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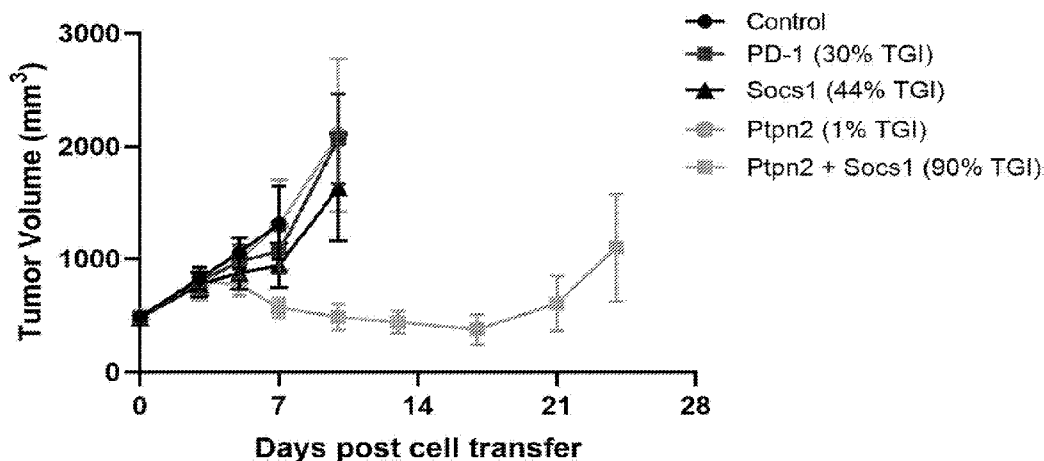
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(54) Title: COMBINATION GENE TARGETS FOR IMPROVED IMMUNOTHERAPY

Fig. 1



(57) Abstract: The present disclosure provides methods and compositions related to the modification of immune effector cells to increase therapeutic efficacy. In some embodiments, immune effector cells modified to reduce expression of one or more endogenous target genes, or to reduce one or more functions of an endogenous protein to enhance effector functions of the immune cells are provided. In some embodiments, immune effector cells further modified by introduction of transgenes conferring antigen specificity, such as exogenous T cell receptors (TCRs) or chimeric antigen receptors (CARs) are provided. Methods of treating a cell proliferative disorder, such as a cancer, using the modified immune effector cells described herein are also provided.



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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/016623

Box No. 1 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:

☒ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

☒ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 7-18 and 185-194 were searched.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/016623

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.: 6-44, 54-58, 64-83, 104-138, 157-160, 198, 201-204, 210-226, 230, 236-240
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:
See extra sheet(s).

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, 2, 45, 46, 49-51, 59-61, 84, 85, 88-93, 139-144, 161-166, 177-180, 187-190, 197, 199, 200, 205-209, 227-229, and 231-235 to the extent that they read on targeting domains complementary to SEQ ID NO:7 and SEQ ID NO:185.

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.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/016623

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 35/17; C12N 5/0783; C12N 9/16; C12N 9/22; C12N 15/90; C12Q 1/68 (2020.01)

CPC - A61K 35/17; C07K 14/70517; C12N 9/22; C12N 15/11; C12N 15/1135 (2020.05)

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	WO 2018/148378 A1 (DANA-FARBER CANCER INSTITUTE, INC. et al) 16 August 2018 (16.08.2018) entire document	1, 2, 45, 46, 49-51, 59-61, 84, 85
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Y		88, 89, 139, 140, 161, 162, 197
X	US 2017/0002426 A1 (FOX CHASE CANCER CENTER) 05 January 2017 (05.01.2017) entire document	177, 178
X	US 2016/0120905 A1 (CELLECTIS) 05 May 2016 (05.05.2016) entire document	187, 188, 199, 200, 205-209, 227-229, 231-235
Y	US 2015/0232881 A1 (GLUCKSMANN et al) 20 August 2015 (20.08.2015) entire document	88, 89, 139, 140, 161, 162, 197
P, X	WO 2019/178422 A1 (KSQ THERAPEUTICS, INC.) 19 September 2019 (19.09.2019) entire document	1, 2, 45, 46, 49-51, 59-61, 84, 85, 88-93, 139-144, 161-166, 177-180, 187-190, 197, 199, 200, 205-209, 227-229, 231-235

☐ 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

06 July 2020

Date of mailing of the international search report

23 JUL 2020

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Continued from Box No. III 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 need to be paid.

Group I+: claims 1-5, 45-53, 59-63, 84-103, 139-156, 161-197, 199, 200, 205-209, 227-229, and 231-235 are drawn to RNA molecules for reducing expression and/or function of SOCS1, PTPN2, and ZC3H12A, and systems, methods, and compositions comprising the same.

