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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(H))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(in))*

Published:

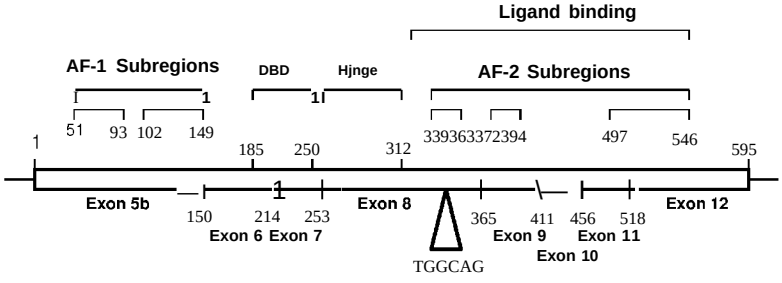
— *with international search report (Art. 21(3))*

— *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))*

(88) Date of publication of the international search report:
6 June 2013

(54) Title: NOVEL ESTROGEN RECEPTOR MUTATIONS AND USES THEREOF

FIG. 1



(57) Abstract: Methods of identifying breast cancer patients resistant to SERM therapy by detecting mutant estrogen receptor are disclosed. The method comprises identifying specific estrogen receptor mutations with a reaction mixture comprising a detection reagent and a target nucleic acid derived from a breast cancer that is estrogen receptor positive, malignant and resistant to treatment with a first or second line SERM therapy.

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INTERNATIONAL SEARCH REPORT

International application No.
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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.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. Ill 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:
Group I: Claims 1-7, 10, 12-15*, 80-85 and 90-93, drawn to a reaction mixture comprising: a detection reagent, or purified or isolated preparation thereof; and a target nucleic acid derived from a breast cancer that is estrogen receptor positive (ER+), malignant, and resistant to treatment with a first or second line SERM therapy, wherein said detection reagent can distinguish a reference sequence from a mutation chosen from one or more of: a missense mutation at nucleotide 1609 according to SEQ ID NO: 1; a missense mutation at nucleotide 1610 according to SEQ ID NO: 1; a missense mutation at nucleotide 1613 according to SEQ ID NO: 1; a 6 nucleotide deletion of nucleotides 1046-1051 (TGGCAG) according to SEQ ID NO: 1, or other deletion, e.g., an in-frame deletion, that includes one or more of nucleotides 1046-1051; a 3 nucleotide insertion between nucleotides 1033 and 1034 according to SEQ ID NO:1; or a mutation at a position identified as mutated in Table 3; AND
A method of making said reaction mixture; AND
"""Continued in Supplemental Box*****

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.:
1-7, 10, 12-15, 80-85 and 90-93

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/US 12/60133

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68; G01 N 33/53 (201 3.01)
USPC - 435/6. 1, 435/6.1 4, 435/6. 17, 435/7. 1

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): C12Q 1/68; G01 N 33/53 (2013.01)
USPC: 435/6.1 , 435/6.14, 435/6.17, 435/7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 435/6.1 1, 435/6.12
(Text Search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (PGPB, USPT, USOC, EPAB, JPAB), PatBase and Google Scholar.
Search Terms: estrogen receptor, ER, 1609, 1610, Y537N, Y537, Y537C, T1609A, A1610G, breast cancer, SERM, selective estrogen receptor modulator, ESR1 , Y537$, D538$, D538, 1609, 1610, 1613.

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 2006/0100168 A1 (RAVID et al.) 11 May 2006 (11.05.2006) para [0005], [0010], [0024], [0027], [0029], [0038], [0047], [0059], [0060], [0062]	80, 82-83, 85, 90 and 92-93 --- 1-7, 10, 12-15, 81, 84 and 91
Y	US 2010/0120039 A1 (FUQUA) 13 May 2010 (13.05.2010) SEQ ID NOs: 6, 9; para [0019], [0082], [0086], [008 η , [0128], [0130], [0136], [0153], [0154], [0157], [0331]	1-7, 10, 12-15, 81 and 91
Y	US 2010/0029498 A1 (GNIRKE et al.) 04 February 201 0 (04.02.2010) para [0106]-[01 11])	84
Y	MAHFOUDI, A. et al. Specific mutations in the estrogen receptor change the properties of antiestrogens to full agonists. Proc. NatL Acad Sci. USA. May 1995, Vol. 92, pages 4206-4210: abstract.	5 and 7

☐ 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 orpriority 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
11 March 2013 (11.03.2013)	22 MAR 2013
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:** Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.

