



- (51) **International Patent Classification:**
C12N 5/0783 (2010.01) C07K 14/705 (2006.01)
- (21) **International Application Number:**
PCT/US2020/041338
- (22) **International Filing Date:**
09 July 2020 (09.07.2020)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
62/871,946 09 July 2019 (09.07.2019) US
- (71) **Applicants:** **THE CHILDREN'S MERCY HOSPITAL** [US/US]; 2401 Gillham Road, Kansas City, Missouri 64108 (US). **THE UNIVERSITY OF KANSAS** (legal representatives of the deceased inventor) [US/US]; 245 Strong Hall, 1450 Jayhawk Boulevard, Lawrence, Kansas 66045 (US).
- (72) **Inventor:** **YANKEE, Thomas** (deceased).
- (72) **Inventors:** **SENG, Amara**; 4239 Leavenworth Road, Kansas City, Kansas 66104 (US). **SZAREJKO, John**;

1012 Canterbury Lane, Liberty, Missouri 64068 (US). **MARKIEWICZ, Mary**; 3908 W 124th Street, Leawood, Kansas 66209 (US). **FISCHER, Ryan**; 22530 W 61st Street, Shawnee, Kansas 66226 (US).

(74) **Agent:** **COOK, Crissa A.**; Hovey Williams LLP, 10801 Mastin Blvd., Suite 1000, Overland Park, Kansas 66210 (US).

(81) **Designated States** (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) **Designated States** (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH,

(54) **Title:** ENGINEERED REGULATORY T CELLS

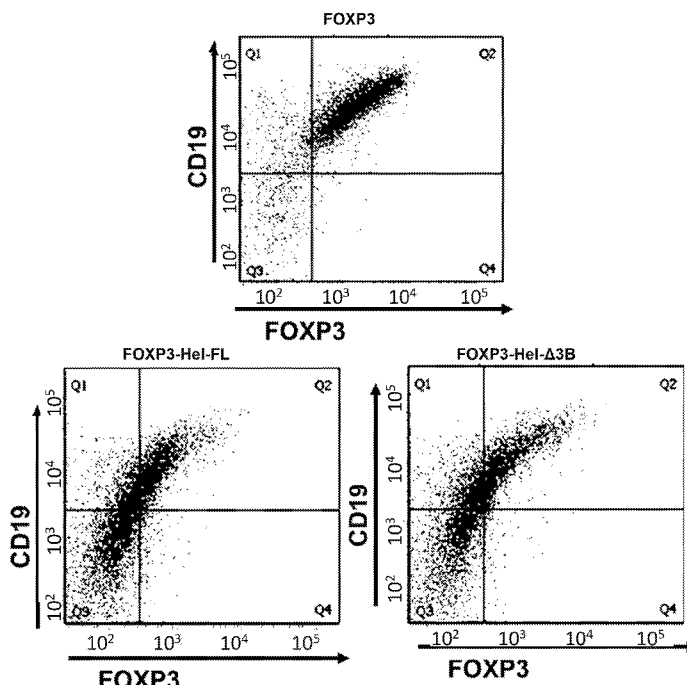


Fig. 1A

(57) **Abstract:** Cell therapy compositions comprising engineered human regulatory T cells (eTregs) characterized by ectopic overexpression of FOXP3 and Helios protein, produced via introduction of separate nucleic acid constructs respectively encoding FOXP3 and Helios (FOXP3+Helios+ eTregs). Cell therapy compositions comprising mixed populations of CD4+ and CD8+ Treg cells each with ectopic overexpression of FOXP3 and Helios. Methods of making and use the same for therapies involving inflammation and/or a disorder of the immune system.



GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, 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))*

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))*
- *with sequence listing part of description (Rule 5.2(a))*

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

25 February 2021 (25.02.2021)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/41338

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.: 29
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 searched, the appropriate additional search fees must be paid.

Group I: claims 1-17, drawn to a cell therapy composition.

Group II: claims 18-28, 30-37 drawn to a method of prophylactically and/or therapeutically treating a disease or condition.

Continued in Supplemental Boxes

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.:
1-17

- 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/US 20/41338

A. CLASSIFICATION OF SUBJECT MATTER
 IPC - C12N 5/0783, C07K 14/705 (2020.01)
 CPC - C12N 5/0637, C07K 14/7155, C12N 2510/00

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.
Y	UGIMOTO et al., Foxp3-dependent and -independent molecules specific for CD25+CD4+ natural regulatory T cells revealed by DNA microarray analysis, International Immunology, 13 June 2006, Vol 18, No 8, pp 1197-1209. Especially pg 1198, col 2, para 2; pg 1207, col 2, para 2; pg 1208, col 1, para 1	1-17
Y	TAKATORI et al. Helios Enhances Treg Cell Function in Cooperation With FoxP3, Arthritis & Rheumatology, June 2015, Vol. 67, No. 6, pp 1491-1502. Especially Abstract; pg 1495, col 1, para 2	1-17
Y	WO 2017/218850 AI (MEMORIAL SLOAN KETTERING CANCER CENTER) 21 December 2017 (21.12.2017) para [0024], para [0081], para [0090], para [0095]	2, 4 and 6
Y	US 2016/0194605 A1 (THE HENRY M. JACKSON FOUNDATION FOR THE ADVANCEMENT OF MILITARY MEDICINE, INC.) 7 July 2016 (07.07.2016) para [0016], para [0020], para [0039], para [0048], para [0052], para [0058], para [0059]	3, 7-12 and 14-15
Y	US 2016/0151465 A1 (BELLICUM PHARMACEUTICALS, INC.) 2 June 2016 (02.06.2016) para [0009], para [0183]	16-17

