# PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

**PCT**

To:
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INVITATION TO PAY ADDITIONAL FEES
AND, WHERE APPLICABLE, PROTEST FEE

(PCT Article 17(3)(a) and Rule 40.1 and 40.2(e))

| | |
|---|---|
| | Date of mailing *(day/month/year)* 24 July 2020 (24-07-2020) |
| Applicant's or agent's file reference 130197-00320 | **PAYMENT DUE** within **ONE MONTH** from the above date of mailing |
| International application No. PCT/US2020/030884 | International filing date *(day/month/year)* 30 April 2020 (30-04-2020) |
| Applicant CHONDRIAL THERAPEUTICS, INC. | |

1. This International Searching Authority

(i) considers that there are 85 *(number of)* inventions claimed in the international application covered by the claims indicated on an extra sheet:

(ii) therefore considers that **the international application does not comply with the requirements of unity of invention** (Rules 13.1, 13.2 and 13.3) for the reasons indicated on an extra sheet:

(iii) [X] has carried out a partial international search (see Annex) [ ] will establish the international search report on those parts of the international application which relate to the invention first mentioned in claims Nos.:

see extra sheet

(iv) will establish the international search report on the other parts of the international application only if, and to the extent to which, additional fees are paid.

2. Consequently, the applicant is hereby **invited to pay,** within the time limit indicated above, the amount indicated below:

| EUR 1.775,00 | x | 84 | = | EUR 149.100,00 |
|---|---|---|---|---|
| Fee per additional invention | | number of additional inventions | | currency/total amount of additional fees |

3. The applicant is informed that, according to Rule 40.2(c), **the payment of any additional fee may be made under protest,** i.e., a reasoned statement to the effect that the international application complies with the requirement of unity of invention or that the amount of the required additional fee is excessive, where applicable, subject to the payment of a protest fee.

Where the applicant pays additional fees under protest, the applicant is hereby invited, within the time limit indicated above, to pay a protest fee (Rule 40.2(e)) in the amount of EUR 910,00 *(currency/amount)*

Where the applicant has not, within the time limit indicated above, paid the required protest fee, the protest will be considered not to have been made and the International Searching Authority will so declare.

4. [ ] Claim(s) Nos. _____ have been found to be unsearchable under Article 17(2)(b) because of defects under Article 17(2)(a) and therefore have not been included with any invention.

| Name and mailing address of the International Searching Authority | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL-2280 HV Rijswijk<br>Tel. (+31-70) 340-2040<br>Fax: (+31-70) 340-3016 | VENCOUROVá, Lenka<br>Tel: +49 (0)89 2399-2105 |

Form PCT/ISA/206 (April 2005)

Registered Mail

INVITATION TO PAY ADDITIONAL FEES

International application No.
PCT/US2020/030884

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 6, 50, 55, 72, 77, 82(completely); 1-5, 7, 13, 19, 20, 22-49, 51-54, 56, 62, 68-71, 73-76, 78-81, 83(partially)

Methods for evaluating effectiveness of Frataxin (FXN) replacement therapy comprising:
(a) determining an FXN replacement expression profile for at least FXN-sensitive genomic marker (FSGMs) CYR61 in a sample from an FXN deficient patient following treatment with FXN replacement therapy;
(b) comparing the patient FXN replacement expression profile with a baseline FXN(-) expression profile; and
(c) using the comparison to determine effectiveness of the FXN replacement therapy.
Compositions, panels, kits thereto.

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2. claims: 1-5, 7-49, 51-54, 56-71, 73-76, 78-81, 83(all partially)

Methods for evaluating effectiveness of Frataxin (FXN) replacement therapy wherein invention 2 is defined by the first marker as depicted in Table 2, namely ABCE1.
Compositions, panels, kits thereto.

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3-85. claims: 1-5, 7-49, 51-54, 56-71, 73-76, 78-81, 83(all partially)

concerns methods for evaluating effectiveness of Frataxin (FXN) replacement therapy wherein inventions 3-85 are defined by the markers as depicted consecutively in Table 2 (with the exception of CYR61 which is sorted to the first invention), namely marker ADAMTS1 for invention 3, ADNP marker for invention 4 and so on until marker TMEM126a for invention 85. Compositions, kits, panels thereto.

