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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))

[Continued on next page]

(54) Title: DOSAGE COMPENSATING TRANSGENES AND CELLS

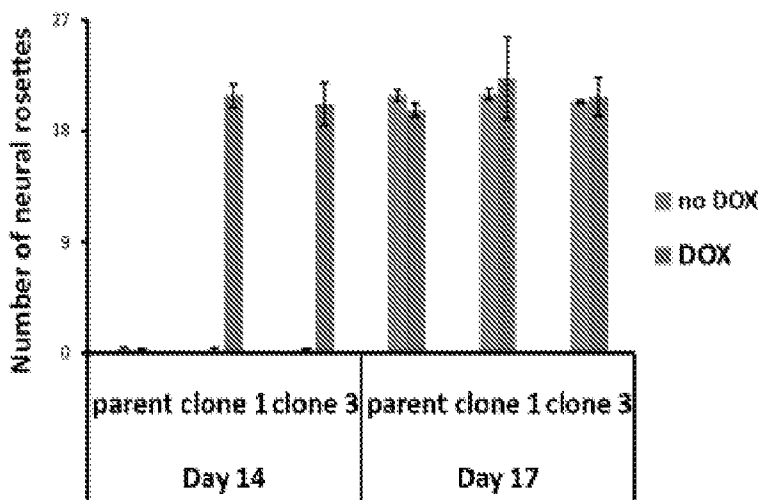


FIG. 6B

(57) Abstract: Methods and compositions for reducing expression of genes on Chromosome 21 ("Chr 21") by targeting an XIST transgene to the Dual specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A) gene or a Regulator of calcineurin 1 (RCAN1) gene, and cells and transgenic animals comprising an XIST transgene inserted into a DYRK1A or RCAN1 allele, e.g., cells and animals trisomic for human Chr 21 and mouse Chr 16.



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

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/027525

A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - C12N 15/63 (2014.01) USPC - 536/24.5 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) IPC(8) - A61K 31/7088, 35/00, 48/00; C07H 21/02, 21/04; C12N 5/10, 15/63, 15/85, 15/90 (2014.01) USPC - 424/93.1; 435/320.1, 325, 455, 463; 514/44R; 536/23.1, 24.31, 24.33, 24.5 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched CPC - A61K 48/00, 48/005; C07K 2319/81; C12N 9/22, 15/85, 15/86, 15/113, 15/907, 2310/11, 2330/10, 2750/14143 (2014.06) Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) PatBase, Google Patents, PubMed		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/0160417 A1 (LAWRENCE et al) 24 June 2010 (24.06.2010) entire document	1-4
A	US 2012/0252123 A1 (LAWRENCE et al) 04 October 2012 (04.10.2012) entire document	1-4, 8
A	US 2012/0142758 A1 (COLLARD et al) 07 June 2012 (07.06.2012) entire document	1-4, 8
A	LEPAGNOL-BESTEL et al. "DYRK1A interacts with the REST/NRSF-SWI/SNF chromatin remodelling complex to deregulate gene clusters involved in the neuronal phenotypic traits of Down syndrome," Human Molecular Genetics, 12 February 2009 (12.02.2009), Vol. 18, No. 8 Pgs. 1405-1414. entire document	1-4, 8
A	CANZONETTA et al. "DYRK1A-Dosage Imbalance Perturbs NRSF/REST Levels, Deregulating Pluripotency and Embryonic Stem Cell Fate in Down Syndrome," The American Journal of Human Genetics, 12 September 2008 (12.09.2008), Vol. 83, Pgs. 388-400. entire document	1-4, 8
P, X	JIANG et al. "Translating dosage compensation to trisomy 21," Nature, 15 August 2013 (15.08.2013), Vol. 500, Pgs. 296-300 and Supplemental Information. entire document	1-4, 8
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/>		
* 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 29 August 2014		Date of mailing of the international search report 12 SEP 2014
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. 571-273-3201		Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2014/027525

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.: 5-7, 9-19
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 first Extra Sheet

- 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.:
1-4 and 8 to the extent that they read on SEQ ID NO:14 (3G/FL/hXIST/DYRK1A).

