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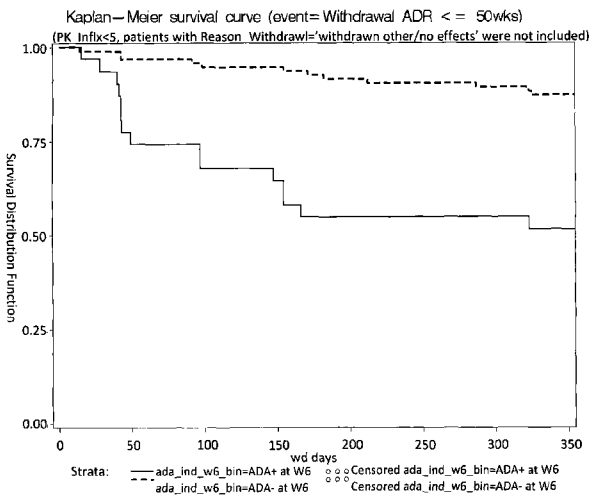
[Continued on next page]

(54) Title: AN ASSAY FOR MEASUREMENT OF ANTIBODIES BINDING TO A THERAPEUTIC MONOCLONAL ANTIBODY

Fig. 2



(57) Abstract: The invention relates to an immunoassay method for determination of an anti- <therapeutic monoclonal antibody> antibody (anti-<TmAB>AB) in vitro in a sample from a patient treated with a therapeutic monoclonal antibody (TmAB). The method comprises the steps of (a) providing an F(ab) fragment of said TmAB bound to a solid phase, (b) incubating the solid phase provided in (a) with the sample, thereby binding the anti-<TmAB>AB to the solid phase via the F(ab) fragment, (c) incubating the solid phase obtained in (b) with a monoclonal antibody that binds to the anti-<TmAB>AB, (d) detecting the monoclonal antibody bound in (c) and thereby determining the anti- <TmAB>AB in the sample. The invention also concerns a method for the determination of antigen specific antibodies of a particular immunoglobulin class by means of an immunoassay in an array format in which the detection of an anti-<TmAB>AB to a TmAB in a sample provided from a patient treated with said TmAB is determined in vitro. Also disclosed is the use of such method for detection of an anti-<TmAB> antibody and in the identification of a patient who is at risk to develop an adverse drug reaction (ADR) during treatment with a TmAB.

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An assay for measurement of antibodies binding to a therapeutic monoclonal antibody

Description

The invention relates to an immunoassay method for determination of an anti-<therapeutic monoclonal antibody> antibody (anti-<TmAB>AB) in vitro in a sample from a patient treated with a therapeutic monoclonal antibody (TmAB). The method comprises the steps of (a) providing an F(ab) fragment of said TmAB bound to a solid phase, (b) incubating the solid phase provided in (a) with the sample, thereby binding the anti-<TmAB>AB to the solid phase via the F(ab) fragment, (c) incubating the solid phase obtained in (b) with a monoclonal antibody that binds to the anti-<TmAB>AB, (d) detecting the monoclonal antibody bound in (c) and thereby determining the anti-<TmAB>AB in the sample. The invention also concerns a method for the determination of antigen specific antibodies of a particular immunoglobulin class by means of an immunoassay in an array format in which the detection of an anti-<TmAB>AB to a TmAB in a sample provided from a patient treated with said TmAB is determined in vitro. Also disclosed is the use of such method for detection of an anti-<TmAB> antibody and in the identification of a patient who is at risk to develop an adverse drug reaction (ADR) during treatment with a TmAB.

Background of the Invention

Since the development of the first monoclonal antibodies by Koehler and Milstein in 1974 a lot of efforts have been dedicated to the development of antibodies which are appropriate for therapy in humans. The first monoclonal antibodies which became available had been developed in mice and rats. In the past ten years an ever growing number of chimeric monoclonal antibodies, humanized monoclonal antibodies or human monoclonal antibodies have reached the market.

Well-known examples of therapeutic monoclonal antibodies (= TmABs) are abciximab (ReoPro®), adalimumab (Humira®), alemtuzumab (Campath®), basiliximab (Simulect®), bevacizumab (Avastin®), cetuximab (Erbitux®), certolizumab pegol (Cimzia®), daclizumab (Zenapax®), eculizumab (Soliris®), efalizumab (Raptiva®), gemtuzumab (Mylotarg®), ibritumomab

tiuxetan (Zevalin®), infliximab (Remicade®), muromonab-CD3 (Orthoclone OKT3®), natalizumab (Tysabri®), omalizumab (Xolair®), palivizumab (Synagis®), panitumumab (Vectibix®), ranibizumab (Lucentis®), rituximab (Rituxan®, MabThera®), trastuzumab (Herceptin®) and tositumomab (Bexxar®).

There are different kinds of TmABs available, some of which might induce adverse drug reactions (ADRs) or secondary treatment failure during treatment of patients.

The various kinds of TmAbs available today, comprise chimeric antibodies, e.g. infliximab (an anti-<TNFα>AB), humanized antibodies, e.g. certolizumab (an anti-<TNFα>AB) and human antibodies, e.g. adalimumab (also an anti-<TNFα>AB) or panitumumab (an anti-<epidermal growth factor receptor>AB).

A quite significant number of chimeric, humanized or human TmAbs is on the market or currently in development and needs to be further investigated. Important criteria in such investigations are induction of auto-antibodies during treatment, adverse drug reactions (ADRs), bio-availability and antibody clearance, just to mention a few of them.

EMEA approval for the treatment of patients with TmAbs will in future also depend on data relating to the formation of anti-<TmAB>AB.

Many different methods have been employed in the prior art for detecting of anti-<TmAB>ABs and have lead to quite different or even conflicting results and implications.

Mire-Sluis, A.R., et al., in J. Immunol. Methods 289 (2004) 1-16, summarize the recommendations for the design and optimization of immunoassays used in the detection of anti drug antibodies of the host against biotechnology produced therapeutic antibodies (anti -<TmAB>ABs). According to Mire-Sluis et al. the well-known anti drug antibody assay formats show considerable disadvantages. Anti drug antibody assays are mentioned, for example, in WO 2005/045058 and WO 90/006515. Anti-idiotypic antibody assays are mentioned, for example, in US 5,219,730; WO 87/002778; EP 0 139 389; and EP 0 170 302. Wadhwa, M., et al., in J. Immunol. Methods

278 (2003) 1-17, report strategies for the detection, measurement and characterization of unwanted antibodies induced by therapeutic biologicals.

Arden et al., Current Opinion in Immunology 20 (2008) 431-435, has reviewed the immunogenicity of anti-<tumor necrosis factor> antibodies and improved methods of anti-<antibody> measurement.

The immune system, e.g. of a mammalian organism produces antibodies which are also called immunoglobulins as a response to foreign (non-self) substances or infectious agents. Such non-self substances are also referred to as antigens. A mammalian organisms uses antibodies to defend itself against the foreign substances or infectious agents.

The immunoglobulins (Ig) can be divided into five different classes. One distinguishes between immunoglobulins of the M, G, A, E, and D classes. These five immunoglobulin classes each differ with respect to the composition of the heavy chain, which is referred to as the μ, γ, α, ε, or δ chain.

Each immunoglobulin class has a different function in the organism. Immunoglobulins of the M class occur when a first contact is made with the antigen, the so-called primary immunization. However, the concentration of these immunoglobulins decreases after such first infection. The immunoglobulins of the G class are first formed slowly during a primary immunization and occur in large amounts when there is a second infection with the same antigen. The immunoglobulins of the A class are found on some of the mucosal surfaces of mammalian tissues and are responsible for the defense processes that occur there. The immunoglobulins of the E class are mainly responsible for allergic reactions. The exact function of the immunoglobulins of the D class is up to now unknown.

The individual immunoglobulin classes occur in blood in very different concentrations. Immunoglobulins of the G class (IgG) are the class with the highest occurrence in human serum, being present in a proportion of about 75%, which corresponds to a serum content of approximately 8 to 18 mg/ml. The second most frequent immunoglobulin class is class A (IgA), whose average serum concentration is usually 0.9 to 4.5 mg/ml. Immunoglobulins of the M class (IgM) normally are present at a concentration of 0.6 to 2.8 mg/ml, and immunoglobulins of class D (IgD) are present at a concentration of

usually 0.003 to 0.4 mg/ml. IgE antibodies are present in the lowest proportion and only occur at a concentration of about 0.02 to 0.05 µg/ml in serum.

For the differential diagnostics of many diseases, it is important to detect the antibodies of one or more particular class of immunoglobulin. A satisfactory diagnosis in the case of viral, bacterial and parasitic infection can only be ensured by means of a class-specific antibody detection and/or by excluding the interfering measurement of certain other immunoglobulin classes (e.g. detection of IgG and IgA antibodies but no detection of IgM antibodies). This is particularly important for differentiating between fresh or acute infections and older infections as well as to clinically monitor the course of an infection. The class-specific detection of antibodies is especially important for HIV, hepatitis A, hepatitis B, toxoplasmosis, rubella and chlamydia infections. The class-specific detection of antibodies that are specific for a certain antigen is also necessary when determining the titer of protecting antibodies, e.g. for checking whether an immunization has been successful.

Antigen-specific antibodies of a particular class are often detected by binding the antigen-specific antibodies comprised in a sample to a solid phase coated with the specific antigen. The immunoglobulins (Ig) specifically bound to the solid phase via the coated antigen are then detected by detection antibodies that are directed specifically against a certain class of human Ig. However, such a test procedure is only possible when all unspecific, non-antigen-bound Ig is removed by washing before the reaction with the class specific labeled antibodies directed against human Ig. Thus, for example, when detecting specific IgG molecules in a sample, relatively large amounts (4-20 mg/ml of serum) of unspecific IgG are present which can bind unspecifically to the solid phase. If a detection antibody against IgG is used, these unspecifically bound immunoglobulins will also be recognized and bound by the detection antibody. This results in elevated background signals and reduced signal-to-noise ratios and last but not least in a reduced sensitivity.

In US2006/0115907A1 immune complex-specific antibodies for increased sensitivity in immunoassay array tests have been described. Immune complex-specific antibodies are rheumathoid factor-like antibodies which preferably bind to aggregated or oligomerized immunoglobulins, but not to single immunoglobulins. EP 1098198 (Berti et al.) concerns a method for the qualitative and quantitative determination of human IgG antibodies in enzyme

immunoassays. In this assay a monoclonal antibody is used which specifically binds human IgG antibodies via a neo-epitope formed upon binding of an antibody to its antigen. A reduction of the background signal due to antibodies bound unspecifically to the solid phase is not described in this method.

One method of reducing background signals is to modify the solid phase in order to avoid unspecific binding of immunoglobulins and to use special buffer additives which are supposed to reduce or prevent unspecific binding of immunoglobulins to the solid phase (examples: HydroGel solid phase (Perkin Elmer), FAST Slides (Schleicher & Schull), detergents, chaotropic salts). The modifications of the solid phase are laborious and expensive. Furthermore, it has emerged that buffer additives can reduce the reactivity of some antibodies and thus also reduce the desired positive signals.

As mentioned, background signals induced by unspecifically bound immunoglobulins increase the blank value, which makes it more difficult to detect the specifically bound antibodies. This is especially the case for miniaturized test systems such as immunoassays in an array format. Such arrays may comprise a plurality of specific tests, in some cases even in different test formats and the test procedure is performed in a single reaction vessel. Thus, for example, addition of a certain detergent can suppress the unspecific binding of antibodies to a first analyte in such an array, but the same detergent can have no effect or even the opposite effect in another test for detection of a second analyte on the same array system.

The use of the coagulation factor C1q, which is a subunit of the first complement component, as a further possibility of reducing background signals in immunoassays is disclosed in EP 0222146 B1. In U.S. Pat. No. 5,698,449 A1, a fragment of C1q is disclosed for selectively removing immune complexes from the blood and for detecting and quantifying the immune complexes. U.S. Pat. No. 4,062,935 A1 describes the addition of rheumatoid factors or C1q to the sample and the binding and quantification of the resulting immune complexes. However, the prior art does not show any application of C1q for immunoassays in an array format.

A characteristic feature of immunoassays in an array format is the solid phase. In such array-based immunoassays the solid phase preferably consists of localized, defined, discrete test areas. These test areas on the solid phase are

preferably spatially separated from one another by inert areas. These localized discrete test areas in most cases are spots and preferably have a diameter of 10 µm to 1 mm and particularly preferably a diameter of 100-200 µm. Array systems are described, for example, in Ekins, R.P. and Chu, F.W. (Clin. Chem. 37 (1995) 1955-1967) and in U.S. Pat. Nos. 5,432,099, 5,516,635 and 5,126,276.

Array systems have the advantage that several analyte determinations can be carried out simultaneously from one sample. The solid phase of these array systems can be preferably coated with a universal binder like streptavidin or avidin as disclosed in EP 0939319 (Hornauer et a1.). It is possible to apply a plurality of binding partners such as antigen-specific antibodies to the individual test areas or spots on the solid phase (solid support). In case e.g. streptavidin is used as a universal binding matrix each binding partner can be biotinylated an easily spotted/bound onto such solid phase. Sample components and in particular IgGs can bind unspecifically to one or more of these binding partners or to the solid phase. In this case it is almost impossible to identify a universal buffer additive to reduce the background signals since each individual binding partner might requires a very particular buffer additive. Buffer additives which have positive effects in the case of one binding partner may even have adverse effects for other binding partners. It is also very difficult to modify the solid phase for numerous different binding partners.

Hence one object of the present invention was to develop a sensitive method for the detection of an anti-<therapeutic monoclonal antibody> antibody (anti-<TmAB>AB) in a sample obtained from a patient treated with said TmAB.

It surprisingly turned out that the use of the immunoassay method according to the present invention allows for the very early detection of anti-<TmAB>AB and thereby will also allow to identify the majority of those patients at risk to develop an adverse drug reaction (ADR) during treatment with a TmAB.

Summary of the Invention

The invention concerns to an immunoassay method for determination of an anti-<therapeutic monoclonal antibody> antibody (anti-<TmAB>AB) in vitro in a sample from a patient treated with a therapeutic monoclonal antibody (TmAB), the method comprising: a) providing an F(ab) fragment of said

TmAB bound to a solid phase, b) incubating the solid phase provided in (a) with the sample thereby binding the anti-<TmAB>AB to the solid phase via the F(ab) fragment, c) incubating the solid phase obtained in (b) with a monoclonal antibody <h-Agg.-IgG>, whereby said monoclonal antibody binds to the anti-<TmAB>AB, and d) detecting monoclonal antibody <h-Agg.-IgG> bound in (c) and thereby determining the anti-<TmAB>AB in the sample.

