



(51) International Patent Classification:

C12Q 1/00 (2006.01) *C12N 5/00* (2006.01)
C12Q 1/68 (2006.01) *C12N 5/02* (2006.01)
G01N 33/53 (2006.01) *G01N 33/92* (2006.01)
G01N 33/00 (2006.01)

(21) International Application Number:

PCT/US2011/044991

(22) International Filing Date:

22 July 2011 (22.07.2011)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/367,023 23 July 2010 (23.07.2010) US

(71) Applicant (for all designated States except US): **PRESIDENT AND FELLOWS OF HARVARD COLLEGE** [US/US]; 17 Quincy Street, Cambridge, Massachusetts 02138 (US).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **KASSIS, Amin, I.** [US/US]; 28 South Street, Chestnut Hill, Massachusetts 02467 (US).

(74) Agent: **IWANICKI, John, P.**; Banner & Witcoff, Ltd., 28 State Street, Suite 1800, Boston, Massachusetts 02109 (US).

(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, 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, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, 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, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, 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, ML, MR, NE, SN, TD, TG).

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:

24 May 2012

(54) Title: METHODS OF DETECTING KIDNEY-ASSOCIATED DISEASES OR CONDITIONS

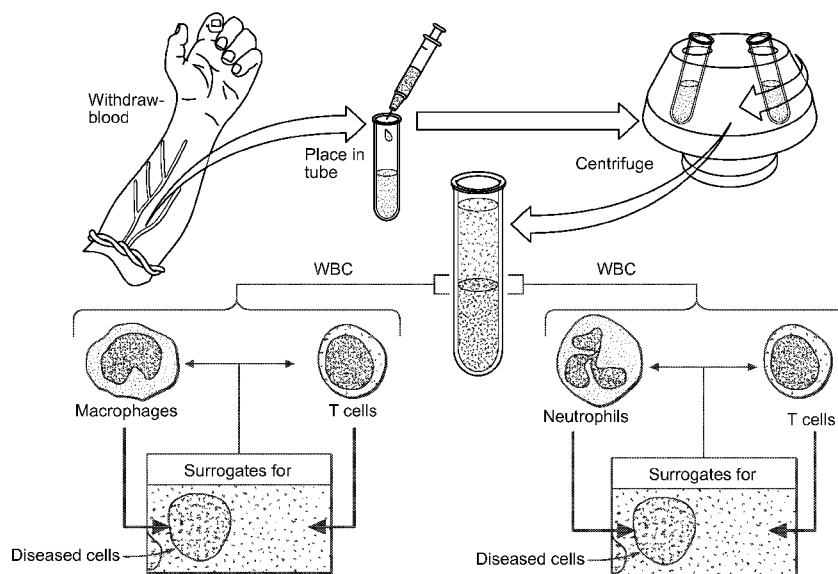


FIG. 1A

(57) Abstract: The disclosure provides methods of using phagocytic cells alone or in combination with non-phagocytic cells in the diagnosis, prognosis, or monitoring of kidney associated diseases or conditions. The disclosure also provides methods of using phagocytic cells alone or in combination with non-phagocytic cells to identify markers of kidney-associated diseases or conditions.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/44991