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NON-UNITY

The Search Authority considers the application to lack unity and identifies inventions 1 to 85 as indicated in the Search Report.
The reasons for the objection as to lack of unity are as follows:
The only identifiable technical feature that all inventions have in common is that they refer to FXN-sensitive genomic markers (expression markers which are reversed after FXN-restoration). This feature cannot represent special technical features as it is known in the art.
D1 (see whole document) describes a Friedreich's Ataxia (FRDA) animal model (FXN-deficient mouse model), which exhibits reduced levels of Fraxantin (FXN). Gene expression changes associated with FXN knock-down were determined. Reverse of gene expression changes of FXN knock-down after FXN restoration was determined. Several pathways are affected due to FXN depletion and shown reversal due to FXN restoration, e.g.

Cacna2Da, Abcc9, Hrc genes were mentioned. Thus, D1 identifies markers genes which are reversed after FXN restoration.
Each of inventions 1-85 differs from D1 in that they refer to a different expression marker and solves the technical problem of providing an alternative FXN-sensitive genomic markers.
Therefore, in view of the fact that D1 already provides a solution to said problem (i.e. provides differentially expressed markers which are restored after FXN replacement), and that the 85 claimed markers do not share any structural features, the search division is of the opinion that there is no single inventive concept underlying the 85 solutions contained in the present application. Consequently there is a lack of unity according to Rule 13(1)(2)(3) PCT.
Two important clarifications with respect to number of inventions and selection of marker for the first invention.
(1) Independent method claim 1 discloses wherein the one or more FSGMs are any one or more markers defined in Table 2, Table 4 and/or Figure 3. It appears that Table 2 encompasses the markers depicted in Table 4 and figure 3. It is thus apparent that the number of inventions is of at least 85. It is indicated that in the case that the present Authority may have overlooked a marker depicted in Table 4 and/or figure 3 (which is not present in Table 2); this marker would represent a further separate invention.
(2) It is of general practice that the first marker disclosed in the independent claim is chosen for the first invention. In the present case, it would have been the first marker depicted in the first table (Table 2), and subsequently for the rest of the markers of table 2 and then markers in table 4 and markers of figure 3.
However, in view of the high amount of markers (high amount of identifiable inventions) and in view of that the application focuses on CYR61 marker gene (e.g. Examples 6 to 8), in contrast to the rest of the markers depicted in the tables for which only differential expression data are provided, this Authority has found more pragmatic and Applicant friendly to choose CYR61 as the first marker for the first invention.

The first invention has been searched.

**Annex to Form PCT/ISA/206**
**COMMUNICATION RELATING TO THE RESULTS OF THE PARTIAL INTERNATIONAL SEARCH**

International Application No
PCT/US2020/030884

1. The present communication is an Annex to the invitation to pay additional fees (Form PCT/ISA/206). It shows the results of the international search established on the parts of the international application which relate to the invention first mentioned in claims Nos.:
see 'Invitation to pay additional fees'
2. This communication is not the international search report which will be established according to Article 18 and Rule 43.

3. If the applicant does not pay any additional search fees, the information appearing in this communication will be considered as the result of the international search and will be included as such in the international search report.

4. If the applicant pays additional fees, the international search report will contain both the information appearing in this communication and the results of the international search on other parts of the international application for which such fees will have been paid.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | VIJAYENDRAN CHANDRAN ET AL: "Inducible and reversible phenotypes in a novel mouse model of Friedreich's Ataxia", ELIFE, vol. 6, 19 December 2017 (2017-12-19), XP055715118, DOI: 10.7554/eLife.30054 page 18; figures 7a, 7b (and supplements 1-6) 8 (with supplement 1) page 21 - page 24 | 1-7,13, 19,20, 22-56, 62,68 |
| | ----- | |
| X | HUMMERT C ET AL: "Creation and Comparison of Different Chip Definition Files for Affymetrix Microarrays", 2011 INTERNATIONAL CONFERENCE ON BIOINFORMATICS & COMPUTATIONAL BIOLOGY. BIOCOMP 2011, 18-21 JULY 2011, LAS VEGAS NV, USA,, vol. 1, 1 January 2011 (2011-01-01), pages 16-22, XP009186218, ISBN: 978-1-60132-170-1 abstract | 69-83 |
| | ----- | |
| | -/-- | |

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° 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 document 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