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Continuation of Box No. III - Observations where unity of invention is lacking:

A detection reagent comprising a nucleic acid molecule, e.g., a DNA, RNA or mixed DNA/RNA molecule, comprising sequence which is complementary with a nucleic acid sequence on a target nucleic acid (the sequence on the target nucleic acid that is bound by the detection reagent is referred to herein as the "detection reagent binding site" and the portion of the detection reagent that corresponds to the detection reagent binding site is referred to as the "target binding site") in which the detection reagent binding site is disposed in relationship to the interrogation position such that binding (or in embodiments, lack of binding) of the detection reagent to the detection reagent binding site allows differentiation of mutant disclosed herein and reference sequences and said target nucleic acid is derived from a breast cancer that is estrogen receptor positive (ER+), malignant and resistant to first or second line SERM treatment; AND
A reaction mixture comprising: a detection reagent, or purified or isolated preparation thereof, e.g., a substrate, e.g., a substrate for phosphorylation or other activity, or an antibody; and a target ESR1 protein derived from a breast cancer that is estrogen receptor positive (ER+), malignant, and resistant to treatment with a first or second line SERM therapy, wherein the detection reagent is specific for a mutation at position 537 or 538 of the amino acid sequence of SEQ ID NO:2 (FIGS. 2A-2B); AND
A nucleic acid molecule suitable as probe, primer, bait or library member that can specifically identify, capture, detect, or isolate, a mutant ESR1, wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR 1; AND
An isolated or purified antibody molecule that binds a mutant ESR1 polypeptide, wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR1.

Group II: Claims 8-9, 11, 66-75, 86-89, drawn to A purified or isolated preparation of a ESR1 nucleic acid from a breast cancer that is estrogen receptor positive (ER+), malignant, and resistant to treatment with a first or second line SERM therapy disposed in a sequencing device, or a sample holder for use in such a device / disposed in a a device for determining a physical or chemical property, e.g., stability of a duplex, e.g., Tm or a sample holder for use in such a device; AND A purified or isolated preparations of an ESR1 nucleic acid, e.g., DNA, e.g., genomic DNA or cDNA, or RNA, containing an interrogation position, useful for determining if a mutation disclosed herein is present, wherein said nucleic acid is derived from a breast cancer that is estrogen receptor positive (ER+), malignant and resistant to first or second line SERM treatment, wherein said ESR1 mutation is chosen from: a missense mutation at nucleotide 1609 according to SEQ ID NO: 1; a missense mutation at nucleotide 1610 according to SEQ ID NO: 1; a missense mutation at nucleotide 1613 according to SEQ ID NO: 1; a 6 nucleotide deletion of nucleotides 1046-1051 (TGGCAG) according to SEQ ID NO: 1, or other deletion, e.g., an in-frame deletion, that includes one or more of nucleotides 1046-1051; a 3 nucleotide insertion between nucleotides 1033 and 1034 according to SEQ ID NO:1; or a mutation at a position identified as mutated in Table 3; and
An isolated or purified nucleic acid molecule that encodes a mutant ESR1 or mutation comprising fragment thereof, wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR1; AND
An isolated or purified mutant ESR1 nucleic acid molecule operatively linked to a native or a heterologous regulatory sequence, wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR1; AND
An isolated or purified vector comprising a nucleic acid molecule that encodes a mutant ESR1 or a breakpoint comprising fragment thereof, wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR1; AND
An isolated or purified mutant ESR1 polypeptide or mutation containing fragment thereof, wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR1.

Group III: Claims 16-38, 63-65, and 110-122, drawn to a method of treating a subject having a breast cancer, comprising: acquiring knowledge of a presence of a mutant estrogen receptor 1 (ESRI) and an Estrogen Receptor positive (ER+) in said subject, wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR 1; and responsive to said knowledge, administering to the subject an effective amount of an anti-cancer agent other than a Selective Estrogen Receptor Modulator (SERM), thereby treating the breast cancer in the subject; and
A method for generating a personalized cancer treatment report, by obtaining a sample from a subject, detecting a mutant ESR1 in the sample, and selecting a treatment based on the mutation identified, wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR1; AND
A method of evaluating a patient, comprising: identifying, selecting, or obtaining information or knowledge that the patient has participated in a clinical trial or has been treated for cancer; acquiring genotype information that identifies a mutant ESR1 as being in the patient, wherein the presence of the mutant ESR1 identifies the patient as having an increased risk for, or having, a cancer associated with the mutant ESR1; and treating the subject with an estrogen inhibitor.

