

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property  
Organization  
International Bureau



(10) International Publication Number  
**WO 2015/196128 A3**

(43) International Publication Date  
23 December 2015 (23.12.2015)

(51) International Patent Classification:

A61K 48/00 (2006.01) A61K 31/70 (2006.01)  
A01N 43/04 (2006.01) C07H 21/02 (2006.01)

(21) International Application Number:

PCT/US2015/036771

(22) International Filing Date:

19 June 2015 (19.06.2015)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/014,620 19 June 2014 (19.06.2014) US  
62/036,880 13 August 2014 (13.08.2014) US

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(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, DK, DM,  
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,  
HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,  
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, SM, ST, SV, SY, TH, TJ, TM, TN,  
TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

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

19 May 2016



WO 2015/196128 A3

(54) Title: ALTERNATIVE NUCLEIC ACID MOLECULES AND USES THEREOF

(57) Abstract: The present disclosure provides alternative nucleosides, nucleotides, and nucleic acids, and methods of using them.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 15/36771

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(8) - A61K 48/00, A01N 43/04, A61K 31/70, C07H 21/02 (2015.01) CPC - A61K 48/00, C07H 19/06, A61K 38/00 According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) IPC(8)- A61K 48/00, A01N 43/04, A61K 31/70, C07H 21/02 (2015.01) CPC- A61K 48/00, C07H 19/06, A61K 38/00 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched USPC- 514/44R, 514/49, 514/50, 536/23.1 (keyword search, terms below) Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) PubWEST (USPT, PGPB, EPAB, JPAB), Google Patents/Scholar Search Terms Used: Modified cytosine, substituted cytosine, pseudouracil, 5-trifluoromethyl cytosone, methylpseudouracil, oligonucleotide, polynucleotide		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2014/0010861 A1 (Bancel et al.) 09 January 2014 (09.01.2014) para [0357], [0359], [1465], [1467], Table 104, claim 1	1
Y	US 2012/0100136 A1 (Patel et al.) 26 April 2012 (26.04.2012) para [0266], [0267]	1
<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 16 November 2015 (16.11.2015)		Date of mailing of the international search report <b>18 DEC 2015</b>
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 W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 15/36771

**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.: 37-43, 54-56, 69-114, 126, 128, 129, 142-159  
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:

- Please see extra sheet for Observations where unity of invention is lacking -

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.:  
claim 1 limited to 5-trifluoromethyl-cytosine and 1-methyl-pseudouracil

**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.

Continuation of:

Box NO III. Observations where unity of invention is lacking (210)

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 must be paid.

Group I+: claims 1-36, 44-53, 57-68, 118-125, 127, 133-141, drawn to a polynucleotide (claims 1-36), an mRNA encoding a polypeptide (claims 44-53, 118-125, 127, 133-141), a compound of formula I (claims 57-68, ), comprising at least two modified nucleobases or at least one modified nucleobase. The polynucleotide, mRNA and compound will be searched to the extent that the at least two modified nucleobases encompasses 5-trifluoromethyl-cytosine and 1-methyl-pseudouracil (the first named modified nucleobase pair in claim 1). It is believed that claim 1 encompasses this first named invention, and thus this claim will be searched without fee to the extent that it encompasses 5-trifluoromethyl-cytosine and 1-methyl-pseudouracil. Additional modified nucleobase(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected modified nucleobase(s). Applicants must further indicate, if applicable, the claims which encompass 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. An exemplary election would be 1,6-dim ethyl-pseudo-uracil (claims 4, 5, 57, 58, (67-68)/(57,58))

Group II: claims 115-117, 130-132 drawn to a mRNA encoding a polypeptide of interest, wherein said mRNA contains an alternative uracil/uridine and alternative cytosine/cytidine in predetermined percentages.

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 polynucleotide comprising at least one modified adenine/adenosine, not required by Group II.

Group II includes the special technical feature of a mRNA containing an alternative uracil/uridine and alternative cytosine/cytidine in predetermined ratio, not required by Group I+.

Another special technical feature of the inventions listed as Group I+ is the specific modified nucleobase(s) recited therein. Each of the inventions of Group I+ requires a unique pair of modified bases or a unique modified base, not required by the other inventions.

#### Common Technical Features

The inventions of Groups I+ and II share the technical feature of a polynucleotide comprising alternative uracil/uridine and/or alternative cytosine/cytidine.

The inventions of Groups I+ share the technical feature of claims 1, 4, 13, 17, 19, 20, 23, 25, 28, 30, 31, 33, 35, 44, 57, 118, 119, 121, 124, 133, 134, 136, and 140.

However, these shared technical feature do not represent a contribution over prior art in view of US 2014/0010861 A1 to Bancel et al. (hereinafter 'Bancel'). Bancel teaches [claim 1] a polynucleotide, comprising at least two modified bases comprising modified cytosine and modified pseudouracil (claim 1, para [1465]. When N1-methyl-pseudouridine is used in combination with 5-methylcytidine a high level of human G-CSF protein is also produced; para [1467], Table 104).

Bancel teaches [claim 4] a polynucleotide comprising at least one modified bases (claim 1, para [1465], 100% N1-methyl-pseudouridine substitution demonstrates the highest level of human G-CSF production; para [1467], Table 104).

Bancel teaches [claim 44] an isolated polynucleotide encoding a polypeptide of interest, said isolated polynucleotide comprising:

- (a) a 5' UTR optionally comprising at least one Kozak sequence;
- (b) a 3' UTR; and
- (c) at least one 5' cap structure, wherein at least one base is a modified nucleobase (claim 1).