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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. |
|---|---|---|
| X | PILARKSY CHRISTIAN P ET AL: "Expression of the extracellular matrix signalling molecule Cyr61 is downregulated in prostate cancer", PROSTATE, WILEY-LISS, NEW YORK, NY, US, vol. 36, no. 2, 1 July 1998 (1998-07-01), pages 85-91, XP009521728, ISSN: 0270-4137, DOI: 10.1002/(SICI)1097-0045(19980701)36:2<85::AID-PROS3>3.3.CO;2-X abstract<br>----- | 69-83 |
| X | SAKAMOTO S ET AL: "Increased expression of CYR61, an extra cellular matrix signaling protein, in human benign prostatic hyperplasia and its regulation by lysophosphatidic acid", ENDOCRINOLOGY, THE ENDOCRINE SOCIETY, US, vol. 45, no. 6, 1 June 2004 (2004-06-01), pages 2929-2940, XP002981701, ISSN: 0013-7227, DOI: 10.1210/EN.2003-1350 abstract<br>----- | 69-83 |
| A | JIUN-I LAI ET AL: "Transcriptional profiling of isogenic Friedreich ataxia neurons and effect of an HDAC inhibitor on disease signatures", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 294, no. 6, 8 February 2019 (2019-02-08), pages 1846-1859, XP055715112, US ISSN: 0021-9258, DOI: 10.1074/jbc.RA118.006515 abstract page 1853 - page 1854<br>----- | 1-7,13, 19,20, 22-56, 62,68 |
| A | JILL SERGESKETTER NAPIERALA ET AL: "Comprehensive analysis of gene expression patterns in Friedreich's ataxia fibroblasts by RNA sequencing reveals altered levels of protein synthesis factors and solute carriers", DISEASE MODELS & MECHANISMS, vol. 10, no. 11, 1 November 2017 (2017-11-01), pages 1353-1369, XP055715105, GB ISSN: 1754-8403, DOI: 10.1242/dmm.030536 abstract<br>----- | 1-7,13, 19,20, 22-56, 62,68 |

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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. |
|---|---|---|
| A | NAPIERALA J ET AL: "Upregulation of mitochondrial aldehyde dehydrogenase activity inhibits lipid peroxidation in friedreich's ataxia cells", NEUROLOGY : OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF NEUROLOGY, LIPPINCOTT WILLIAMS & WILKINS, US, vol. 90, no. 15, Supplement 1, 22 April 2018 (2018-04-22), XP009521707, ISSN: 1526-632X [retrieved on 2018-04-09] the whole document<br>---- | 1-7,13, 19,20, 22-56, 62,68 |
| A | ABRAHAO AGESSANDRO ET AL: "Gene Expression Profile in Peripheral Blood Cells of Friedreich Ataxia Patients", CEREBELLUM, TAYLOR AND FRANCIS, GB, vol. 15, no. 3, 15 July 2015 (2015-07-15), pages 306-313, XP035959447, ISSN: 1473-4222, DOI: 10.1007/S12311-015-0700-X [retrieved on 2015-07-15] abstract<br>---- | 1-7,13, 19,20, 22-56, 62,68 |

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Application no:
Demande nº:
Anmelde-Nr: PCT/US2020/030884

DISCLAIMER

The attached provisional opinion on the patentability of the first invention searched serves only as information.
A reply addressing the points raised in the opinion is **not** required and will **not** be taken into account when issuing the final search report and opinion on patentability.

AVERTISSEMENT

L'avis provisoire ci-joint sur la brevetabilité de la première invention recherchée ne sert qu'à titre d'information.
Une réponse abordant les points soulevés dans l'avis n'est **pas** nécessaire et ne sera **pas** prise en compte lors de l'établissement du rapport final de la recherche et de l'avis sur la brevetabilité.

DISCLAIMER

Die beigefügte vorläufige Stellungnahme zur Patentierbarkeit der ersten geprüften Erfindung dient lediglich zur Information.
Eine Antwort auf die erhobenen Punkte in der Stellungnahme ist **nicht** erforderlich und bleibt bei der Erstellung des endgültigen Recherchenberichts und der Stellungnahme zur Patentierbarkeit **unberücksichtigt**.

## Re Item I

### Basis of the opinion

**First invention**: claims 6, 50, 55, 72, 77, 82 in full; claims 1-5, 7, 13, 19, 20, 22-49, 51-54, 56, 62, 68-71, 73-76, 78-81, 83 partial.

## Re Item IV

### Lack of unity of invention

The Search Authority considers the application to lack unity and identifies the inventions 1 to 85 as follows.

Invention 1:

concerns methods for evaluating effectiveness of Frataxin (FXN) replacement therapy comprising:

(a) determining an FXN replacement expression profile for at least FXN-sensitive genomic marker (FSGMs) CYR61 in a sample from an FXN deficient patient following treatment with FXN replacement therapy;

(b) comparing the patient FXN replacement expression profile with a baseline FXN(-) expression profile; and

(c) using the comparison to determine effectiveness of the FXN replacement therapy.

Compositions, kits, panels thereto.

Invention 2

concerns methods for evaluating effectiveness of Frataxin (FXN) replacement therapy wherein invention 2 is defined by the first marker as depicted in Table 2, namely ABCE1. Compositions, kits, panels thereto.

