



(43) International Publication Date
23 March 2017 (23.03.2017)

(10) International Publication Number
WO 2017/048121 A1

- (51) **International Patent Classification:**
G01N 33/68 (2006.01) *G01N 33/94* (2006.01)
- (21) **International Application Number:**
PCT/NL2016/050634
- (22) **International Filing Date:**
14 September 2016 (14.09.2016)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
2015442 15 September 2015 (15.09.2015) NL
- (71) **Applicant:** ERASMUS UNIVERSITY MEDICAL
CENTER ROTTERDAM [NL/NL]; Dr. Molewaterplein
50, 3015 GE Rotterdam (NL).
- (72) **Inventor:** VAN DER SPEK, Petrus Johannes; c/o Dr.
Molewaterplein 50, 3015 GE Rotterdam (NL).
- (74) **Agent:** JANSEN, C.M.; V.O., Carnegieplein 5, 2517 KJ
Den Haag (NL).
- (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, BN, 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, IR, IS, JP, KE, KG, KN, KP, KR,
KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME,
MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,
OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA,
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, RW, SD, SL, ST, SZ,
TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, 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, KM, 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))

(54) **Title:** IL17 RELATED ASSAYS

(57) **Abstract:** The present invention relates to a method for predicting adverse effects of pharmaceutically active compounds, preferably monoclonal antibodies, by a) determining binding specificity of said pharmacologically active compound to IL17 receptor(s); and b) determining the level of IL-17RB expression in insular brain tissue, where binding to IL-17 receptor(s) predicts pharmaceutical activity and an increase in insular brain IL-17RB indicates adverse side effects. The invention further comprises a method for predicting side effects of pharmaceutically active compounds, preferably monoclonal antibodies, by determining the binding of said pharmaceutically active compounds to IL- 7RB, or to IL-17B and/or IL-25 or compounds in the signaling pathway downstream of IL-17B or IL-25. Also comprised in the invention is a method of therapy against a psychotropic disorder, especially depression, more particularly depression accompanied by suicidal ideation, wherein the person suffering from the psychotropic disorder is administered a compound that is found to decrease the level of expression of the IL-17B receptor in the insular cortex.



WO 2017/048121 A1

Title: IL17 related assays

The invention relates to the field of pharmaceuticals, more specifically relating to side effects of pharmaceuticals, in particular side effects of pharmaceuticals that interact with the IL-17 receptor pathway, more particular anti-IL-17 monoclonal antibodies, such as brodalumab and similar biologicals.

IL-17 (interleukin 17) is a cytokine (or, actually, a family of cytokines) that acts as a potent mediator in delayed-type hypersensitivity reactions by increasing chemokine production in various tissues to recruit monocytes and neutrophils to the site of inflammation, similar to interferon- γ . IL-17 is produced by T-helper cells and is induced by IL-23 which results in destructive tissue damage in delayed-type hypersensitivity reactions. Interleukin 17 as a family functions as a pro-inflammatory cytokine that responds to the invasion of the immune system by extracellular pathogens and induces destruction of the pathogen's cellular matrix. Interleukin 17 acts synergistically with tumor necrosis factor and interleukin-1.

Because of its involvement in immune regulatory functions, IL-17 inhibitors are being investigated as possible treatments for autoimmune diseases such as rheumatoid arthritis, psoriasis and inflammatory bowel disease. Based on emerging evidence from animal models, IL-17 has been suggested as a target for anti-inflammatory therapies to improve recovery post-stroke and to reduce the formation of skin cancer. IL-17 has also been implicated in multiple sclerosis.

Members of the IL-17 family include IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (also called IL-25), and IL-17F.

To elicit its functions, IL-17 binds to a type I cell surface receptor called IL-17R, which also forms a family of receptors with cell-specific signaling cascades. The IL-17 receptor family consists of five, broadly distributed receptors (IL-17RA, RB, RC, RD and RE) each having individual

ligand specificities. Within this family of receptors, IL-17RA is the best-described. IL-17RA binds both IL-17A and IL-17F and is expressed in multiple tissues: vascular endothelial cells, peripheral T cells, B cell lineages, fibroblast, lung, myelomonocytic cells, and marrow stromal cells. Signal transduction for both IL-17A and IL-17F requires the presence of a heterodimeric complex consisting of both IL-17RA and IL-17RC and the absence of either receptor results in ineffective signal transduction. This pattern is reciprocated for other members of the IL-17 family such as IL-17E, which requires an IL-17RA-IL-17RB complex (also known as IL-17Rh1, IL-17BR or IL-25R) for effective function.

Another member of this receptor family, IL-17RB, binds both IL-17B and IL-17E. Furthermore, it is expressed in the kidney, pancreas, liver, brain, and intestine. IL-17RC is expressed by the prostate, cartilage, kidney, liver, heart, and muscle, and its gene may undergo alternate splicing to produce a soluble receptor in addition to its cell membrane-bound form. In similar manner, the gene for IL-17RD, which is the most different from IL-17RA, may undergo alternative splicing to yield a soluble receptor. This feature may allow these receptors to inhibit the stimulatory effects of their yet-undefined ligands. The least-described of these receptors, IL-17RE, is known to be expressed in the pancreas, brain, and prostate.

Signal transduction by these receptors is as diverse as their distribution. These receptors do not exhibit a significant similarity in extracellular or intracellular amino acid sequence when compared to other cytokine receptors. Transcription factors such as TRAF6, JNK, Erk1/2, p38, AP-1 and NF- κ B have been implicated in IL-17 mediated signaling in a stimulation-dependent, tissue-specific manner.

Recently, it has been discovered that one of the IL-17 inhibitors, the monoclonal antibody brodalumab which inhibits the IL-17RA receptor, caused side effects that showed in suicidal ideation and behavior.