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	"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
"D" document cited by the applicant in the international application	"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
"E" earlier application or patent but published on or after the international filing date	"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
"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)	"&" document member of the same patent family
"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	

Date of the actual completion of the international search
 30 October 2020

Date of mailing of the international search report
 15 JAN 2021

Name and mailing address of the ISA/US
 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 Young
 Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/US 20/41338

Continuation of:

Box No. III. Observations where unity of invention is lacking

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 includes the special technical feature of a composition which differs from the special technical feature of a method, as disclosed by Group II.

Common Technical Features

The inventions of Groups I and II share the technical feature of FOXP3+Helios+ eTregs.

However, these shared technical features do not represent a contribution over prior art in view of the article "Foxp3-dependent and -independent molecules specific for CD25+CD4+ natural regulatory T cells revealed by DNA microarray analysis" to Sugimoto et al. (hereinafter 'Sugimoto') (International Immunology, 2006, Vol. 18, No. 8, pp. 1197-1209) in view of the article "Helios Enhances Treg Cell Function in Cooperation With FoxP3" to Takatori et al. (hereinafter 'Takatori') (Arthritis & Rheumatology, June 2015, Vol. 67, No. 6, pp 1491-1502.).

Sugimoto teaches (instant claim 1) a cell composition comprising engineered human regulatory T cells (eTregs) characterized by ectopic overexpression of nucleic acid construct encoding FOXP3 (FOXP3+ eTregs) (pg 1198, col 2, para 2 - 'For Foxp3 retroviral transduction, sorted CD25-CD4+ T cells were activated with anti-CD3.....and IL-2.....in the presence of X-irradiated (18 Gy) BALB/c splenocytes as antigen presenting cells (APCs) for 24 h, and were infected with the retroviral supernatant of Foxp3/MIGR1 or MIGR1 transfected Plat-E packaging cells as described previously.'). Sugimoto further teaches eTregs characterized by ectopic overexpression of nucleic acid constructs encoding Helios (Helios+ eTregs) (pg 1207, col 2, para 2 - 'The forced gene expression resulted in variable in vitro survival depending upon which gene was introduced (data not shown). For instance, Helios- and to a lesser extent Socs2-transduced cells had significant low survival after transduction.').

Sugimoto does not specifically teach eTregs characterized by ectopic overexpression of separate nucleic acid constructs respectively encoding FOXP3 and Helios (FOXP3+Helios+ eTregs). Given that Helios expression is independent of that of FOXP3 and appears to be upstream of FOXP3 expression (Sugimoto, pg 1208, col 1, para 1 - 'We surmise that Foxp3-independent expression of some Treg-specific genes might be related to their unique mode of activation in the thymus and periphery (e.g. possible co-stimulation via CTL associated molecule-4) and their high self-activity, rather than Foxp3-regulated suppression. The expression of helios appears to be upstream of Foxp3 expression since helios is transcribed in more immature thymocytes than those expressing Foxp3. '), it would have been obvious to one of ordinary skill in the art to have provided eTregs characterized by ectopic overexpression FOXP3 and Helios in separate development stages using separate nucleic acid constructs encoding FOXP3 and Helios, respectively.

Sugimoto does not specifically teach a cell therapy composition. However, Takatori teaches forced expression of Helios in FOXP3+ Treg cell enhanced the therapeutic function of FOXP3+ Treg cells (Abstract - 'The forced expression of Helios enhanced the expression of various Treg cell related molecules and the suppressive function in murine induced Treg cells. Helios-mediated enhancement of the suppressive function of induced Treg cells was obvious in FoxP3-sufficient CD4+ T cells but not in FoxP3-deficient CD4+ T cells. Conclusion. Our findings indicate that Helios enhances induced Treg cell function in cooperation with FoxP3. '; pg 1498, col 2, para 2 to pg 1499, col 1, para 1, We next evaluated the suppressive effect of Helios+ induced Treg cells and control induced Treg cells on the proliferation of naive CD4+ T cells. As shown in Figure 5B, Helios+ induced Treg cells exhibited stronger suppressive effects on the proliferation of responder cells than control induced Treg cells did.'). Given that the expression of both Helios and FOXP3 are essential for the suppressive functions of Treg, as taught by Takatori, one of ordinary skill in the art would apply the eTreg comprising ectopic overexpression of FOXP3 and Helios of Sugimoto in the treatment of arthritis by TCZ, since Helios expression was significantly increased in RA patients who showed good clinical response to TCZ (Figure 1) (Takatori, pg 1501, col 1, para 3).

As said technical features were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the groups

Groups I and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Item 4 (continued):

Claim 29 is an improper multiple dependent claim because it is a dependent claim and is not drafted in accordance with the second and third sentences of Rule 6.4(a).