Group IV, Claims 39-62, drawn to a method of determining the presence of a mutant ESR1, comprising: directly acquiring knowledge that a mutant ESR nucleic acid or polypeptide is present in a sample from a subject; and (optionally) acquiring knowledge of the Estrogen Receptor status, wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR1, wherein the mutant ESR1 comprises a missense mutation at position 537, 538, 311, 341, 350, 394, 414, 433, or 503; a deletion of nucleotides 1046-1051 of SEQ ID NO:3, or an insertion between amino acids 344 and 345, of the amino acid sequence of SEQ ID NO:2 (FIGS. 2A-2B).

Group V: Claims 76-79, drawn to a nucleic acid molecule that specifically reduces or inhibits the expression of a nucleic acid molecule that encodes a mutant ESR1, wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR1.

Group VI: Claims 94-101, drawn to a method of reducing an activity of a mutant ESR1 comprising: optionally, acquiring knowledge of the presence of the mutant ESR1; and contacting the mutant ESR1, or a mutant ESR1-expressing cell, with an agent that inhibits an activity or expression of mutant ESR1, wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR1.

Group VII: Claims 102-109, drawn to a method for screening for an agent that modulates, e.g., inhibits, the expression or activity of a mutant ESRI, comprising: optionally, determining if a mutant ESR1 is present; contacting a mutant ESR1 with a candidate agent; and detecting a change in a parameter associated with a mutant ESR1.

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The inventions listed as Groups I-VII 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:

Groups I-VI do not include the inventive concept of a method for screening for an agent that modulates, e.g., inhibits, the expression or activity of a mutant ESRI, as required by Group VII.

Groups I-V and VII do not include the inventive concept of a method of reducing an activity of a mutant ESR1 comprising contacting the mutant ESR1 , or a mutant ESR1 -expressing cell, with an agent that inhibits an activity or expression of mutant ESR1 , as required by Group VI.

Groups I-IV and VI-VII do not include the inventive concept of a nucleic acid molecule that specifically reduces or inhibits the expression of a nucleic acid molecule that encodes a mutant ESR1 , as required by Group V.

Groups I-II and V not include the inventive concept of a method of determining the presence of a mutant ESR1 , comprising: directly acquiring knowledge that a mutant ESR nucleic acid or polypeptide is present in a sample from a subject; and (optionally) acquiring knowledge of the Estrogen Receptor status, as required by Group IV.
Groups VI-VII do not include the inventive concept of a method of determining the presence of a mutant ESR1 , comprising: (optionally) acquiring knowledge of the Estrogen Receptor status, as required by Group IV.

Groups I-II and IV-VII do not include the inventive concept of a method of treating a subject having a breast cancer, and A method for generating a personalized cancer treatment report, by obtaining a sample from a subject, detecting a mutant ESR1 in the sample, and selecting a treatment based on the mutation identified, wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR1 ; AND A method of evaluating a patient, wherein the presence of the mutant ESR1 identifies the patient as having an increased risk for, or having, a cancer associated with the mutant ESRI ; and treating the subject with an estrogen inhibitor, as required by Group III.

Groups III and V-VII do not include the inventive concept of A purified or isolated preparation of a ESR1 nucleic acid from a breast cancer that is estrogen receptor positive (ER+), malignant, and resistant to treatment with a first or second line SERM therapy disposed in a sequencing device, or a sample holder for use in such a device / disposed in a device for determining a physical or chemical property, e.g., stability of a duplex, e.g., Tm or a sample holder for use in such a device; AND A purified or isolated preparations of an ESR1 nucleic acid, e.g., DNA, e.g., genomic DNA or cDNA, or RNA, containing an interrogation position, useful for determining if a mutation disclosed herein is present, wherein said nucleic acid is derived from a breast cancer that is estrogen receptor positive (ER+), malignant and resistant to first or second line SERM treatment, wherein said ESR1 mutation is chosen from: a missense mutation at nucleotide 1609 according to SEQ ID NO: 1; a missense mutation at nucleotide 1610 according to SEQ ID NO: 1; a missense mutation at nucleotide 1613 according to SEQ ID NO: 1; a 6 nucleotide deletion of nucleotides 1046-1051 (TGGCAG) according to SEQ ID NO: 1, or other deletion, e.g., an in-frame deletion, that includes one or more of nucleotides 1046-1051; a 3 nucleotide insertion between nucleotides 1033 and 1034 according to SEQ ID NO:1 ; or a mutation at a position identified as mutated in Table 3, as required by Group II.