The first invention of Group I+ is restricted to RNA molecules for reducing expression and/or function of SOCS1 and PTPN2, and systems, methods, and compositions comprising the same, wherein the RNA molecule for targeting SOCS1 is selected to be SEQ ID NO:7 and the RNA molecule for targeting PTPN2 is selected to be SEQ ID NO:185. It is believed that claims 1, 2, 45, 46, 49-51, 59-61, 84, 85, 88-93, 139-144, 161-166, 177-180, 187-190, 197, 199, 200, 205-209, 227-229, and 231-235 read on this first named invention and thus these claims will be searched without fee to the extent that they read on RNA molecules of SEQ ID NO:7 and SEQ ID NO:185.

Applicant is invited to elect additional RNA molecules, each with specified SEQ ID NO, to be searched in a specific combination by paying an additional fee for each set of election. An exemplary election would be RNA molecules for reducing expression and/or function of SOCS1 and ZC3H12A, and systems, methods, and compositions comprising the same, wherein the RNA molecule for targeting SOCS1 is selected to be SEQ ID NO:8 and the RNA molecule for targeting ZC3H12A is selected to be SEQ ID NO:208. Additional RNA molecules 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 for producing a modified immune effector cell, requiring the selection of alternatives for the RNA molecules, where "at least one SOCS1-targeting gRNA molecule comprising a targeting domain sequence that binds to a target DNA sequence selected from the group consisting of SEQ ID NOs: 7-151" and "at least one PTPN2-targeting gRNA molecule comprising a targeting domain sequence that binds to a target DNA sequence selected from the group consisting of SEQ ID NOs: 185-207" and "at least one ZC3H12A-targeting gRNA molecule comprising a targeting domain sequence that binds to a target DNA sequence selected from the group consisting of SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575".

Additionally, even if Groups I+ were considered to share the technical features of a modified immune effector cell comprising a gene-regulating system capable of reducing expression and/or function of at least two endogenous target genes selected from SOCS1, PTPN2, and ZC3H12A, wherein the reduced expression and/or function of the at least two endogenous target genes enhances an effector function of the immune effector cell; a modified immune effector cell, comprising one or more exogenous polynucleotides encoding at least two nucleic acid inhibitors of at least two endogenous target genes selected from SOCS1, PTPN2, and ZC3H12A; a gene-regulating system capable of reducing expression of at least two endogenous target genes in a cell selected from SOCS1, PTPN2, and ZC3H12A, comprising (i) a nucleic acid molecule; (ii) an enzymatic protein; or (iii) a nucleic acid molecule and an enzymatic protein; a composition comprising a plurality of gRNA molecules, wherein the plurality of gRNA molecules comprises at least one gRNA molecule targeting a first target gene and at least one gRNA molecule targeting a second target gene, wherein the first and second target gene are selected from SOCS1, PTPN2, and ZC3H12A; a polynucleotide molecule encoding a plurality of siRNA or shRNA molecules, wherein the plurality of siRNA or shRNA molecules comprises at least one siRNA or shRNA molecule targeting a first target gene and at least one siRNA or shRNA molecule targeting a second target gene, wherein the first and second target gene are selected from SOCS1, PTPN2, and ZC3H12A; a polynucleotide molecule encoding at least one TALEN, zinc finger, or meganuclease protein targeting a first target gene and at least one TALEN, zinc finger, or meganuclease protein targeting a second target gene, wherein the first and second target gene are selected from SOCS1, PTPN2, and ZC3H12A; these shared technical features do not represent a contribution over the prior art.

Specifically, US 2016/0120905 A1 to Collectis discloses a modified immune effector cell (the inventors have achieved the production of genetically modified T-cells, Para. [0012]) comprising a gene-regulating system capable of reducing expression and/or function of at least two endogenous target genes selected from SOCS1, PTPN2, and ZC3H12A (the inactivation of the expression of immune checkpoint genes in T cells that allow the production of engineered allogeneic T cellswas made possible by gene inactivation using specific TALE-nucleases directed against ...genes encoding immune checkpoint proteins, Para. [0012]; Table 1 ...shows immune checkpoint genes that can be inactivated according to the teaching of the present invention in order to improve the efficiency and fitness of the engineered T-cells, Para. [0085]; Scos1, Ptpn2, Table 1), wherein the reduced expression and/or function of the at least two endogenous target genes enhances an effector function of the immune effector cell (i) immune checkpoint moleculeswhen engaged by its ligands ...on antigen presenting cells, T-cell activation and effector function are inhibited. Thus the present invention relates to a method of engineering T-cells, especially for immunotherapy, comprising genetically modifying T-cells by inactivating at least one protein involved in the immune check-point, Para. [0083]; a modified immune effector cell (the inventors have achieved the production of genetically modified T-cells, Para. [0012]), comprising one or more exogenous polynucleotides encoding at least two nucleic acid inhibitors of at least two endogenous target genes selected from SOCS1, PTPN2, and ZC3H12A (the inactivation of the expression of immune checkpoint genes in T cells that allow the production of engineered allogeneic T cellswas made possible by gene inactivation using specific TALE-nucleases directed against ...genes encoding immune checkpoint proteins, Para. [0012]; Table 1 ...shows immune checkpoint genes that can be inactivated according to the teaching of the present invention in order to improve the efficiency and fitness

