Abstract: The disclosure provides a composition comprising polyclonal antibodies that specifically bind to human tumor necrosis factor (hTNF) wherein the polyclonal antibodies are derived from the serum, milk or colostrum of a bovine animal that has been immunized with hTNF or an immunogenic portion thereof. Further provided are the methods for antigenic specificity analysis of the anti-hTNF polyclonal antibodies.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

<table>
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<tr>
<th>IPC(8)</th>
<th>C07K 16/00; A61K 39/00; A61K 39/395 (2013.01)</th>
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<td>530/389.1, 530/389.2, 424/157.1, 424/158.1, 530/387.1, 424/130.1, 424/145.1</td>
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELD SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

<table>
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<th>IPC(8)</th>
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</table>

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

<table>
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<tr>
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</tbody>
</table>

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatWest(USPT,PGPB,EPAB,JPAB); PatBase; Medline; Google; polyclonal antibody, human tumor necrosis factor alpha, hTNF, TNF-epitope, fragment, serum, milk, colostrom, bovine, stability, neutralizing, primate, rhesus monkey, cynomologus, canine, apoptosis, peripheral, mononuclear, L929, recombinant, immunize, adjuvant, Quil A, Montanide ISA 201 VG,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tbody>
<tr>
<td><strong>Y</strong> US 2002/0106372 A1 (LE et al.) 08 August 2002 (08.08.2002), para [0040], [0080], [0081], [0086], [0100], [0106], and SEQ ID NO: 1</td>
<td>1-2, 6-7</td>
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<td><strong>Y</strong> US 2010/0266007 A1 (Fox) 21 October 2010 (21.10.2010), Abstract, para [0014], [0021], [0034], [0043], [0088], [0089], [0128], [0139], [0141]. and [0142], and [0166]</td>
<td>1-2, 6-7</td>
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<td><strong>Y</strong> US 2010/0040064 A1 (SALFELD et al.) 18 February 2010 (18.02.2010), para [0019], [0064]. and [0191]</td>
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<td><strong>Y</strong> US 2008/0193473 A1 (ZAGURY et al.) 14 August 2008 (14.08.2008), para [0124], [0125], [0174], [0177], [0178], [0179], [0181], [0361], and [0426]</td>
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</table>


**"A"** 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
11 January 2013 (11.01.2013)

Date of mailing of the international search report
28 JAN 2013

Name and mailing address of theISA/US
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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/2 10 (second sheet) (July 2009)
INTERNATIONAL SEARCH REPORT

PCT/US 12/49207

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. 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 1-, claims 1-7, 19-24, drawn to a composition comprising polyclonal antibodies that specifically bind to human tumor necrosis alpha (hTNF) wherein the polyclonal antibodies are derived from the serum, milk or colostrum of a bovine animal that has been immunized with hTNF or an immunogenic portion thereof wherein the polyclonal antibodies comprise one or more of the following features, as indicated in claim 1. The first named invention (claims 1-2, 6-7), is limited to wherein the polyclonal antibodies also bind and neutralize canine TNF. """"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. 1-2, 6-7, limited to wherein the polyclonal antibodies also bind and neutralize canine TNF.

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.

Form PCT/ISA/2 10 (continuation of first sheet (2)) (July 2009)
Continuation of:
Box No III (unity of invention is lacking)

(Continuation of Group I) Applicant is invited to elect (an) additional feature(s), including wherein the polyclonal antibodies have about 2% or less cross reactivity with murine TNF as compared to hTNF (claim 3), wherein the polyclonal antibodies bind canine TNF to a greater degree than cynomolgus macaque TNF and neutralize cynomolgus macaque TNF to a greater degree than canine TNF (claim 4), the composition neutralizes human TNF cytotoxicity in a standard in vitro L929 assay with an EC50 of 0.03 mg/ml or less (claim 5), the composition contains less than about 1 mg of lactoferrin per gram of total protein present in the composition (claim 19), or/and wherein the preparation of the composition comprises the steps of: (a) filtering the whey derived from the colostrum of the bovine through an anion exchange column or a cationic exchange column; (b) collecting the flow through of the column in step (a); and (c) concentrating the flow through of step (b) by ultrafiltration, or steps as indicated in claim 21 (claims 20-24), to be searched by paying additional fee for each election.