A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - C12Q 1/00; C12Q 1/68; G01N 33/53; G01N 33/00; C12N 5/00; C12N 5/02; G01N 33/92 USPC - 435/4; 435/6.1; 435/7.1; 435/7.24; 435/325; 435/374; 435/375; 436/71; 436/811 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 1/00; C12Q 1/68; G01N 33/53; G01N 33/00; C12N 5/00; C12N 5/02; G01N 33/92; G01N 33/53 (2012.01) USPC - 435/4; 435/6.1; 435/7.1; 435/7.24; 435/325; 435/374; 435/375; 436/71; 436/811 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched IPC(8) - C12Q 1/00; C12Q 1/68; G01N 33/53; G01N 33/00; C12N 5/00; C12N 5/02; G01N 33/92; G01N 33/53 (2012.01) - see below USPC - 435/4; 435/6.1; 435/7.1; 435/7.24; 435/325; 435/374; 435/375; 436/71; 436/811 - see keyword below Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) PubWEST (USPT, PGPB, EPAB, JPAB); Medline, Google: phagocytic cells, phagocyte, monocytes, macrophages, inflammation, autoimmune, kidney, renal, glomerulonephritis, interstitial nephritis, non-diabetic kidney, diabetic nephropathy, polycystic, primary, parameter, absence, increase, inhibit, expression, marker, sequencing, RT-PCR, profile, T cell, B ce		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2008/0108509 A1 (H. UPL et al.) 08 May 2008 (08.05.2008), Abstract, para [0001], [0013], [0016], [0018], [0019], [0037], [0038], [0043], [0044], [0047], [0049], [0051], [0055], [0057], [0058], [0059], [0063], [0088], [0091], [0120], [0171], [0176], [0181], [0184], Table 1, Table 2A, Table 2B, and Table 5	1, 3-5, 15-16, 19, 21, 26, 35-36, 38-41, 64-66, 72-77, 83-90, 165-167
Y	US 2010/0143951 A1 (KRONENBERG et al.) 10 June 2010 (10.06.2010), Abstract, para [0004], [0013], [0017], [0022], [0023], [0024], [0029], [0032], [0037], [0043], [0054], [0076], and [0085]	1, 3-5, 15-16, 19, 21, 26, 35-36, 38-41, 64-66, 72-77, 83-90, 165-167
Y	US 2009/0263796 A1 (WOHLGEMUTH et al.) 22 October 2009 (22.10.2009), Abstract, para [0005], [0010], [0064], [0090], [0093], [0109], [0158], [0242], [0356], and [0410]	16, 19, 77
A	SEGELMARK et al. Autoimmune kidney diseases. Autoimmun Rev. 2010 Mar, Vol. 9(5), p. A368-71. Epub 2009 Nov 10. Abstract	1, 3-5, 15-16, 19, 21, 26, 35-36, 38-41, 64-66, 72-77, 83-90, 165-167
A	US 2009/0104605 A1 (SIUZDAK et al.) 23 April 2009 (23.04.2009), para [0063], and [0113]	1, 3-5, 15-16, 19, 21, 26, 35-36, 38-41, 64-66, 72-77, 83-90, 165-167
A	US 2004/0038201 A1 (NAU et al.) 26 February 2004 (26.02.2004), para [0004], [0005], [0013], [0057], [0059], [0076], [0079], and [0088]	1, 3-5, 15-16, 19, 21, 26, 35-36, 38-41, 64-66, 72-77, 83-90, 165-167
☒ 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 30 January 2012 (30.01.2012)		Date of mailing of the international search report 16 MAR 2012
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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/44991

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BITTERMAN et al. Alveolar Macrophage Replication One Mechanism for the Expansion of the Mononuclear Phagocyte Population in the Chronically Inflamed Lung. J Clin Invest. 1984, Vol. 74(2), p. 460-9. Abstract; pg 462, col 1, para 2, and col 2, para 3 and 4; pg 463, col 2, lower para and Fig 2; pg 464, Fig 4; pg 466, col 2, para 2; and pg 468, Ref 34	1, 3-5, 15-16, 19, 21, 26, 35-36, 38-41, 64-66, 72-77, 83-90, 165-167
A	NCBI_Bookshelf ID_ NBK7572, Chapter 2Chromosomes in cells, 1999, [online]. [Retrieved on 2011.11.18]. Retrieved from the Internet: <URL: http://www.ncbi.nlm.nih.gov/books/NBK7572/ >, Under 2.1.3, para 1 and 2	1, 3-5, 15-16, 19, 21, 26, 35-36, 38-41, 64-66, 72-77, 83-90, 165-167
A	Zamora, The Hematologist, Scientific Psychic, Poster, 2007, [online]. [Retrieved on 2012.01.27]. Retrieved from the Internet: <URL: http://www.scientificpsychic.com/mind/whitecells.html > white blood cells - monocyte, lymphocyte	1, 3-5, 15-16, 19, 21, 26, 35-36, 38-41, 64-66, 72-77, 83-90, 165-167

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/44991

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.: 22-25, 28-31, 42-49, 54-63, 102-103, 105-106, 118-125, 129-139, 142-148, and 168
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:

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, 3-5, 15-16, 19, 21, 26, 33-41, 64-90, 165-167, drawn to a method for diagnosing or assessing the risk of developing or prognosing a kidney-associated disease or condition in a subject, comprising: a) determining a first profile of one or more markers of the disease or condition from a population of phagocytic cells; b) determining a second profile of at least one of the one or more markers from a population of non-phagocytic cells; and c) identifying a difference between the first and second profiles of at least one or more of said markers. The first named invention (claims 1, 3-5, 15-16, 19, 21, 26, 35-36, 38-41, 64-66, 72-77, 83-90, 165-167) is limited to wherein phagocytic cells are professional phagocytic cells, and wherein one or more markers are nucleic acids that are DNAs. Applicant is invited to elect an additional step (claims 33-34), non-professional phagocytic cells (claim 37), markers with associated profile are RNAs (claim 67), proteins (claims 68, 78-80), lipid (claims 69 and 81), carbohydrates (claims 70 and 82) and/or metabolites (claim 71) to be searched by paying additional fee for each election.