In an embodiment the present invention relates to the use of the immunoassay method for identification of a patient who is at risk to develop an adverse drug reaction (ADR) by determination of an anti-<TmAB>AB in vitro in a sample from a patient treated with a therapeutic monoclonal antibody (TmAB). This method comprises: a) providing an F(ab) fragment of said TmAB bound to a solid phase, b) incubating the solid phase provided in (a) with the sample thereby binding the anti-<TmAB>AB to the solid phase via the F(ab) fragment, c) incubating the solid phase obtained in (b) with a monoclonal antibody <h-Agg.-IgG>, whereby said monoclonal antibody binds to the anti-<TmAB>AB, and d) detecting monoclonal antibody <h-Agg.-IgG> bound in (c) and thereby determining the anti-<TmAB>AB in the sample during treatment with a TmAB, wherein the patient testing positive for anti-<TmAB>AB in the method is at risk of developing an ADR.

In a further embodiment the present invention relates to a method for selecting an alternative therapeutic antibody for a patient under treatment with a first TmAB, wherein at least a first and one or more alternative TmAB are available, comprising: a) determining in vitro an anti-<TmAB>AB to the first TmAB in a sample from a patient treated with said first TmAB, and b) selecting an alternative TmAB for future therapy, if an anti-<TmAB>AB to said first TmAB is present.

Detailed Description of the Invention

The practicing of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, "Molecular Cloning: A Laboratory Manual", second edition (Sambrook et al., 1989); "Oligonucleotide Synthesis" (M. J. Gait, ed., 1984); "Animal Cell Culture" (R. 1. Freshney, ed., 1987);

"Methods in Enzymology" (Academic Press, Inc.); "Current Protocols in Molecular Biology" (F. M. Ausubel et al., eds., 1987, and periodic updates); "PCR: The Polymerase Chain Reaction", (Mullis et al., eds., 1994).

Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton, P. and Sainsburg, D. et al., Dictionary of Microbiology and Molecular Biology 2nd ed., J. Wiley & Sons, New York, N.Y. (1994); March, Advanced Organic Chemistry Reactions, Mechanisms and Structure 4th ed., John Wiley & Sons (New York, N.Y. 1992); Lewin, B., Genes V, published by Oxford University Press (1994), ISBN 0-19-854287 9); Kendrew, J. et al. (eds.), The Encyclopedia of Molecular Biology, published by Blackwell Science Ltd. (1994), ISBN 0-632-02182-9); and Meyers, R.A. (ed.), Molecular Biology and Biotechnology: a Comprehensive Desk Reference, published by VCH Publishers, Inc. (1995), ISBN 1-56081-569 8) provide one skilled in the art with a general guide to many of the terms used in the present application.

All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

Definitions

As used herein, each of the following terms has the meaning associated with it in this section.

The articles "a" and "an" are used herein to refer to one or to more than one (i.e. to at least one) of the grammatical object of the article. By way of example, "an antibody" means one antibody or more than one antibody. The term “at least” is used to indicate that optionally one or more further objects may be present. By way of example, an array comprising at least two discrete areas may optionally comprise two or more discrete test areas.

The expression “one or more” denotes 1 to 50, preferably 1 to 20 also preferred 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, or 15.

The expression “of interest” denotes an analyte or substance of possible relevance that shall be analyzed or determined.

"Detection" includes any means of detecting, including direct and indirect detection. The term "detection" is used in the broadest sense to include both qualitative and quantitative measurements of an analyte, herein measurements of an analyte such as an anti-<therapeutic antibody> antibody. In one aspect, a detection method as described herein is used to identify the mere presence of an analyte of interest in a sample. In another aspect, the method can be used to quantify an amount of analyte in a sample.

By "correlate" or "correlating" is meant comparing, in any way, the performance and/or results of a first analysis or protocol with the performance and/or results of a second analysis or protocol. For example, one may use the results of a first analysis or protocol in carrying out a second protocols and/or one may use the results of a first analysis or protocol to determine whether a second analysis or protocol should be performed.

To "reduce" or "inhibit" is to decrease or reduce an activity, function, and/or amount as compared to a reference. By reduce or inhibit is meant the ability to cause an overall decrease preferably of 20% or greater, more preferably of 50% or greater, and most preferably of 75%, 85%, 90%, 95%, or greater. Reduce or inhibit can refer to the symptoms of the disorder being treated.

The term "sample" or "test sample" as used herein refers to a biological sample obtained for the purpose of evaluation in vitro obtained from a patient. The sample may comprise antibodies that bind to the antibody or drug with which the patient has been treated, such as human anti-<chimeric antibody> (HACA) or human anti-<human antibody> (HAHA), both being anti-<TmAB>ABs. The term sample or test sample includes biological samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components, such as proteins or polynucleotides. Typically, the sample is a liquid sample.

The biological sample may for example be whole blood, serum, antibodies recovered from the patient or plasma. The sample is preferably whole blood, serum or plasma. The biological sample may comprise antibodies recovered from the patient. In one embodiment, the sample is a clinical sample. In another embodiment, the sample is used in a diagnostic assay.

In an embodiment, a sample is obtained from a subject or patient prior to therapeutic monoclonal antibody (TmAB) therapy. In an embodiment, a

sample is obtained from a subject or patient under TmAB therapy. In one embodiment, a sample is obtained from a subject or patient after at least one treatment with a TmAB.

If a sample is stated herein to be taken at week 2, the sample can be taken from the 9th day to the 21st day after initiation of therapy with said TmAB. If a sample is stated herein to be taken at week 6, the sample can be taken from the 28th day to the 64th day after initiation of therapy. If a sample is stated herein to be taken at week 14, the sample can be taken from week 13 to week 16 after initiation of therapy.

A "reference sample" as used herein, refers to any sample, standard, or level that is used for comparison purposes. In one embodiment, a reference sample is obtained from an untreated subject or patient. In another embodiment, a reference sample is obtained from a healthy and/or non-diseased individual who is not the subject or patient. In another embodiment, a reference sample is obtained from an untreated individual who is not the subject or patient. In certain embodiments, a reference sample is a single sample or combined multiple samples from the same subject or patient that are obtained at one or more different time points than when the test sample is obtained. For example, a reference sample is obtained at an earlier time point from the same subject or patient than when the test sample is obtained. In certain embodiments, a reference sample includes all types of biological samples as defined above under the term "sample" that is obtained from one or more individuals who is not the subject or patient. In certain embodiments, a reference sample is a combined multiple samples from one or more healthy individuals who are not the subject or patient. In certain embodiments, a reference sample is a combined multiple samples from one or more individuals with a disease or disorder (e.g., rheumathoid arthritis) who are not the subject or patient. In certain embodiments, a reference sample is pooled plasma or serum samples from one or more individuals who are not the subject or patient. In certain embodiments, a reference sample is pooled plasma or serum samples from one or more individuals with a disease or disorder who are not the subject or patient.

As the skilled artisan will appreciate, the immunoassay method according to the present invention is performed in vitro. The patient sample is discarded afterwards. The patient sample is solely used for the in vitro diagnostic method

of the invention and the material of the patient sample is not transferred back into the patient's body.

The term "antibody" is used in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments.

The "light chains" of antibodies (immunoglobulins) from many vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda. This classification and nomenclature is based on the amino acid sequences of their constant domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3 and IgG4.

The notation of an antibody is written in that the antigen, which is bound specifically by the antibody, is denoted in "<...>", e.g. an antibody against the antigen "X" is denoted as an "anti-<X> antibody".

"Antibody fragments" comprise a portion of an intact antibody, preferably comprising the antigen-binding or variable region thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$ and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

The "Fab" fragment contains the variable domains of the antibody light an heavy chains, respectively but also the constant domain of the light chain and the first constant domain (CH1) of the heavy chain.

"Fab'" fragments differ from Fab fragments by having in addition a few amino acid residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab' antibody fragments originally are produced as pairs of Fab' fragments ($F(ab')_2$) which have a hinge cystine bridge between them. The Fab'-monomer is obtained from $F(ab')_2$ by reduction of the cysteine bridge.

"Single-chain Fv" or "scFv" antibody fragments comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv, see Plueckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404 097; WO 93/11161; and Holliger, P. et al., Proc. Natl. Acad. Sci. USA 90 (1993) 6444-6448.

A "F(ab) fragment" according to the present invention, includes Fab, Fab', scFv and diabodies. Fab or Fab' fragments of a TmAB are produced by processing of said TmAB, e.g. by digestion of the TmAB into Fab or $F(ab')_2$-fragments and an Fc part, respectively. In case a therapeutic antibody is an scFv or a diabody, these molecules do not to be further digested but can be used as such in the immunoassay method according to the present invention.

The term "monoclonal antibody" (MAb) as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody

preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Koehler, G. et al., Nature 256 (1975) 495-497, or may be made by recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson, T. et al., Nature 352 (1991) 624-628 and Marks, J.D. et al., J. Mol. Biol. 222 (1991) 581-597, for example.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; and Morrison, S.L. et al., Proc. Natl. Acad. Sci. USA 81 (1984) 6851-6855).

"Humanized" forms of non-human (e.g., murine) antibodies are chimeric antibodies which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains,

in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones, P.T. et al., Nature 321 (1986) 522-525; Riechmann, L. et al., Nature 332 (1988) 323-327; and Presta, L.G., Curr. Op. Struct. Biol. 2 (1992) 593-596.

A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art. In one embodiment, the human antibody is selected from a phage library, where that phage library expresses human antibodies (Vaughan, T.J. et al., Nature Biotechnology 14 (1996) 309-314; Sheets, M.D. et al., Proc. Natl. Acad. Sci. 95 (1998) 6157-6162; Hoogenboom, H.R. and Winter, G., J. Mol. Biol. 227 (1992) 381-388; Marks, J.D. et al., J. Mol. Biol., 222 (1991) 581). Human antibodies can also be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks, J.D. et al., Bio/Technology 10 (1992) 779-783; Lonberg, N. et al., Nature 368 (1994) 856-859; Morrison, S.L., Nature 368 (1994) 812-813; Fishwild, D.M. et al., Nature Biotechnology 14 (1996) 845-851; Neuberger, M., Nature Biotechnology 14 (1996) 826; Lonberg, N. and Huszar, D., Intern. Rev. Immunol. 13 (1995) 65-93. Alternatively, the human antibody may be prepared via immortalization of human B lymphocytes producing an antibody directed against a target antigen (such B lymphocytes may be recovered from an individual or may have been immunized in vitro). See, e.g., Cole et al., Monoclonal Antibodies and Cancer

Therapy, Alan R. Liss, p. 77 (1985); Boerner, P. et al., J. Immunol. 147 (1991) 86-95; and U.S. Pat. No. 5,750,373.

The term "therapeutic antibody" denotes an antibody which is tested in clinical studies for approval as human therapeutic and which can be administered to an individual for the treatment of a disease. In one embodiment the therapeutic antibody is a monoclonal antibody. In a further embodiment the therapeutic antibody is obtained from a great ape or an animal transformed with a human antibody locus or a human monoclonal antibody or a humanized monoclonal antibody. In one embodiment the therapeutic antibody is a human monoclonal antibody. In a further embodiment the therapeutic antibody is a humanized monoclonal antibody. Therapeutic antibodies are being used widely for the treatment of various diseases such as oncological diseases, immunological diseases, central nervous diseases, vascular diseases, chronic inflammatory diseases, or infectious diseases. Such antibodies are, for instance, antibodies against CD20, CD22, HLA-DR, CD33, CD52, EGFR, G250, GD3, HER2, PSMA, CD56, VEGF, VEGF2, CEA, Levis Y antigen, IL-6 receptor (IL6R), TNFα, or IGF-1 receptor (IGF1R). Therapeutic antibodies are also described by Groner, B., et al., Curr. Mol. Meth. 4 (2004) 539-547; and Harris, M., Lancet Oncol. 5 (2004) 292-302.

As used herein, an "anti-<therapeutic antibody> antibody" is an antibody that binds a therapeutic antibody. An "anti-<therapeutic monoclonal antibody> antibody" (anti-<TmAB>AB) is an antibody that binds a therapeutic monoclonal antibody. For example, an anti-<infliximab>antibody is an antibody that binds infliximab, a therapeutic monoclonal antibody, targeting TNFα.

An antibody that "specifically binds to" or is "specific for" a particular polypeptide or an epitope on a particular polypeptide is one that binds to that particular polypeptide or epitope on a particular polypeptide without substantially binding to any other polypeptide or polypeptide epitope.

An "isolated" polypeptide or "isolated" antibody is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the polypeptide or antibody, and may include enzymes, hormones, and other proteinaceous or

nonproteinaceous solutes. In preferred embodiments, the polypeptide or antibody will be purified (1) to greater than 95% by weight of polypeptide or antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated polypeptide or antibody includes the polypeptide or antibody in situ within recombinant cells since at least one component of the polypeptide's natural environment will not be present. Ordinarily, however, isolated polypeptide or antibody will be prepared by at least one purification step.

By "subject" or "patient" is meant a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, ovine, or feline. Preferably, the subject or patient is a human.

As used herein, "treatment" refers to clinical intervention in an attempt to alter the natural course of the individual or cell being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments the methods of the invention are useful in attempts to delay development of a disease or disorder, especially of an adverse drug reaction.

An "effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. A "therapeutically effective amount" of a therapeutic agent may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the therapeutic agent are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease,

the prophylactically effective amount will be less than the therapeutically effective amount.

The term "diagnosis" is used herein to refer to the identification of a molecular or pathological state, disease or condition or to refer to identification of a patient who may benefit from a particular treatment regimen. The term "prognosis" is used herein to refer to the prediction of the likelihood of clinical benefit from a therapy. The term "prediction" is used herein to refer to the likelihood that a patient will respond either favorably or unfavorably to a particular therapy. In one embodiment, the prediction relates to the extent of those responses. In one embodiment, the prediction relates to whether and/or the probability that a patient will survive or improve following treatment, for example treatment with a particular therapeutic agent, and for a certain period of time without disease recurrence. The predictive methods of the invention can be used clinically to make treatment decisions by choosing the most appropriate treatment modalities for any particular patient. The predictive methods of the present invention are valuable tools in predicting if a patient is likely to respond favorably to a treatment regimen, such as a given therapeutic regimen, including for example, administration of a given therapeutic agent or combination, surgical intervention, steroid treatment, etc., or whether long-term survival of the patient, following a therapeutic regimen is likely. The term “selecting” and “selection” is used herein to refer to a choice from a number of alternatives. As an example a “selection” is the process to choose one TmAB, from two or more available TmABs available for treatment of a disease.

"Patient response" can be assessed using any endpoint indicating a benefit to the patient, including, without limitation, (1) inhibition, to some extent, of disease progression, including slowing down and complete arrest; (2) reduction in lesion size; (3) inhibition (i.e., reduction, slowing down or complete stopping) of disease cell infiltration into adjacent peripheral organs and/or tissues; (4) inhibition (i.e. reduction, slowing down or complete stopping) of disease spread; (5) relief, to some extent, of one or more symptoms associated with the disorder; (6) increase in the length of disease-free presentation following treatment; and/or (7) decreased mortality at a given point of time following treatment.