of the engineered T-cells, Para. [0085]; Scos1, Ptpn2, Table 1; the present invention relates to the polypeptides and the polynucleotides, which encode the rare-cutting endonucleases, to precisely target the above genes of interest, in particular ...immune checkpoint genes, thereby enabling the genetic modification of the T-cells for immunotherapy. ...more particularly specific target sequences within these genes and TALE-nucleases designed to respectively target those genes, Para. [0020]; [t]he isolated cells of the present invention or cell lines can further comprise exogenous recombinant polynucleotides, Para. [0021]; a gene-regulating system (specific TALE-nucleases directed against ...genes encoding immune checkpoint proteins, Para. [0012]) capable of reducing expression of at least two endogenous target genes in a cell selected from SOCS1, PTPN2, and ZC3H12A, comprising (i) a nucleic acid molecule; (ii) an enzymatic protein; or (iii) a nucleic acid molecule and an enzymatic protein (the inactivation of the expression of immune checkpoint genes in T cells that allow the production of engineered allogeneic T cells ...was made possible by gene inactivation using specific TALE-nucleases directed against ...genes encoding immune checkpoint proteins, Para. [0012]; Table 1 ...shows immune checkpoint genes that can be inactivated according to the teaching of the present invention in order to improve the efficiency and fitness of the engineered T-cells, Para. [0085]; Scos1, Ptpn2, Table 1; the present invention relates to the polypeptides and the polynucleotides, which encode the rare-cutting endonucleases, to precisely target the above genes of interest, in particular ...immune checkpoint genes, thereby enabling the genetic modification of the T-cells for immunotherapy. ...more particularly specific target sequences within these genes and TALE-nucleases designed to respectively target those genes, Para. [0020]; [t]he isolated cells of the present invention or cell lines can further comprise exogenous recombinant polynucleotides, Para. [0021]; the genetic modification step of the method further comprises a step of introduction into cells an exogenous nucleic acid comprising at least a sequence homologous to a portion of the target nucleic acid sequence, such that homologous recombination occurs between the target nucleic acid sequence and the exogenous nucleic acid, Para. [0159]; a polynucleotide molecule encoding at least one TALEN, zinc finger, or meganuclease protein (the present invention relates to the polypeptides and the polynucleotides, which encode the rare-cutting endonucleases, Para. [0020]; said rare-cutting endonuclease can be a meganuclease, a Zinc finger nuclease or a TALE-nuclease, Para. [0152]) targeting a first target gene and at least one TALEN, zinc finger, or meganuclease protein targeting a second target gene (the present invention relates to the polypeptides and the polynucleotides, which encode the rare-cutting endonucleases, to precisely target the above genes of interest, in particular ...immune checkpoint genes, thereby enabling the genetic modification of the T-cells for immunotherapy. ...more particularly specific target sequences within these genes and TALE-nucleases designed to respectively target those genes, Para. [0020]), wherein the first and second target gene are selected from SOCS1, PTPN2, and ZC3H12A (Table 1 ...shows immune checkpoint genes that can be inactivated according to the teaching of the present invention in order to improve the efficiency and fitness of the engineered T-cells, Para. [0085]; Scos1, Ptpn2, Table 1).

US 2015/0232881 A1 to Glucksmann et al. discloses a composition (a composition, Para. [0071]) comprising a plurality of gRNA molecules (a composition, e.g., a pharmaceutical composition, comprising ...a governing gRNA molecule ...Cas9 molecule ...a second gRNA molecule, Paras. [0071]-[0075]), wherein the plurality of gRNA molecules comprises at least one gRNA molecule targeting a first target gene and at least one gRNA molecule targeting a second target gene (two gRNAs ...two gRNAs complex with Cas9 ...on either side of the target position, e.g., the first gRNA is used to target upstream (i.e., 5') of the target position and the second gRNA is used to target downstream (i.e., 3') of the target position, Para. [1106]), wherein the first and second target gene are selected from SOCS1, PTPN2, and ZC3H12A ([e]xemplary metabolic targets disclosed herein may be modulated using CRISPR/Cas9 as described herein, Para. [1429]; SOCS1, Table VII-16).

US 2017/0002426 A1 to Fox Chase Cancer Center discloses a polynucleotide molecule encoding a plurality of siRNA or shRNA molecules (a custom panel of siRNAs, Para. [0082]; an siRNA agent or vector or transgene encoding same, Para. [0053]), wherein the plurality of siRNA or shRNA molecules comprises at least one siRNA or shRNA molecule targeting a first target gene and at least one siRNA or shRNA molecule targeting a second target gene, wherein the first and second target gene are selected from SOCS1, PTPN2, and ZC3H12A (the custom siRNA library targeting 638 human genes was designed and synthesized with two siRNA duplexes for each gene target, Para. [0059]; sequences for the siRNA molecules disclosed herein are provided in Table 3, Para. [0009]; SEQ ID NO:857, SOCS1; SEQ ID NO:618, PTPN2, Table 3).

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