*****Continued in the extra sheet*****

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, 3-5, 15-16, 19, 21, 26, 35-36, 38-41, 64-66, 72-77, 83-90, 165-167, as defined in Group I

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 (unity of invention is lacking)

Group II+, claims 2, 6-8, 15-16, 19-21, 26, 33-41, 64-90, 167, drawn to a method for assessing the efficacy of a treatment or a method for monitoring the progression or regression of a kidney-associated disease or condition in a subject, or method for identifying a compound capable of treating of an autoimmune or immune-related disease or condition in a subject, comprising: a) determining a first profile of one or more markers of the disease or condition from a population of phagocytic cells from the subject before the treatment; determining a second profile of at least one of the one or more markers from a population of non-phagocytic cells from the subject before the treatment, as indicated in claim 6; b) determining a third profile of the one or more markers from a population of phagocytic cells from the subject after the treatment; determining a fourth profile of at least one of the one or more markers from a population of non-phagocytic cells from the subject after the treatment, as indicated in claim 6; and c) identifying a difference between the first difference and the second difference. The first named invention (claim 2, 6-8, 15-16, 19-21, 26, 35-36, 38-41, 64-66, 72-77, 83-90, 167) is limited to wherein phagocytic cells are professional phagocytic cells, and wherein one or more markers are nucleic acids that are DNAs. Applicant is invited to elect an additional step (claims 33-34), non-professional phagocytic cells (claim 37), markers with associated profile are RNAs (claim 67), proteins (claims 68, 78-80), lipid (claims 69 and 81), carbohydrates (claims 70 and 82) and/or metabolites (claim 71) to be searched by paying additional fee for each election.

Group III+, claims 1, 9-11, 17-19, 27, 32-37, 50-53, 64-90, 165-167, drawn to a method for diagnosing or assessing the risk of developing or prognosing a kidney-associated disease or condition in a subject comprising: a) determining a first profile of one or more markers of the disease or condition from a population of phagocytic cells having a DNA content more than $2n$ ($>2n$ phagocytic cells); b) determining a second profile of at least one of the one or more markers from a population of phagocytic cells having a DNA content of $2n$ ($=2n$ phagocytic cells); and c) identifying a difference between the first and second profiles of at least one or more of said markers, wherein the difference is indicative of the presence of said disease or condition in the subject. The first named invention (claims 1, 9-11, 17-19, 27, 32, 35-36, 50-53, 64-66, 72-77, 83-90, 165-167) is limited to wherein phagocytic cells are professional phagocytic cells, and wherein one or more markers are nucleic acids that are DNAs. Applicant is invited to elect an additional step (claims 33-34), non-professional phagocytic cells (claim 37), markers with associated profile are RNAs (claim 67), proteins (claims 68, 78-80), lipid (claims 69 and 81), carbohydrates (claims 70 and 82) and/or metabolites (claim 71) to be searched by paying additional fee for each election.

Group IV+, claims 2, 12-14, 17-20, 27, 32-37, 50-53, 64-90, 167, drawn to a method for assessing the efficacy of a treatment or a method for monitoring the progression or regression of a kidney-associated disease or condition in a subject, or method for identifying a compound capable of treating of a kidney-associated disease or condition in a subject, comprising: a) determining a first profile of one or more markers of the disease or condition from a population of $>2n$ phagocytic cells from the subject before the treatment; determining a second profile of at least one of the one or more markers from a population of $=2n$ phagocytic cells from the subject before the treatment; as indicated in claim 12; b) determining a third profile of the one or more markers from a population of $>2n$ phagocytic cells from the subject after the treatment; determining a fourth profile of at least one of the one or more markers from a population of $=2n$ phagocytic cells from the subject after the treatment; as indicated in claim 12; and c) identifying a difference between the first difference and the second difference. The first named invention (claims 2, 12-14, 17-20, 27, 32, 35-36, 50-53, 64-66, 72-77, 83-90, 167), is limited to wherein phagocytic cells are professional phagocytic cells, and wherein one or more markers are nucleic acids that are DNAs. Applicant is invited to elect an additional step (claims 33-34), non-professional phagocytic cells (claim 37), markers with associated profile are RNAs (claim 67), proteins (claims 68, 78-80), lipid (claims 69 and 81), carbohydrates (claims 70 and 82) and/or metabolites (claim 71) to be searched by paying additional fee for each election.