Prediction of the occurrence of such side effects in view of possible therapeutic compounds that target IL-17 or its receptor(s) is highly needed. There are currently no tests, other than clinical trials, to determine if a drug potentially increases the risk of suicidal behavior, suicidal thinking (suicidality) or suicidal ideation.

The invention therefore comprises methods as specified in the claims. Comprised therein is a method for predicting adverse effects of pharmaceutically active compounds, preferably monoclonal antibodies, by a) determining binding specificity of said pharmacologically active compound to IL17 receptor(s); and b) determining the level of IL-17RB expression in insular brain tissue, where binding to IL-17 receptor(s) predicts pharmaceutical activity and an increase in insular brain IL-17RB indicates adverse side effects. The invention further comprises a method for predicting side effects of pharmaceutically active compounds, preferably monoclonal antibodies, by determining the binding of said pharmaceutically active compounds to IL-17RB, or to IL-17B and/or IL-25 or compounds in the signaling pathway downstream of IL-17B or IL-25. Also comprised in the invention is a method of therapy against a psychotropic disorder, especially depression, more particularly depression accompanied by suicidal ideation, wherein the person suffering from the psychotropic disorder is administered a compound that is found to decrease the level of expression of the IL-17B receptor in the insular cortex.

Further, the invention also relates to use of IL-25 in the treatment of psychotropic conditions, preferably depression, more particularly depression accompanied by suicidal ideation.

LEGENDS TO THE FIGURES

Fig. 1 shows expression data in trauma, cocaine abuse and cocaine delirium individuals as described in Example 1. High expression is indicated by an

increase in whiteness, while low expression is indicated by an increase in blackness.

DETAILED DESCRIPTION

In this application the term 'biomarker' is used for a distinctive biological or biologically derived indicator of a process, event or condition. Biomarkers can be used in methods of diagnosis, e.g. clinical screening, and prognosis assessment and in monitoring the results of therapy. They also can be used for identifying patients that are most likely to respond to a certain treatment, for drug screening and for development in medicine. Biomarkers and their uses are therefore valuable for identification of new patients at risk or patients at need to lower dose of treatment or even, more importantly, at risk for severe side-effects such as suicidal ideation. They are also valuable for drug treatments and for discovery of new targets for drug treatment. Further they are valuable for exploring dosage regimes and drug combinations.

The term "quantifying" or "quantify", as used herein, refers to any method for obtaining a quantitative measure. For example, quantifying a biomarker can include determining its abundance, relative abundance, intensity, and/or concentration etc. Quantifying a biomarker may encompass determining its concentration in a sample, preferably a clinical sample such as blood, serum or urine.

The term "reference protein" refers to a protein used in an assay as a control molecule. Preferably, said reference protein is used as a positive control. A positive control is used in a procedure to determine the nature or the amount of a detection signal in an assay known to give a positive result. A positive control is preferably used in a known amount and detected under the same or comparable conditions as the unknown amount of the same or comparable molecule to be detected.

In the present invention and/or embodiments thereof, the sample in the method and/or use is a biological sample, preferably a sample selected from the group consisting of blood, serum, urine, spinal fluid, broncho-alveolar lavage fluid, synovial fluid, saliva, biopsies, especially brain tissue specimens, and sputum. Preferably the sample is selected from the group consisting of blood, serum, urine, spinal fluid, broncho-alveolar lavage fluid, and synovial fluid. Preferably the sample is selected from the group consisting of blood, serum, and urine.

The term "blood," as used herein, means the blood derivatives plasma and serum.

The term "subject," as used herein, means a human or non-human animal, including but not limited to mammals such as a dog, cat, horse, cow, pig, rabbit, guinea pig, sheep, goat, primate, rat, and mouse.

The term "antibody" as used herein, or "immunoglobulin", refers to a molecule comprising two heavy chains linked together by disulfide bonds and two light chains, each light chain being linked to a respective heavy chain by disulfide bonds in a "Y" shaped configuration.

It should be understood that when the terms "antibody" or "antibodies" are used, this is intended to include intact antibodies, such as polyclonal antibodies or monoclonal antibodies (mAbs), as well as proteolytic fragments thereof such as the Fab or F(ab')₂ fragments. Further included within the scope of the invention are chimeric antibodies; human and humanized antibodies; recombinant and engineered antibodies, fragments thereof, fragments produced by a Fab expression library, anti-idiotypic antibodies and epitope-binding fragments. Furthermore, the DNA encoding the variable region of the antibody can be inserted into the DNA encoding other antibodies to produce chimeric antibodies. Single chain antibodies fall within the scope of the present invention. Methods of production of such single chain antibodies are known.

The term "epitope" is meant to refer to that portion of any molecule capable of being bound by an antibody or a fragment thereof which can also be recognized by that antibody. Epitopes or antigenic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and have specific three-dimensional structural characteristics as well as specific charge characteristics.

By reference to an "antibody that specifically binds" to another molecule is meant an antibody that binds the other molecule, and displays no substantial binding to other naturally occurring proteins other than those sharing the same antigenic determinants as the other molecule.

Unless otherwise defined, all technical terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

It is generally known that psoriasis and related autoimmune disorders have mental comorbidities such as depression and suicidal ideation (Dalgard, J. et al., 2015, J. Invest. Dermatol. 135(4):984-991).

It was found by the current inventors that the reported side-effect of suicidal ideation in the use of anti-IL-17 receptor therapeutic compound brodalumab, can be explained as an off-target effect of said drug by interference with the IL-17B and/or IL-17E (IL-25) signaling pathways. Especially, the binding of the compound to the IL-17B receptor (IL-17RB) instead of the intended binding of the antibody to the IL-17RA molecule, seems important in this aspect.

Incidental findings in relation to interferon-based treatments have been reported previously to induce suicidal ideation. No relation with the IL-17RB cascade has been established yet.