Inventions 3-85:

concerns methods for evaluating effectiveness of Frataxin (FXN) replacement therapy wherein inventions 3-85 are defined by the markers as depicted consecutively in Table 2 (with the exception of CYR61 which is sorted to the first invention), namely marker ADAMTS1 for invention 3, ADNP marker for invention 4 and so on until marker TMEM126a for invention 85. Compositions, kits, panels thereto.

The reasons for the objection as to lack of unity are as follows:

The only identifiable technical feature that all inventions have in common is that they refer to FXN-sensitive genomic markers (expression markers which are reversed after FXN-restoration). This feature cannot represent special technical features as it is known in the art.

D1 (see whole document) describes a Friedreich's Ataxia (FRDA) animal model (FXN-deficient mouse model), which exhibits reduced levels of Fraxantin (FXN). Gene expression changes associated with FXN knock-down were determined. Reverse of gene expression changes of FXN knock-down after FXN restoration was determined. Several pathways are affected due to FXN depletion and shown reversal due to FXN restoration, e.g. Cacna2Da, Abcc9, Hrc genes were mentioned. Thus, D1 identifies markers genes which are reversed after FXN restoration.

Each of inventions 1-85 differs from D1 in that they refer to a different expression marker and solves the technical problem of providing an alternative FXN-sensitive genomic markers.

Therefore, in view of the fact that D1 already provides a solution to said problem (i.e. provides differentially expressed markers which are restored after FXN replacement), and that the 85 claimed markers do not share any structural features, the search division is of the opinion that there is no single inventive concept underlying the 85 solutions contained in the present application. Consequently there is a lack of unity according to Rule 13(1)(2)(3) PCT.

Two important clarifications with respect to number of inventions and selection of marker for the first invention.

(1) Independent method claim 1 discloses wherein the one or more FSGMs are any one or more markers defined in Table 2, Table 4 and/or Figure 3. It appears that Table 2 encompasses the markers depicted in Table 4 and figure 3. It is thus apparent that the number of inventions is of at least 85. It is indicated that in the case that the present Authority may have overlooked a marker depicted in Table 4 and/or figure 3 (which is not present in Table 2); this marker would represent a further separate invention.

(2) It is of general practice that the first marker disclosed in the independent claim is chosen for the first invention. In the present case, it would have been the first marker depicted in the first table (Table 2), and subsequently for the rest of the markers of table 2 and then markers in table 4 and markers of figure 3.

However, in view of the high amount of markers (high amount of identifiable inventions) and in view of that the application focuses on CYR61 marker gene (e.g. Examples 6 to 8), in contrast to the rest of the markers depicted in the tables for which only differential expression data are provided, this Authority has found more pragmatic and Applicant friendly to choose CYR61 as the first marker for the first invention.

The first invention has been searched.

**Re Item V**

**Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

Reference is made to the following documents:

D1 VIJAYENDRAN CHANDRAN ET AL: "Inducible and reversible phenotypes in a novel mouse model of Friedreich's Ataxia",ELIFE, vol. 6, 19 December 2017 (2017-12-19), XP055715118, DOI: 10.7554/eLife.30054

D2 JILL SERGESKETTER N ET AL: "Comprehensive analysis of gene expression patterns in Friedreich's ataxia fibroblasts by RNA sequencing reveals altered levels of protein synthesis factors and solute carriers", DISEASE MODELS & MECHANISMS, vol. 10, no. 11,(2017-11-01), pages 1353-1369, XP055715105, ISSN: 1754-8403

D3 HUMMERT C ET AL: "Creation and Comparison of Different Chip Definition Files for Affymetrix Microarrays",2011 INT. CON. ON BIOINFORMATICS & COMPUTATIONAL BIOLOGY. BIOCOMP 2011, 18-21 JULY 2011, vol. 1, 1 January 2011, pages 16-22,XP009186218, ISBN: 978-1-60132-170-1

D4 PILARKSY CHRISTIAN P ET AL: "Expression of the extracellular matrix signalling molecule Cyr61 is downregulated in prostate cancer", PROSTATE,vol. 36, no. 2, 1 July 1998 (1998-07-01), pages 85-91

D5 SAKAMOTO SHINJI ET AL: "Increased expression of CYR61, an extracellular matrix signaling protein, in human benign prostatic hyperplasia and its regulation by lysophosphatidic acid",ENDOCRINOLOGY,vol. 145, no. 6, (2004-06), pages 2929-2940, ISSN: 0013-7227

## V.1. NOVELTY (Article 33(2) PCT)

1 Methods for evaluating effectiveness of Fraxatin replacement therapy according to invention 1 have not been described in the prior art.

