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(54) Title: MACROPHAGE-SPECIFIC PROMOTERS AND USES THEREOF

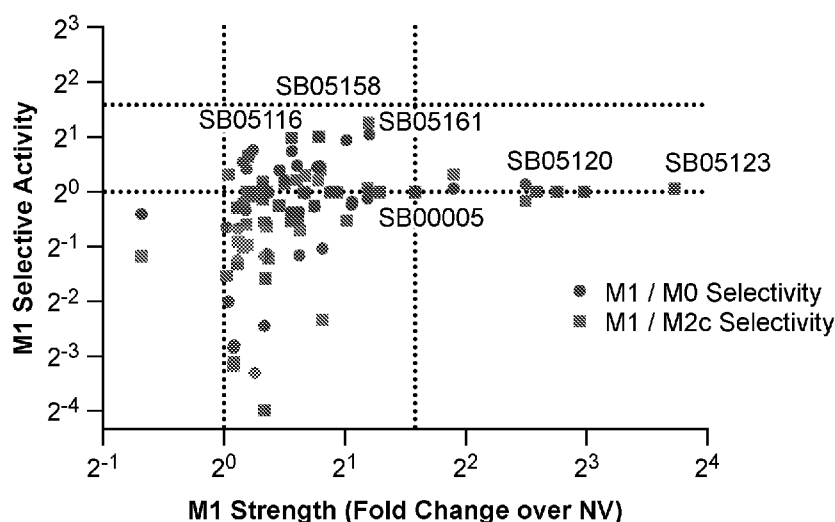


FIG. 1

(57) Abstract: Described herein are compositions and methods for regulating expression of effector molecules using engineered macrophage-specific promoters. Immunoresponsive cells (such as macrophages) comprising the same are also described.

SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN,
GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
- *of inventorship (Rule 4.17(iv))*

Published:

- *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))*

(88) Date of publication of the international search report:

25 July 2024 (25.07.2024)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2023/082548

A. CLASSIFICATION OF SUBJECT MATTER

IPC: A61K 39/00 (2024.01); C12N 5/00 (2024.01); C12N 15/113 (2024.01); C12N 15/85 (2024.01)

CPC: C12N 15/113; A61K 39/4614; C12N 5/0645; C12N 15/85; A61K 39/4631; A61K 39/4632; A61K 39/4633; A61K 39/4635; A61K 39/4637; C12N 2310/14; C12N 2830/008

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	US 2007/0161031 A1 (TRINKLEIN et al.) 12 July 2007 (12.07.2007) entire document	1, 6
A	PIETILA et al. "Multiple NF-kappaB and IFN regulatory factor family transcription factors regulate CCL19 gene expression in human monocyte-derived dendritic cells," Journal of Immunology, 01 January 2007 (01.01.2007), Vol. 178, No. 1, Pgs. 253-261. entire document	1, 6
A	US 2021/0207096 A1 (THUNDER BIOTECH INC.) 08 July 2021 (08.07.2021) entire document	1, 6
A	WO 2022/072712 A1 (VOR BIOPHARMA INC.) 07 April 2022 (07.04.2022) entire document	1, 6
A	US 2021/0393682 A1 (ZHU) 23 December 2021 (23.12.2021) entire document	1, 6



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

"D" document cited by the applicant in the international application

"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

12 April 2024 (12.04.2024)

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04 June 2024 (04.06.2024)

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2023/082548

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☐ forming part of the international application as filed.
 - b. ☒ furnished subsequent to the international filing date for the purposes of international search (Rule 13ter.1(a)),
☒ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

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.: **8-20**
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:

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 need to be paid.

Group I+: claims 1-7 are drawn to an engineered macrophage-specific promoter systems.

The first invention of Group I+ is restricted to an engineered macrophage-specific promoter system comprising a regulatory element comprising SEQ ID NO: 132 and a heterologous payload. The first named invention has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines. Specifically, the first named invention was selected based on the first listed compound species presented in the claims (see claims 1 and 6). It is believed that claims 1 and 6 read on this first named invention and thus these claims will be searched without fee to the extent that they read on an engineered macrophage-specific promoter system comprising a regulatory element comprising SEQ ID NO: 132 and a heterologous payload.