Independent from the drug-receptor interaction with relation to IL-17 it was found that the mRNA coding for said IL-17RB is significantly increased in the insular cortex of cocaine delirium patients, as compared to the expression level of this mRNA in the insular cortex of cocaine (ab)users and drug-free victims of trauma control subjects. It is envisaged that the psychotic disorders, mood disorders, and anxiety disorders that hallmark the severe cocaine-induced intoxication are related to the compound-induced suicidal ideation observed in the clinical trials of brodalumab. Of course it is known that the insular cortex is involved in cognitive-emotional processes that are specific to primates, such as empathy and self-aware emotional feelings. This is supported by functional imaging results showing that the structure and function of the right frontal insula are correlated with the ability to feel one's own heartbeat, or to empathize with the pain of others.

It is believed that in case of anti-IL-17 or anti-IL-17 receptor compounds signaling through the ligands IL-17 or especially IL-25 (which molecule is small enough to pass the blood brain barrier) is a possible mechanism for affecting the brain, especially the insular cortex located expression of the IL-17B receptor. In this case, the (level of) expression of IL-17RB functions as a biomarker. However, also the compounds IL-17 or IL-25 can then be recognized as biomarker for psychotropic conditions.

This finding opens up an arsenal of possible assays that could benefit drug research and could signal unwanted side-effects already early in the development of a pharmaceutical compound. Moreover, generic testing for depression related conditions now becomes feasible. One of those assays is an assay for measuring expression of the biomarker IL-17B receptor in insular brain tissue in a mammal after administration of a pharmaceutically active compound and compare this expression to a normal control. If expression of the biomarker is found to be elevated, there is a risk that the compound will have psychotropic side effects, one of those side

effects being suicidal ideation. Preferably, humanized animal (mouse) models will be used to study the receptor-ligand interactions, both *in vivo* and *in vitro*.

Such an assay may be performed on candidate drugs that are in the pipelines of the pharmaceutical companies, such as secukinumab (AIN457), ixekizumab (Ly2439821), ustekinumab, CNTO 1959, MK-3222; SCH 900222, SCH 900117, RG 4934, NI-1401; RG7624 and other such compounds. However, it will of course be beneficial that such an assay is performed before the start of clinical studies or will be used to detect patients at risk for severe side-effects, such as suicidal ideation.

In a similar way, the invention comprises a method for selecting a potential pharmaceutical compound by assaying it for activity with respect to IL-17 and assaying it for binding to IL-17RB, more specifically the IL-17RB that is present in the insular cortex. In this way drug candidates that have the desired pharmacological activity (e.g. inhibition of IL-17A signaling) can be selected that do not have the undesired side effect of increasing suicidal ideation. Such an assay has the advantage that it can be included in the primary screening for anti-IL-17 compounds.

Next to brodalumab which is an antibody that binds to the IL-17 receptor and which now has been shown to introduce suicidal ideation, it is known that also interferon, especially interferon-alpha has the property of causing suicides, depression and suicidal ideation side effects. Thus also in those circumstances where a medicament is supposed to bring an effect that is similar to interferon, it would be advantageous to perform an assay as described above. Further, interferon-alpha has been reported to control IL-17 expression (Moschen, A.R. et al., 2008, Immunobiol. 213:779-787), which shows that there is also a functional relation between interferon and the IL-17 signaling pathway.

A second assay that can be contemplated is an assay in which the binding specificity of antibodies or other therapeutically effective compounds towards IL-17 or IL-17 receptor targets is tested, where specific reactivity with the biomarker IL-17RB forms part of the test panel. In such an assay both agonists and antagonists will be discovered. Therefore, compounds retrieved in this assay may be used to modulate the mood- and inflammatory pathways in both directions. Such an assay may function as a first screen in drug research, which should then be followed by the earlier described assay for full determination of psychotropic side effects. The reactivity with the IL-17RB not only could detect compounds that would cause or aggravate psychotropic disorders, but it could also lead to find compounds that would ameliorate these kind of disorders. It would therefore be essential that if such compounds are detected, also an assay as described earlier is performed, in which the effects on the level of expression of the IL-17RB in the insular cortex are measured. This assay then will tell whether the reactivity towards IL-17RB will cause suicidal ideation or will prohibit or decrease suicidal ideation.

In stead of assays that measure the reactivity towards, or the binding with the IL-17B receptor, also assays that are measuring the biomarkers IL-17B and/or IL-25, which are ligands for the IL-17RB, or compounds in the signaling pathway downstream of IL-17B or IL-25 can be used in the present invention. It is hypothesized that the amount of ligand relates to the level of expression of the IL-17RB, especially to the amount of IL-17RB in the insular cortex. An increase in the amount of IL-17B or IL-25 will decrease the need for the IL-17RB, since all available receptor molecules will be stimulated. Since IL-25 is able to pass the blood brain barrier, this would also be true for the insular cortex. Conversely, a low level of circulating IL-17B or IL-25 will increase the need for the receptor molecule, since otherwise the chances of missing a signal will be high, and

missing such signals could lead to undesired immunologic conditions. The same would apply to downstream compounds of IL-17B and IL-25, which downstream compounds then thus also would qualify as biomarkers.

Detection and/or quantification of the above mentioned biomarkers in the above mentioned assays may be performed using an immunologic method, involving an antibody, or fragment thereof, capable of specific binding to the biomarker. Suitable immunologic methods include sandwich immunoassays, such as sandwich ELISA, in which the detection of the biomarkers is performed using two antibodies which recognize different epitopes on the biomarker; radioimmunoassay (RIA), direct, indirect or competitive enzyme linked immunosorbent assays (ELISA), enzyme immunoassays (EIA), fluorescence immunoassays (FIA), chemiluminescent immunoassays, western blotting, immunoprecipitation and any particle based immunoassay (e.g. using gold, silver or latex particles, magnetic particles, or Q-dots). Immunologic methods may for example be performed on a microtiter plate or on test strips. It is also possible to perform the analysis with concurrent lab-on-a-chip techniques. These immunoassays and/or the individual components thereof (such as antibodies that recognise the IL-17RB, antibodies that recognize IL-25) are commercially available.

