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(71) Applicants: DANA-FARBER CANCER INSTITUTE, INC. [US/US]; 450 Brookline Avenue, Boston, MA 02215-5450 (US). UNIVERSITY OF PENNSYLVANIA [US/US]; 3160 Chestnut Street, Suite 200, Philadelphia, PA 19104 (US). THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 1111 Franklin Street, Twelfth Floor, Oakland, CA 94607-5200 (US).

(72) Inventors; and

(71) Applicants: HAINING, William, Nicholas [GB/US]; 100 Homer Street, Newton, MA 02459 (US). SEN, Debatama [BD/US]; 210 Prospect Street, Apt. 4, Cambridge, MA 02139-1255 (US). WHERRY, E., John [US/US]; 110 Myrtle Avenue, Havertown, PA 19083 (US). YOSEF, Nir [—/US]; 1520 Laurel Avenue, Richmond, CA 94805 (US). KAMINSKI, James [US/US]; 1919 Curtis Street, Apt 6, Berkeley, CA 94702 (US).

(74) Agent: SMITH, DeAnn, F. et al.; Foley Hoag LLP, Seaport West, 155 Seaport Blvd., Boston, MA 02210-2600 (US).

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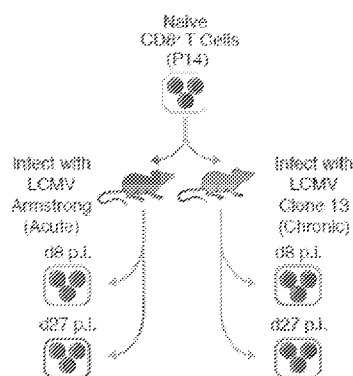
- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

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(54) Title: T-CELL EXHAUSTION STATE-SPECIFIC GENE EXPRESSION REGULATORS AND USES THEREOF

Figure 1 A



(57) Abstract: The present invention is based on the identification of T-cell exhaustion state-specific gene expression regulators, as well as uses thereof.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/23404

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 15/113, 15/63, 15/79, 9/16, 9/22 (2017.01)

CPC - C12N 5/0636, 5/0638, 2510/00, 2310/20, 15/113, 9/16, 9/22

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)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	Oestreich et al. NFATc1 regulates PD-1 expression upon T cell activation. J Immunol 1 October 2008 Vol 181 No 7 Pages 4832-4839. Especially abstract, pg 4834 col 1 para 2, pg 4835 fig 3, 4; pg 4837 fig 8.	1, 4, 5, 6/(1,4,5), 10 ----- 2, 3, 6/(2,3)
Y	Kao et al. Transcription factor T-bet represses expression of the inhibitory receptor PD-1 and sustains virus-specific CD8+ T cell responses during chronic infection. Nat Immunol 29 May 2011 Vol 12 No 7 Pages 663-671. Especially abstract, "pg 670 col 1 para 3.	2, 3, 6/(2,3)
A	Schumann et al. Generation of knock-in primary human T cells using Cas9 ribonucleoproteins. Proc Nat Acad Sci 18 August 2015 Vol 112 No 33 Pages 10437-10442. Especially abstract, Pg 10441 fig 4A, C.	1
A, P	Sen et al. The epigenetic landscape of T cell exhaustion. Science Epub 27 October 2016 Vol 354 No 6316 Pages 1165-1169. Especially entire article.	1-6, 10

☐ 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 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 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

15 June 2017

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Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300

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-----Continuation of Box III (Lack of Unity of Invention)-----

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: Claims 1-6, 10, drawn to an engineered mammalian T cell composition.

Group II: Claims 28-33, 37, drawn to a method of modulating the expression of at least one gene in a mammalian T cell.

The inventions listed as Groups I and II 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:

Special Technical Features:

Group I has the special technical feature of an engineered mammalian T cell composition, not required by Group II.

Group II has the special technical feature of specific method steps to genetically modify a genomic regulatory region that modulates expression of at least one gene, not required by Group I.

Common Technical Feature:

Groups I and II share the common technical features of:

1. a genomic region that regulates the gene expression of at least one gene.
2. the genomic region is selectively chromatin accessible within the genome of an exhausted CD8+ T cell.
3. a genetic modification that modulates the expression of one gene.

However, said common technical feature does not represent a contribution over the prior art, and is obvious over the publication titled "T-bet and Eomes are differentially linked to the exhausted phenotype of CD8+ T cells in HIV Infection" by BUGGERT et al. (hereinafter "Buggert") [published 1/ July, 2014, PLoS Pathogens, Vol 10, No 7, Pages e1004251, 1-15], in view of the publication titled "ATAC-seq: A Method for Assaying Chromatin Accessibility Genome-Wide" by BUENROSTRO (hereinafter "Buenrostro") [published 5 January, 2015, Curr Protoc Mol Biol, Vol 109, No 21., 29.1-21.29.9]; the publication titled "A genome-wide regulatory network identifies key transcription factors for memory CD8+ T-cell development" by Hu et al. (hereinafter 'Hu') [Published 12 Dec, 2013, NATURE COMMUNICATIONS, 4:2830] and the publication titled "Generation of knock-in primary human T cells using Cas9 ribonucleoproteins" by SCHUMANN et al. (hereinafter "Schumann") [published 18 August, 2015, Vol 112, No. 33, Pages 10437-10442].