Group II, claims 8-9, drawn to a pharmaceutical composition comprising the composition of claim 1 and a pharmaceutically acceptable carrier or excipient.

Group III, claims 10-18, drawn to method of treating an inflammatory disorder in a patient comprising administering to the patient a therapeutically effective amount of the pharmaceutical composition of claim 8. The first named invention (claims 10 and 15) is limited to treating inflammatory bowel disease (IBD). Applicant is invited to elect an additional method(s); including the method for treating oral or intestinal mucositis (claims 11-13, 15), or the method for treating gastrointestinal acute radiation syndrome (GI-ARS) (claims 14-18), to be searched by paying additional fee for each election. Note: The scope of claim 15 will be searched will depend upon the election.

The inventions listed as Groups I-Ill do not relate to a single general inventive concept under PCT Rule 13.2 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups I+II do not include the inventive concept of administering to a patient a therapeutically effective amount of the pharmaceutical composition, as required by Group III.

Groups I do not include the inventive concept of a pharmaceutically acceptable carrier or excipient, as required by Group II.

Among Group I, different features: bind and neutralize canine TNF (claim 2), cross reactivity with murine TNF (claim 3), neutralize cynomolgus macaque TNF (claim 4), neutralizes human TNF cytotoxicity in a standard in vitro L929 assay with an EC50 of 0.03 mg/ml or less (claim 5), contains less than about 1 mg of lactoferrin per gram of total protein present in the composition (claim 19), each requires a different method for characterization, and each does not include the inventive concept of filtering the whey derived from the colostrum of the bovine through an anion exchange column or a cationic exchange column, etc. as required by claims 20-24.

Furthermore, among Group III, inflammatory bowel disease (claim 10) is pathologically different from oral or intestinal mucositis (claim 11), or gastrointestinal acute radiation syndrome (GI-ARS) (claims 14 and 16), and vice versa; each requires a different treatment regimen.

The inventions of Groups I through III share the technical feature of a composition comprising polyclonal antibodies that specifically bind to human tumor necrosis alpha (hTNF) wherein the polyclonal antibodies are derived from the serum, milk or colostrum of a bovine animal that has been immunized with hTNF or an immunogenic portion thereof wherein the polyclonal antibodies comprise one or more of the following features, as indicated in claim 1. However, this shared technical feature does not represent a contribution over prior art as being obvious over US 2002/0166372 A1 to LE et al. (hereinafter 'Le'), in view of US 2010/0266607 A1 to Fox as follows:

Le discloses a composition comprising polyclonal antibodies that specifically bind to human tumor necrosis alpha (hTNF) (para [0040]) - provides anti-TNF antibodies in the form of pharmaceutical compositions; Abstract - Anti-TNF antibodies, specific for human tumor necrosis factor-alpha. (TNF.alpha.) - para [0080] - The term ‘antibody’ is meant to include polyclonal antibodies'

wherein the polyclonal antibodies are derived from the serum of an animal that has been immunized with hTNF or an immunogenic portion thereof (para [0081]) - Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen; para [0086] - 'Such antibodies ... can include those generated by immunization using purified recombinant hTNFalpha. ... or peptide fragments thereof'

- wherein the polyclonal antibodies comprise one or more of the following features:
  b) bind to at least one epitope on hTNF wherein at least one epitope comprises an amino acid sequence selected from all or a portion of the amino acid sequence of: SEQ ID NO: 2; SEQ ID NO: 3 SEQ ID NO: 4; SEQ ID NO: 5; and SEQ ID NO: 6 (para [0086] - 'Such antibodies ... can include those generated by immunization using purified recombinant hTNFalpha. (SEQ ID NO:1) or peptide fragments thereof, wherein SEQ ID NO: 1 is 100% identical to the claimed SEQ ID NO: 1 of the application and comprises a region between amino acid residues 1-13, that is 100% identical to the claimed SEQ ID NO: 2; a region between amino acid residues 61-65, that is 100% identical to the claimed SEQ ID NO: 3, a region between amino acid residues 10-18, that is 100% identical to the claimed SEQ ID NO: 4, a region between amino acid residues 91-95, that is 100% identical to the claimed SEQ ID NO: 5, and a region between amino acid residues 131-140, that is 100% identical to the claimed SEQ ID NO: 6, para [0100] - 'anti-TNF antibodies of the present invention recognize... the epitope comprises at least 5 amino acids from residues 87-108 of hTNFalpha. (SEQ ID NO:1), wherein at least 5 amino acids from residues 87-108 of hTNFalpha. (SEQ ID NO:1) comprising SEQ ID NO: 5 that is between the amino acid residues of 91-95 of the SEQ ID NO: 1).