However, the invention is not limited to immunoassays. Analysis of the biomarkers in the sample of the subject may be carried out with chemical analytical methods (like mass spectrometry, MALDI-TOF, micro-Raman spectrometry), with magnetic radio imaging, flow cytometric analyses and all other quantitative analysis systems that are suitable for detecting proteins in fluids. The assays may also be performed on nucleic acids that encode for the (protein) markers or for enzymes that are in the biosynthesis pathways of the protein markers. Such assays are preferably RT-PCR assays in which mRNA is measured from the sample. In such a case, the sample may be any cell material derived from the body. Also

receptor-based assays, using a receptor that is normally present in a biological system for the mentioned biomarker, as analytical tool may be used. Of course also RT-PCR assays for the nucleic acid that encodes the receptor, are usable. Apart from RT-PCR also hybridization techniques (northern blotting, microarray hybridization etc.) and sequencing techniques can be used to determine variants of said (protein) markers and/or their receptors. Those variants could predispose for severe side-effects such as suicidal ideation.

Recently, scientists have focused on epigenetic variation, and specifically changes in DNA methylation as a promising class of biomarker that may apply to a range of disorders (Petronis, 2010; Portela&Esteller, 2010). Methylation refers to the addition of methyl (CH₃) groups to the cytosine of CpGs in their promoter regions, and in most normal cells these CpG “islands” are unmethylated. Methylation of CpG islands in promoter regions can dramatically alter gene expression. For many years research focused heavily on the role of DNA methylation in cancer, but scientists have increasingly focused on the role of DNA methylation in psychiatric disorders (Tsankova et al., 2007; Bredy et al., 2010). In fact, the mechanisms of action for some existing psychiatric medications (e.g. valproic acid) may involve epigenetic alterations. Epigenetic changes have been linked to changes in gene regulation in neurons and downstream processes such as memory and cognition. The overall conclusion is that epigenetic changes may play an important role in terms of long-term neurological changes (or “molecular and cellular memory”). Thus, DNA methylation can serve as important biomarker of treatment target.

An important way to determine a biomarker is to determine the level of expression of the mRNA encoding the biomarker protein. This is especially useful in those assays where the level of expression of the IL-17RB is measured, and predominantly when this is measured in the brain. In such a case brain tissue, preferably tissue from the insular cortex, is

obtained and the transcriptome is determined through transcriptome analysis techniques, such as described by Tang, F. et al. (Nature Meth. 6:377-382, 2009), Lao, K.Q. et al. (J. Biomed. Tech. 20(5):266-271, 2009) or Kojima, K. et al. (PLoS ONE 9: e86961. doi:10.1371/journal.pone.0086961). It is accepted that antibodies do not cross the blood-brain-barrier. Therefore, the off-target effects induced by increasing levels of IL-25 (or other ligands) that can cross the blood-brain-barrier indirectly can trigger signaling of the brain located IL-17RB and can induce suicidal ideation.

The assays that are useful in the present invention are preferably quantitative assays, in which the concentration of biomarker in the sample can be determined, preferably where the sample is peripheral blood or is derived therefrom. This can – in principle – be achieved with all of the above mentioned detection methods. While interpreting the results of such an assay, various determinants such as sex, age, smoking status, urbanicity, food and alcohol intake should be taken into account.

The biomarkers of the present invention are recognised by 'biosensors', which may comprise a ligand or ligands capable of specific binding to the biomarker. Such biosensors are useful in detecting and/or quantifying the biomarker, preferably in quantifying the biomarker in a sample, such as a blood sample or tissue extract of the patient.

Especially useful biosensors are antibodies. The term 'antibody' as further defined above, also refers to immunoglobulin and T-cell receptor molecules, i.e. molecules that contain an antigen-binding site that specifically binds an antigen. The immunoglobulin molecules can be of any class (e.g. IgA, IgD, IgE, IgG and IgM) or subclasses thereof. If the biomarker is a nucleic acid, a specific probe can be used as 'biosensor'. Preferably in such a case, the target DNA is first amplified in a PCR reaction with suitable primer sequences.

Appropriate diagnostic tools such as biosensors can be developed in methods and uses of the invention; and detection and quantification of the biomarker can be performed using a biosensor in a micro-analytical system, a micro-engineered system, a micro-separation system, an immunochromatography system or other suitable analytical devices (such as Raman or mass spectrography and the like). The biosensor may be incorporated in an immunological method for detection of the biomarker(s), or via electrical, thermal, magnetic, optical (e.g. hologram) or acoustic technologies. Using these techniques, it is possible to detect the target biomarker(s) at the anticipated concentrations found in biological samples. Thus, according to a further aspect of the invention there is provided an apparatus for methods of diagnosing or monitoring as specified in the claims which comprises one or more biosensors in a micro-analytical, micro-engineered, micro-separation and/or immunochromatography system configured to detect and/or quantify any of the biomarkers defined herein.

One of the most common forms of a diagnostic tool is an antibody microarray. Antibody microarrays can be produced in two forms, either by a sandwich assay or by direct labelling approach. The sandwich assay approach utilizes two different antibodies that recognize two different epitopes on the biomarker target molecule (protein). One antibody is immobilized on a solid support and captures its target molecule from the biological sample. Using the appropriate detection system, the labelled second antibody detects the bound targets. The main advantage of the sandwich assay is its high specificity and sensitivity. High sensitivity is achieved by a strong reduction of background, yielding a high signal-to noise ratio. In addition, only minimal amounts of labelled detection antibodies are applied in comparison to the direct labelling approach where a large amount of labelled proteins are present in a sample. The sandwich immunoassay format can also be easily applied to the field of microarray technology, and such immunoassays can be applied to the protein microarray format to

quantify the biomarkers as described herein in conditioned media and/or patient sera.