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/US2014/027525

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 1-4 and 8 are drawn to a silencing vector.

The first invention of Group I+ is restricted to a silencing vector, comprising: a silencing element comprising a silencing sequence flanked by first and second targeting sequences, wherein each of the first and second targeting sequences are selected to be homologous to dual specificity tyrosinephosphorylation-regulated kinase 1A (DYRK1A) gene; and a promoter operably linked to the silencing element; further, wherein the silencing vector is selected to be SEQ ID NO:14 (3G/FL/hXIST/DYRK1A). It is believed that claims 1-4 and 8 read on this first named invention and thus these claims will be searched without fee to the extent that they read on SEQ ID NO:14 (3G/FL/hXIST/DYRK1A).

Applicant is invited to elect additional silencing vectors with specified SEQ ID NO to be searched in a specific combination by paying additional fee for each set of election. An exemplary election would be to a silencing vector, comprising: a silencing element comprising a silencing sequence flanked by first and second targeting sequences, wherein each of the first and second targeting sequences are selected to be homologous to dual specificity tyrosinephosphorylation-regulated kinase 1A (DYRK1A) gene; and a promoter operably linked to the silencing element; further, wherein the silencing vector is selected to be SEQ ID NO:16. Additional silencing vectors 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, requiring the selection of alternatives for the silencing vector where the "silencing vector comprising the sequence shown in SEQ ID NOs: 14, 16, 17, 18, 19, 20, 21, 22, or 23" and "silencing sequence flanked by first and second targeting sequences, wherein each of the first and second targeting sequences are homologous to at least 50 bp in, or within 1 MB of, the dual specificity tyrosinephosphorylation-regulated kinase 1A (DYRK1A) gene or the Regulator of calcineurin 1 (RCAN1) gene".

The Groups I+ share the technical features of a silencing vector comprising: a silencing element comprising a silencing sequence flanked by first and second targeting sequences, wherein each of the first and second targeting sequences are homologous to at least 50 bp in, or within 1 MB of, the dual specificity tyrosinephosphorylation-regulated kinase 1A (DYRK1A) gene or the Regulator of calcineurin 1 (RCAN1) gene; and a promoter operably linked to the silencing element. However, these shared technical features do not represent a contribution over the prior art. Specifically, US 2010/0160417 A1 to Lawrence et al. discloses a silencing vector (nucleic acid construct or vector that silences a targeted chromosomal region, Para. [0019]) comprising: a silencing element (nucleic acid constructs that include a transgene, e.g., a silencing sequence encoding an Xist RNA or other non-coding RNA that silences a segment of a chromosome, Para. [0008]); a nucleotide sequence that encodes an RNA that silences a chromosome or a segment or region thereof, Para. [0010]) comprising a silencing sequence flanked by first and second targeting sequences (first and second sequences that direct insertion of the silencing sequence into a targeted chromosome, Para. [0008]), wherein each of the first and second targeting sequences are homologous to at least 50 bp in, or within 1 MB of, the dual specificity tyrosinephosphorylation-regulated kinase 1A (DYRK1A) gene (silencing sequence flanked by sequences, typically 400 bp-5 kb in length, homologous to the desired site of integration can be inserted e.g. by homologous recombination into the site cleaved by the endonuclease, thereby achieving a targeted insertion, Para. [0085]; first and second sequences that direct insertion of the silencing sequence into a targeted chromosome, Para. [0008]; targeting the Xist transgene to a trisomic chromosome ...using as preferred targeting sites some of the genes important to the pathology of Down's syndrome, e.g. Dyrk1A, Para. [0106]); and a promoter operably linked to the silencing element (the expression vector must have a promoter, Para. [0068]; tet promoter controlling Xist expression, and Xist-mediated silencing, Para. [0124]).

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