pro Thr Ser Gln Ala Arg Gly Asp
65 70 75 80

Pro Thr Gly Pro Lys Glu
85

What is claimed:

1. A composition comprising an anti-human immunodeficiency virus (HIV) drug, wherein the anti-HIV drug comprises an attenuated transactivator of transcription ("Tat") protein, having an amino acid sequence shown as SEQ NO: 1, SEQ NO: 2, SEQ NO: 3, or SEQ NO: 4.

2. An attenuated transactivator of transcription ("Tat") protein, characterized in that, an amino acid sequence of the attenuated Tat protein is shown as SEQ NO: 1, SEQ NO: 2, SEQ NO: 3, or SEQ NO: 4.

3. A method for preparing the attenuated Tat protein according to claim **2**, characterized in that,

firstly selecting a sites for site-directed mutagenesis in genes of a Tat protein,

performing site-directed mutagenesis on these Tat protein site,

removing apoptotic and other active attenuated proteins from the mutated Tat protein, and

retaining trans-activation functions of the mutated Tat protein to form the attenuated Tat protein.

* * * * *

中华人民共和国国家知识产权局

510620

广东省广州市天河区体育西路中石化大厦B塔3912室
广州粤高专利商标代理有限公司 单香杰

发文日：

2016年08月11日

申请号或专利号：201610652995.9　　发文序号：**2016081101022330**

专利申请受理通知书

根据专利法第28条及其实施细则第38条、第39条的规定，申请人提出的专利申请已由国家知识产权局受理。现将确定的申请号、申请日、申请人和发明创造名称通知如下：

申请号：201610652995.9
申请日：2016年08月10日
申请人：中山大学
发明创造名称：一种VC-CAR分子及在清除 IIIV-1 感染细胞中的应用

经核实，国家知识产权局确认收到文件如下：
发明专利请求书 每份页数:4页 文件份数:1份
权利要求书 每份页数:1页 文件份数:1份 权利要求项数： 10项
说明书 每份页数:8页 文件份数:1份
说明书附图 每份页数:4页 文件份数:1份
说明书摘要 每份页数:1页 文件份数:1份
摘要附图 每份页数:1页 文件份数:1份
专利代理委托书 每份页数:2页 文件份数:1份
费用减缓请求书 每份页数:1页 文件份数:1份
费用减缓证明 每份页数:1页 文件份数:1份
核苷酸或氨基酸序列表计算机可读载体 每份页数:0页 文件份数:1份
实质审查请求书 每份页数:1页 文件份数:1份
说明书核苷酸和氨基酸序列表 每份页数:7页 文件份数:1份

提示：

1. 申请人收到专利申请受理通知书之后，认为其记载的内容与申请人所提交的相应内容不一致时，可以向国家知识产权局请求更正。

2. 申请人收到专利申请受理通知书之后，再向国家知识产权局办理各种手续时，均应当准确、清晰地写明申请号。

审 查 员：卢婕虹(电子申请)　　审查部门：专利局初审及流程管理部-08

中华人民共和国国家知识产权局 专利申请受理章

电子提交收据

兹确认,一件PCT国际申请,已通过国家知识产权局安全电子提交软件收到。收到时，已自动给予申请号和收到日(PCT行政规程第7部分)。

提交号:	104507
申请号:	PCT/CN2017/072266
收到日:	2017年 1月 23日
受理局:	中国国家知识产权局
申请人或代理人档案号:	P17002SXJ
申请人:	中山大学
申请人数目:	4
发明名称:	一种VC-CAR分子及在清除 HIV-1感染细胞中的应用

所提交文件:

P17002SXJ-abst.txt	601	2017年 1月 23日 18:28:38
P17002SXJ-appb-P000001.pdf	180381	2017年 1月 23日 18:11:38
P17002SXJ-appb-P000002.pdf	74322	2017年 1月 23日 17:17:24
P17002SXJ-appb-P000003.pdf	39539	2017年 1月 23日 17:36:02
P17002SXJ-appb-P000004.pdf	648264	2017年 1月 23日 17:42:04
P17002SXJ-appb.xml	836	2017年 1月 23日 18:28:40
P17002SXJ-fees.xml	2049	2017年 1月 23日 18:28:40
P17002SXJ-poat-000001.xml	478	2017年 1月 23日 18:28:40
P17002SXJ-poat-I000001.pdf	283446	2017年 1月 23日 17:50:30
P17002SXJ-requ.xml	6912	2017年 1月 23日 18:31:06
P17002SXJ-seql.app	7431	2016年 8月 10日 16:16:58
P17002SXJ-vlog.xml	1831	2017年 1月 23日 18:28:40
pct101.GML	2202	2017年 1月 23日 18:28:38

签署人:	EMAILADDRESS=zhongjun.zhu@yogoip.com, CN=zhongjun zhu, OU="www.verisign.com/repository/CPS Incorp. by Ref.,LIAB.LTD(c)99", OU=WIPO Customer CA V2, O=World Intellectual Property Organization
收到日期和时间:	2017年 1月 23日 18:29
提交件的正式信息摘要:	38:3D:D2:BE:45:32:F2:7C:87:B7:FB:DC:5D:61:35:EA:8D:B4:97:05

/北京，RO/CN/

中华人民共和国国家知识产权局

510627

广东省广州市黄埔大道西100号富力盈泰广场A栋910
广州嘉权专利商标事务所有限公司 胡辉,许飞

发文日：

2016年08月22日

申请号或专利号：201610692755.1　　发文序号：**2016082200524630**

专利申请受理通知书

根据专利法第28条及其实施细则第38条、第39条的规定，申请人提出的专利申请已由国家知识产权局受理。现将确定的申请号、申请日、申请人和发明创造名称通知如下：

申请号：201610692755.1

申请日：2016年08月18日

申请人：中山大学

发明创造名称：可溶性蛋白BAFF在B细胞体外培养及扩增的应用

经核实，国家知识产权局确认收到文件如下：

实质审查参考资料 每份页数:2页 文件份数:1份
实质审查请求书 每份页数:1页 文件份数:1份
说明书核苷酸和氨基酸序列表 每份页数:5页 文件份数:1份
发明专利请求书 每份页数:4页 文件份数:1份
权利要求书 每份页数:1页 文件份数:1份 权利要求项数： 9项
说明书 每份页数:9页 文件份数:1份
说明书附图 每份页数:2页 文件份数:1份
说明书摘要 每份页数:1页 文件份数:1份
专利代理委托书 每份页数:2页 文件份数:1份
费用减缓请求书 每份页数:1页 文件份数:1份
费用减缓证明 每份页数:1页 文件份数:1份
核苷酸或氨基酸序列表计算机可读载体 每份页数:0页 文件份数:1份

提示：

1.申请人收到专利申请受理通知书之后，认为其记载的内容与申请人所提交的相应内容不一致时，可以向国家知识产权局请求更正。

2.申请人收到专利申请受理通知书之后，再向国家知识产权局办理各种手续时，均应当准确、清晰地写明申请号。

审 查 员：黄瑞敏(电子申请)　　审查部门：专利局初审及流程管理部-08

纸件申请，回函请寄：100088 北京市海淀区蓟门桥西土城路6号 国家知识产权局受理处收
电子申请，应当通过电子专利申请系统以电子文件形式提交相关文件。除另有规定外，以纸件等其他形式提交的文件视为未提交。

专 利 合 作 条 约

发信人:受理局

收信人:

510627

中国广东省广州市天河区黄埔大道西 100 号富力盈泰广场 A 栋 910 张萍

广州嘉权专利商标事务所有限公司

PCT

国际申请号和国际申请日通知书

(PCT 细则 20.2(c))

		发文日（日/月/年） 28. 11 月 2016(28. 11. 2016)
申请人或代理人档案号 PCT16-0101GZ		重 要 通 知
国际申请号 PCT/CN2016/104807	国际申请日（日/月/年） 07. 11 月 2016(07. 11. 2016)	优先权日（日/月/年） 18. 8 月 2016(18. 08. 2016)
申请人 中山大学		
发明名称 可溶性蛋白 BAFF 在 B 细胞体外培养及扩增的应用		