"Adverse drug reactions" (ADRs) describe harm associated with the use of given medications at a normal dose. ADRs may be local, i.e. limited to a certain location, or systemic, where a medication has caused ADRs throughout the organism and is e.g. measureable from the circulation. ADRs may be classified by cause (Type A: augmented pharmacologic effects - dose dependent and predictable (intolerance, side effects), Type B: bizarre effects (or idiosyncratic) - dose independent and unpredictable, Type C: chronic effects, Type D: delayed effects, Type E: end-of treatment effects or Type F: failure of therapy), or by severity. The American FDA defines a serious "adverse drug reaction" (ADR) as one when the patient outcome is one of the following: death, life-threatening, hospitalization (initial or prolonged), disability (significant, persistent, or permanent change, impairment, damage or disruption in the patient's body function/structure, physical activities or quality of life), congenital anomaly, requires intervention to prevent permanent impairment or damage. While no official scale exists yet to communicate overall drug risk, the iGuard Drug Risk Rating System (www.iguard.org) is a five color rating scale: red (high risk), orange (elevated risk), yellow (guarded risk), blue (general risk), Green (low risk). ADRs also comprise infusion reactions. These infusion reactions, e.g. include urticaria, low blood pressure, chest tightness, flushing or decreased blood pressure.

"Lack of efficacy" (LOE) is defined as high disease activity despite treatment with under conditions otherwise considered to be adequate, e.g. with the usually effective amount of a therapeutic agent.

"Treatment Efficacy" is a measure of the ability of an intervention to produce a desired beneficial clinical effect in average conditions of application, usually determined in non-randomized outcome studies. The treatment efficacy could be affected by LOE and/or patients compliance.

The term "benefit" is used in the broadest sense and refers to any desirable effect and specifically includes clinical benefit as defined herein. Clinical benefit can be measured by assessing various endpoints, e.g., inhibition, to some extent, of disease progression, including slowing down and complete arrest; reduction in the number of disease episodes and/or symptoms; reduction in lesion size; inhibition (i.e., reduction, slowing down or complete stopping) of disease cell infiltration into adjacent peripheral organs and/or tissues; inhibition (i.e. reduction, slowing down or complete stopping) of

disease spread; decrease of auto-immune response, which may, but does not have to, result in the regression or ablation of the disease lesion; relief, to some extent, of one or more symptoms associated with the disorder; increase in the length of disease-free presentation following treatment, e.g., progression-free survival; increased overall survival; higher response rate; and/or decreased mortality at a given point of time following treatment.

As used herein, the term "Immunoassay" (IA) means a specific binding assay in which an analyte is detected by use of at least one antibody as a specific binding partner or agent. Immunoassay includes, but is not limited to, radioimmunoassay (RIA), fluoroluminescence assay (FLA), chemiluminescence assay (CLA), electrochemiluminescence assay (ECLA), and enzyme linked immunosorbant assay (ELISA). ELISA methods are described, for example, in WO 2001/36972.

The term "detection agent" refers to an agent that binds to an analyte and is detectably labeled. Examples of detection agents include, but are not limited to, an antibody, antibody fragment, soluble receptor, receptor fragment, and the like. Detection of a detection agent is either possible directly, i.e. via a label directly linked to the agent or indirectly via a labeled second binding partner, such as a further antibody or receptor that specifically binds the detection agent.

The term “label” as used herein refers to any substance that is capable of producing a detectable signal, whether visibly or by using suitable instrumentation. Various labels suitable for use in the present invention include, but are not limited to, chromogens, fluorescent, chemiluminescent or electrochemiluminescent compounds, catalysts, enzymes, enzymatic substrates, dyes, colloidal metallic and nonmetallic particles, and organic polymer latex particles.

A “directly detectable label” is for example a chromogen (fluorescent or luminescent group and dye), an NMR-active group or a metal particle. Metal chelates which can be detected by electrochemoluminescence are a preferred signal-emitting groups, with particular preference being given to ruthenium chelates, e.g. a ruthenium $(bispyridyl)_3^{2+}$ chelate. Suitable ruthenium labeling groups are described, for example, in EP 0 580 979, WO 90/05301, WO 90/11511 and WO 92/14138.

The term “luminescence” refers to any emission of light that does not derive energy from the temperature of an energy source (for example, a source of electromagnetic radiation, a chemical reaction, mechanical energy). In general, the source causes an electron of an atom to move from a lower energy state into an “excited” higher energy state; then the electron releases that energy in the form of emitted light when it falls back to a lower energy state. Such emission of light usually occurs in the visible or near-visible range of the electromagnetic spectrum. The term “luminescence” includes, but is not limited to, such light emission phenomena such as phosphorescence, fluorescence, bioluminescence, radioluminescence, electroluminescence, electrochemiluminescence and thermo-luminescence.

The term “luminescent label” refers to a label that generates a luminescent signal, e.g. an emission of light that does not derive energy from the temperature of the emitting source. The luminescent label may be, for example, a fluorescent molecule, a phosphorescent molecule, a radioluminescent molecule, a luminescent chelate, a phosphor or phosphor-containing compound, or a quantum dot.

An “electrochemiluminescence assay” or "ECLA" is an electrochemical assay in which bound analyte molecule is detected by a label linked to a detecting agent (target molecule). An electrode electrochemically initiates luminescence of a chemical label linked to a detecting agent. Light emitted by the label is measured by a photodetector and indicates the presence or quantity of bound analyte molecule/target molecule complexes. ECLA methods are described, for example, in U.S. Patent Nos. 5,543,112; 5,935,779; and 6,316,607. Signal modulation can be maximized for different analyte molecule concentrations for precise and sensitive measurements.

In an ECLA procedure microparticles can be suspended in the sample to efficiently bind to the analyte. For example, the particles can have a diameter of 0.05 µm to 200 µm, 0.1 µm to 100 µm, or 0.5 µm to 10 µm, and a surface component capable of binding an analyte molecule. In one frequently used ECLA-system (Elecsys, Roche Dagnsotics, Germany), the microparticles have a diameter of about 3 µm. The microparticles can be formed of crosslinked starch, dextran, cellulose, protein, organic polymers, styrene copolymer such as styrene/butadiene copolymer, acrylonitrile/butadiene/styrene copolymer, vinylacetyl acrylate copolymer, vinyl chloride/acrylate copolymer, inert

inorganic particles, chromium dioxide, oxides of iron, silica, silica mixtures, proteinaceous matter, or mixtures thereof, including but not limited to sepharose beads, latex beads, shell-core particles, and the like. The microparticles are preferably monodisperse, and can be magnetic, such as paramagnetic beads. See, for example, U.S. Patent Nos. 4,628,037; 4,965,392; 4,695,393; 4,698,302; and 4,554,088. Microparticles can be used in an amount ranging from about 1 to 10,000 µg/ml, preferably 5 to 1,000 µg/ml.

A "detection limit" for an analyte molecule in a particular assay is a minimum concentration of the analyte molecule that can be detected above background levels for that assay. For example, in IA and ECLA, the detection limit for an analyte molecule that specifically binds a target molecule can be the concentration at which the analyte molecule produces an IA signal or ECLA signal above that produced by a control antibody that does not bind, or non-specifically binds, the target antigen. Molecules that have an IA response less than the IA detection limit are IA^-. Molecules that have an IA response equal to or greater than the IA detection limit are IA^+. Molecules that have an ECLA response less than the ECLA detection limit are $ECLA^-$. Molecules that have an ECLA response equal to or greater than the ECLA detection limit are $ECLA^+$. Detection limits can be raised or lowered to achieve a desired assay result.

A “solid phase”, also known as “solid support”, is insoluble, functionalized, polymeric material to which library members or reagents may be attached or covalently bound (often via a linker) to be immobilized or allowing them to be readily separated (by filtration, centrifugation, washing etc.) from excess reagents, soluble reaction by- products, or solvents. Solid phases for the immunoassays according to the invention are widely described in the state of the art (see, e.g., Butler, J.E., Methods 22 (2000) 4-23). The term "solid phase" means a non-fluid substance, and includes particles (including microparticles, beads, magnetic beads, metallic or non-metallic particles) made from materials such as polymer, metal (paramagnetic, ferromagnetic particles), glass, and ceramic; gel substances such as silica, alumina, and polymer gels; capillaries, which may be made of polymer, metal, glass, and/or ceramic; zeolites and other porous substances; membranes; electrodes; microtiter plates; solid strips; and cuvettes, tubes, chips or other spectrometer sample containers. A solid phase component of an assay is distinguished from inert solid surfaces with which the assay may be in contact in that a "solid phase" contains at least one

moiety on its surface, which is intended to interact with the capture antibody or capture molecule. A solid phase may be a stationary component, such as a tube, strip, cuvette, chip or microtiter plate, or may be non-stationary components, such as beads and microparticles. Microparticles can also be used as a solid phase for homogeneous assay formats. A variety of microparticles that allow either non-covalent or covalent attachment of proteins and other substances may be used. Such particles include polymer particles such as polystyrene and poly(methylmethacrylate); gold particles such as gold nanoparticles and gold colloids; and ceramic particles such as silica, glass, and metal oxide particles. See for example Martin, C.R., et al., Analytical Chemistry-News & Features 70 (1998) 322A-327A, which is incorporated herein by reference.

The terms “chip”, “bio-chip”, “polymer-chip” or “protein-chip” are used interchangeably and refer to a collection of a large number of probes, markers or biochemical markers arranged on a shared substrate (e.g. a solid phase) which could be a portion of a silicon wafer, a nylon strip, a plastic strip, or a glass slide.

The term ”discrete test area” according to the present invention is used to contain a single type of capture molecule. Neighboured discrete test areas on a stationary component solid phase, e.g. an array or a chip, don’t overlap each other. In case the solid phase is e.g. an array or a chip, the discrete test areas might be adjacent to each other. Also a spacing in between at least two “discrete test areas” on a stationary component is possible. Discrete test areas on a stationary component solid phase, e.g. on an array or a chip, may be arranged in geometrically patterns. If a solid phase is a non-stationary component, such as beads and microparticles, the term “discrete test area” means that on each non-stationary component one type of capture molecule is immobilized.

An “array”, “macroarray” or “microarray” is an intentionally created collection of substances, such as molecules, markers, openings, microcoils, detectors and/or sensors, attached to or fabricated on a substrate or solid surface, such as glass, plastic, silicon chip or other material forming an array. The arrays can be used to measure the levels of large numbers, e.g., tens, thousands or millions, of reactions or combinations simultaneously. An array may also contain a small number of substances, e.g., one, a few or a dozen.

The substances in the array can be identical or different from each other. The array can assume a variety of formats, e.g., libraries of soluble molecules, libraries of immobilized molecules, libraries of immobilized antibodies, libraries of compounds tethered to resin beads, silica chips, or other solid phases. The array could either be a macroarray or a microarray, depending on the size of the pads on the array. A macroarray generally contains pad sizes of about 300 microns or larger and can be easily imaged by gel and blot scanners. A microarray would generally contain pad sizes of less than 300 microns.

Method:

Therapeutic monoclonal antibodies (TmABs) are increasingly used for combating a broad variety of diseases. Application of a TmAB to tumor necrosis factor (<TNFα>) or CD20 (<CD20>), respectively, is of paramount importance for many patients having a diagnosis of a chronic inflammatory disease such as rheumatoid arthritis (RA). These TmABs are also frequently used for treatment of Crohn's disease (CD), ankylosing spondylitis (AS), polyarticular juvenile idiopathic arthritis (JIA), psoriatic arthritis (PsA), Morbus Bechterew or chronic plaque psoriasis (Ps), as well as other diseases. Several TmABs used in therapy of chronic inflammatory disease belong to the group of anti-<TNFα> antibodies.

As discussed in detail above, TmABs are either mouse-human chimeric TmABs (e.g. infliximab) or human TmABs (e.g. adalimumab). TmABs contain elements that might be “foreign” to the immune system of the patient. Such anti-<TmAB>ABs may occur during treatment which said TmAB as an immune defense reaction of a patient (Pan, Y., et al., FASEB J. 9 (1995) 43-49).

In case the immune system treats an element of a TmAB as foreign, it is to be expected that administration of this protein will elicit an immune response. Anti-<TmAB>ABs can be directed against any region of the TmAB, like the variable region, the constant region or the glycostructure of the TmAB. The variable domain regions comprising rare sequence elements, those are domains that may well cause an immune response by the immune system of a patient treated with a TmAB.

We developed an immunoassay method for the detection of anti-<therapeutic monoclonal antibody> antibodies (anti-<TmAB>ABs) against a therapeutic monoclonal antibody (TmAB).

In an embodiment the present invention relates to an immunoassay method for determination of an anti-<therapeutic monoclonal antibody> antibody (anti-<TmAB>AB) in vitro in a sample from a patient treated with a therapeutic monoclonal antibody (TmAB), the method comprising: a) providing a F(ab) fragment of said TmAB bound to a solid phase, b) incubating the solid phase provided in (a) with the sample thereby binding the anti-<TmAB>AB to the solid phase via the F(ab) fragment, c) incubating the solid phase obtained in (b) with a monoclonal antibody <h-Agg.-IgG>, whereby said monoclonal antibody binds to the anti-<TmAB>AB, and d) detecting monoclonal antibody <h-Agg.-IgG> bound in (c) and thereby determining the anti-<TmAB>AB in the sample.

The subject or patient can be any mammalian species. In a preferred embodiment the subject or patient is a human. In one embodiment a human anti-<TmAB>AB is determined in the immunoassay method.

In an embodiment the sample will be selected from the group consisting of a liquid sample like antibodies recovered from the patient, whole blood, plasma, or serum. In a further embodiment the sample will be selected from the group consisting of whole blood, plasma or serum, with serum being most preferred. In one embodiment the sample is derived from a human.

The antigen bound to a solid phase for the determination of anti-<TmAB>AB to a TmAB is selected from the group consisting of an Fab' fragment of a TmAB, an Fab fragment of a TmAB, an scFv representing a TmAB and a diabody representing a TmAB. In one preferred embodiment the F(ab) fragment of said TmAB is selected from the group consisting of an Fab' fragment of said TmAB and an Fab fragment of said TmAB. In one preferred embodiment the antigen bound to a solid phase for the determination of an anti-<TmAB>AB is a Fab fragment of the TmAB of interest. In one preferred embodiment the antigen bound to a solid phase for the determination of an anti-<TmAB>AB is a Fab' fragment of the TmAB of interest.

Surprisingly the inventors have found, that an immunoassay method based on the use of an F(ab) fragment of a TmAB of interest bound to a solid phase can

overcome at least some of the current limitations concerning the specificity and sensitivity of the detection of an anti-<TmAB>AB in a sample from a patient treated with said TmAB.