2 With respect to product claims:

Kit claim is defined as kit "for detecting one or more frataxin-sensitive genomic marker (FSGM) in a biological sample from a subject exhibiting frataxin (FXN) deficiency or being treated for FXN deficiency", said kit claim must be construed as meaning merely kit suitable for the detection of the marker. In view of that, the expression in brackets does not have a limiting effect on the scope of the present claim.

Thus, kit claims are characterized by one or more reagents for for measuring the level of at least CYR61. The same applies *mutatis mutandis* to composition / panel claims which are characterized by reagents for the detection of at least CYR61.

It is further pointed out that the intended use of a product is not a technical feature of the product per se, e.g. in the present case a kit containing a primer for detecting a marker gene. Therefore, any means, e.g. any primers / probes / antibodies suitable for the detection of CYR61 destroys the novelty of the product claims according to invention 1. For example comprehensive Affymetrix human GeneChips of D3, or means for detecting CYR61 of D4 or D5.

## V.2. INVENTIVE STEP (Article 33(3)PCT)

Friedriech's Ataxia (FRDA) is characterized by reduced levels of Fraxantin (FXN). Gene expression analysis in Friedriech's Ataxia (FRDA) fibroblasts has been performed in e.g. D2. RNA sequencing of the fibroblasts revealed up-regulated expression of protein synthesis factors and solute carriers and down-

regulation in genes encoding enzymes involved in cytoplasmatic and mitochondrial protein synthesis. Therefore, the skilled-person was aware at the date of filing that FXN deficient patients exhibit differential gene expression.

D1 has been considered the closest prior art since it describes a Friedreich's Ataxia (FRDA) animal model (FXN-deficient mouse model) and expression / analysis after Fraxatin-replacement (FXN-restoration).

The present invention differs from D1 in that it discloses the marker gene CYR61.

The problem to be solved could be regarded as the provision of markers which are suitable for evaluating the effectiveness of Fraxatin replacement therapy. The solution provided is the determination of expression levels of marker CYR61 gene as indicative of effectiveness of FXN replacement therapy.

Example 6 of application (see Fig. 7) indicates that the levels of the secreted CYR61 protein from the knock-down cells (hFXN-KD) are very high and that the levels of the secreted CYR61 protein significantly decreased to control levels after treatment with FXN fusion protein. Example 7 (see fig. 8) shows that the amount of secreted CYR61 protein is decreased by the transient expression of hFXN in the cells. Thus, it is shown that after fraxatin replacement treatment the levels of CYR61 are reversed.

D1 (see pages 11, 17-18, 21-24, figures 7a, 7b and its supplements 1-6, figure 8 and supplement 1) does not mention CYR61. D1 however, studies gene expression changes in FXN-deficient mouse model and provides a list of gene expression candidate biomarkers associated with fraxatin knock-down. It further analyses rescue of behavioral, pathological and molecular changes due to fraxatin restoration. It determines reverse of gene expression changes of FXN knock-down after FXN restoration. It describes that several pathways are affected due to FXN depletion and shows complete reversal due to FXN restoration, e.g. Cacna2Da, Abcc9, Hrc genes were mentioned.

Therefore, the identification of genes that are contrary regulated by FXN gene ablation followed by FXN protein replacement has been described in D1, thus, these gene expression changes induced by FXN replacement treatment could

be used as indicative of treatment effectiveness in patients treated with FXN replacement therapy. D1 already identifies markers genes which are reversed after FXN restoration and thus the provision of a further marker (CYR61) does appear to represent a contribution over the markers of D1.

The provision of an alternative marker can only be considered to involve inventive step if it provides a surprising / additional technical effect, this does not seem to be the case. The application must provide a technical contribution over the prior art. In view of the above argumentations, the present application does not seem to fulfill the requirements of Article 33 (3) PCT.

## Re Item VIII

### Clarity and Support

Claims 51-54, 56, 62, 68 (according to the first invention) relates to methods of treatment of a mitochondrial disease, the method comprising: providing a sample from a subject suffering from FXN deficiency and initiating, increasing or decreasing the dosage of FXN replacement therapy to be administered to the subject based on the classification of the sample FXN expression profile.

Claims comprising the expressions "taking a biological sample from the individual..." and "administering a treatment to the individual...", relate to subject-matter considered by the present Authority to be covered by the provisions of Rule 39.1(iv) and Rule 67.1 (iv) PCT. The patentability depend upon the formulation of the claims, thus the EPO does not recognize as patentable claims directed to surgical and therapeutic treatment of individuals, this shall be taken into consideration when entering the Regional Phase.