1. 通知申请人，对国际申请给出上述国际申请号和国际申请日。
2. 通知申请人，国际申请的登记本：
   - ☒ 已于 28. 11 月 2016(28. 11. 2016) 传送给国际局。
   - ☐ 由于下列原因，尚未传送给国际局；本通知书副本已寄给国际局*：
     - ☐ 尚未获得必要的国家安全审查许可。
     - ☐ 原因（具体的理由）：

* 国际局监视受理局传送登记本，并将收到该登记本情况通知申请人(用 PCT/IB/301 表)。如果自优先权日起 14 个月期满，尚未收到国际申请登记本，国际局将把该情况通知申请人(细则 22. 1(c))。

受理局的名称和邮寄地址 中华人民共和国国家知识产权局(RO/CN) 中国北京市海淀区蓟门桥西土城路 6 号 100088 传真号：（86—10）62019451	受权官员： 黄筱筱 电话号码： (86-10)62088349

中华人民共和国国家知识产权局 专利审查业务章

PCT/RO/105 表（2008 年 7 月）

中华人民共和国国家知识产权局

510627

广东省广州市黄埔大道西100号富力盈泰广场A栋910
广州嘉权专利商标事务所有限公司 许飞

发文日：

2016年09月18日

申请号或专利号：201610826488.2　　发文序号：**2016091800689310**

专利申请受理通知书

根据专利法第28条及其实施细则第38条、第39条的规定，申请人提出的专利申请已由国家知识产权局受理。现将确定的申请号、申请日、申请人和发明创造名称通知如下：

申请号：201610826488.2

申请日：2016年09月14日

申请人：中山大学

发明创造名称：白介素-21在制备干细胞样记忆T细胞体外扩增诱导剂中的应用

经核实，国家知识产权局确认收到文件如下：

发明专利请求书 每份页数:4页 文件份数:1份

权利要求书 每份页数:1页 文件份数:1份 权利要求项数： 7项

说明书 每份页数:3页 文件份数:1份

说明书附图 每份页数:2页 文件份数:1份

说明书摘要 每份页数:1页 文件份数:1份

专利代理委托书 每份页数:2页 文件份数:1份

实质审查请求书 每份页数:1页 文件份数:1份

提示：

1. 申请人收到专利申请受理通知书之后，认为其记载的内容与申请人所提交的相应内容不一致时，可以向国家知识产权局请求更正。

2. 申请人收到专利申请受理通知书之后，再向国家知识产权局办理各种手续时，均应当准确、清晰地写明申请号。

审 查 员：周晓鸣(电子申请)　　审查部门：专利局初审及流程管理部-08

纸件申请，回函请寄：100088 北京市海淀区蓟门桥西土城路6号 国家知识产权局受理处收

电子申请，应当通过电子专利申请系统以电子文件形式提交相关文件。除另有规定外，以纸件等其他形式提交的文件视为未提交。

专 利 合 作 条 约

发信人:受理局

收信人:
510620
中国广东省广州市天河区体育西路191号中石化大厦B塔3912室
广州粤高专利商标代理有限公司

PCT

国际申请号和国际申请日通知书

(PCT 细则20.2(c))

	发文日（日/月/年） 04.7月2014(04.07.2014)
申请人或代理人档案号 P14051MES	重 要 通 知

国际申请号	国际申请日（日/月/年）	优先权日（日/月/年）
PCT/CN2014/080199	18.6月2014(18.06.2014)	

申请人
中山大学

发明名称
一种嵌合载体及其制备方法和应用

1. 通知申请人，对国际申请给出上述国际申请号和国际申请日。
2. 通知申请人，国际申请的登记本：
   - ☒ 已于04.7月2014(04.07.2014)传送给国际局。
   - ☐ 由于下列原因，尚未传送给国际局；本通知书副本已寄给国际局*：
     - ☐ 尚未获得必要的国家安全审查许可。
     - ☐ 原因（具体的理由）：