Group V, claims 91-101, 104, 107-117, 126-128, 140-141, 149-164, 169-170, drawn to a method for identifying one or more markers for a kidney-associated disease or condition comprising: a) determining a first profile of analytes from phagocytic cells from a subject having said disease or condition; determining a second profile of analytes from non-phagocytic cells from the subject having said disease or condition; as indicated in claim 91; b) determining a third profile of analytes from phagocytic cells from a control subject not having said disease or condition; determining a fourth profile of analytes from non-phagocytic cells from the control subject not having said disease or condition; as indicated in claim 91; c) identifying one or more analytes specific to the first set of differences relative to the second set of differences, the identified analytes being markers of said disease or condition; or the method comprising steps as indicated in claims 93, 95, 97, or 99; or a kit comprising a plurality of marker detection agents that detect at least one or more of the markers identified; or a method of administering to a subject an agent that modulates the activity or expression of at least one or more of the markers identified.

The inventions listed as Groups I+-V 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+-IV+ do not include the inventive concept of identifying one or more markers for a kidney-associated disease or condition comprising determining profile from a control subject, as required by Group V. Groups I+-II+ do not include the inventive concept of a) determining a first profile of one or more markers of the disease or condition from a population of phagocytic cells having a DNA content more than $2n$ ($>2n$ phagocytic cells); b) determining a second profile of at least one of the one or more markers from a population of phagocytic cells having a DNA content of $2n$ ($=2n$ phagocytic cells), as required by Groups III+ and IV+. Groups III+-IV+ do not include the inventive concept of a) determining a first profile of one or more markers of the disease or condition from a population of phagocytic cells; b) determining a second profile of at least one of the one or more markers from a population of non-phagocytic cells, as required by Groups I+ and II+. Groups I+, III+, and V do not include the inventive concept of determining a profile before and after treatment, as required by Group II+ and IV+.

Furthermore, between each groups of Groups I+-IV+, professional phagocytic cells and non-professional phagocytic cells are structurally and functionally different; markers of different types including DNAs, RNAs, proteins, lipids, carbohydrates or metabolites are structurally and functionally different and determining a profile of DNAs, RNAs, proteins, lipids, carbohydrates or metabolites, each requires different methods for detection.

The inventions of Groups I+-V share the technical feature of a kidney-associated disease or condition in a subject; Groups I+-II+ and part of V share the technical feature of a) determining a first profile of one or more markers of the disease or condition from a population of phagocytic cells; b) determining a second profile of at least one of the one or more markers from a population of non-phagocytic cells; and c) identifying a difference between the first and second profiles of at least one or more of said markers. However, these shared technical features do not represent a contribution over prior art as being obvious over US 2008/0108509 A1 to HAUPL et al. (hereinafter 'Haupt'), in view of an article entitled 'Autoimmune kidney diseases' by SEGELMARK et al. (hereinafter 'Segelmark'; Autoimmun Rev. 2010 Mar, Vol. 9(5), p. A366-71. Epub 2009 Nov 10), as follows: *****Continued in the next extra sheet*****

Continuation of:

The previous extra sheet - Box No. III (unity of invention is lacking)

Haupt discloses a method for diagnosing or aiding in the diagnosis of an autoimmune or immune-related disease or condition in a subject (Abstract - 'making available at least one suitable expression profile...to the application of the process...in the diagnosis, prognosis and/or tracking of a disease'; para [0044] - 'expression profiles, that characterize ... inflammation,... autoimmune reaction') comprising:

a) determining a first profile of one or more markers of the disease or condition from a population of phagocytic cells (Abstract - 'determining the complex expression profile of the biological sample'; para [0047] - 'For the detection of different ... marker genes for monocytes'; para [0037] - 'The typical "expression profiles" ... of defined influences and/or conditions are also named "signatures" signatures for the various cell types are necessary, e.g., for monocytes', wherein 'monocytes' are phagocytic cells; Specification: para [0033] - 'professional phagocytic cells (e.g., neutrophils, macrophages, monocytes, dendritic cells, foam cells, mast cells, eosinophils)')

