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(54) Title: SYNTHETIC INTRONS FOR TARGETED GENE EXPRESSION

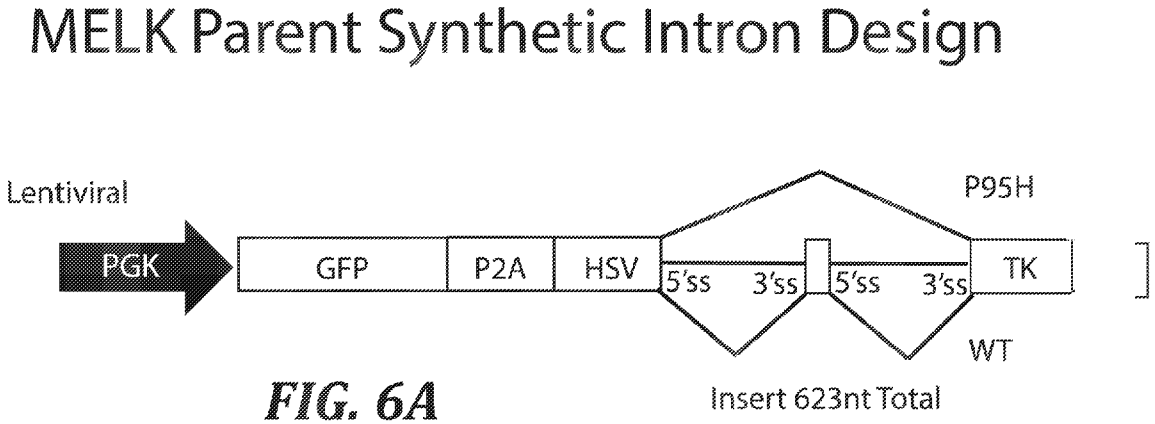

FIG. 6A

(57) Abstract: The disclosure provides artificial nucleic acid introns configured for selective splicing in cells with aberrant RNA splicing activity, *e.g.*, neoplastic cells. The artificial intron can comprise an upstream flanking exon, an upstream intron, an alternatively spliced "cassette" exon, a downstream intron, and a downstream flanking exon. Also provided are constructs integrating the artificial introns with exons in a configuration that, when the artificial intron is spliced out by the aberrant RNA splicing factors, encode a functional protein. Also disclosed are methods that employ the disclosed platform of selective expression, including, targeted gene therapy methods (*e.g.*, in cancers), diagnostics and imaging, and drug screening.

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[Continued on next page]

LV, MC, ME, 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 May 2023 (25.05.2023)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 22/78615

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. C12N 15/63, C12N 15/67, C12N 15/85 (2023.01)
ADD. A61P 35/00, C12N 9/12 (2023.01)

CPC - INV. C12N 15/63, C12N 15/67, C12N 15/85
ADD. C12N 2830/42, A61P 35/00, C12N 9/1205, A61K 38/465

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 --- A	WO 2020/033473 A1 (CHILDREN'S HOSPITAL OF PHILADELPHIA) 13 February 2020 (13.02.2020). Especially para [0075], sheet 4 fig 4A.	1, 2, 20-22 --- 3-5, 7-16, (17,18)/(1-5,7-16) --- 6, (17,18)/6
Y	PERRIN et al. Two novel mutations affecting mRNA splicing of the neurofibromatosis type 1 (NF1) gene. Hum Mutat. 1996;7(2):172-175. Especially pg 173 fig 1B, pg 173 col 2 para 4, pg 174 col 1 para 1, pg 174 figs 2, 3.	3, 10-14, (17,18)/(3,10-14)
Y --- A	ZHANG et al. Disease-associated mutation in SRSF2 misregulates splicing by altering RNA-binding affinities. Proc Natl Acad Sci U S A, 25 August 2015, Vol 112, No 34, E4726-4734. Especially abstract, pg E4726 col 1 para 2, pg E4729 table 1, pg E4731 fig 5A. pg E4731 col 1 para 3 continued to pg E4731 col 2 para 1.	4-5, 7-9, 15, 16, (17,18)/(1-5,7-16) --- 6, (17,18)/6
Y	US 2010/120022 A1US 2010/120022 A1 (AYALON-SOFFER et al.) 13 May 2010 (13.05.2010) Especially SEQ ID NO: 2053.	5, 7-9, (17,18)/(5,7-9)
A	WO 2016/149455 A2 (THE GENERAL HOSPITAL CORPORATION) 22 September 2016 (22.09.2016). Especially SEQ ID NO: 16555	6, (17,18)/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	Date of mailing of the international search report
23 February 2023	APR 07 2023
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 Kari Rodriquez Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 22/78615

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	WO 2022/087427 A1 (FRED HUTCHINSON CANCER RESEARCH CENTER) 28 April 2022 (28.04.2022). Especially claims 1, 9-12, 16-17	1-3, 7-18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 22/78615

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 fi led.

   b. ☐ furnished subsequent to the international fi ling 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:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 22/78615

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.: 19, 23-80
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 1-18, 20-22, limited to intron 10 of MELK (SEQ ID NO: 22) and MELK synthetic intron construct SEQ ID NO: 15

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.