Groups II-VII do not include the inventive concept of a reaction mixture comprising: a detection reagent, or purified or isolated preparation thereof; and a target nucleic acid derived from a breast cancer that is estrogen receptor positive (ER+), malignant, and resistant to treatment with a first or second line SERM therapy, wherein said detection reagent can distinguish a reference sequence from a mutation chosen from one or more of: a missense mutation at nucleotide 1609 according to SEQ ID NO: 1; a missense mutation at nucleotide 1610 according to SEQ ID NO: 1; a missense mutation at nucleotide 1613 according to SEQ ID NO: 1; a 6 nucleotide deletion of nucleotides 1046-1051 (TGGCAG) according to SEQ ID NO: 1, or other deletion, e.g., an in-frame deletion, that includes one or more of nucleotides 1046-1051 ; a 3 nucleotide insertion between nucleotides 1033 and 1034 according to SEQ ID NO:1 ; or a mutation at a position identified as mutated in Table 3; AND A method of making said reaction mixture; AND A detection reagent comprising a nucleic acid molecule, e.g., a DNA, RNA or mixed DNA/RNA molecule; AND A reaction mixture comprising: a detection reagent, or purified or isolated preparation thereof, e.g., a substrate, e.g., a substrate for phosphorylation or other activity, or an antibody; and a target ESR1 protein derived from a breast cancer that is estrogen receptor positive (ER+), malignant, and resistant to treatment with a first or second line SERM therapy, wherein the detection reagent is specific for a mutation at position 537 or 538 of the amino acid sequence of SEQ ID NO:2 (FIGS. 2A-2B); AND A nucleic acid molecule suitable as probe, primer, bait or library member that can specifically identify, capture, detect, or isolate, a mutant ESR1 , wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR 1; AND An isolated or purified antibody molecule that binds a mutant ESR1 polypeptide, wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR1 , as required by Group I.

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Groups I-II share the technical feature of a nucleic acid derived from a breast cancer that is estrogen receptor positive (ER+), malignant, and resistant to treatment with a first or second line SERM therapy, wherein the nucleic acid comprises a mutation chosen from one or more of: a missense mutation at nucleotide 1609 according to SEQ ID NO: 1; a missense mutation at nucleotide 1610 according to SEQ ID NO: 1; a missense mutation at nucleotide 1613 according to SEQ ID NO: 1; a 6 nucleotide deletion of nucleotides 1046-1051 (TGGCAG) according to SEQ ID NO: 1, or other deletion, e.g., an in-frame deletion, that includes one or more of nucleotides 1046-1051; a 3 nucleotide insertion between nucleotides 1033 and 1034 according to SEQ ID NO:1; or a mutation at a position identified as mutated in Table 3; AND An ESR1 protein derived from a breast cancer that is estrogen receptor positive (ER+), malignant, and resistant to treatment with a first or second line SERM therapy, wherein the ESR1 comprises a mutation in the ligand binding domain of the ESR1.

Groups I-III share the technical feature of a presence of a mutant estrogen receptor 1 (ESRI) and an Estrogen Receptor positive (ER+) breast cancer in said subject, wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR 1.

Groups I and IV share the technical feature of a mutant ESR nucleic acid or polypeptide is present in a sample from a subject, wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR1, wherein the mutant ESR1 comprises a missense mutation at position 537 or 538 of the amino acid sequence of SEQ ID NO:2 (FIGS. 2A-2B).

Groups I-II and V-VII share the technical feature of a mutant ESR1, wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR1.

Groups II and IV share the technical feature of a mutant ESR nucleic acid or polypeptide present in a sample from a subject with an Estrogen Receptor status, wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR1.

Groups II and VI share the technical feature of a nucleic acid molecule that encodes a mutant ESR1, wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR1.

Groups III-IV share the technical feature of a method comprising acquiring knowledge that a mutant ESR nucleic acid or polypeptide is present in a sample from a subject; and (optionally) acquiring knowledge of the Estrogen Receptor status, wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR1.