b) determining a second profile of at least one of the one or more markers from a population of non-phagocytic cells (Abstract - 'determining the complex expression profile of the biological sample'; para [0047] - 'For the detection of different ... marker genes for ... T cells'; para [0037] - 'The typical "expression profiles" ... of defined influences and/or conditions are also named "signatures" signatures for the various cell types are necessary, e.g., for...T cells', wherein T cells are 'non-phagocytic cells'; Specification: para [0033] - 'the non-phagocytic cells are T cells') and

c) identifying a difference between the first and second profiles of at least one or more of said markers (para [0049] - 'detection of expression profile of a T-cell, monocyte... expression profile'; para [0051] - 'using signatures that characterize different cell types... using normal signatures... an expected profile ... is calculated. ... the functional signature that is adjusted by cell type, ... assessed quantitatively in the difference between virtual profile and actually measured profile', wherein 'using signatures that characterize different cell types...functional signature that is adjusted by cell type, the proportion of this signature can be assessed quantitatively in the difference between virtual profile and actually measured profile' indicating marker genes chosen express differentially in different cell types; Abstract - 'calculation of the difference from the actually measured complex expression profile ... determination of the quantitative composition of the complex expression profile based on the determined differences').

Although Haupt does not expressly teach wherein the difference is indicative of the presence of said disease or condition in the subject, one of ordinary skill in the art at the time the invention was made would have known to make such conclusion because it is a routine practice in the art as indicated by Haupt (para [0001] - 'determining the quantitative ... expression profile .. determined differences. ... in the diagnosis, prognosis ...a disease'; para [0063] - diagnosis, prognosis ... is known to one skilled in the art').

Haupt further teaches diagnosing a kidney-associated disease or condition in a subject comprising determining associated profile (para [0013] - 'Quantitative Detection of T-Cell Activation Markers by Real-Time PCR in Renal Transplant Rejection').

Haupt does not expressly teach using the method for diagnosing a kidney-associated disease or condition in a subject. Segelmark discloses the second most common cause of chronic kidney failure is immune-related kidney disease (Abstract - 'The second most common cause of chronic renal failure is glomerulonephritis, which is a collective term used for numerous diseases with the common denominator of histological renal inflammation ...Whether all forms of glomerulonephritis should be considered as autoimmune disease is debatable, but immunemechanisms are important in all of them').

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Haupt and Segelmark, to obtain a method for diagnosing or aiding in the diagnosis of an autoimmune or immune-related disease or condition in a subject, comprising: a) determining a first profile of one or more markers of the disease or condition from a population of phagocytic cells; b) determining a second profile of at least one of the one or more markers from a population of non-phagocytic cells; c) identifying a difference between the first and second profiles of at least one or more of said markers, wherein the difference is indicative of the presence of said disease or condition in the subject, based on the teaching of Haupt, and further wherein the method is for diagnosing a kidney-associated disease or condition in a subject, based on the combination of Haupt and Segelmark, in order to use the method taught by Harple for diagnosing different immune-related disease including the kidney diseases that contribute to the second common cause of kidney failure for facilitating earlier treatment. Without a shared special technical feature, the inventions lack unity with one another.

The inventions of Groups III+IV+ and part of Group V further share the technical feature of determining a first profile of one or more markers of the disease or condition from a population of phagocytic cells having a DNA content more than 2n (>2n phagocytic cells); b) determining a second profile of at least one of the one or more markers from a population of phagocytic cells having a DNA content of 2n (=2n phagocytic cells); and c) identifying a difference between the first and second profiles of at least one or more of said markers, wherein the difference is indicative of the presence of said disease or condition in the subject. However, this shared technical feature does not represent a contribution over prior art as being obvious over US 2004/0038201 A1 to NAU et al. (hereinafter 'Nau'), in view of an article entitled 'Alveolar Macrophage Replication One Mechanism for the Expansion of the Mononuclear Phagocyte Population in the Chronically Inflamed Lung' by BITTERMAN et al. (hereinafter 'Bitterman'; J Clin Invest. 1984, Vol. 74(2), p. 460-9) as follows:

Nau discloses a method for diagnosing an immune-related disease or condition in a subject (para [0076] - 'diagnosing infection by a specific pathogen', wherein 'infection' is 'immune-related disease'; para [0057] - 'selective immune response to a specific pathogen ...providing additional targets for diagnosis and therapy'; para [0013] - 'a disorder selected from the group consisting of pathogenic infection, sepsis and autoimmunity') comprising:

a) determining a first profile of one or more markers of the disease or condition from a population of phagocytic cells exposed to an immune stimulator (para [0005] - 'Gene expression profiles of human macrophages differ depending on the organism to which the macrophages are exposed, providing clues to diagnosis...identifies biomarkers ... whose expression is altered in response to pathogens. ...useful as diagnostics', wherein 'pathogens' is 'an immune stimulator', and 'macrophages' are 'phagocytic cells'; para [0088] - 'Stimuli (e.g., pathogens) ... elicits an immune response when exposed to macrophages'; para [0076] - 'isolating mRNA from one or more monocytes or macrophages in a mammal; determining a gene expression profile of at least one stimulus-specific gene', wherein 'monocytes or macrophages' are phagocytic cells; para [0004] - 'Macrophage phagocytosis of microorganisms is important in host immunity'; Specification: para [0033] - 'professional phagocytic cells ... macrophages, monocytes')

b) determining a second profile of at least one of the one or more markers from a population of phagocytic cells unexposed to the immune stimulator (para [0076] - 'isolating mRNA from one or more monocytes or macrophages in a mammal; determining a gene expression profile of at least one stimulus-specific gene ...The profile of the stimulus-specific gene can be positive or negative with respect to a reference state, e.g., an uninfected state...relative to a control gene expression profile', wherein 'a control gene expression profile' is obtained from 'an uninfected state' of phagocytic cells'; para [0059] - 'Stimulus-specific genes are identified ... by satisfying the following criteria: the ratio of gene expression score in response to the pathogens differs by greater than 2.5-fold compared to control media', wherein 'control media' is non-immune stimulator); and

*****Continued in the next extra sheet*****

Continuation of:

The previous extra sheet - Box No. III (unity of invention is lacking)

c) identifying a difference between the first and second profiles of at least one or more of said markers (para [0005] - 'biomarkers whose expression is altered in response to pathogens. ...useful as diagnostics', wherein 'biomarkers whose expression is altered in response to pathogen' indicates the different expression of biomarkers in phagocytic cells after cells exposed to an immune stimulator in comparison with phagocytic cells have not been exposed to the immune stimulator; para [0076] - 'The profile of the stimulus-specific gene can be positive or negative with respect to a reference state, e.g., an uninfected state'), ---wherein the difference is indicative of the presence of said disease or condition in the subject (para [0076] - 'wherein expression of a stimulus-specific gene is indicative of infection by a pathogen to which the stimulus-specific gene is specific'; para [0059] - 'the gene expression profiles described herein and identified by the methods of the present invention can be used to identify a pathogen or pathogen class to which a macrophage has been exposed').