Applicant is invited to elect additional macrophage-specific promoter systems to be searched in a specific combination by paying additional fee for each set of election. An exemplary election would be an engineered macrophage-specific promoter system comprising a regulatory element comprising SEQ ID NO: 133 and a heterologous payload. Additional macrophage-specific promoter systems will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ 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 Groups I+ formulas do not share a significant structural element responsible for forming a macrophage-specific promoter system requiring the selection of alternative regulatory elements "wherein the regulatory element comprises a sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NO: 132 – 138" and "comprising at least one regulatory element comprising a nucleotide sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%,

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

at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 1-29, 81-82, 88-97, 119-122, 132-138, 142 - 163, 97- 313, 139-141, 314 - 371, 390, 392 - 393, and 420-443.”

Additionally, even if Groups I+ were considered to share the technical features of An engineered macrophage-specific promoter system comprising: a. a regulatory element, wherein the regulatory element is derived from a promoter of a gene selected from the group consisting of CCL19, CCR7, CXCL 11, GBP5, IDO1, UBD, and UNQ6494.1; and b. a heterologous payload, optionally wherein the heterologous payload is selected from the group consisting of transcriptions factors, cytokines, receptors, enzymes, chemokines, antibodies, fragments of antibodies, miRNAs, and shRNAs, wherein the regulatory element exhibits greater activity in an M1 macrophage compared to an M2 or MO macrophage, and wherein the regulatory element is or comprises an enhancer region that is derived from a promoter of a gene that is more highly expressed in M1 macrophage compared to M2 or MO macrophages, optionally wherein M2 macrophages are selected from the group consisting of M2a macrophages, M2b macrophages, and M2c macrophages; An engineered macrophage-specific promoter system comprising: a. a regulatory element, wherein the regulatory element is derived from a promoter of a gene selected from the group consisting of CD28, SOCS3, PLXDC1, IL7R ZNF704, LNCAROD, MRCJ, and ID3; and b. a heterologous payload, optionally wherein the heterologous payload is selected from the group consisting of transcriptions factors, cytokines, receptors, enzymes, chemokines, antibodies, fragments of antibodies, miRNAs, and shRNAs, wherein the regulatory element exhibits greater activity in an M2 macrophage compared to an M1 or MO macrophage, and wherein the regulatory element is or comprises an enhancer region that is derived from a promoter of a gene that is more highly expressed in M2 macrophage compared to M1 or MO macrophages, optionally wherein M2 macrophages are selected from the group consisting of M2a macrophages, M2b macrophages, and M2c macrophages; An engineered macrophage-specific promoter comprising an ablation of at least one nucleotide motif, wherein the ablation increases specific activity of the engineered macrophage-specific promoter in M1 macrophages, as compared to activity of a corresponding macrophage-specific promoter lacking the ablation in M1 macrophages, optionally wherein the corresponding macrophage-specific promoter lacking the ablation in M1 macrophages is a wildtype macrophage promoter, optionally wherein the ablation comprises a substitution or deletion of one or more nucleotides of the at least one nucleotide motif; An engineered macrophage-specific promoter comprising at least one regulatory element, wherein the regulatory element exhibits greater activity in an M1 macrophage compared to an M2 or MO macrophage or exhibits greater activity in an M2 macrophage compared to an M1 or MO macrophage, optionally wherein the engineered macrophage-specific promoter comprises at least 2, at least 3, at least 4, or at least 5 regulatory elements, optionally wherein each of the regulatory elements are the same or different, optionally wherein M2 macrophages are selected from the group consisting of M2a macrophages, M2b macrophages, and M2c macrophages; and an engineered macrophage-specific promoter comprising at least one regulatory element; optionally wherein the regulatory element or the engineered macrophage-specific promoter is operably linked to a minimal promoter, wherein optionally the minimal promoter comprises a sequence of a promoter selected from minP, NFkB response element, CREB response element, NFAT response element, SRF response element 1, SRF response element 2, API response element, TCFLEF response element promoter fusion, Hypoxia responsive element, SMAD binding element, STAT3 binding site, minCMV, YB TATA, minTK, SCP3, YB-SCP3, inducer molecule responsive promoters, CMV, EFS, SFFV, SV40, MND, PGK, UbC, hEFlaV1, hCAGG, hEFlaV2, hACTb, heJF4A1, hGAPDH, hGRP78, hGRP94, hHSP70, hKINb, hUBIb, and tandem repeats thereof, optionally wherein the engineered macrophage-specific promoter system further comprises a translation initiator site, optionally wherein the translation initiator site is or comprises a Kozak sequence, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2021/0393682 A1 to Zhu teaches an engineered macrophage-specific promoter system comprising: a. a regulatory element and b. a heterologous payload (a macrophage... a reporter gene operably linked to a Hom-1 promoter, Para. [0007]), wherein the regulatory element exhibits greater activity in an M1 macrophage compared to an M2 or MO macrophage, and wherein the regulatory element is or comprises an enhancer region that is derived from a promoter of a gene that is more highly expressed in M1 macrophage compared to M2 or MO macrophages (We found that Hom-1 promotes and is required for M1 activation of human macrophages, but not required for the expression of critical genes involved in M2 activation, Para. [0037]); an engineered macrophage-specific promoter system comprising: a. a regulatory element and b. a heterologous payload (a macrophage... a reporter gene operably linked to a Hom-1 promoter, Para. [0007]), wherein the regulatory element exhibits greater activity in an M1 macrophage compared to an M2 or MO macrophage, and wherein the regulatory element is or comprises an enhancer region that is derived from a promoter of a gene that is more highly expressed in M1 macrophage compared to M2 or MO macrophages