The antigen (e.g. an F(ab) fragment) provided in the immunoassay method in one embodiment is bound to the solid phase by a binding system selected from the group consisting of covalent binding, direct attachment and affinity interaction. A covalent binding of an antigen (e.g. an F(ab) fragment) provided in the immunoassay method can be done for example by an epoxy-, NHS-, carboxymethyl-activation of the solid phase and a subsequent reaction with an appropriate functional group of the antigen. A direct attachment of an antigen (e.g. an F(ab) fragment) provided in the immunoassay method can be based for example on hydrophobic or hydrophilic interactions, chelate binding or adsorptive interactions. A affinity interaction of an antigen (e.g. an F(ab) fragment) can be based for example on biotin/streptavidin-, biotin/avidin-, tag/anti-tag-, lecitin/antibody-, or biotin-anti-<biotin> antibody interactions.

In one embodiment the antigen provided in the immunoassay method is bound to the solid phase by a binding system selected from the group consisting of biotin/streptavidin, biotin/avidin, and biotin-anti-<biotin> antibody. To allow such binding the antigen is biotinylated (e.g. F(ab)-Bi fragment). In a preferred embodiment an F(ab) fragment provided in the method is bound to the solid phase by a binding system selected from the group consisting of biotin/streptavidin and biotin/avidin. In a further preferred embodiment an Fab fragment provided in the method is bound to the solid phase by a binding system selected from the group consisting of biotin/streptavidin and biotin/avidin. In a further preferred embodiment an Fab' fragment provided in the method is bound to the solid phase by a binding system selected from the group consisting of biotin/streptavidin and biotin/avidin.

Methods for biotinylation are known to the skilled artisan. A detailed description of reaction variants and reaction conditions for conjugating of antibody fragments as well as other proteins and biomolecules is given in G. T. Hermanson: Bioconjugate Techniques, Elsevier/AP, (2008); 2nd edition (ISBN: 978-0-12-370501-3). The method for the production of a biotin conjugated Fab fragment (Fab-Bi) of a TmAB according to the present invention is described in Example 1.

The attachment of the antigen (e.g. the F(ab) fragment) to the solid phase can be accomplished under side controlled conditions so that the antigen binding domain is presented outwards the surface of the solid phase providing the highest accessibility of the antigen using (i) site specific conjugation (e.g. a conjugation in the hinge-region of an F(ab) fragment or a tag-assisted conjugation) or (ii) site a specific interaction with the solid phase (e.g. a specific sterically oriented interaction of an antigen with a lecitin coated solid phase).

In an embodiment the hinge region of an F(ab) fragment is conjugated to the solid phase. In an embodiment the hinge region of an Fab fragment is conjugated to the solid phase. In an embodiment the hinge region of an Fab' fragment is conjugated to the solid phase.

In an embodiment an F(ab) fragment is conjugated on the solid phase by a sterically oriented interaction of said F(ab) fragment with a lecitin coated solid phase. In an embodiment an Fab fragment is conjugated on the solid phase by a sterically oriented interaction of said Fab fragment with a lecitin coated solid phase. In an embodiment an F(ab) fragment is conjugated on the solid phase by a sterically oriented interaction of said Fab' fragment with a lecitin coated solid phase.

A stochastic sterically non directed coupling of an F(ab) fragment to the solid phase provides equivalent results as the sterically directed coupling. However, without wanted to be bound to this theory, the directed coupling can be under some circumstances advantageous.

In one embodiment the method according of the present invention is practiced with a TmAB of interest selected from the group consisting of chimeric antibodies (CA) and humanized antibodies (HA).

In one embodiment the method according of the present invention is practiced with a biotinylated F(ab) (F(ab)-Bi) fragment of a therapeutic monoclonal antibody (TmAB) selected from the group consisting of abciximab, adalimumab, alemtuzumab, basiliximab, bevacizumab, cetuximab, certolizumab pegol, daclizumab, eculizumab, efalizumab, gemtuzumab, ibritumomab tiuxetan, infliximab, muromonab-CD3, natalizumab, omalizumab, palivizumab, panitumumab, ranibizumab, rituximab, tositumomab and trastuzumab. In an preferred embodiment the method

according of the present invention is practiced with a F(ab)-Bi fragment of a therapeutic monoclonal antibody (TmAB) selected from the group consisting of infliximab, adalimumab, certolizumab and rituximab. In another preferred embodiment the method according of the present invention is practiced with a F(ab)-Bi fragment of a therapeutic monoclonal antibody (TmAB) selected from the group consisting of infliximab and adalimumab. In another preferred embodiment the method according of the present invention is practiced with a F(ab)-Bi fragment of the therapeutic monoclonal antibody (TmAB) infliximab.

In one embodiment the method according of the present invention is practiced with a biotinylated Fab (Fab-Bi) fragment of a therapeutic monoclonal antibody (TmAB) selected from the group consisting of abciximab, adalimumab, alemtuzumab, basiliximab, bevacizumab, cetuximab, certolizumab pegol, daclizumab, eculizumab, efalizumab, gemtuzumab, ibritumomab tiuxetan, infliximab, muromonab-CD3, natalizumab, omalizumab, palivizumab, panitumumab, ranibizumab, rituximab, tositumomab and trastuzumab. In an preferred embodiment the method according of the present invention is practiced with a Fab-Bi fragment of a therapeutic monoclonal antibody (TmAB) selected from the group consisting of infliximab, adalimumab, certolizumab and rituximab. In another preferred embodiment the method according of the present invention is practiced with a Fab-Bi fragment of a therapeutic monoclonal antibody (TmAB) selected from the group consisting of infliximab and adalimumab. In another preferred embodiment the method according of the present invention is practiced with a Fab-Bi fragment of the therapeutic monoclonal antibody (TmAB) infliximab.

In one embodiment the method according of the present invention is practiced with a biotinylated Fab' (Fab'-Bi) fragment of a therapeutic monoclonal antibody (TmAB) selected from the group consisting of abciximab, adalimumab, alemtuzumab, basiliximab, bevacizumab, cetuximab, certolizumab pegol, daclizumab, eculizumab, efalizumab, gemtuzumab, ibritumomab tiuxetan, infliximab, muromonab-CD3, natalizumab, omalizumab, palivizumab, panitumumab, ranibizumab, rituximab, tositumomab and trastuzumab. In an preferred embodiment the method according of the present invention is practiced with a Fab'-Bi fragment of a therapeutic monoclonal antibody (TmAB) selected from the group consisting of infliximab, adalimumab, certolizumab and rituximab. In another preferred embodiment the method according of the present invention is practiced with a

Fab'-Bi fragment of a therapeutic monoclonal antibody (TmAB) selected from the group consisting of infliximab and adalimumab. In another preferred embodiment the method according of the present invention is practiced with a Fab'-Bi fragment of the therapeutic monoclonal antibody (TmAB) infliximab.

It is known to a person skilled in the art that after binding the anti-<TmAB>AB to a solid phase via the F(ab) fragment forming an anti-<TmAB>AB – F(ab) fragment complex, unspecific loosely bound compounds may be removed, e.g. by a washing step.

The anti-<TmAB>AB to be determined binds specifically to the F(ab) fragment of the TmAB of interest. In a preferred embodiment the antibody of interest is an anti-<TNFα> TmAB. In an other preferred embodiment the antibody of interest is selected from the group consisting of infliximab, adalimumab, certolizumab and rituximab. In an other preferred embodiment the antibody of interest is selected from the group consisting of infliximab and adalimumab. In an other preferred embodiment the antibody of interest is infliximab. An "anti-<TmAB>AB – F(ab) fragment complex" is formed, if an anti-<TmAB>AB is present in a sample taken from a patient treated with a TmAB and binds to the F(ab) fragment of said TmAB bound to the solid phase. As obvious to the skilled artisan, the F(ab) fragment of the TmAB and the anti-<TmAB>AB are incubated under conditions allowing for the formation of an anti-<TmAB>AB – F(ab) complex.

Enzyme-linked immunosorbent assay (ELISA) technique is a common assay type in the investigation of an immunogenic answer of a patient to a TmAB. There are several different ELISA formats known to the skilled artisan, e.g. "indirect assay", "sandwich assay", "competitive assay", "double antigen bridging assay (DAGS)" or "reverse assay". Mire-Sluis, A. R., et al., J. Immunol. Methods 289 (2004) 1-16, summarize the recommendations for the design and optimization of immunoassays using detection of host antibodies, e.g. anti-<TmAB>ABs against biotechnology products (e.g. TmABs).

In a preferred embodiment the method according to the present invention is performed in an indirect assay format. Surprisingly in the indirect assay format Fab fragments result in a much better differentiation between negative and truly positive results, as shown in Examples 4 and 5. In such indirect assay

format a monoclonal antibody <h-Agg.-IgG> is used as detecting monoclonal antibody.

The immunoassay method according to the present invention in one embodiment is practiced with a detection antibody <h-Agg.-IgG> having a low affinity for binding the antigen-specific antibodies (anti-<TmAB>ABs). The affinity of an antibody for an epitope is defined as the strength of all non-covalent interactions between the individual antigen-binding site on an antibody and the individual epitope. Antibodies with a low affinity bind weakly and dissociate rapidly whereas high affinity antibodies bind more strongly and remain bound for a longer period of time. The affinity at a binding site does not always reflect the true strength of an antigen-antibody interaction. For example in the case of complex antigens with many repeated antigenic determinants and with complementary antibodies having several low affinity binding sites nonetheless a rather strong binding is observed due to cooperative binding phenomena. The interaction of an antigen and an antigen binding site of an antibody at a first site increases the probability of a reaction at a second antigen binding site of the same antibody. The strength of such multiple interactions between the multivalent antibody and an antigen is referred to as avidity. A high avidity compensates for a low affinity as for example in the case of the pentameric immunoglobulin IgM. In the method according to the invention an antibody with a low affinity for the antigen-specific antibody is preferably used which has several i.e. at least two, preferably at least four and also preferred ten and more paratopes such as the IgM or IgG immunoglobulins that are cross-linked with one another. Examples of this are rheumatoid factors which are usually composed of IgM molecules and more rarely also of IgG, IgA and IgE molecules.

A person skilled in the art knows that the value for the affinity of a binding partner, preferably an antibody is determined by the affinity coefficient defined by the model of Langmuir. A molecule with a high dissociation rate constant (K_{dissoc}) is likely to have low affinity, as the equilibrium dissociation constant, $K_D = K_{dissoc}/K_{assoc}$. It predicts that the affinity coefficient for a very high binding affinity is about 10^{-9} to 10^{-11}, for a medium binding affinity about 10^{-8}, for a low binding affinity about 10^{-7} and for a very low binding affinity about 10^{-6}. The detecting monoclonal antibody <h-Agg.-IgG> of the present invention possesses a low binding affinity. In an embodiment the detecting monoclonal antibody <h-Agg.-IgG> used in the immunoassay of the present

invention is an antibody having a K_D value of about 10^{-6} mol/l to 10^{-8} mol/l. In a preferred embodiment the detecting monoclonal antibody <h-Agg.-IgG> is an antibody having a K_D value of about 10^{-7} mol/l to 10^{-8} mol/l.

As discussed before the determination of the anti-<TmAB>AB in the sample bound in step (c) of the immunoassay method disclosed in the present invention is performed by a detecting monoclonal antibody <h-Agg.-IgG>. In one embodiment this detecting monoclonal antibody is of the IgM immunoglobulin class. Preferably the monoclonal antibody <h-Agg.-IgG> binds antibodies of the immunoglobulin class IgG that have bound to their antigen in a specific manner. This monoclonal antibody only recognizes the densely packed and specifically bound anti-<TmAB>AB, i.e those anti-<TmAB>AB that have bound to the F(ab) fragments of the TmABs of interest spotted onto the solid phase. This detection antibody does not react with unspecifically bound or adsorbed IgG.

In an embodiment the method according to the present invention is practiced using a labeled monoclonal antibody <h-Agg.-IgG>. In a preferred embodiment the monoclonal antibody <h-Agg.-IgG> is labeled with Dig (<h-Agg.-IgG>-Dig). This Dig-labeled monoclonal antibody <h-Agg.-IgG>-Dig is easily detected via an anti-<Dig> antibody conjugated to a detectable label. Such detectable label, e.g. can be selected from luminescent labels, chemiluminescent labels, electrochemiluminescent labels, fluorescent labels or radioactive labels.

The monoclonal antibody <h-Agg.-IgG> used in the method according to the present invention in an embodiment is specific for the selected Ig class to be determined. In an preferred embodiment the monoclonal antibody <h-Agg.-IgG> used in the method according to the present invention is specific for the IgG class. In an embodiment the monoclonal antibody <h-Agg.-IgG> is capable of detecting all IgG sub-classes.

Surprisingly the inventors could show, that the use of a detection monoclonal antibody <h-Agg.-IgG> in an immunoassay method as disclosed in the present invention leads to an improved detection of anti-<TmAB>AB in a sample and overcomes at least some of the current limitations in the early detection of anti-<TmAB>AB. Surprisingly the combination of an F(ab) fragment of the TmAB of interest spotted onto the solid phase with the detection antibody <h-

Agg.-IgG> in an indirect immunoassay format allows the inventors the very early detection of anti-<TmAB>AB of the IgG class of said TmAB. It is possible to detect anti-<TmAB>AB of the IgG class in vitro in a sample from a patient treated with a TmAB from 2 weeks onwards after first administration of said TmAB. In a preferred embodiment the combination of an Fab fragment of the TmAB of interest spotted onto the solid phase with the detection antibody <h-Agg.-IgG> in an indirect immunoassay format allows the detection of anti-<TmAB>AB of the IgG class of said TmAB from 2 weeks onwards after first administration of said TmAB. In a also preferred embodiment the combination of an Fab' fragment of the TmAB of interest spotted onto the solid phase with the detection antibody <h-Agg.-IgG> in an indirect immunoassay format allows the detection of anti-<TmAB>AB of the IgG class of said TmAB from 2 weeks onwards after first administration of said TmAB.

In an embodiment the monoclonal antibody <h-Agg.-IgG> used in the method according to the present invention is selected from the group consisting of MAb <h-Agg.-IgG>M-3.022.5-IgM (DSM ACC2873), MAb <h-Agg.-IgG>M-1.010.2-IgM and MAb <h-Agg.-IgG>M-1.1.7-IgM (shown in Table 1). In a preferred embodiment the detecting monoclonal antibody <h-Agg.-IgG> is the MAb <h-Agg.-IgG>M3.022.5-IgM-Dig (DSM ACC2873). A characteristic feature of the MAb <h-Agg.-IgG>M3.022.5-IgM-Dig is that immunoglobulins bound unspecifically to the solid phase, i.e. those that are not specifically bound to an antigen, are not recognized or only recognized to a negligible extend. Without wanted to be bound to this theory, the use of MAb <h-Agg.-IgG>M3.022.5-IgM-Dig might substantially reduce the background signal in the immunoassay and thereby keep it at a constant low level independent from possibly interfering IgG comprised in a sample to be analyzed.