In the direct labelling approach, all proteins and protein fragments in a sample are labelled with a fluorophore. Labelled proteins and protein fragments that bind to the protein microarray such as to an antibody microarray are then directly detected by fluorescence. Also, proteins from two different biological samples may be labelled with different fluorophores. These two labelled samples are then mixed together in an unknown ratio and applied to an antibody microarray. This approach allows (direct) comparisons between diseased and healthy, or treated and untreated samples. Direct labelling has the advantage that the direct labelling method only requires one specific antibody to perform an assay.

Miniaturized and multiplexed immunoassays may also be used to screen a biological sample for the presence or absence of proteins or DNA variants.

In a preferred embodiment of aspects of the invention, the detection or capture agents, preferably antibodies are immobilized on a solid support, preferably on a polystyrene surface. In another most preferred embodiment, the detection or capture agents are spotted or immobilized in duplicate, triplicate or quadruplicate onto the bottom of a well of a microplate.

The biomarker(s) of the invention can also be detected using a biosensor incorporating technologies based on "smart" holograms, or high frequency acoustic systems, such systems are particularly amenable to "bar code" or array configurations.

In smart hologram sensors (Smart Holograms Ltd, Cambridge, UK), a holographic image is stored in a thin polymer film that is sensitized to react specifically with the biomarker. On exposure, the biomarker reacts with the polymer leading to an alteration in the image displayed by the hologram.

The test result read-out can be a change in the optical brightness, image, color and/or position of the image. For qualitative and semi-quantitative applications, a sensor hologram can be read by eye, thus removing the need for detection equipment. A simple color sensor can be used to read the signal when quantitative measurements are required. Opacity or color of the sample does not interfere with operation of the sensor. The format of the sensor allows multiplexing for simultaneous detection of several substances. Reversible and irreversible sensors can be designed to meet different requirements, and continuous monitoring of a particular biomarker of interest is feasible.

Suitably, methods for detection of one or more biomarkers according to the invention combine biomolecular recognition with appropriate means to convert detection of the presence or quantity of the biomarker in the sample into a signal.

Biosensors to detect one or more biomarkers can also be detected by acoustic, plasmon resonance, holographic and microengineered sensors. Imprinted recognition elements, thin film transistor technology, magnetic acoustic resonator devices and other novel acousto-electrical systems may be employed for detection of the one or more biomarkers of the invention.

Methods involving detection and/or quantification of one or more biomarkers of the invention can be performed on bench-top instruments, or can be incorporated onto disposable, diagnostic or monitoring platforms that can be used in a non-laboratory environment, e.g. in the physician's office or at the patient's bedside. Suitable platforms for performing methods of the invention include "credit" cards with optical or acoustic readers. The sensor systems can be configured to allow the data collected to be electronically transmitted to the physician for interpretation and thus can form the basis for remote diagnosis or can be used as a companion diagnostic test.

Methods of the invention can be performed in array format, e.g. on a chip, or as a multi well array. This enables testing for several

biomarkers or for only one biomarker in multiple subjects or samples simultaneously. Methods can be adapted into platforms for single tests, or multiple identical or multiple non-identical tests and can be performed in high throughput format. Methods of the invention may comprise performing one or more additional, different tests to confirm or exclude diagnosis, and/or to further characterize a condition.

Preferably, antibodies that are used are monoclonal. The mAbs that may be used in the present invention may be of any immunoglobulin class including IgG, IgM, IgE, IgA, and any subclass thereof. Methods for producing mAbs are well known in the art and include the use of hybridomas. A hybridoma producing a mAb may be cultivated *in vitro* or *in vivo*. High titers of mAbs can be obtained in *in vivo* production where cells from the individual hybridomas are injected intraperitoneally into mice, preferably pristine-primed Balb/c mice, to produce ascites fluid containing high concentrations of the desired mAbs. MAb of isotype IgM or IgG may be purified from such ascites fluids, or from culture supernatants, using column chromatography methods well known to those of skill in the art.

Besides the conventional method of raising antibodies *in vivo*, antibodies can be generated *in vitro* using phage display technology. Such *in vitro* methods for the production of recombinant antibodies are much faster compared to conventional antibody production and recombinant antibodies can be generated against an enormous number of antigens.

To generate recombinant monoclonal antibodies one can use various methods based on phage display libraries to generate a large pool of antibodies with different antigen recognition sites. Such a library can be made in several ways: One can generate a synthetic repertoire by cloning synthetic CDR3 regions in a pool of heavy chain germline genes and thus generating a large antibody repertoire, from which recombinant antibody fragments with various specificities can be selected. One can use the lymphocyte pool of humans as starting material for the construction of an

antibody library. It is possible to construct naive repertoires of human IgM antibodies and thus create a human library of large diversity. Protocols for bacteriophage library construction and selection of recombinant antibodies are well known.

Next to immunological and molecular biological methods for detection and quantification of the biomarkers as previously described, the biomarkers may also be detected through other methods.

In another embodiment of the present assay methods, the step of detecting the biomarker is performed by using Mass spectrometry (MS), preferably by tandem mass spectrometry (MS-MS) or by Matrix-assisted laser desorption/ionization (MALDI). Mass spectrometry provides a powerful means of determining the structure and identity of complex organic molecules, including proteins and peptides. A sample compound is bombarded with high-energy electrons causing it to fragment in a characteristic manner. The fragments, which are of varying weight and charge, are then passed through a magnetic field and separated according to their mass/charge ratios. The resulting characteristic fragmentation signature pattern of the sample compound is used to identify and quantitate that compound. A typical MS procedure comprises the following steps:

1. a sample is loaded onto the MS instrument, by applying the sample optionally (in case of a special form of MS called MALDI) together with a matrix on a mass spectrometric sample support and drying the sample or the mixture on the support by evaporation of the solvents.
2. the components of the sample are ionized by one of a variety of methods (e.g., by impacting them with an electron beam), which results in the formation of charged particles (ions),
3. the positive ions are then accelerated by an electric field,

4. computation of the mass-to-charge ratio (m/z) of the particles based on the details of motion of the ions as they transit through electromagnetic fields, and

5. detection of the ions, which in step 4 were sorted according to m/z .

