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

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5 November 2015

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(54) Title: ENOLASE 1 (ENO1) COMPOSITIONS AND USES THEREOF

(57) Abstract: The invention provides compositions comprising Eno1 for delivery to a muscle. Further, the invention provides a method for normalizing blood glucose in a subject with elevated blood glucose, comprising administering to the subject enolase 1 (Eno1), thereby normalizing blood glucose in the subject. The invention also provides methods of treating one or more conditions including impaired glucose tolerance, insulin resistance, pre-diabetes, and diabetes, especially type 2 diabetes in a subject, comprising administering to the subject enolase 1 (Eno1), thereby treating the condition in the subject. In certain methods of the invention, the Eno1 is delivered to muscle.

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A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61P 3/08; A61K 38/28, 38/46; C07K 1/00; C12N 15/00 (2015.01)
CPC - A61K 38/43, 38/46, 38/00; C07K 2319/01; C12N 15/86
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)
CPC: A61K 38/43, 38/46, 38/00; C07K 2319/01; C12N 15/86

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC: A61K 38/43, 38/46, 38/00; C07K 2319/01; C12N 15/86 (text search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Electronic Data Bases: PatBase; Google Scholar; Google Patents Search terms: ENO1 (i.e. enolase 1, alpha enolase, EC 4.2.1.11), diabetes, hyperglycemia, HbA1c, pharmaceutical composition, delivery, muscle targeting peptide (MTP) (e.g. ASSLNIA; WDANGKT; GETRAPL; CGHHPVYAC; and HAIYPRH), viral vector, liposome, measurable enzyme activity, gluc

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	US 2008/0138913 A1 (JEON et al.) 12 JUNE 2008 (12.06.2008). Especially para [0020], [0025], [0026], [0029].	1, 3/1, 4/1, 7/1 ---------- 2, (3,4,7)/2, 20, 22, 24, 26-28, 30, 32, 34, 35, 37, 39
X ---- Y	THANGARAJAH et al. The molecular basis for impaired hypoxia-induced VEGF expression in diabetic tissues. Proc Nat Acad Sci 11 August 2009 Vol 106 No 32 Pages 13505. Especially pg 13506 col 1 para 2, pg 13507 fig 2A, pg 13507 col 2 para 3.	47, 50, 59, 60, 65, 67-69 ---------- 48, 49,51-58, 61, 66, 70-72
Y	US 2010/0184948 A1 (HEEMSKERK et al) 22 July 2010 (22.07.2010). Especially para [0010], [0027], [0028].	2, (3,4,7)/2
Y	JP2004081111 (YOICHI) 18 March 2004 (18.03.2004). Especially abstract.	2
Y	US2004/0204356 A1 (GUENZLER-PUKALL et al.) 14 October 2004 (14.10.2004). Especially [0018-0020], [0080], [0082], [0084], [0096], [0191], [0199], [0201-0202].	20, 22, 24, 26-28, 30, 32, 34, 35, 37, 39, 48, 49, 61, 66, 70-80
Y	US 2007/0162985 A1 (MOSE LARSEN et al). 12 July 2007 (12.07.2007). Especially [0008], [0021], [0144] [0145], pg 24 table 1b.	51-58, 73-80

☒ Further documents are listed in the continuation of Box C. ☐

* 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	Date of mailing of the international search report
6 May 2015 (06.05.2015)	04 JUN 2015

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KOVES et al. Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. January 2008 Vol 7 No 1 Pages 45-56. Especially pg 45 col 2 para 2.	39

INTERNATIONAL SEARCH REPORT

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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.: 5,6,8-19,21,23, 25,29,31,33,36,38,40-46,62-64
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.:

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.

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------continuation of Box III (Lack of Unity of Invention)----------

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 1-4, 7 drawn to a pharmaceutical composition comprising a therapeutically effective amount of Eno1 [i.e. alpha enolase, enolase 1] or a fragment thereof.