The immunoassay method according to the present invention in one embodiment is carried out in an array format, e.g. on a chip or bio-chip. In such array format the F(ab) fragment(s) of one or more TmABs are immobilized on discrete areas of the solid phase, which are defined as test areas that are spatially separated from one another. Methods for immobilizing the capture binding partners (e.g. F(ab) fragment(s)) are familiar to a person skilled in the art and are, for example, disclosed in EP 0 929 319 (Hornauer et al.).

Test areas comprising one or more spots containing the same capture binding partner may be present on the solid phase. In one embodiment patterns consisting of several identical spots may be formed.

It is the advantage of such an immunoassay in an array format (e.g. on a chip or bio-chip) that different analytes can be simultaneously determined. In an embodiment each of the various discrete areas or spots in an array format contains F(ab) fragments of one of the different TmABs of interest, that are able to specifically bind with an anti-<TmAB>AB to be determined. In an embodiment such array comprises at least two discrete areas, wherein in each area a different F(ab) fragment of an TmAB of interest (capture molecule) is present. It is also possible to have a combination of F(ab) fragments derived from different TmABs of interest and several spots each containing the F(ab) fragment of one of these different TmABs, respectively, on one array. In an embodiment at least two different F(ab) fragments derived from TmABs of interest each having two or more individual spots are present on such array. In an embodiment the array used in the method preferably consists of a support made of metal, glass, a plastic, or polysterene. Polystyrene supports are preferably used in the method according to the invention which are known to a person skilled in the art and described, for example, in EP 0939319 (Hornauer et al.). The use of the <h-Agg.-IgG>M3.022.5-IgM-Dig antibody in said method performed in an array format enables that several to a large number of different tests for anti-<TmAB>ABs of interest can be combined on an array. A major advantage of an array assay format is that only one buffer composition is required in each handling step.

In an embodiment the method according to the present invention is performed using a sample provided from a patient no later than 14 weeks after first administration of a TmAB. In an embodiment a detection of anti-<TmAB>AB performed from 2 weeks onwards after first administration of a TmAB. In an embodiment a detection of anti-<TmAB>AB is performed at week 2 to 6 after first administration of a TmAB. In an embodiment a detection of anti-<TmAB>AB is performed at 6 weeks after first administration of a TmAB.

The methods according to the present invention are also of a value for the selection of an appropriate TmAB therapy.

Lack of effectivity (LOE) of a TmAB therapy is a rare but known phenomenon in patients under treatment with a TmAB, e.g. for patients under treatment with an anti-<TNFα> antibody. The determination of anti-<TmAB>ABs in a patient under treatment with a TmAB has been used in attempts to predict LOE of a TmAB therapy. It has been shown in the prior art that anti-<TmAB>ABs can reduce the effective amount of available TmAB in the circulation of the patient under treatment with said TmAB. It is currently unclear what determines the magnitude of the anti-<TmAB>AB response in a patient treated with said TmAB (Aarden, L. et al., Current Opinion in Immunology 20 (2008) 431-435). In some therapeutic approaches the dosage of said TmAB has been raised after a LOE diagnosis to compensate the available TmAB in the circulation of the patient, opening new scientifically and clinically relevant questions. Determining the serum level of TmABs is currently the gold standard to monitor the treatment efficacy of a TmAB therapy. It is proposed by Aarden et al. that patients should be frequently monitored for serum TmAB levels, and the level of antibodies could guide in deciding whether to increase the dose or to switch to another TmAB/drug.

Also known from the prior art are side effects (adverse effects, ADRs) during TmAB therapy, e.g. during anti-<TNFα> therapy. It is known to the skilled artisan that patients under TmAB therapy are at risk to develop an ADR. However, it would appear that no method is available to assess such risk early after initiation of a TmAB-based therapy, e.g. before severe ADRs set in.

Surprisingly it has been found that an in vitro determination of antibodies against a TmAB of interest in a sample from a patient under treatment with said TmAB allowed us to predict which patients are at increased risk to develop an ADR during treatment with a TmAB before an ADR occurs.

In an further embodiment an in vitro determination of antibodies against a TmAB in a sample from a patient under treatment with said TmAB is used to identify patients at risk to develop an ADR during treatment with a TmAB, wherein the patient testing positive for an anti-<TmAB>AB is at risk to develop an ADR. Without wanting to be bound to this theory, it may well be that in a case where a patient tested positive for anti-<TmAB>ABs against a certain TmAB is treated with a higher dosage of said TmAB, the risk to develop an ADR later on is much higher. Therefore a change of therapy after determination of anti-<TmAB>AB to the administered first TmAB to another

(second) TmAB should be seriously considered in order to reduce the risk for an ADR later on.

In an embodiment the method of the current invention is used to determine whether a patient is at risk to develop an ADR during treatment with a TmAB. In this embodiment a patient testing positive for anti-<TmAB>AB is at risk of developing an ADR. A patient testing positive for anti-<TmAB>AB in the method disclosed herein is at increased risk of developing an ADR. Such risk can be determined as a relative risk using mathematical methods known to the skilled artisan. As shown in the examples, early development of anti-<TmAB>AB precedes later development of an ADR and/or drop-out of the patients from the study. In an embodiment the risk of developing an ADR is a relative risk of at least 40%, in an preferred embodiment the risk is a relative risk of at least 45%.

Anti-<TmAB>AB time series plots of Fig. 2 and Fig. 3 show the difference between patients not withdrawn and those withdrawn from study due to an ADR. Study data of Example 5 are represented in Fig. 2, showing the results for patients treated with infliximab as Kaplan-Meier (KM)-curves with respect to anti-<TmAB>AB status at week 6. In Fig. 3 the results for patients treated with infliximab as KM-curves with respect to anti-<TmAB>AB status at week 14 are shown. In both Figures for patients withdrawn due to an ADR (or for patients withdrawn due to no effect of treatment) the KM-curves of anti-<TmAB>AB positive (anti-<TmAB>AB+) patients are lower than the KM-curves of anti-<TmAB>AB negative (anti-<TmAB>AB-) patients. This difference is even more visible at week 6 than in week 14.

In an embodiment the present invention relates to a method for selecting an alternative therapeutic antibody for a patient under treatment with a first TmAB, wherein at least a first and one or more alternative TmAB are available, comprising: a) determining in vitro an anti-<TmAB>AB to the first TmAB in a sample from a patient treated with said first TmAB, and b) selecting an alternative TmAB for future therapy, if an anti-<TmAB>AB to said first TmAB is present.

In an embodiment the method for selecting an alternative therapeutic antibody is practiced using a sample obtained from a patient having a diagnosis of a chronic inflammatory disease. In an embodiment the chronic inflammatory

disease is selected from the group consisting of rheumatoid arthritis (RA), Crohn's disease (CD), ankylosing spondylitis (AS), polyarticular juvenile idiopathic arthritis (JIA), psoriatic arthritis (PsA), Morbus Bechterew and or chronic plaque psoriasis (Ps). In a preferred embodiment the patient has a diagnosis of rheumatoid arthritis (RA).

In an embodiment the method for selecting an alternative therapeutic antibody is practiced using a sample obtained from a human patient. In an embodiment the determined anti-<TmAB>AB is an anti-<TNFαAB>AB.

In an embodiment the present invention relates to a method for selecting an alternative TmAB for a patient under treatment with a first TmAB, wherein at least a first and one or more alternative TmAB are available, comprising: a) determining in vitro anti-<TmAB>AB of the IgG class to the first TmAB in a sample from a patient treated with said first TmAB, and b) selecting an alternative TmAB for future therapy, if anti-<TmAB>AB to said first TmAB are present.

In an embodiment the method for selecting an alternative therapeutic antibody is performed using a sample provided from a patient no later than 14 weeks after first administration of a TmAB. In an embodiment a detection of anti-<TmAB>AB is performed from 2 weeks onwards after first administration of a TmAB. In an embodiment a detection of anti-<TmAB>AB is performed at week 2 to 6 after first administration of a TmAB. In an embodiment a detection of anti-<TmAB>AB is performed at 6 weeks after first administration of a TmAB.

It is known to the skilled artisan how to select an alternative TmAB for future therapy, if an anti-<TmAB>AB to said first TmAB is present in a sample obtained from a patient under treatment of said first TmAB. In an embodiment the alternative TmAB is selected from the group consisting of an anti-<TNFα> monoclonal antibody and rituximab. In an embodiment the alternative TmAB is selected from the group consisting of infliximab, adalimumab, certolizumab and rituximab. In an embodiment the alternative TmAB is an anti-<TNFα> monoclonal antibody. In an embodiment the alternative TmAB is an anti-<CD20> antibody. In an embodiment the alternative TmAB is rituximab. In an embodiment the first TmAB is an anti-<TNFα> monoclonal antibody and the alternative TmAB is an anti-<CD20> antibody. In an embodiment the first

TmAB is an anti-<TNFα> monoclonal antibody and the alternative TmAB is rituximab.

Use:

The method according to the present invention can generally be used for detection of anti-<TmAB>ABs, both in clinical trial as well as in clinical routine. In an embodiment the present invention relates to the use of the immunoassay method of the present invention for detection of anti-<TmAB>ABs.

In an embodiment the present invention relates to the use of the immunoassay method as disclosed herein for identification of a patient who is at risk to develop an adverse drug reaction (ADR) by determination of an anti-<TmAB>AB in vitro in a sample from a patient treated with a therapeutic monoclonal antibody (TmAB).

In an embodiment the present invention relates to the use of an immunoassay method for determination of an anti-<TmAB>AB in vitro, wherein anti-<TmAB>AB is detected using a sample provided from a patient no later than 14 weeks after first administration of a TmAB. In an embodiment a detection of anti-<TmAB>AB performed from 2 weeks onwards after first administration of a TmAB. In an embodiment a detection of anti-<TmAB>AB is performed at week 2 to 6 after first administration of a TmAB. In an embodiment a detection of anti-<TmAB>AB is performed no later than 6 weeks after first administration of a TmAB.

The method according to the present invention can be used to monitor patients treated with a therapeutic monoclonal antibody (TmAB) who are at risk to develop an ADR. The method is used in an embodiment to investigate the frequency of development of anti-<TmAB>AB in patients during treatment with TmAB, and to determine if development of such anti-<TmAB>AB was associated with early ADR and/or treatment failure.

The following examples and the figure are provided to aid the understanding of the present invention, the true scope of which is set forth in the appended claims. It is understood that modifications can be made in the procedures set forth without departing from the spirit of the invention.

Description of the Figures

Figure 1 Fig. 1 shows the indirect immunoassay test format as described in example 3. anti-<TmAB>AB = anti-<therapeutic monoclonal antibody> antibody to be detected in a sample to be investigted; MAb <h-Agg.-IgG> Dig = Dig-labeled monoclonal antibody <h-Agg.-IgG>; $F(ab')_2$-Bi and Fab-Bi = on solid phase immobilized biotinylated antigens specifically binding anti-<TmAB>AB.

Figure 2 Fig. 2 shows the Kaplan-Meier Survival Curves for patients treated with infliximab which are withdrawl from the study due to ADR <= 50 weeks. X-axis = withdrawl from study in days (wd_days); Y-axis = survival distribution function; Lower line = Patients with detected anti drug antibodies (anti-<TmAB>AB+) at week 6: total = 31, failed = 15; Upper line = Patients without detected anti drug antibodies (anti-<TmAB>AB-) at week 6: total = 94, failed = 12; p-value Log-Rank test =< 0.0001; hazard-ratio = 5.06, 95% Cl hazard-ratio = [2.36, 10.84]. Data shown in Table 4.

Figure 3 Fig. 3 shows the Kaplan-Meier Survival Curves for patients treated with infliximab which are withdrawl from the study due to ADR <= 50 weeks. X-axis = withdrawl from study in days (wd_days); Y-axis = survival distribution function; Lower line = Patients with detected anti drug antibodies (anti-<TmAB>AB+) at week 14 total = 43, failed = 16; Upper line = Patients without detected anti drug antibodies (ADA-) at week 14: total = 88, failed = 12; p-value Log-Rank test =< 0.0009; hazard-ratio = 3.30, 95% Cl hazard-ratio = [1.56, 6.99]. Data shown in Table 4.

Figure 4 Fig. 4 shows a sandwich immunoassay format as mentioned in the description. Anti-<TmAB>AB = anti-<therapeutic monoclonal antibody> antibody to be detected in a sample to be investigated; $F(ab')_2$-Dig = Dig-labeled $F(ab')_2$ fragment; $F(ab')_2$-Bi or Fab-Bi = on solid phase immobilized biotinylated antigens specifically binding anti-<TmAB>AB.

Figure 5a Fig. 5a shows the results of $F(ab')_2$-Bi infliximab fragments as capture antibody. TN = serum samples taken from

apparently healthy human blood donors (true negatives). TP = serum samples taken from RA patients treated with infliximab (true positives). Columns of 50, 500, and 150000 are truncated in height.

Figure 5b Fig. 5b shows the results of Fab-Bi infliximab fragments as capture antibody. TN = serum samples taken from apparently healthy human blood donors (true negatives). TP = serum samples taken from RA patients treated with infliximab (true positives). Columns of 50, 75000, 100000 and 150000 are truncated in height

Example 1

Preparation of biotin conjugated Fab and F(ab')$_2$ fragments of the specific therapeutic monoclonal antibody

Fab fragment: The full length therapeutic monoclonal antibody of the immunoglobulin class G (IgG) in 100 mM phosphate, 2 mM EDTA buffer, pH 7.0 was incubated with papain in the presence of 10-20 mM cysteine (5 to 20 mU papain per mg IgG). The fragmentation was analyzed by analytical gel permeation chromatography and stopped after 60-120 minutes by addition of iodacetamide solution (ad 10 mM).

F(ab')$_2$ fragment: The full length therapeutic antibody of the immunoglobulin class G (IgG) in 100 mM sodium citrate buffer, pH 3.7 was incubated with pepsin (1 to 15 µg pepsin per mg IgG). The fragmentation was analyzed by analytical gel permeation chromatography and stopped after 90 minutes by adjusting the pH value to 6.5 by the addition of potassium phosphate.

Purification: Both fragmentation mixtures were each dialysed against 10 mM sodium phosphate buffer with 10 mM sodium chloride, pH 5.5, the solution was applied to an SP-sepharose chromatography column, the isolated fractions eluted in a salt gradient were analyzed individually by analytical gel filtration. The pool containing the antibody Fab or F(ab)'$_2$ fragments were applied to an affinity matrix with immobilized polyclonal antibodies against human Fcg to eliminate trace amounts of Fcg fragments, the flow through was pooled and analyzed to a residual Fcg content. The affinity purification procedure was repeated at least three times until the residual Fcg concentration fell below 0.5

ppm. The product was concentrated to about 10 mg/ml and finally applied to a gel filtration column (Superdex 200).