As to common technical features #1 and #2, Buggert teaches the transcription factor Eomes upregulates PD-1 expression in chronically infected HIV positive subjects (abstract; Two key transcription factors governing CD8+ T cell differentiation, T-bet and Eomesodermin (Eomes), have previously been shown in mice to differentially regulate T cell exhaustion in part through direct modulation of PD-1. Here, we examined the relationship between these transcription factors and the expression of several inhibitory receptors (PD-1, CD160, and 2B4), functional characteristics and memory differentiation of CD8+ T cells in chronic and treated HIV infection. The expression of PD-1, CD160, and 2B4 on total CD8+ T cells was elevated in chronically infected individuals and highly associated with a T-bet.sup.dim Eomes.sup.hi expressional profile.). Although Buggert does not specifically teaches that Eomes is in a selectively chromatin accessible region within the genome of exhausted CD8+ T cells, Buggert teaches "both resting and activated HIV-specific CD8+ T cells in chronic infection were almost exclusively Tbet.sup.dim, Eomes.sup.hi cells, while CMV-specific CD8+ T cells displayed a balanced expression pattern of T-bet and Eomes" (abstract). Thus, the cognate genomic region wherein Eomes binds is a selectively chromatin accessible region in exhausted CD8+ T cells and meets claim limitation: "selectively chromatin accessible within the genome of an exhausted CD8+ T cell from the mammal".

Buggert does not specifically teach methods to identify the selectively chromatin accessible genomic region where Eomes binds, however, there are means to identify selectively chromatin accessible region are well established. For example Buenrostro (abstract; "This unit describes Assay for Transposase-Accessible Chromatin with high throughput sequencing (ATAC-seq), a method for mapping chromatin accessibility genome-wide. This method probes DNA accessibility with hyperactive Tn5 transposase, which inserts sequencing adapters into accessible regions of chromatin."). Furthermore, Hu teaches mapping transcription factor EOMES genomic binding regions using chromatin immunoprecipitation assays (ChIP assay) (pg 2 col 2 para 4; To verify this regulatory module, chromatin immunoprecipitation (ChIP) was performed for the top three TFs, Sox4, Tcf7 and Eomes, in CD8+ T cells followed by PCR amplification of promoter regions (within 1 kb upstream of the transcription-starting site) of randomly selected MSGs that were predicted to be regulated by Sox4 or Tcf7 or Eomes. As shown in Fig.1c,d (Supplementary Fig. S15)"; pg 11 col 1 para 1; The network approach and methodologies developed here can be applied to any phenotypic transition, such as effector T cells and exhausted T cells, to identify novel transcriptional modules and TFs"). By combining Buggert, Buenrostro, and Hu, it would have been obvious to one of ordinary skill in the art to have determined the selectively chromatin accessible regions in the exhausted CD8+ T cells, more specifically the chromatin region where Eomes transcription factor binds to its cognate genomic region that affect PD-1 expression.

-----continued on next sheet-----

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/23404

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims 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. ☒ Claims Nos.: 7-9, 11-27, 34-36, 38-81
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

-----Go to Extra Sheet for continuation-----

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 additional fees, this Authority did not invite payment of additional fees.
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.:
Claims 1-6, 10

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

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As to common technical feature #3, Buggert does not teach modifying the Eomes genomic region in order to modulate and downregulate PD-1. Schumann teaches using CRISPR/Cas9 genome editing tools to knock-out PD-1 expression (abstract; "Importantly, Cas9 RNPs paired with homology-directed repair template oligonucleotides generated a high frequency of targeted genome modifications in primary T cells. Targeted nucleotide replacement was achieved in CXCR4 and PD-1 (PDCD1), a regulator of T-cell exhaustion that is a validated target for tumor immunotherapy"). It would have been obvious for one of ordinary skill in the art to combine Buggert, Buenrostro, and Hu with Schumann and to have used the CRISPR/Cas9 gene editing tools to knock-down expression of the EOMES genomic region in order to modulate expression of PD-1, without undue effort, and a high probability of success, in order to control CD8+ T cell exhaustion.

As the common technical features were known in the art at the time of the invention, they cannot be considered common special technical features that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Groups I and II lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature

Note concerning item 4: Claims 7-9, 11-27, 34-36, 38-81 are multiple dependent claims and are not drafted according to the second and third sentences of PCT Rule 6.4(a).

Note concerning claim 29: Methods claim 29 is written to depend from composition claim 27, but logically should depend from claim 28, because claim 28 is a method claim and also has proper antecedent precedent for "gene expression regulator region". For the purposes of the international search and opinion, claim 29 is interpreted to depend from claim 28.