Groups III and V-VII share the technical feature of a mutant ESR1, wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR 1.
Groups III and VI-VII share the technical feature of optionally, acquiring knowledge of the presence of the mutant ESR1.

Groups IV-VII share the technical feature of a mutant ESR1, wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR1.
Groups IV and VI-VII further share the technical feature of acquiring knowledge of the presence of the mutant ESR1.

Groups V-VI share the technical feature of an agent that inhibits an activity or expression of mutant ESR1, wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR1.
Groups V and VII share the technical feature of an agent that inhibits an activity or expression of mutant ESR1.
Groups VI-VII share the technical feature of a method regarding an agent that inhibits an activity or expression of a mutant ESR1 comprising: acquiring knowledge of the presence of the mutant ESR1; and contacting the mutant ESR1 with an agent that inhibits an activity or expression of mutant ESR1.

However, these shared technical features do not represent a contribution over the prior art, specifically, US 2006/0100168 A1 to Ravid et al. (hereinafter 'Ravid') teaches a nucleic acid derived from a breast cancer that is estrogen receptor positive (ER+), malignant, and resistant to treatment with a first or second line SERM therapy, wherein said nucleic acid comprising a mutation chosen from one or more of: a missense mutation at nucleotide 1609 of estrogen receptor alpha (ESR1) (para [0029]-'a method of treating breast cancer with an estrogen receptor alpha mutation Tyr 537 to Asn (T 1609 A), by administering an estrogen receptor down-regulating amount of an adenosine analog to the individual with cells having the mutation. This mutation has been identified in approximately 1 of 30 metastatic breast cancers (http://www.ncbi.nlm. nih.gov/entrez/dispomim. cgi?id=133430). This substitution confers constitutive transcriptional activity to estrogen receptor and its activity cannot be antagonized with antiestrogens such as tamoxifen and pure antiestrogen ICI 164384', [0047]-'the individual affected with breast cancer which is unresponsive to tamoxifen, 4-OH-tamoxifen, raloxifene, or ICI 164,384 therapy', [0005]-'The most widely used SERM in breast cancer is tamoxifen, which is a partial estrogen receptor agonist/antagonist', [0010]-'a novel method for treating individuals affected with cancers associated with estrogen receptor expression, such as estrogen receptor positive cancers, including breast and ovarian cancers').
Ravid does not expressly disclose that said estrogen receptor alpha comprises SEQ ID NO: 1. However, US 2010/0120039 A1 (Fuqua) teaches that estrogen receptor alpha comprises SEQ ID NO: 1 (SEQ ID NO: 6, 100% identical; para [0157]-'the estrogen receptor alpha wildtype and/or mutant nucleic acid comprises at least one nucleic acid segment of SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or at least one biologically functional equivalent thereof.').
Thus one of ordinary skill in the art would have found it obvious to use said estrogen receptor alpha sequence disclosed by Fuqua to design primers/probes to detect the mutant ESR1 disclosed by Ravid so as to design proper treatment for a patient based on the detected genotype of ESR1 in said patient.

**********Continued in Supplemental LOU Statement Continuation of Form 210*** **********

Continuation of Previous Page:

Ravid further teaches an ESR1 protein derived from a breast cancer that is estrogen receptor positive (ER+), malignant, and resistant to treatment with a first or second line SERM therapy, wherein the ESR1 comprises a mutation in the ligand binding domain of the ESR1 , wherein said mutation comprises a missense mutation at position 537 or 538 of the amino acid sequence of said ESR1 (para [0029]-'a method of treating breast cancer with an estrogen receptor alpha mutation Tyr 537 to Asn (T 1609 A), by administering an estrogen receptor down-regulating amount of an adenosine analog to the individual with cells having the mutation. This mutation has been identified in approximately 1 of 30 metastatic breast cancers (http://www.ncbi. nlm.nih.gov/entrez/dispomim.cgi?id=133430). This substitution confers constitutive transcriptional activity to estrogen receptor and its activity cannot be antagonized with antiestrogens such as tamoxifen and pure antiestrogen ICI 164384 ', [said 'Tyr 537 to Asn mutation is a mutation in the ligand binding domain.] [0047]-'the individual affected with breast cancer which is unresponsive to tamoxifen, 4-OH-tamoxifen, raloxifene, or ICI 164,384 therapy', [0005]-'The most widely used SERM in breast cancer is tamoxifen, which is a partial estrogen receptor agonist/antagonist', [0010]-'a novel method for treating individuals affected with cancers associated with estrogen receptor expression, such as estrogen receptor positive cancers, including breast and ovarian cancers').