Conjugation: the purified Fcg-free fragments were conjugated using NHS activated biotin labels at pH value of 8.2 to 8.4. The reaction stoichiometry was 1:5 (IgG:label), the reaction was stopped by addition of 1 M lysine solution after 1 hour and the raw conjugates were purified on a gel filtration column (Superdex 200).

Preparation of biotin conjugated Fab fragment of the specific therapeutic antibody:

The purified $F(ab')_2$ fragment was incubated with 5 mM cysteamine for 1 hour, the reduction to a Fab fragment was monitored by analytical gel permeation chromatography. The raw product was applied to a gel filtration column (Superdex 200) and the pooled Fab fractions were immediately conjugated with MEA activated biotin labels (stoichiometry 1:10, 1 hour). The final analytical characterization was performed by ESI-MS in order to confirm the conjugation site and yield, respectively.

Example 2

Production of monoclonal mouse IgM antibodies with rheumatoid factor-like specificity

Immunogen: H-IgG polymer:

10 mg human IgG1 (Sigma Company) is dissolved in 0.6 ml 25 mM bicarbonate buffer pH 9.5. After adding 3.5 µl 12.5% glutardialdehyde solution, it is incubated for 2 hours at room temperature. Subsequently it is cooled in an ice bath, adjusted to pH 8.3 with 50 mM triethanolamine solution pH 8.0 and 0.15 ml freshly prepared sodium boron hydride solution (8 mg boron hydride/ml water) is added. After 2.5 hours at 0°C the preparation is dialysed for 16 hours at 4°C against 10 mM potassium phosphate buffer/0.2 M NaCl, pH 7.5. The dialysate containing IgG polymer is stored in aliquots at -80°C or used for immunization and for specificity tests in culture supernatants of hybridoma cells.

H-IgG3 polymer is produced in a similar manner starting from human IgG3 (Sigma Company).

Immunization of mice:

12 week old, female Balb/c mice are firstly immunized intraperitoneally with 100 µg H-IgG1 or IgG3 polymer together with the adjuvant CFA (complete Freund's adjuvant). After 8 days a further immunization is carried out with 100 µg of the respective IgG polymer in CFA. 13 days after the initial immunization, 200 µg of the respective polymer is administered intraperitoneally without adjuvant, 14 and 15 days after the initial immunization 100 µg was administered in each case intraperitoneally and intravenously. The fusion is carried out after 16 days.

Production of hybridoma clones:

Fusion and cloning:

Spleen cells of an immunized mouse are fused with myeloma cells following the method of Galfré, G., Methods in Enzymology 73 (1981) 3-46. Approximately 1 x 108 spleen cells of the immunized mouse are mixed with 2 x 107 myeloma cells (P3X63-Ag8-653, ATCC CRL 1580) and centrifuged (10 min at 300 g and 4°C). The cells are then washed once with RPMI-1640 medium without foetal calf serum (FCS) and again centrifuged at 400 g in a 50 ml conical tube. 1 ml PEG (polyethylene glycol) (molecular weight 4000, Merck, Darmstadt) is added and mixed by pipetting. After 1 min in a water bath at 37°C, 5 ml RPMI 1640 without FCS is added dropwise, mixed, filled up to 50 ml with medium (RPMI 1640 + 10% FCS) and subsequently centrifuged. The sedimented cells are taken up in RPMI 1640 medium containing 10% FCS and sown in hypoxanthine-azaserine selection medium (100 mmol/l hypoxanthine, 1 µg/ml azaserine in RPMI 1640 + 10% FCS). Interleukin 6 (100 U/ml) is added to the medium as a growth factor. After about 10 days the primary cultures were tested for specific antibody synthesis. Primary cultures which show a positive reaction with aggregated human IgG1 but no cross-reaction with monomeric IgG are cloned by means of a fluorescence-activated cell sorter in 96-well cell culture plates. Interleukin 6 (100 U/ml) is added to the medium as a growth additive.

The following hybridoma clones were obtained in this manner:

Table 1:

Monoclonal antibody name	Immunogen	Subclass specificity
MAb <h-Agg.-IgG>M-3.022.5-IgM	h-IgG1 polymer	IgG1>IgG3>IgG4>IgG2
MAb <h-Agg.-IgG>M-1.010.2-IgM	h-IgG1 polymer	IgG1>IgG3>IgG4>IgG2
MAb <h-Agg.-IgG>M-1.1.7-IgM	h-IgG3 polymer	IgG1>IgG3>IgG2>IgG4

Screening test for monoclonal antibodies having specificity for aggregated, human IgG.

Streptavidin-coated MTPs are coated with biotinylated human IgG1 or IgG3. Afterwards they are incubated with the monoclonal antibody in the cell culture supernatant. Subsequently the bound antibodies are detected in the usual manner using an anti-<mouse-IgM>-POD by reaction with a POD substrate.

Determination of the subclass specificity using human IgG bound to a solid phase:

In order to determine the specificity of the antibodies in the culture supernatant of the hybridoma cells, MTPs coated with recombinant streptavidin (MicroCoat Company, Order No. 12-K 96 N) are coated with 1 µg/ml biotinylated h-IgG (=h-IgG-Bi) of subclass 1 or 2 or 3 or 4 in incubation buffer. Since IgG bound via biotin to a solid phase behaves like aggregated, polymeric IgG, this experimental approach can be used to determine the subclass specificity. For this 100 µl h-IgG-Bi solution per well is incubated for 60 minutes at room temperature while shaking and subsequently washed 3 times with 0.9% NaCl / 0.05% Tween ® 20.

In the next step 100 µl of the antibody solution to be examined (culture supernatant) is added to a coated well and incubated for 1 hour at room temperature while shaking. After washing 3 times with 0.9% sodium chloride / 0.05% Tween® 20, 100 µl of a POD-labeled Fab fragment of a polyclonal antibody from the goat against mouse IgM (Dianova Company, Order No. 115-036-075, concentration used 0.16 µg/ml incubation buffer) is added in each case to

detect bound antibody from the sample, incubated for 1 hour at room temperature while haking and subsequently washed 3 times with 0.9% sodium chloride / 0.05% Tween® 20.

Finally 100 µl / well ABTS® substrate (Roche Diagnostics GmbH, Order No. 1684 302) is added and the absorbance at 405/492 nm is measured after 30 min at room temperature in an MR700 Microplate reader from the Dynatech Company.

Incubation buffer:

40 mM Na phosphate, pH 7.4, 200 mM Na tartrate, 0.1% Tween® 20, 0.2% bovine serum albumin.

Determination of the reactivity / cross-reaction with monomeric, human IgG1:

In order to determine the reactivity / cross-reaction with monomeric, non-aggregated H-IgG1, the monoclonal antibody to be examined is pre-incubated in the test described above with monomeric, non-aggregated IgG1 in increasing concentrations or in excess. If the measured signal remains unchanged at a high level, there is no cross-reaction. If the measured signal decreases, a cross-reaction has occurred.

For this microtitre plates (MTP) (MicroCoat Company, Order No. 12-K 96 N) coated with recombinant streptavidin are coated with 1 µg/ml biotinylated H-IgG1 (=H-IgG1-Bi) in incubation buffer. 100 µl of the H-IgG1-Bi solution is used per well and incubated for 60 min at room temperature while shaking and subsequently washed 3 times with 0.9% NaCl / 0.05% Tween® 20.

The monoclonal antibody to be tested for cross-reaction is pre-incubated with serial concentrations of up to 1 µg/ml monomeric, non-aggregated IgG1. The pre-incubation takes place in uncoated 96-well MTPs for 1 hour at room temperature while shaking.

In the next step 100 µl of this solution (antibody + non-aggregated, monomeric IgG1 in excess) is added to a coated well and incubated for 1 hour at room temperature while shaking. After washing 3 times with 0.9% sodium chloride / 0.05% Tween® 20, 100 µl of a POD-labeled Fab fragment of a polyclonal antibody from the goat against mouse IgM (Dianova Company,

Order No. 115-036-075, concentration used 0.16 µg/ml incubation buffer) is added in each case to detect bound antibody from the sample, incubated for 1 hour at room temperature while shaking and subsequently washed 3 times with 0.9% sodium chloride / 0.05% Tween® 20.

Finally 100 µl / well ABTS® substrate (Roche Diagnostics GmbH, Order No. 1684 302) is added and the absorbance at 405/492 nm is measured after 30 min at room temperature in an MR700 Microplate reader from the Dynatech Company.

The monoclonal rheumatoid factor-like binding antibodies that are suitable in the sense of the invention recognize all human IgG subclasses and exhibit less than 10% cross-reaction with monomeric h-IgG in a competition test. If H-IgG1 polymer is used to determine the reactivity, the measured signal is greatly reduced. Table 1 shows the major properties of the monoclonal antibodies that were found.

Fermentation of hybridoma clones to isolate monoclonal antibodies:

The hybridoma cells that are obtained are sown at a density of 1 x 105 cells per ml in RPMI 1640 medium containing 10% FCS and propagated for 7 days in a fermenter (Thermodux Company, Wertheim/_Main, model MCS-104XL, Order No. 144-050). Average concentrations of 100 µg monoclonal antibody per ml are reached in the culture supernatant.

Isolation of monoclonal MAb <h-Agg.-IgG>M-3.022.5-IgM:

5 mg MAb <h-Agg.-IgG>M-3.022.5-IgM (DSM ACC2873) is adjusted to a total volume of 2 ml with 0.1 M sodium phosphate buffer, pH 8.6. 50 µl of a 1.11 mM solution of digoxigenin-3-O-methyl-carbonyl-e-aminocaproic acid-N-hydroxysuccinimide ester in dimethyl sulfoxide is added to this solution and subsequently stirred for 60 min at 25°C. The ratio of IgM to activated digoxigenin is 1:10. The IgM-digoxigenin that forms is dialysed against 20 mM potassium phosphate buffer / 0.1 M NaCl / 3% sucrose, pH 7.5. The dialysed IgM-Dig is stored in aliquots at -80°C.

Example 3

Fully automated ELISA assay on a multi parameter biochip platform

A multiparameter biochip platform is described in Hornauer, H. et al., BIOspectrum, Special Proteomics 10 (2004) 564-565 and Hornauer, H. et al., Laborwelt 4 (2004) 38-39.

A streptavidin coating is applied over the whole area of a test area of about 2.5 x 6 mm on a black-stained polystyrene support (solid phase). Lines of identical spots of approximately 10 to 20 per line consisting of biotinylated fragments of the therapeutic antibody are applied to the test area in an ink-jet procedure; the diameter per spot is about 150 µm.

The following test-specific reagents were used:

Sample dilution buffer:
50 mM Tris, pH 7.6; 150 mM NaCl; 0.1% detergent (polydocanol); 0.6% BSA; 0.2% preservative (oxypyrion and methylisothiazolone hydrochloride (MIT))

Wash buffer:
10 mM Tris, 0.01% polydocanol, 0.001% oxypyrion, 0.001% MIT

Samples:
human sera, positive samples were obtained by screening study populations which were treated with the respective therapeutic antibody; the negative samples are healthy blood donors not treated with the respective therapeutic antibody.

Infliximab Fab fragments were used as biotinylated antigens. Auto-antibodies (anti-<TmAB>AB) against these antigens were detected in an indirect test format. 50 µg/ml of the respective biotinylated antigen was used in each spot solution.

Description of the test procedure:

The samples were diluted 1:50 with the sample dilution buffer for the measurement. The diluted samples were incubated for 12 min at 37°C. After aspirating the sample and washing the test field with wash buffer, they were incubated with the MAb <h-Agg.-IgG>M-3.022.5-IgM (DSM ACC2873), an

antibody labeled with digoxin (Dig-labeled monoclonal antibody <h-Agg.-IgG>), for 6 min at 37°C with a subsequent washing step. After incubation with a fluorescently labeled <Dig> antibody for 3 min at 37°C and subsequently washing and suction drying the test field, the signals were detected by a CCD camera.

Example 4

Comparison of F(ab')$_2$ and Fab fragments in an indirect assay format

Biotinylated infliximab, as fragments F(ab')$_2$-Bi or Fab-Bi, is spotted each onto a distinct area on a chip surface (solid phase). Digoxigenated anti-<human IgG> is used as detection reagents. As infliximab is a humanized IgG1, the detection antibody would bind directly to the spotted antibody. Therefore only the use of infliximab fragments (in more general anti-<TNFα antibody fragments>) F(ab')$_2$-Bi or Fab-Bi is possible in this assay format.

In total 100 serum samples from apparently healthy blood donors (TN) as well as 155 serum samples from rheumatoid arthritis (RA) patients treated with infliximab (TP) were taken to compare the specificity of the two different assays, using infliximab fragments Fab-Bi or F(ab')$_2$-Bi as capture antibodies. The use of biotinylated infliximab as F(ab')$_2$-Bi results in falsely elevated signals in samples taken from several apparently healthy blood donors (TN) which were almost as strong as the signals of samples taken from truly positive (TP) rheumatoid arthritis patients trated with infliximab (Results are shown in Table 2 and a graphical representation of the results is given in Figure 5a).

Table 2:

Infliximab fragement F(ab')$_2$-Bi									
Counts	50	500	5000	10000	25000	50000	75000	100000	150000
TN (n=100)	58	26	9	1	3	2	0	0	0
TP (n=155)	0	0	0	0	0	6	1	9	145

The use of biotinylated Fab-Bi fragment of infliximab as capture antibody allowed us for a much better differentiation between true positive (TP) and

true negative (TN) samples. Results are shown in Table 3 and a graphical representation of the results is given in Figure 5b.

Table 3:

Infliximab fragment Fab-Bi									
Counts	50	500	5000	10000	25000	50000	75000	100000	150000
TN (n=100)	87	10	2	0	0	0	0	0	0
TP (n=155)	0	0	0	0	0	6	33	34	82

Example 5

Screening assays for detection of anti-<TNFα antibody> antibodies (anti-<TNFαAB>ABs)

Study data are based on samples from the Copenhagen Cohort. Blood samples were taken from a total of 218 patients with rheumatoid arthritis (RA) treated with infliximab. This blood samples were analyzed for the presence of anti-<TmAB>ABs to infliximab (anti-<TNFαAB>ABs). A baseline sample (reference sample) is taken at week 0, before first administration of a TmAB. If a sample is stated herein to be taken at week 2 after first administration of a TmAB, the sample can be taken from the 9th day to the 21st day. If a sample is stated herein to be taken at week 6 after first administration of a TmAB, the sample can be taken from the 28th day to the 64th day. If a sample is stated herein to be taken at week 14 after first administration of a TmAB, the sample can be taken from week 13 to week 16.

Anti-<TmAB>AB to infliximab are determined using an indirect assay format as described. A complete overview of rare reagents, buffers, calibrators and controls are given in Example 3.

Indirect Assay Format:

Biotinylated infliximab, as Fab-Bi, is spotted onto a chip surface. Digoxigenated anti-<human IgG> is used as detection reagents. As infliximab is a humanized IgG1, the detection antibody would bind directly to the spotted antibody. Therefore only the use of infliximab fragments (in more general

anti-<TNFα antibody fragments>) F(ab')$_2$-Bi or Fab-Bi is possible in this assay format.