Nau does not specifically teach determining the first profile from a population of phagocytic cells having a DNA content more than $2n$ ($>2n$ phagocytic cells), and determining a second profile from a population of phagocytic cells having a DNA content of $2n$ ($=2n$ phagocytic cells). Bitterman discloses an increased DNA synthesis is observed in phagocytic cells that are exposed to an immune stimulator (Abstract - 'chronically inflamed tissue...an increased number of macrophages...alveolar macrophages from normals ...and individuals with chronic lung inflammation', wherein 'macrophages' are 'phagocytic cells' as discussed above; pg 462, col 2, para 3 - 'Alveolar macrophages were clearly capable of actively synthesizing DNA ... the cells to rapidly phagocytose opsonized iron'; pg 463, Fig 2), with an increased phagocytic cell division as evident by increased phagocytic cells in S-M phase (Abstract ? 'While the proportion of replicating macrophages in normals was very low, there was a 2- to 15-fold increase in this proportion in patients with chronic lung inflammation'; pg 463, col 2, lower para - 'the average the replicating macrophages were larger than the nonreplicating macrophages...the majority of the alveolar macrophages in the S + (G2 + M) phases of the cell cycle'; pg 464, Fig 4), which are phagocytic cells having a DNA content more than $2n$ ($>2n$ phagocytic cells) as determined by the inherent property of cell cycle regulation (pg 466, col 2, para 2 - 'studies support the concept that tissue macrophages are capable of replicating...macrophages can synthesize DNA ...macrophage DNA synthesis has been shown during murine liver regeneration .? macrophages not only are capable of DNA synthesis, but also are able to divide'; please also see: NCBI Bookshelf ID _NBK7572: Fig. 2.2 - 'Interphase comprises G1 + S + G2. Chromosomes contain one DNA double helix from anaphase of mitosis right through until the DNA has duplicated'), while little DNA synthesis or cell division is observed in phagocytic cells that are not exposed to immune stimulator (Abstract ? 'the proportion of replicating macrophages in normals was very low'; pg 462, para 4 - 'In normal individuals, less than 1 in 200 alveolar macrophages was actively synthesizing DNA'; pg 463, Fig 2), which are phagocytic cells having a DNA content of $2n$ ($=2n$ phagocytic cells) as determined by the inherent property of cell cycle regulation at G1 stage (please see: NCBI Bookshelf ID _NBK7572: Under 2.1.3, para 1 - 'Most cells of humans are diploid...The DNA content of diploid cells is $2C$ and they have $2n$ chromosomes'; para 2 - 'Interphase can be divided into S phase (DNA synthesis), ... G2 phase (gap between S phase and M phase). From anaphase of mitosis right through until DNA duplication in S phase, a chromosome of a diploid cell contains a single DNA double helix and the total DNA content is $2C$. G1 is the normal state of a cell, and the long-term end state of nondividing cells. Cells enter S phase only if they are committed to mitosis; nondividing cells remain in a modified G1 stage, sometimes called G0'; Note: normal phagocytic cells such as macrophages are diploid cells; Specification: para [0004] - 'Phagocytes from healthy animals do not replicate and are diploid'). Bitterman further discloses determining proportions of cells in different phases using flow cytometry for cell sorting (pg 462, col 1, para 2 - 'Determination of the proportion of cells in the various phases of the cell cycle (G1, S, or G2 + M) was carried out assuming G, and G2 + M were Gaussian populations according to standard methods (34)'; pg 468, Reference 34 - 'Flow Cytometry and Sorting. 1979. M. R. Melamed, P. F. Mullaney, and M. L. Mendelsohn, editors. Wiley, New York').

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Nau and Bitterman, to obtain a method for diagnosing an immune-related disease or condition in a subject, a) determining a first profile of one or more markers of the disease or condition from a population of phagocytic cells exposed to an immune stimulator, b) determining a second profile of at least one of the one or more markers from a population of phagocytic cells unexposed to the immune stimulator, as taught by Nau, and further wherein the first profile is determined from a population of phagocytic cells having a DNA content more than $2n$ ($>2n$ phagocytic cells), and the second profile is determined from a population of phagocytic cells having a DNA content of $2n$ ($=2n$ phagocytic cells), based on the combination of Bitterman and Nau, in order to determining profiles from a population of phagocytic cells that have actually exposed to immune stimulator (committed dividing cells; $>2n$ phagocytic cells), and a population of phagocytic cells that actually have not exposed to immune stimulator (normal cell; $=2n$ phagocytic cells), based on DNA replication using standard method for cell sorting, for reducing background influence because of existing cells in different phases for facilitating diagnosis. Without a shared special technical feature, the inventions lack unity with one another.

The inventions of Groups II+ and IV+, further share the technical feature of determining profile before and after treatment. However, this shared technical feature does not represent a contribution over prior art. Specifically, Nau further discloses deter (para [0079] - 'a strong correlation between a gene-profile and a disease for which treatment is available, detection of a stimulus-responsive gene expression profile in a human or animal patient can justify immediate administration of treatment, ... regular monitoring of the patient. ...Identification of a stimulus-responsive gene expression profile in a patient correlated with enhanced receptiveness to one of several treatment regimes for a disease indicates that this treatment regime should be followed', wherein 'regular monitoring of the patient. ...Identification of a stimulus-responsive gene expression profile in a patient correlated with enhanced receptiveness to one of several treatment regimes for a disease indicates that this treatment regime should be followed' indicates the method comprising 'determining profile before and after treatment'. Without a shared special technical feature, the inventions lack unity with one another.

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

Note re item 4: Claims 22-25, 28-31, 42-49, 54-63, 102-103, 105-106, 118-125, 129-139, 142-148, and 168 are not drafted in accordance with the second and third sentences of Rule 6.4 (a). These claims are improper multiple dependent claims.