Box 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 searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-18, 20-22, drawn to a synthetic intron construct.
The synthetic intron construct will be searched to the extent that it is derived the first named component, intron 10 of human MELK (claim 4)[instant application SEQ ID NO: 22, see claim 5], and the construct comprises the first named (in claim 6(i)): (i) a total of 623 nucleotides (nt); the first 50 nt of MELK intron 10 [from instant application SEQ ID NO: 22], the last 200 nt of MELK intron 10 [from instant application SEQ ID NO: 22], 123 nt of endogenous MELK exon 10 [from instant application SEQ ID NO: 10], the first 50 nt of MELK intron 11 [from instant application SEQ ID NO: 24], and the last 200 nt of MELK intron 11[from instant application SEQ ID NO: 24] [fully comprised by SEQ ID NO: 15, see instant application pg 80-81] This 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.
It is believed that claims 1-18, 20-22 read on this first named invention and thus these claims will be searched without fee to the extent that they encompass intron component derived from intron 10 of human MELK, SEQ ID NO: 22, and the synthetic intron construct comprises a total of 623 nucleotides (nt); the first 50 nt of MELK intron 10 [from instant application SEQ ID NO: 22], the last 200 nt of MELK intron 10 [from instant application SEQ ID NO: 22], 123 nt of endogenous MELK exon 10 [from instant application SEQ ID NO: 10], the first 50 nt of MELK intron 11 [from instant application SEQ ID NO:24], and the last 200 nt of MELK intron 11[from instant application SEQ ID NO: 24] [fully comprised by SEQ ID NO: 15].
Additional synthetic intron constructs will be searched upon payment of additional fees. Applicant must specify the claims that encompass any additional elected intronic sequences. 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. An exemplary election would be: derived from component intron 34 of human GTF3C1, construct comprises (in claim 6(ii)): a total of 325 nt: the first 125 nt of GTF3C1 intron 34 [from instant application SEQ ID NO: 25], the last 200 nt of GTF3C1 intron 34 (claims 1-18, 20-22).

The inventions listed as Group I+ inventions 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:
Among the inventions listed as Groups I+ are the specific intron and exon components that comprise the synthetic intron construct, recited therein. Each invention requires a specific intron and exon components not required by any other inventions.

No technical features are shared between the polypeptide sequences of Group I+ and, accordingly, this group lacks unity a priori.

Common Technical Feature:
Additionally, even if Groups I+ inventions were considered to share the technical features of:

Group I+ inventions share claim 1.

However, said common technical feature does not represent a contribution over the prior art, and is disclosed by WO 2020/033473 A1 to Children's Hospital of Philadelphia (hereinafter "CHHP").

As to the common technical feature (claim 1), CHHP discloses an artificial nucleic acid intron construct (para [0075]; "FIGS. 4a-c show modified SMN2/transactivator minigenes express spliced RNA transcript isoforms to constitutively exclude (CSI3) or include (CSI5) exon7 ... the SMN2 minigene comprising exons 6-7 and the 5' end of exon 8, and minimal intronic intervening sequences necessary to recapitulate SMN2 splicing. The 3' and 5' Exon7 splicing sites in the SMN2/transactivator minigene were modified to constitutively exclude (CSI3, 3' modified) or include (CSI5, 5' modified) exon 7. FIG. 4b shows that for CSI, 10% of the transcripts include exon 7, which for CSI3 and CSI5 exon 7 is either included or excluded"; SEE SHEET 4 FIG 4A), comprising an intron comprising:
(i) an upstream flanking exon (sheet 4 Fig 4A; e6: exon 6 of SMN2)
(ii) an upstream intron (sheet 4 fig 4A; i7: intron i1 of SMN2)
(iii) an alternatively spliced cassette exon (sheet 4 fig 4A: e7; exon 7 of SMN2)
(iv) a downstream intron (sheet 4 fig 4A: i2: intron i2); and
(v) a downstream flanking exon (sheet 4 fig 4A: e8: exon 8 of SMN2).

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

Therefore, Group I+ inventions lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Item 4 (continued): Claims 19, 23-80 are dependent claims and are not drafted according to the second and third sentences of PCT Rule 6.4(a).