As shown by the results from Example 4, the use of Fab fragments is preferred. In the indirect assay format Fab fragments result in a much better differentiation between negative and truly positive results.

Table 4: anti-<TmAB>AB indirect Assay (optimized for sensitivity)

Time point	**week 2**	**week 6**	**week 14**
no of patients with anti-<TmAB>AB+ test result	4	31	43
no of patients withdrawn due to ADR before week 50	3	15	16
Percentage withdrawn	**75%**	**48%**	**37%**
no of patients with anti-<TmAB>AB- test result		94	88
no of patients withdrawn due to ADR before week 50		12	12
Percentage withdrawn		**13%**	**14%**
Hazard ratio (95% CI)		**5.06 (2.36 – 10.84)**	**3.30 (1.54-6.99)**
p value		**< 0.0001**	**0.0009**

The anti-<TmAB>AB indirect assay format shown in Figure 1 with a superior sensitivity detects anti-<TmAB>ABs in samples taken from patients as early as 2 weeks after first therapeutic administration of infliximab.

Later on withdrawal from the Study due to an ADR can be predicted by anti-<TmAB>AB determination:

Early development of anti-<TmAB>AB at week 2 or week 6 let us predict a later ADR and drop-out of the patients from the study with 75% and 48% probability, respectively (data shown in Table 4).

Treatment with a TmAB, e.g. infliximab lead to anti-<TmAB>AB formation against said TmAB in a minority but still significant number of patients. Most importantly a high number of these patients left the study due to ADRs at a later point in time. These findings might indicate that anti-drug antibodies – assessed in a method according to the present invention - can be detected before ADRs set in and thus it may be possible to use a positive test for anti-<TmABs> antibodies to better direct therapy, e.g. to switch from a first TmAB to a second, alternative TmAB.

Patent Claims

1. An immunoassay method for determination of an anti-<therapeutic monoclonal antibody> antibody (anti-<TmAB>AB) in vitro in a sample from a patient treated with a therapeutic monoclonal antibody (TmAB), the method comprising:

   a) providing an F(ab) fragment of said TmAB bound to a solid phase,
   b) incubating the solid phase provided in (a) with the sample thereby binding the anti-<TmAB>AB to the solid phase via the F(ab) fragment,
   c) incubating the solid phase obtained in (b) with a monoclonal antibody <h-Agg.-IgG>, whereby said monoclonal antibody binds to the anti-<TmAB>AB, and
   d) detecting monoclonal antibody <h-Agg.-IgG> bound in (c) and thereby determining the anti-<TmAB>AB in the sample.

2. The method according to claim 1, wherein the sample is whole blood, serum or plasma.

3. The method according to any of claims 1 to 2, wherein the TmAB is selected from the group consisting of chimeric antibodies (CA) and humanized antibodies (HA).

4. The method according to any of claims 1 to 3, wherein the TmAB is selected from the group consisting of infliximab, adalimumab, certolizumab and rituximab.

5. The method according to any of claims 1 to 4, wherein the F(ab) fragment is bound to the solid phase by a binding system selected from the group consisting of biotin/steptavidin, biotin/avidin, and biotin-anti-<biotin> antibody.

6. The method according to any of claims 1 to 5, wherein the monoclonal antibody is an antibody having a dissociation constant (=K_D) value of about 10^{-6} mol/l - 10^{-8} mol/l.

7. The method according to any of claims 1 to 6, wherein the monoclonal antibody <h-Agg.-IgG> is labeled.

8. The method according to any of claims 1 to 7, wherein the monoclonal antibody <h-Agg.-IgG> is labeled with Dig.

9. The method according to claim 8, wherein the Dig-labeled monoclonal antibody <h-Agg.-IgG> is detected by incubating with an anti-<Dig> antibody conjugated to a detectable label.

10. The method according to claim 9, wherein the detectable label is selected from the group consisting of luminescent labels, chemiluminescent labels, electrochemiluminescent labels, fluorescent labels, and radioactive labels.

11. Use of the immunoassay method according to any of claims 1 to 10 for detection of anti-<TmAB> antibodies.

12. Use of a method according to any of claims 1 to 10 for an identification of a patient who is at risk to develop an adverse drug reaction (ADR) during treatment with a TmAB, wherein the patient testing positive for an anti-<TmAB>AB in the method is at risk of developing an ADR.

13. The use according to claim 12, wherein an anti-<TmAB>AB is detected in a sample taken from a patient no later than 14 weeks after first administration of said first TmAB.

14. A method for selecting an alternative therapeutic antibody for a patient under treatment with a first TmAB, wherein at least a first and one or more alternative TmAB are available, comprising:

a) determining in vitro an anti-<TmAB>AB to the first TmAB in a sample from a patient treated with said first TmAB, and
b) selecting an alternative TmAB for future therapy, if an anti-<TmAB>AB to said first TmAB is present.

15. The method according to claim 14, wherein the anti-<TmAB>AB can be determined in vitro within a sample provided from a patient no later than 14 weeks after first administration of said first TmAB.

16. The method according to any of claims 14 to 15, wherein the alternative TmAB is selected from the group consisting of an anti-<TNFα> monoclonal antibody and rituximab.

17. The method according to any of claims 14 to 16, wherein the alternative TmAB is selected from the group consisting of infliximab, adalimumab, certolizumab and rituximab.

18. The method according to any of claims 14 to 17, wherein the alternative TmAB is an anti-<TNFα> monoclonal antibody.

19. The method according to any of claims 14 to 18, wherein the alternative TmAB is selected from the group consisting of infliximab, adalimumab and certolizumab.

20. The method according to any of claims 14 to 19, wherein the first TmAB is an anti-<TNFα> monoclonal antibody and the alternative TmAB is rituximab.

Fig. 1

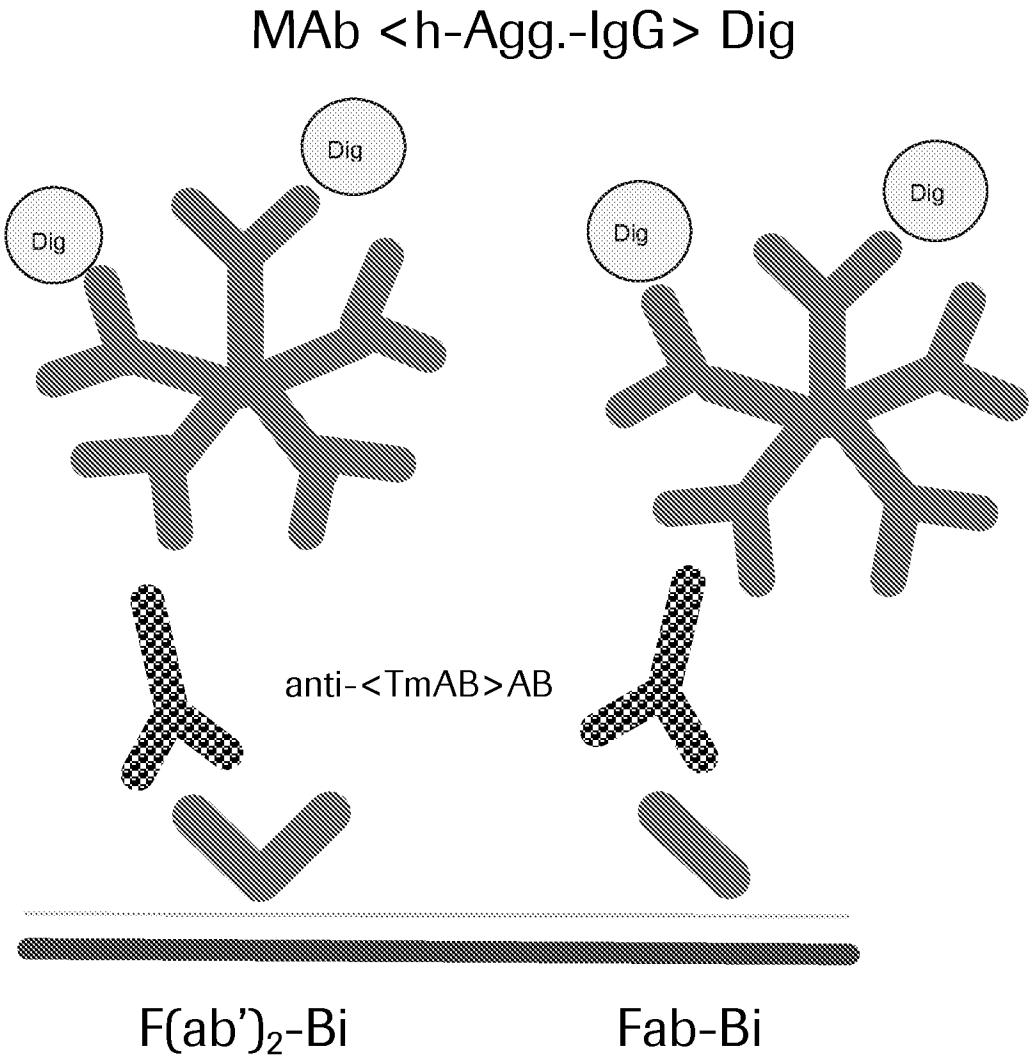
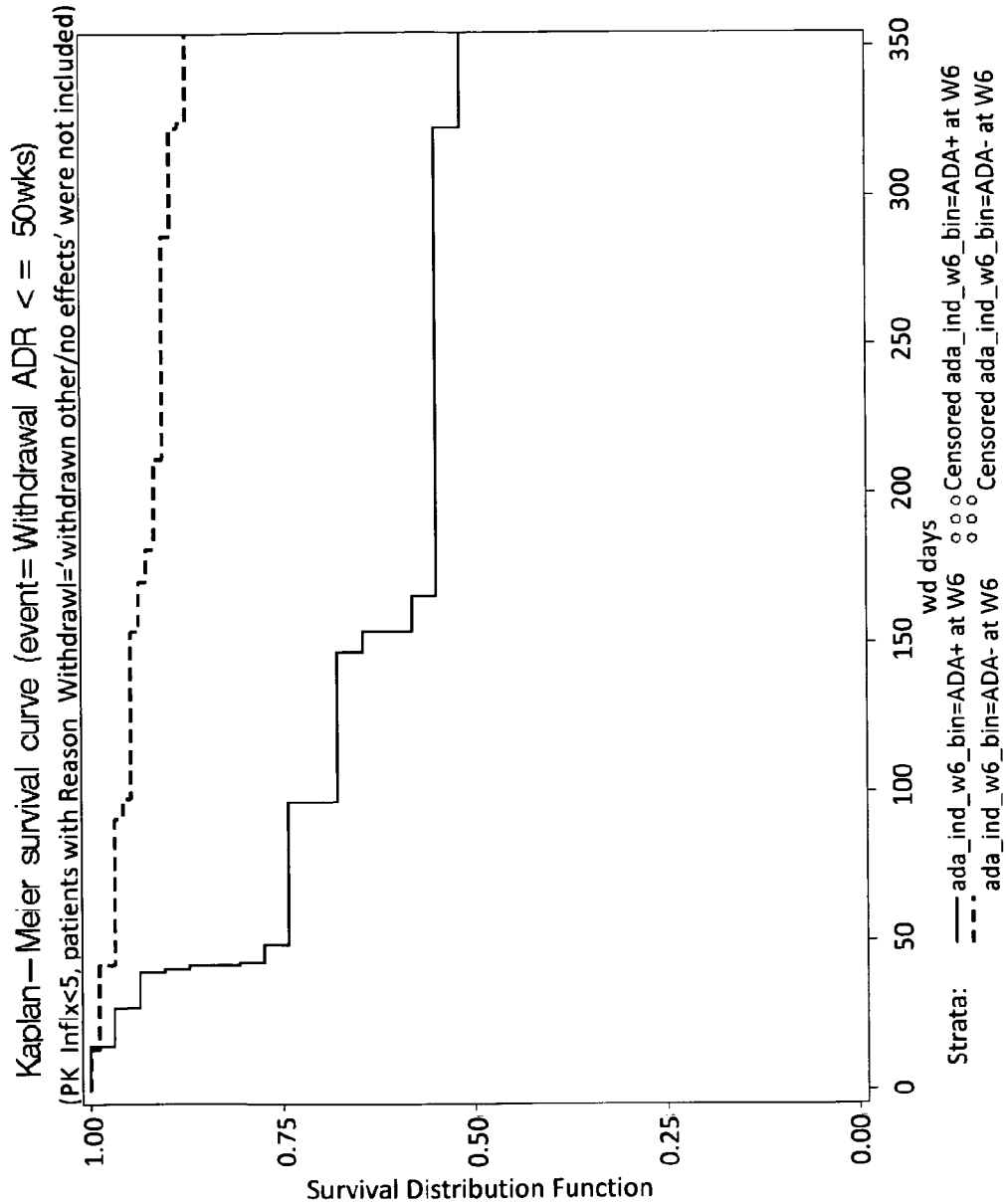