Ravid does not specifically teach that said ESR1 comprises SEQ ID NO: 2. However, Fuqua teaches that ESR1 (estrogen receptor alpha) comprises the amino acid sequence of SEQ ID NO: 2 (SEQ ID No: 9, 100% identical; para [0154]-'substitution include NP.sub.-0001 16.2 (SEQ ID NO:8); AAF65451 .1 (SEQ ID NO:9); AAD23565.1 (SEQ ID NO:10); AAB001 15.1 (SEQ ID NO:11); AAA52399.1 (SEQ ID NO:12); and CAA27284.1 (SEQ ID NO:13)').

Ravid further discloses a nucleic acid encoding a mutant ESR1 protein, wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR1 , wherein the mutant ESR1 comprises a missense mutation at position 537 or 538 of the amino acid sequence of said ESR1 protein (para [0029]-'a method of treating breast cancer with an estrogen receptor alpha mutation Tyr 537 to Asn (T 1609 A), by administering an estrogen receptor down-regulating amount of an adenosine analog to the individual with cells having the mutation').

Thus one of ordinary skill in the art would have found it obvious to use said ESR1 amino acid sequence disclosed by Fuqua to design detecting agents such as antibody for the T537N ESR1 mutation disclosed by Ravid in a patient so as to design personalized treatment plan for said patient based on the result of said detecting.

Ravid further teaches a method of treating a subject having estrogen receptor positive (ER+) breast cancer comprising: administering to a subject an effective amount of an anti-cancer agent other than Selective Estrogen Receptor Modulator (SERM), thereby treating the breast cancer in the subject, wherein the subject has the present of a mutant ESR1 , wherein the mutant ESR1 comprises a mutation in the ligand binding domain of ESR1 , wherein said mutation comprises a mutation at 537 of said ESR1 polypeptide, (para [0029]-'a method of treating breast cancer with an estrogen receptor alpha mutation Tyr 537 to Asn (T 1609 A), by administering an estrogen receptor down-regulating amount of an adenosine analog to the individual with cells having the mutation. This mutation has been identified in approximately 1 of 30 metastatic breast cancers (http://www. ncbi.nlm. nih.gov/entrez/dispomim. cgi?id=1 33430). This substitution confers constitutive transcriptional activity to estrogen receptor and its activity cannot be antagonized with antiestrogens such as tamoxifen and pure antiestrogen ICI 164384') [said 'Tyr 537 to Asn' mutation is a mutation in the ligand binding domain.], [0010]-'a novel method for treating individuals affected with cancers associated with estrogen receptor expression, such as estrogen receptor positive cancers, including breast and ovarian cancers').

Ravid does not specifically teach that the method further comprises acquiring knowledge that said mutant ESR nucleic acid or polypeptide is present in a sample from a subject; and (optionally) acquiring knowledge of the Estrogen Receptor status and that said ESR1 polypeptide comprises SEQ ID NO: 2. However, Ravid teaches antibodies for detecting the presence of ESR1 in a sample (para [0059]-'a monoclonal antibody or fragment thereof that specifically binds estrogen receptor to enable detection of downregulation of estrogen receptors in the cells'). Based on this disclosure, one of ordinary skill in the art would have known to use said antibody to detect and acquire knowledge of whether the breast cancer in the subject is ER+.

Further Fuqua teaches a method of detecting the present of a specific mutant ESR nucleic acid in a sample from a subject using mutation specific primers (para [0019]-'a kit for identifying resistance to hormonal breast cancer therapy and/or sensitivity to breast cancer chemotherapy comprising reagents suitable for detecting an A908G mutation in an estrogen receptor alpha nucleic acid sequence and/or for detecting a K303R mutation in an estrogen receptor alpha amino acid sequence. In particular embodiments, the kit comprises at least one primer to conduct PCR amplification of the mutation, and/or at least one primer suitable for primer extension such that the primer extension identifies the mutation. The mutation can be detected using standard sequencing techniques, or mass spectroscopy. In specific embodiments, the mutation can be detected by primer extension, sequencing, single stranded conformation polymorphism, mismatch oligonucleotide mutation detection, mass spectroscopy, DNA microarray, HPLC, microarray, SNP PCR genotyping, or a combination thereof, [0082]-'a kit for diagnosing an A908G mutation in an estrogen receptor alpha nucleic acid sequence, comprising at least one primer suitable for use in primer extension to detect the mutation').