The technique can be used for identifying compounds in the sample as a protein fragment of IL-17RB, IL-17 or IL-25, or any compound downstream in the signaling pathway, as described herein, by determining the structure of the protein fragment from its fragmentation pattern.

An advantage of the MALDI technique is that it is a soft ionization technique used in mass spectrometry, which is specifically suitable for the analysis of the protein fragment as described above, which tend to be fragile and fragment when ionized by more conventional ionization methods. The matrix as described above is used to protect the protein fragment as described above from being destroyed by direct laser beam and to facilitate vaporization and ionization.

The matrix consists of crystallized molecules, of which the three most preferably used are 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid), α -cyano-4-hydroxycinnamic acid (α -cyano or α -matrix) and 2,5-dihydroxybenzoic acid (DHB). The matrix solution is mixed with the protein fragment-sample. The organic solvent allows hydrophobic molecules to dissolve into the solution, while the water allows for water-soluble (hydrophilic) molecules to do the same. This solution is spotted onto a MALDI plate (usually a metal plate designed for this purpose). The solvents evaporate, leaving only the recrystallized matrix, together with the protein fragments dispersed throughout the crystals. The matrix and the protein fragments are thus co-crystallized in a MALDI spot.

Other MS applications include MALDI-TOF MS mass spectrometry, MALDI-FT mass spectrometry, MALDI-FT-ICR mass spectrometry, MALDI Triple-quad mass spectrometry.

Next to being the subject of an assay for finding side effects of (candidate) drug compounds, the identification of the present biomarkers as being related to and predictive for depression and more particularly depression accompanied by suicidal ideation permits integration of diagnostic procedures and therapeutic regimes. Currently effectiveness of drug treatment or psychotherapy is difficult to test, and it has thus far not been possible to perform rapid assessment of therapy response. Traditionally, many anti-depressant therapies require treatment lasting weeks to months for a given therapeutic approach. Detection of a biomarker of the invention can be used to screen subjects prior to their participation in clinical trials or prior to treatment. The biomarkers provide the means to indicate therapeutic response, failure to respond, unfavourable side-effect profile, and degree of medication compliance. The biomarkers may be used for patient stratification and to prevent or stop treatment in non-responders at a very early stage. They can also be used to fine-tune dosage, minimize the number of prescribed medications, and to reduce the delay in attaining effective therapy. Thus by monitoring a biomarker of the invention, patient care can be tailored precisely to match the needs determined by the disorder and the pharmacogenomic profile of the patient. The biomarker can thus be used to titrate the optimal dose and to identify a positive therapeutic response.

Biomarker-based tests, such as provided by the present invention, provide a first line assessment of 'new' patients. Furthermore, diagnostic biomarker tests, such as provided by the present invention, are useful to identify family members or patients at high risk of developing depression with suicidal ideation. This permits initiation of appropriate therapy, or preventive measures, e.g. managing risk factors. These approaches are recognized to improve outcome and may prevent overt onset of the disorder.

Biomarker monitoring methods, biosensors and kits are also vital as patient monitoring tools. If pharmacological treatment is assessed to be inadequate, then therapy can be reinstated or increased; a change in therapy can be given if appropriate. As the biomarkers are sensitive to the state of the disorder, they provide an indication of the impact of drug therapy.

Diagnostic kits for the diagnosis and monitoring of a mood disorder, preferably depression, most preferably major depressive disorder, are described herein. A method of diagnosis or monitoring the biomarkers may comprise quantifying the biomarker in a sample from the patient and comparing the level of the biomarker present in said sample with one or more controls. For monitoring, the control may be a test sample of the same patient at an earlier point in time.

One of the main advantages of the present invention is that it provides an easy and reliable way to monitor initiation or progress of a psychotropic disease, especially depression and more particularly depression accompanied by suicidal ideation, and/or effectiveness of a therapy. To this end, the values for one or more of the above identified biomarkers for a subject are determined at a certain moment (null-value) and after an amount of time this procedure is repeated. Over the time, several repeat measurements can be performed. In the mean time therapy can e.g. be started or changed. Change(s) in the levels of biomarker will then indicate the effectiveness of the therapy or the progress of the disease.

Suitably, the time elapsed between taking samples from a subject undergoing monitoring will be several days, a week, two weeks, a month, several months or longer. Samples may be taken prior to and/or during and/or following antidepressant therapy. Samples can be taken at intervals

over the remaining life, or a part thereof, of a patient.

The current invention also comprises a method of testing a drug candidate against a psychotropic condition, specifically wherein said condition would be (major) depression, more particularly wherein said depression is accompanied by suicidal ideation. In such a method of testing basically a similar assay is conducted as described above wherein the drug candidate is administered to a subject and the expression of the IL-17B receptor in the insular cortex is assessed. In the same manner it will follow that a decrease found in the level of expression of the insular cortex IL-17B receptor will indicate that the drug will have a beneficial effect on the suicidal ideation.

Further, the invention also comprises a method of therapy against a psychotropic disorder, particularly (major) depression, and especially where said depression is joined by suicidal ideation. Such a method of therapy would benefit from a compound that has been established, e.g. through one of the assays as described above, to decrease the level of expression of the IL-17B receptor in the insular cortex tissue. More specifically such a compound would be IL-25 or IL-17B or any compound in the signaling pathway downstream of IL-17B or IL-25 that is capable to achieve the desired effect on the insular cortex level of the IL-17B receptor.