* 国际局监视受理局传送登记本，并将收到该登记本情况通知申请人(用PCT/IB/301表)。如果自优先权日起14个月期满，尚未收到国际申请登记本，国际局将把该情况通知申请人(细则22.1(c))。

中华人民共和国国家知识产权局(RO/CN) 北京市海淀区蓟门桥西土城路6号 100088 传真号：（86—10）62019451	受权官员： 于建华 电话号码： (86-10)62088251

中华人民共和国国家知识产权局 专利审查业务章

PCT/RO/105表（2008年7月）

中华人民共和国国家知识产权局

510627

广东省广州市黄埔大道西100号富力盈泰广场A栋910
广州嘉权专利商标事务所有限公司 许飞

发文日：

2016年10月08日

申请号或专利号：201610870886.4　　发文序号：**2016100800112030**

专利申请受理通知书

根据专利法第28条及其实施细则第38条、第39条的规定，申请人提出的专利申请已由国家知识产权局受理。现将确定的申请号、申请日、申请人和发明创造名称通知如下：

申请号：201610870886.4

申请日：2016年09月30日

申请人：中山大学

发明创造名称：一种干细胞样记忆性T细胞体外诱导剂及方法

经核实，国家知识产权局确认收到文件如下：

发明专利请求书 每份页数:4页 文件份数:1份

权利要求书 每份页数:1页 文件份数:1份 权利要求项数： 10项

说明书 每份页数:5页 文件份数:1份

说明书附图 每份页数:3页 文件份数:1份

说明书摘要 每份页数:1页 文件份数:1份

专利代理委托书 每份页数:2页 文件份数:1份

实质审查参考资料 每份页数:3页 文件份数:1份

实质审查请求书 每份页数:1页 文件份数:1份

提示：

1. 申请人收到专利申请受理通知书之后，认为其记载的内容与申请人所提交的相应内容不一致时，可以向国家知识产权局请求更正。

2. 申请人收到专利申请受理通知书之后，再向国家知识产权局办理各种手续时，均应当准确、清晰地写明申请号。

审 查 员：牛晨蕾(电子申请)　　审查部门：专利局初审及流程管理部-08

纸件申请，回函请寄：100088 北京市海淀区蓟门桥西土城路6号 国家知识产权局受理处收

电子申请，应当通过电子专利申请系统以电子文件形式提交相关文件。除另有规定外，以纸件等其他形式提交的文件视为未提交。

专 利 合 作 条 约

发信人:受理局

收信人：
510627
中国广东省广州市天河区黄埔大道西 100 号富力盈泰广场 A 栋 910 张萍
广州嘉权专利商标事务所有限公司

PCT

国际申请号和国际申请日通知书

(PCT 细则 20.2(c))

		发文日（日/月/年） 10.11 月 2016(10.11.2016)
申请人或代理人档案号 PCT16-0095GZ		重 要 通 知
国际申请号 PCT/CN2016/102480	国际申请日（日/月/年） 19.10 月 2016(19.10.2016)	优先权日（日/月/年）
申请人 中山大学		
发明名称 一种干细胞样记忆性 T 细胞体外诱导剂及方法		

1. 通知申请人，对国际申请给出上述国际申请号和国际申请日。
2. 通知申请人，国际申请的登记本：
   - ☒ 已于 10.11 月 2016(10.11.2016) 传送给国际局。
   - ☐ 由于下列原因，尚未传送给国际局；本通知书副本已寄给国际局*：
     - ☐ 尚未获得必要的国家安全审查许可。
     - ☐ 原因（具体的理由）：

* 国际局监视受理局传送登记本，并将收到该登记本情况通知申请人(用 PCT/IB/301 表)。如果自优先权日起 14 个月期满，尚未收到国际申请登记本，国际局将把该情况通知申请人(细则 22.1(c))。

受理局的名称和邮寄地址 中华人民共和国国家知识产权局(RO/CN) 中国北京市海淀区蓟门桥西土城路 6 号 100088 传真号：（86—10）62019451	受权官员： 黄筱筱 电话号码： (86-10)62088340

中华人民共和国国家知识产权局 专利审查业务章

PCT/RO/105 表（2008 年 7 月）