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

(We found that Hom-1 promotes and is required for M1 activation of human macrophages, but not required for the expression of critical genes involved in M2 activation, Para. [0037]); an engineered macrophage-specific promoter comprising at least one regulatory element, wherein the regulatory element exhibits greater activity in an M1 macrophage compared to an M2 or MO macrophage or exhibits greater activity in an M2 macrophage compared to an M1 or MO macrophage (a macrophage... a reporter gene operably linked to a Hom-1 promoter, Para. [0007]; We found that Hom-1 promotes and is required for M1 activation of human macrophages, but not required for the expression of critical genes involved in M2 activation, Para. [0037]); and an engineered macrophage-specific promoter comprising at least one regulatory element (a macrophage... a reporter gene operably linked to a Hom-1 promoter, Para. [0007]; We found that Hom-1 promotes and is required for M1 activation of human macrophages, but not required for the expression of critical genes involved in M2 activation, Para. [0037]).

Further, WO 2022/072712 A1 to Vor Biopharma Inc. teaches a CCR7 promoter (a CCR7 promoter, Para. [0082]); a CD28 promoter (a CD28 promoter, Para. [0082]).

Further, US 2021/0207096 A1 to Thunder Biotech Inc. teaches an engineered macrophage-specific promoter comprising an ablation of at least one nucleotide motif (Activation of the intracellular portion of the chimeric receptor may polarize the macrophage into an M1... macrophage, Para. [0050]; a nucleic acid encoding a chimeric receptor may be operably linked to a promoter, Para. [0096]; mutations in the regulatory and/or promoter sequences of polypeptide expression, especially leading to a modification of the rate of expression of the polypeptide, Para. [0120]).

Further, it would have been obvious to one of ordinary skill in the art before the priority date for the ablation to increase specific activity of the engineered macrophage-specific promoter in M1 macrophages, as compared to activity of a corresponding macrophage-specific promoter lacking the ablation in M1 macrophages, since where the general conditions of the claim are disclosed in the prior art, discovering the optimum or workable ranges involves only routine skill in the art.

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.

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.: **1, 6**

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.