---------------------------------------- Continued on the next sheet ----------------------------------------
Continuation of:
Box No III (unity of invention is lacking)

(Continuation of the discussion for Groups I+ through III+)
Le does not specifically teach wherein the polyclonal antibodies are derived from the milk or colostrum of a bovine animal. Fox discloses a composition comprising polyclonal antibodies that specifically bind to tumor necrosis alpha (hTNF) [para [0036]] - 'a composition comprising a therapeutically effective amount of an antibody specific for tumor necrosis factor (TNF)...the anti-TNF antibody is a polyclonal antibody'; para [0021] The term TNF as used herein is used to describe the cytokine TNF-alpha,' wherein the polyclonal antibodies are derived from the milk or colostrum of a bovine animal [para [0142] - 'the polyclonal antibody is isolated from the milk or colostrum of a bovine', with an increased antibody stability and mucosal permeability as well as a high antibody production [para [0142] - 'the polyclonal antibody is isolated from the milk or colostrum of a bovine, preferably an immunized cow...cows secrete a large bolus of antibody into the colostrum immediately after parturition and approximately 50% of the protein in colostrum is immunoglobulin'; para [0034] - 'immunoglobulin from colostrum is fractionated on the basis of displayed carbohydrate to provide a preparation of antibody with improved stability to degradation in the digestive tract'; para [0068] - 'heterogeneity is also observed in polyclonal antibodies isolated from animals...differences in antibody glycosylation can affect the stability of antibodies to gastric digestion...differences in antibody glycosylation can further affect the mucosal permeability of antibodies'; para [0043] - 'the presence of carbohydrates associated with stability to gastric or oral degradation' for treating a digestive tract disorder with an increased antibody stability and mucosal permeability (Abstract - 'use of antibodies within the digestive tract is provided...to treat disorders associated with altered permeability of the digestive tract'; para [0086] - 'a method of treating mucositis in a patient comprising administering to the patient a composition comprising a therapeutically effective amount of an antibody specific for tumor necrosis factor (TNF)...the anti-TNF antibody is a stabilized antibody...with enhanced mucosal permeability; [0166] - 'GI mucositis'; para [0014] - 'the "gastrointestinal tract", or "GI tract"...include the stomach, small intestine...large intestine'.

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 Le and Fox, to obtain a composition comprising polyclonal antibodies that specifically bind to human tumor necrosis alpha (hTNF), wherein the polyclonal antibodies are derived from the serum of an animal that has been immunized with hTNF or an immunogenic portion thereof, wherein the polyclonal antibodies comprise one or more of the following features: b) bind to at least one epitope on hTNF wherein at least one epitope comprises an amino acid sequence selected from all or a portion of the amino acid sequence of: SEQ ID NO: 2; SEQ ID NO: 3; SEQ ID NO: 4; SEQ ID NO: 5; and SEQ ID NO: 6, as taught by Le, and further wherein the polyclonal antibodies are derived from the milk or colostrum of a bovine animal, based on the combination of Fox and Le, in order to produce a large quantity of the polyclonal antibodies derived from the milk or colostrum of a bovine animal with an increased stability and mucosal permeability for treating digestive tract disorder with desired effects. Without a shared special technical feature, the inventions lack unity with one another.