The formulation and mode of administration of such a pharmaceutical agent is known to the skilled person or can be established on an empirical basis. If the effective compound is IL-17B or IL-25, it is submitted that this compound can pass the blood brain barrier and provide the desired effect. The dose of the compound and the therapeutic regime will be easily established by the skilled person. It further follows from the present disclosure that the effect of the therapy may be monitored by performing any of the assays as described herein.

EXAMPLES

Analysis was made of the RNA expression profiles found in insular cortex tissue (*post mortem*) of 5 control individuals (victim of trauma), 5 individuals known with cocaine abuse and 5 individuals known with cocaine delirium (derived from Affymetrix U133Plus 2.0 microarrays on patient samples licensed from Genelogic Inc.). Expression data of receptor subunits of IL-17RA, IL-17RB, IL-17RC, IL17-RD, IL-17RE, the IFITM1, IFITM2 and IFITM3 receptors (interferon induced transmembrane protein) and IL-25 were depicted (see Figure 1) for each individual. Data were normalized according to established procedures (quantile normalization, see e.g. WO 2005/080601) and the log2 transformed geometrical mean value was taken in order to discriminate subtle expression level changes. The heatmap depicted in Figure 1 visualizes the relative high expression levels of IL-17RB and the IFITM receptors for the cocaine delirium subjects.

Cocaine delirium is a psychotropic condition which resembles depression and is known to induce suicidal ideation.

Claims

1. Method for predicting adverse effects of pharmaceutically active compounds, preferably monoclonal antibodies, by
 - a) determining binding specificity of said pharmacologically active compound to IL17 receptor(s); and
 - b) determining the level of IL-17RB expression in insular brain tissue, where binding to IL-17 receptor(s) predicts pharmaceutical activity and an increase in insular brain IL-17RB indicates adverse side effects.
2. Method for selecting pharmaceutically active compounds, preferably monoclonal antibodies, with less side effects by
 - a) determining binding specificity of said pharmacologically active compound to IL17; and
 - b) determining the level of IL-17RB expression in insular brain tissue, where binding to IL-17 predicts pharmaceutical activity and an increase in insular brain IL-17RB indicates adverse side effects, and selecting said compounds that do have pharmaceutical activity and that do not have side effects.
3. Method for predicting side effects of pharmaceutically active compounds, preferably monoclonal antibodies, by determining the binding of said pharmaceutically active compounds to IL-17RB.
4. Method for predicting side effects of pharmaceutically active compounds, preferably monoclonal antibodies, by determining the binding of said pharmaceutically active compounds to IL-17B and/or IL-25 or compounds in the signaling pathway downstream of IL-17B

or IL-25.

5. Method according to any of the previous claims, wherein the pharmaceutically active compound is intended to have an inhibitory effect on IL-17A or on the IL-17A receptor, preferably wherein the pharmaceutically active compound is an IL-17 antagonist.
6. Method to monitor therapy in depressed patients by determining the levels of IL-17B and IL-25 at various points during therapy and record changes in those levels, whereby an increase in the levels of IL-17B and/or IL-25 relates to effectiveness of said therapy.
7. Method to monitor therapy, wherein said therapy is a therapy directed to autoimmune diseases related to IL-17A and or the IL-17A receptor, wherein said monitoring comprises measuring the levels of IL-17B and/or IL-25.
8. Method to monitor therapy, wherein said therapy is a therapy against a psychotropic disease, especially depression or addiction, wherein said monitoring comprises measuring the levels of IL-17B and/or IL-25.
9. Method for testing drug candidates against a psychotropic disorder, especially depression, more particularly depression accompanied by suicidal ideation, wherein said drug candidate is administered to the test subject and the expression of the IL-17B receptor in the insular cortex is measured, wherein the drug candidate would be suitable for decreasing suicidal ideation if the expression is found to be decreased.

10. Method of therapy against a psychotropic disorder, especially depression, more particularly depression accompanied by suicidal ideation, wherein the person suffering from the psychotropic disorder is administered a compound that is found to decrease the level of expression of the IL-17B receptor in the insular cortex.
11. IL-25 for use in the treatment of psychotropic disorders, preferably depression, more particularly depression accompanied by suicidal ideation.
12. Method for detecting genetically based variant nucleic acids coding for IL-17RB which cause a predisposition for psychotropic conditions comprising sequencing nucleic acids from control and diseased patients and comparing these sequences.
13. Method for predicting the disposition of a subject for a psychotropic disorder by detection of a genetically based variant nucleic acid coding for IL-17RB which is known to be related to such psychotropic condition.