Group II: Claims 20, 22, 24, 26-28, 30, 32, 34, 35, 37, 39, drawn to a method of modulating a diabetes-related parameter(s), comprising administering to the subject a pharmaceutical composition comprising Eno1 or a fragment thereof. The diabetes-related parameter may be decreasing blood glucose, increasing glucose tolerance, improving insulin response, treating diabetes, improving blood glucose level control, increasing glucose flux, increasing glycolytic flux, increasing mitochondrial free fatty acid flux.

Group III: Claims 47-61, drawn to a method for diagnosing an elevated blood glucose level in a subject, comprising: (a) detecting a level of Eno1 in a biological sample from the subject, and (b) comparing the level of Eno1 in the biological sample with a PREDETERMINED threshold value.

Group IV: Claims 65-72, drawn to a method for monitoring elevated blood glucose in a subject, the method comprising: (1) determining a level of Eno1 in a first biological sample obtained at a first time from a subject having elevated blood glucose; (2) determining a level of Eno1 in a second biological sample obtained from the subject at a second time, wherein the second time is later than the first time; and (3) comparing the level of Eno1 in the second sample with the level of Eno1 in the first sample, wherein a change in the level of Enol is indicative of a change in elevated blood glucose status in the subject.

Group V: Claims 73-79, drawn to a composition or kit of detection reagents for Eno1, HbA1c and glucose.

Group VI: Claim 80, drawn to a use of a panel comprising a plurality of detection reagents specific for detecting markers of elevated blood glucose in a method for diagnosing and/or treating elevated blood glucose, where one detection reagent is specific for detecting Eno1, and the remaining one or more detection reagents are specific for detecting an indicator of elevated blood glucose marker selected from HbA1c and glucose.

The inventions listed as Groups I-VI 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:

Groups I and V are compositions, not required by Groups II-IV, VI.

Groups II-IV, VI are methods, not required by Groups I or V.

Group I has the special technical feature of a pharmaceutical composition comprising a therapeutically effective amount of Eno1 or a fragment thereof, not required by Group V.

Group V has the special technical feature of a composition comprising detection reagents for Eno1 and HbA1c and glucose, not required by Group I.

Group II has the special technical feature of administering a pharmaceutical composition comprising Eno1 or a fragment thereof, not required by Groups III, IV or VI.

Group III has the special technical feature of a method of comparing the level of detecting the level of Eno1 in a biological sample and comparing it to a predetermined threshold level, not required by Groups II, IV or VI.

Group IV has the special technical feature of a method of monitoring blood glucose levels in a subject by determining a level of Eno1 in a first biological sample at a first time, determining a level of Eno1 in a second biological sample at a second time, and comparing them to determine a change in elevated blood glucose status in a subject, nor required by Groups II, III or VI.

Group VI has the special technical feature of use of panel comprising a plurality of detection reagents for detecting markers of elevated glucose in a method of diagnosing and/or treating elevated blood glucose, not required by Groups II-IV.

----------continued on next sheet----------

continued from previous sheet

Common Technical Features:

1. Groups I-VI share the common technical feature of Eno1 [i.e. alpha enolase, enolase 1].

2. Groups I and II share the common technical feature of a pharmaceutical composition comprising Eno1 or a fragment thereof.

3. Groups III-VI share the common technical feature of detecting a level of Eno1 in a biological sample

4. Groups V and VI share the common technical feature of reagents for detecting Eno1, HbA1c and glucose.

5. Group II and III share the common technical feature of elevated blood glucose level and Eno1.

However, said common technical features do not represent a contribution over the prior art, and is obvious over US 2007/0162985 A1 to MOSE LARSEN et al. (hereinafter "Larsen"), in view of US 2004/0204356 A1 to GUENZLER-PUKALL (hereinafter "Guenzler").