Bancel teaches [claim 57] a compound of formula I: A-B, wherein A is Formula II (para [0359], see exemplary compound BB-30 on pg 88)

wherein the dashed line represents an optional double bond;

U is O;

Each of R1", R2", R4, R5 is, independently, H, hydroxy;

R3 is H;

m' is 0 and m" is 1; q is 0, r is 1 (para [0359], see exemplary compound BB-30 on pg 88);

each of Y1, and Y3, is, independently, O;

each of Y4 and Y6 is, independently, hydroxyl;

Y5 is optionally substituted C1-C6 alkylene (para [0357], see exemplary compound BB-1 on pg 85); wherein B is a modified base (para [0359], see exemplary compound BB-30 on pg 88, N1-methyl-pseudouridine).

- Please see extra sheet for continuation -

Continuation of:

Box NO III. Observations where unity of invention is lacking

Bancel teaches [claims 118, 121, and 124] an mRNA encoding polypeptide of interest, comprising modified nucleobases (claim 1, para [1465]). When N1-methyl-pseudouridine is used in combination with 5-methylcytidine a high level of human G-CSF protein is also produced; para [1467], Table 104).

Bancel teaches [claim 119] an mRNA encoding a polypeptide of interest, wherein an alternative uracil represents about 25%-100% of the uracils in the mRNA (claim 1, para [1465]), 100% N1-methyl-pseudouridine substitution demonstrates the highest level of human G-CSF production; para [1467], Table 104).

Bancel teaches [claims 133, 136, and 140] an mRNA encoding a polypeptide of interest, comprising modified nucleosides (claim 1, para [1465]). When N1-methyl-pseudouridine is used in combination with 5-methylcytidine a high level of human G-CSF protein is also produced; para [1467], Table 104).

Bancel teaches [claim 134] an mRNA encoding a polypeptide of interest, wherein an alternative uridine represents about 25%-100% of the uridine (claim 1, para [1465]), 100% N1-methyl-pseudouridine substitution demonstrates the highest level of human G-CSF production; para [1467], Table 104).

Bancel teaches Formula b15 [claim 13] wherein T4, and T4" combine to form O (oxo) (para [0422], [0424]); V5 is N or CRvd; V6 is CRvd; each Rvd is, independently hydrogen; and R18, R19a, and R19b are each hydrogen, provided that, when V5 is N, Rvd is not hydrogen, and, V5 and V6 are not both CH (para [0425]).

PubChem entry CID 57336529 (hereinafter 'CID 57336529') [retrieved on 23 September 2015 from <https://pubchem.ncbi.nlm.nih.gov/compound/10630529#section=Names-and-Identifiers>; published on 2006-10-25] teaches Formula b45 [claim 17] wherein R35 is hydrogen (pg 3).

Bancel teaches Formula b16 [claim 19] wherein T5' and T5" combine to form O (oxo); R21 is hydrogen, bromo, chloro, iodo, nitro, or optionally substituted C1-C6 alkyl; and each of R22, R23, and R24 is, independently hydrogen or optionally substituted C1-C6 alkyl, wherein said base does not have the structure as illustrated (para [0422], [0424], [0426], Formula b16).

PubChem entry CID 10374519 (hereinafter 'CID 10374519') [retrieved on 23 September 2015 from <https://pubchem.ncbi.nlm.nih.gov/compound/10374519#section=Top>; published on 2006-10-25] teaches Formula b3 [claim 20] wherein T2', and T2" combine to form O (oxo); R11 is H; and R12c combines with R10 to form an optionally substituted C2-C9 heterocycl (pg 3).

Bancel teaches Formula b30 [claim 23] wherein each of T1 and T2 is O (oxo); R12a is H; and R12b is optionally substituted amino or optionally substituted C1-6 hydroxyalkyl (para [0389], [0392], Formula b30).

Bancel teaches Formula b29 [claim 25] wherein each of T1 and T2 is O (oxo); R12a is H; and RVb' is optionally substituted C6-C10 aryl, optionally substituted C2-C9 heteroaryl, optionally substituted C1-C6 aminoalkyl, optionally substituted C1-C6 alkoxyalkyl, or optionally substituted C1-C6 hydroxyalkyl (para [0449], Formula b23).

US 2010/0183639 A1 to Uhlmann et al. (hereinafter 'Uhlmann') teaches Formula b44 [claim 28] and Formula b46 [claim 30], (para [0020]), a universal base ?. e.g. benzimidazole or fluorobenzene).

PubChem entry CID 10630529 (hereinafter 'CID 10630529') [retrieved on 23 September 2015 from <https://pubchem.ncbi.nlm.nih.gov/compound/10630529#section=Names-and-Identifiers>; published on 2006-10-25] teaches Formula b48 [claim 31] and Formula b8 [claim 33] (pg 3).

PubChem entry CID 22086948 (hereinafter 'CID 2208694') [retrieved on 23 September 2015 from <https://pubchem.ncbi.nlm.nih.gov/compound/22086948#section=2D-Structure>; published on 2007-12-05] teaches Formula b47 [claim 35] (pg 3).

WO 03/051881 A1 to AN et al. (hereinafter 'An') teaches Formula b20 [claim 36] (pg 30, para 1, Formula 2b).

As said technical features were known in the art at the time of the invention, this cannot be considered special technical feature 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.

Continuation of Section 4:

Claims 37-43, 54-56, 69-114, 126, 128, 129, 142-159 are improper multiple dependent claims because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).