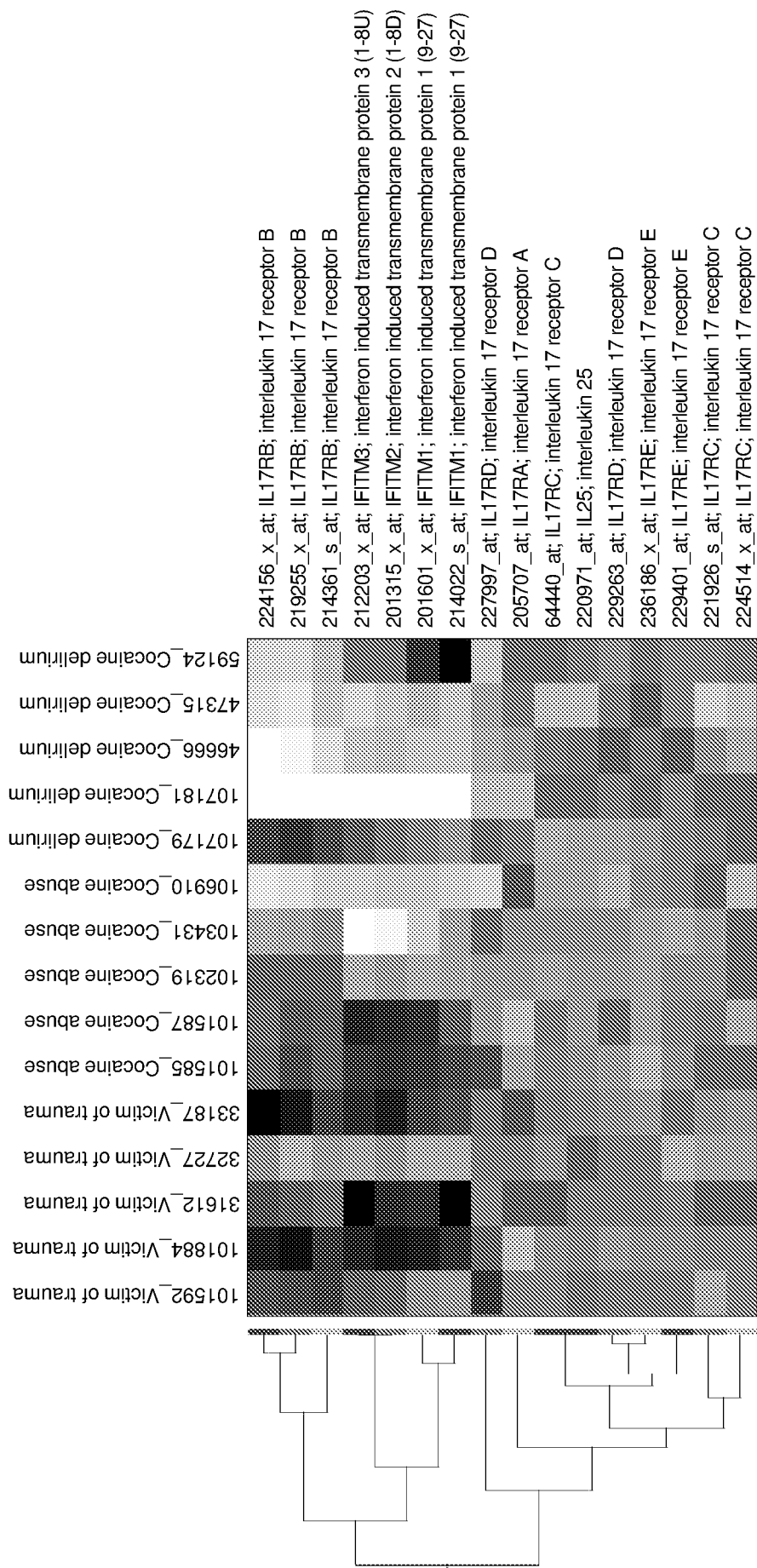


FIG. 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2016/050634

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/68 G01N33/94
ADD.

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)
G01N

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

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

EPO-Internal, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/097631 A1 (CAMBRIDGE ENTPR LTD [GB]; BAHN SABINE [GB]; SCHWARZ EMANUEL [GB]) 2 September 2010 (2010-09-02) claims; table 3 -----	12,13
X	US 2014/200151 A1 (BAHN SABINE [GB] ET AL) 17 July 2014 (2014-07-17) paragraph [0013]; claim 37 -----	6,8,11
X	WO 2012/085555 A2 (CAMBRIDGE ENTPR LTD [GB]; BAHN SABINE [GB]; SCHWARZ EMANUEL [GB]) 28 June 2012 (2012-06-28) claims 8-11 -----	6,8,12, 13
X	US 2004/185474 A1 (ROKUTAN KAZUHITO [JP] ET AL) 23 September 2004 (2004-09-23) paragraphs [0045], [0046] ----- -/-	9,10



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier 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

31 October 2016

Date of mailing of the international search report

18/01/2017

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Luis Alves, Dulce

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL2016/050634

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:

see additional 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 an 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.:

1-6, 8-13

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/NL2016/050634

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FAN LU ET AL: "Erlofinib promotes endoplasmic reticulum stress-mediated injury in the intestinal epithelium.", TOXICOLOGY AND APPLIED PHARMACOLOGY 1 JUL 2014, vol. 278, no. 1, 1 July 2014 (2014-07-01), pages 45-52, XP002763652, ISSN: 1096-0333 abstract -----	4,5
A	US 2007/020735 A1 (CHEN JIAN [US] ET AL) 25 January 2007 (2007-01-25) claims 40-41 -----	1-6,8-13

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/NL2016/050634

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010097631 A1	02-09-2010	CA 2790094 A1 EP 2401615 A1 US 2012071340 A1 WO 2010097631 A1	02-09-2010 04-01-2012 22-03-2012 02-09-2010
US 2014200151 A1	17-07-2014	CA 2821406 A1 EP 2656080 A2 US 2014200151 A1 WO 2012085555 A2	28-06-2012 30-10-2013 17-07-2014 28-06-2012
WO 2012085555 A2	28-06-2012	CA 2821406 A1 EP 2656080 A2 US 2014200151 A1 WO 2012085555 A2	28-06-2012 30-10-2013 17-07-2014 28-06-2012
US 2004185474 A1	23-09-2004	JP 2004208547 A US 2004185474 A1	29-07-2004 23-09-2004
US 2007020735 A1	25-01-2007	US 7771719 B1 US 2007020735 A1 US 2008160021 A1 US 2010028343 A1 US 2012058909 A1 US 2012064073 A1 US 2013064827 A1 US 2015141283 A1 US 2015266953 A1	10-08-2010 25-01-2007 03-07-2008 04-02-2010 08-03-2012 15-03-2012 14-03-2013 21-05-2015 24-09-2015

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-6, 8-13

Methods of predicting adverse effects of a drug, methods of monitoring therapy in depression and psychotropic diseases, methods of testing drug candidates for such diseases and methods of treating such diseases based on the binding of the drug to IL-17 or IL-17 receptors.

2. claim: 7

Methods of monitoring autoimmune disease therapy, in which the disease is related to IL-17 or IL-17 receptor, based on the levels of IL-17B or IL-25.