The inventions of Groups II-III+ further share the technical feature of a pharmaceutical composition comprising the composition of claim 1 and a pharmaceutically acceptable carrier or excipient. However, this shared technical feature does not represent a contribution over prior art. Specifically, Fox further discloses a pharmaceutical composition comprising a composition comprising a polyclonal antibody that specifically binds to tumor necrosis alpha (hTNF) and a pharmaceutically acceptable carrier or excipient [para [0145]]. The pharmaceutical compositions...comprise a therapeutically effective amount of an antibody of the present invention formulated together with one or more pharmaceutically acceptable carriers or excipients'; para [0086] - 'a composition comprising a therapeutically effective amount of an antibody specific for tumor necrosis factor (TNF)...the anti-TNF antibody is a polyclonal antibody'; para [0021]. The term TNF as used herein is used to describe the cytokine TNF-alpha, for treating a digestive tract disorder (Abstract - 'use of antibodies within the digestive tract is provided...to treat disorders associated with altered permeability of the digestive tract'). One of ordinary skill in the art at the time the invention was made would have known to obtain a pharmaceutical composition comprising the composition of claim 1 and a pharmaceutically acceptable carrier or excipient, based on the combination of Fox and Le (see quotations above), as discussed above. Without a shared special technical feature, the inventions lack unity with one another.

The inventions of Groups III+ further share the technical feature of treating an inflammatory disorder in a patient comprising administering to the patient a therapeutically effective amount of the pharmaceutical composition of claim 8. However, this shared technical feature does not represent a contribution over prior art. Specifically, Fox further discloses a method of treating inflammatory bowel disease (IBD) in a patient comprising administering to the patient a therapeutically effective amount of the pharmaceutical composition comprising a polyclonal anti-TNF antibody [para [0121] - 'a method of treating inflammatory bowel disease (IBD)...in a patient comprising administering to the patient a composition comprising a therapeutically effective amount of an antibody specific for tumor necrosis factor (TNF)...the anti-TNF antibody is a polyclonal antibody'; para [0021]. The term TNF as used herein is used to describe the cytokine TNF-alpha,). One of ordinary skill in the art at the time the invention was made would have known to obtain a method of treating inflammatory bowel disease (IBD) in a patient comprising administering to the patient a therapeutically effective amount of the pharmaceutical composition of claim 8, based on the combination of Fox and Le (see quotations above), as discussed above. Without a shared special technical feature, the inventions lack unity with one another.

**************************************************Continued in the next extra sheet**************************************************
Continuation of:
Box No III (unity of invention is lacking)

The inventions of claims 2 and 4 of Group I further share the technical feature of polyclonal antibodies that specifically bind to human tumor necrosis alpha (hTNF) and also bind and neutralize canine TNF. However, this shared technical feature does not represent a contribution over prior art. Specifically, a technical datasheet entitled 'MONOCLONAL ANTIBODY TO HUMAN TUMOR NECROSIS FACTOR ALPHA (TNF-alpha) clone 5N' by HycultBiotech (Modified: 27 October 2009, [online]. [Retrieved on 2012:10:1]). Retrieved from: URL: http://www.hycultbiotech.com/doc4371.dx) discloses a monoclonal antibody specifically binds to human tumor necrosis alpha (hTNF) (Title: 'MONOCLONAL ANTIBODY TO HUMAN TUMOR NECROSIS FACTOR ALPHA (TNF-alpha) clone 5N'), which also binds and neutralizes canine TNF (Description: para 3 - "The monoclonal antibody 5N cross-reacts and cross-neutralizes ...With affinity about two orders lower it recognizes ...canine TNF-alpha'). Furthermore, please note: whether or not an anti-human TNF antibody cross-neutralizes with TNF from another species, it is determined by the epitope(s) the antibody recognizes. Since Le discloses a human TNF sequence (para [0086]) - 'Such antibodies ... can include those generated by immunization using purified recombinant hTNF.alpha. (SEQ ID NO:1) or peptide fragments thereof, which is 100% identical to the SEQ ID NO: 1 of the application and comprises all epitopes of the claimed SEQ ID NOs: 2-6 (Specification: pg 6, In 14-21 - 'the polyclonal antibodies bind at least one epitope on hTNF within approximately the amino acid positions selected from: amino acids 1-15 of SEQ ID NO: 1; ... wherein the hTNF epitope comprises an amino acid sequence selected from: SEQ ID NO: 2; ... and SEQ ID NO: 6'), and therefore, the sequence disclosed by Le also comprises the epitope that cross-reacts with an epitope of canine TNF (Specification: pg 66, In 28-32 - 'VRSSRTPSDKPVAH (SEQ ID NO: 2) ...the binding of this epitope by the polyclonal antibody of the invention results in neutralization is low because the sequence of residues 3-15 of canine ...TNF is almost identical to human TNF'). Without a shared special technical feature, the inventions lack unity with one another.

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