Further Fuqua teaches that ESR1 (estrogen receptor alpha) comprises the amino acid sequence of SEQ ID NO: 2 (SEQ ID No: 9, 100% identical; para [0154]-'substitution include NP.sub.-0001 16.2 (SEQ ID NO:8); AAF65451 .1 (SEQ ID NO:9); AAD23565.1 (SEQ ID NO:10); AAB001 15.1 (SEQ ID NO:11); AAA52399.1 (SEQ ID NO:12); and CAA27284.1 (SEQ ID NO:13)').

Thus one of ordinary skill in the art would have found it obvious to design primers specific for T1609A ESR1 mutation disclosed by Ravid based on the teaching of Fuqua so as to determine whether said T1609 A mutant ESR1 (encoding Y537N ESR1 mutant protein) is present in the subject in order to determine whether an anti-cancer therapy other than SERM should be provided.

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Continuation of Previous page:

Ravid further teaches a method of reducing activity or expression of a mutant ESR1 comprising: contacting a mutant ESR1 with an agent that inhibits an activity or expression of mutant ESR1 , wherein the mutant ESR1 comprises a mutation in the ligand binding domain (para [0029]-'a method of treating breast cancer with an estrogen receptor alpha mutation Tyr 537 to Asn (T 1609 A), by administering an estrogen receptor down-regulating amount of an adenosine analog to the individual with cells having the mutation. This mutation has been identified in approximately 1 of 30 metastatic breast cancers (http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=1 33430). This substitution confers constitutive transcriptional activity to estrogen receptor and its activity cannot be antagonized with antiestrogens such as tamoxifen and pure antiestrogen ICI 164384') [said 'Tyr 537 to Asn' mutation is a mutation in the ligand binding domain.], [0018]-'In the most preferred embodiment, the estrogen receptor down-regulating adenosine analog is IB-MECA or a functional, estrogen receptor down-regulating derivative thereof. Preferably, the estrogen receptor is estrogen receptor alpha', [0091]-[0092]-'IB-MECA treatment decreases estrogen receptor a in MCF-7 cells', 'As a consequence of a downregulation of ER.alpha. mRNA, ER.alpha, protein should also show a time-dependent decrease in IB-MECA-treated cells. Indeed, Western blot analyses indicated that ER.alpha. protein in MCF-7 cells decreased after IB-MECA treatment').
Ravid does not specifically teach acquiring knowledge of the present of said mutant ESR1 . However, Fuqua teaches a method of detecting the present of a specific mutant ESR nucleic acid in a sample from a subject using mutation specific primers (para [0019]-'a kit for identifying resistance to hormonal breast cancer therapy and/or sensitivity to breast cancer chemotherapy comprising reagents suitable for detecting an A908G mutation in an estrogen receptor alpha nucleic acid sequence and/or for detecting a K303R mutation in an estrogen receptor alpha amino acid sequence. In particular embodiments, the kit comprises at least one primer to conduct PCR amplification of the mutation, and/or at least one primer suitable for primer extension such that the primer extension identifies the mutation. The mutation can be detected using standard sequencing techniques, or mass spectroscopy. In specific embodiments, the mutation can be detected by primer extension, sequencing, single stranded conformation polymorphism, mismatch oligonucleotide mutation detection, mass spectroscopy, DNA microarray, HPLC, microarray, SNP PCR genotyping, or a combination thereof, [0082]-'a kit for diagnosing an A908G mutation in an estrogen receptor alpha nucleic acid sequence, comprising at least one primer suitable for use in primer extension to detect the mutation').
Thus one of ordinary skill in the art would have found it obvious to design primers specific for T 1609A ESR1 mutation disclosed by Ravid based on the teaching of Fuqua so as to determine whether said T1609 A mutant ESR1 (encoding Y537N ESR1 mutant protein) is present in a subject in order to determine whether the ESR1 in said subject should be inhibited by said inhibiting agent disclosed by Ravid.

As said shared technical features were known or would have been obvious to one of ordinary skill in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the groups.

Groups I-VII therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

•NOTE:

Claim 15 is object to because it lacks proper antecedent basis for the term "The kit", for the purpose of this IRS, this term is replaced with the term "The kit of claim 14'.