Fig. 2


Kaplan–Meier survival curve (event= Withdrawal ADR <= 50wks)
(PK Inflx<5, patients with Reason_Withdrawl='withdrawn other/no effects' were not included)
Survival Distribution Function
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0.75
0.50
0.25
0.00
0
50
100
150
200
250
300
350
wd days
Strata:
ada_ind_w6_bin=ADA+ at W6
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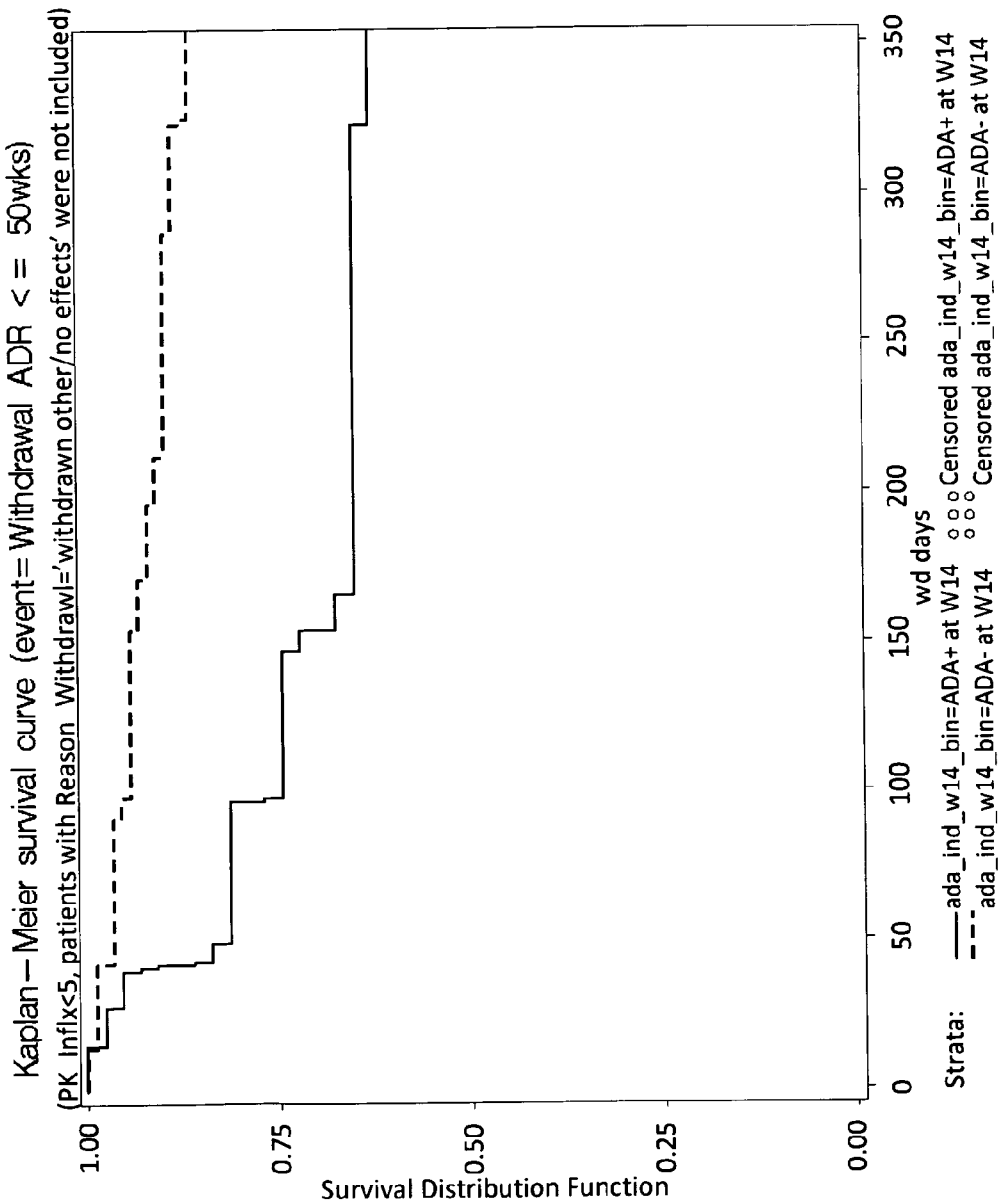
Fig. 3


Kaplan–Meier survival curve (event= Withdrawal ADR < = 50wks)
(PK_Inflx<5, patients with Reason_Withdrawl='withdrawn other/no effects' were not included)
1.00
0.75
0.50
0.25
0.00
Survival Distribution Function
0
50
100
150
200
250
300
350
wd days
Strata:
ada_ind_w14_bin=ADA+ at W14
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Censored ada_ind_w14_bin=ADA+ at W14
Censored ada_ind_w14_bin=ADA- at W14

Fig. 4

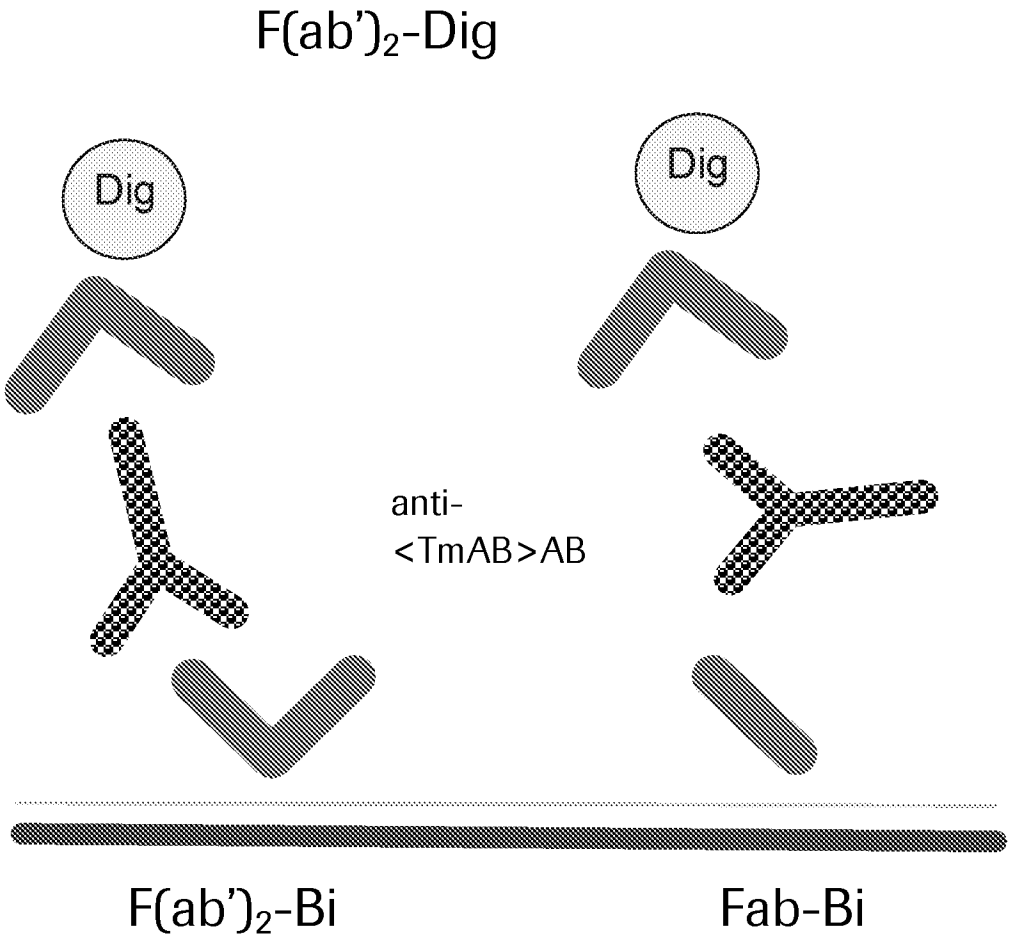
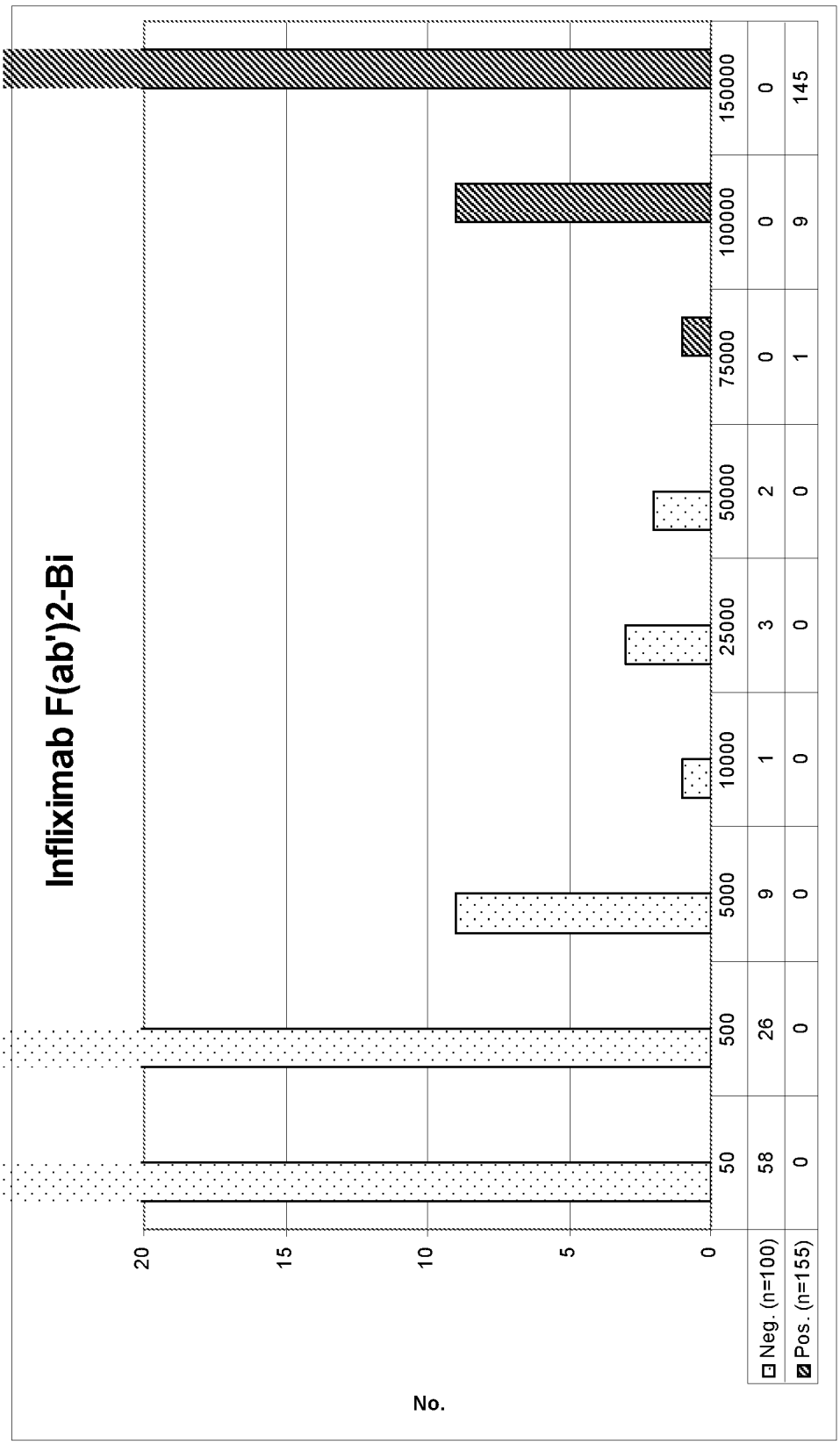


Fig. 5a


Infliximab F(ab')2-Bi
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10
5
0
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Pos. (n=155)
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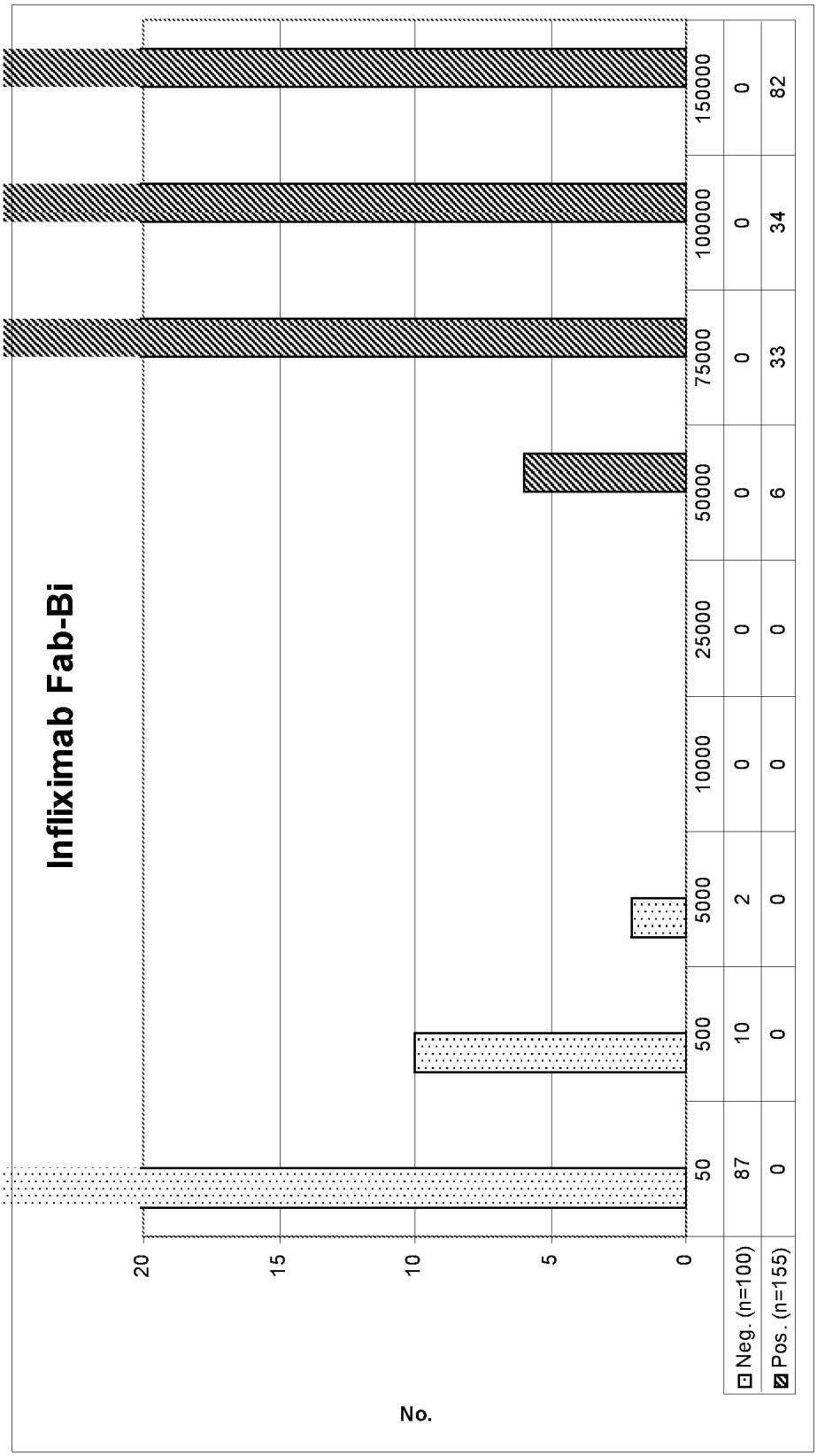
Fig. 5b


Infliximab Fab-Bi
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Pos. (n=155)
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50000
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75000
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33
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/064178

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/68
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 practical, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KNIGHT DAVID M ET AL: "The immunogenicity of the 7E3 murine monoclonal Fab antibody fragment variable region is dramatically reduced in humans by substitution of human for murine constant regions", MOLECULAR IMMUNOLOGY, vol. 32, no. 16, 1995, pages 1271-1281, XP002601367, ISSN: 0161-5890 the whole document -----	1-20
X	WO 2009/003082 A2 (UNIV VANDERBILT [US]; POHLMANN PAULA R [US]; MERNAUGH RAY [US]; ARTEAG) 31 December 2008 (2008-12-31) page 27 example 7 claims 1-38 -----	1-20
	-/--	

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

[X] 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

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"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

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 September 2011	23/09/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bayer, Martin

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/064178

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 1 653 233 A1 (ROCHE DIAGNOSTICS GMBH [DE]; HOFFMANN LA ROCHE [CH]) 3 May 2006 (2006-05-03) cited in the application example 1 claims 1-10 -----	1-20

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2011/064178

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		JP 2010181421 A	19-08-2010
		US 2006115907 A1	01-06-2006