As to shared common technical feature #1, Larsen teaches a purified composition of Eno1 (para [0008]; "Accordingly, in one aspect the invention features substantially purified diabetes-mediating proteins exhibiting an altered expression during development of diabetes relative to expression in the absence of diabetes development. The purified diabetes-mediating proteins of the invention are selected from the proteins listed in Tables 1 and 2; pg 24 table 1B: alpha enolase [i.e. Eno1]) para [0100]; "The present invention encompasses several aspects including: (1) diabetes-mediating proteins identified by differential expression in the presence and absence of the development of diabetes; (2) patterns and combinations of DM proteins useful for predicting the development of diabetes and for identifying a compound able to effect a combination of DM proteins in a desired manner; (3) protective diabetes-mediating proteins").

Concerning shared common technical feature #2, Larsen teaches a composition of substantially pure Eno1 (i.e. alpha enolase) (para [0008], pg 24 table 1B). Larsen does not specifically teach a pharmaceutical composition of Eno1. However, Guenzler teaches a method of treating diabetes comprising providing a compound that increases the expression of Eno1 (para [0011]; "The present invention relates to methods and compounds for regulating glucose metabolism and achieving glucose homeostasis. Methods for decreasing blood glucose levels, reducing insulin resistance, decreasing glycated hemoglobin levels, and improving glycemic control in a subject are also provided. Methods for treating or preventing diabetes, hyperglycemia, and other conditions associated with increased blood glucose levels are provided, as are methods for treating or preventing conditions"; para [0018]- "In one embodiment, the present invention provides a method for increasing expression of a glucose regulatory factor in a subject, by stabilizing HIFalpha in the subject or by administering to the subject an effective amount of a compound of the invention, thereby increasing expression of the glucose regulatory factor in the subject. In further embodiments, the glucose regulatory factor is [...] enolase-1"; para [0019-0020]). Based on the observation of Guenzler, an artisan of ordinary skill in the art would have recognized that a pharmaceutical composition, prepared using methods well-known in the art, of a nucleic acid encoding enolase-1 or enolase-1 polypeptide could have been used to treat diabetes and establish glucose homeostasis.

Concerning shared common technical feature #3, detecting a level of Eno1 in a biological sample in a subject, Larsen teaches (para [0144]; "For purposes of the invention, an antibody or nucleic acid probe specific for a diabetes-mediating protein may be used to detect the diabetes-mediating protein (using antibody) or encoding polynucleotide (using nucleic acid probe) in biological fluids or tissues"; para [0008]-detecting diabetes mediating protein, as indicated in Table 1; pg 24 table 1b-alpha enolase)

Concerning share common technical feature #4, reagents for detecting Eno1, HbA1c and glucose, Larsen teaches reagents for detecting Eno1 (para [0144], para [0008], pg 24 table 1b). Larsen does not teach reagents for detecting HbA1c or glucose. However, reagents for the detection of HbA1c were well known in the art, as is exemplified by use of a kit in Guenzler (para [0201]; " Glycated hemoglobin is formed by the attachment of various sugars (most commonly glucose) to the hemoglobin molecule, and is formed at a rate that is directly proportional to the blood glucose concentration"; para [0202]; The effect of compound administration on glycated hemoglobin levels was examined using a mouse model of diabetes as follows [...] Prior to study initiation and at weeks 4 and 8 following treatment, blood samples were collected from the tail vein and HbA1c levels were measured using an HbA1cNOW kit"). Guenzler also teaches reagents for measurement of glucose (para [0199]- Oral Glucose Tolerance Test (OGTT), [0080]). An artisan of ordinary skill in the art would have known how to combine the reagents for measurement of Eno1, HbA1c and glucose because they indicators of blood glucose and glucose homeostasis (based on the teaching of Guenzler para [0018-0020]). An artisan would have also known how to assemble a kit and provide instructions for their use, with the aim of simplifying use of the claimed compositions

As to common technical feature #5, elevated blood glucose level and Eno1, Guenzler teaches elevated blood glucose level (para [0019-0020] and Eno1 (para [0018]).

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, Groups I-VI lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Note concerning item 4: Claims 5, 6, 8-19, 21, 23, 25, 29, 31, 33, 36, 38, 40-46, 62-64 are multiple dependent claims and are held unsearchable because they are not drafted according to the second and third sentences of PCT Rule 6.4(a).