B7-H6 therapeutically active monoclonal antibody against B7-H6 polypeptide

The present invention is concerned with diagnostic methods and means. Specifically, it relates to an antibody which specifically binds to a portion of the extracellular domain of the B7-H6 polypeptide. Moreover, said antibody is provided for use in the treatment or diagnosis of cancer or inflammatory disease. Furthermore, provided are a method for diagnosing cancer in a sample of a subject suspected to suffer from cancer or an inflammatory disease. Further, the present invention concerns a device and a kit for diagnosing cancer or inflammation.

BACKGROUND

Until today, cancer is one of the leading causes of deaths in the United States, even though progress has been made in reducing incidence and mortality rates and improving survival (see Jemal et al. 2010, CA Cancer J Clin. Sep-Oct 60(5):277-300). Further progress can be accelerated by improving diagnostic methods and means due to the fact that cancer development is often associated with the lack of specific recognition of tumor cells by the immune system.

Targeted cancer therapy comprises medication which interferes with specific targeted molecules (e.g., monoclonal or polyclonal antibodies) to directly block the growth of cancer cell. Thus, targeted cancer therapy may be more effective than traditional therapeutic approaches (e.g., resection, radiation, chemotherapy) and may be less harmful to normal cells. Monoclonal antibodies (mAb) can be designed to specifically bind to an extracellular domain or to a cell surface target of the target cell to stimulate the immune system of the patient. Monoclonal antibodies can also be created for numerous serious diseases (e.g., inflammatory diseases or different types of cancers). Thus, monoclonal antibodies may provide reliable and efficient therapeutic and diagnostic methods and means to e.g., detect early developmental stages of these diseases or to offer therapeutic approaches.

Natural killer cells (NK cells) constitute a major component of the innate immune system shaping the inflammatory and adaptive immune response (see Vivier et al. 2008, Nat. Immuno. 9:503-510) and playing a crucial role in the rejection of transformed and virally

infected cells (see Smyth et al. 2002, Nat. Rev. Cancer 2:850-861; Lanier 2005, Annu. Rev. Immunol. 23:225-274). NK cells survey target cells for expression of major histocompatibility complex (MHC) class I (see Parham 2005, Nat. Rev. Immunol. 5:201-204) which protects the target cell from NK cell activation and from NK cell attack. Target cells which lack MHC class I are directly killed by NK cells due to the induction of apoptosis (programmed cell death). The discovery of NK-activating receptors (e.g., the natural cytotoxicity receptor (NCR) family like NKp30) revealed that also activation signals are necessary for the activation of NK cells and tumor cell lysis (see Pende et al. 1999, Cancer Res. 62:6178-6186; Moretta et al. 2001, Annu. Rev. Immunol. 19:197-223).

Recently, it could be shown that the human NKp30 directly interacts with the B7 family member B7-H6 whose expression on tumor cells induces NKp30-dependent cell activation and cytotoxity (see Brandt et al. 2009, J. Exp. Med. 206(7):1495-1503; US 2011/0081346). Hereby, the extracellular domain of NKp30 directly interacts with the extracellular domain of B7-H6 which is exclusively expressed on the surface of several tumor cell lines (see Brandt et al. 2009, J. Exp. Med. 206(7):1495-1503).

SUMMARY OF THE INVENTION

The present invention relates to an antibody which specifically binds to an epitope formed by a portion of the extracellular domain of the B7-H6 polypeptide, said portion having an amino acid sequence as shown in SEQ ID NO: 22. Preferably, said sequence represents an IgV-like domain.

In a preferred embodiment of the antibody of the invention, said antibody comprises complementarity determining regions (CDRs) as shown in SEQ ID NOs: 5, 7, 9, 15, 17, and 19. Nucleic acid sequences of the above mentioned CDRs were annotated according to the IMGT-ONTOLOGY (see Giudicelli and Lefranc 1999, Bioinformatics 15:1047-1054).

In a preferred embodiment of the antibody of the invention, said antibody is a monoclonal antibody. More preferably, said antibody is the antibody deposited under accession number DSM ACC 3117 at the DSMZ, Braunschweig, Germany under the Budapest treaty on February 02, 2011.

The present invention contemplates an antibody of the invention for use in the treatment or diagnosis of cancer. Preferably, the cancer is T cell lymphoma, myeloid leukemia, colon carcinoma, B cell lymphoma, melanoma, or cervical carcinoma.

The present invention, furthermore, contemplates an antibody of the invention for use in the treatment or diagnosis of inflammatory disease. Preferably, the inflammatory disease is a viral infection.

The present invention relates to a method for diagnosing cancer in a sample of a subject suspected to suffer from cancer comprising:

a) contacting the sample with the antibody of the invention under conditions which allow for binding of said antibody to its epitope on the B7-H6 polypeptide; and

b) determining binding of the antibody to the said epitope, whereby cancer is diagnosed.

In a preferred embodiment of the method of the invention, the cancer is T cell lymphoma, myeloid leukemia, colon carcinoma, B cell lymphoma, melanoma, or cervical carcinoma.

The present invention also relates to a method for diagnosing an inflammatory disease in a sample of a subject suspected to suffer from an inflammatory disease comprising:

a) contacting the sample with the antibody of the invention under conditions which allow for binding of said antibody to its epitope on the B7-H6 polypeptide; and

b) determining binding of the antibody to the said epitope, whereby the inflammatory disease is diagnosed.

In a preferred embodiment of the method of the invention, said sample is a tissue or body fluid sample.

Encompassed by the invention is also a device for diagnosing cancer or an inflammatory disease in a sample comprising:

a) an analyzing unit comprising the antibody of the invention; and

b) a detector which detects binding of the antibody in the analyzing unit to its epitope on the B7-H6 polypeptide.

In a preferred embodiment of the device of the invention, said sample is a tissue or body fluid sample.

The present invention finally relates to a kit for diagnosing cancer or an inflammatory disease comprising the antibody of the invention and, preferably, an agent for detection of binding of said antibody to its epitope on the B7-H6 polypeptide.

FIGURES

Figure 1 shows the nucleic acid and amino acid sequences of the B7-H6-Ig-fusionprotein. Italic nucleic acid and amino acid sequences indicate enzymatic restriction sites. Nucleic acid and amino acid sequences of the extracellular domain of human B7-H6 are underlined bold, whereby said sequences of Fcm are dotted underlined.

Figure 2 shows the amino acid sequence of the extracellular domain of the human B7-H6 polypeptide and indicates the IgV-like domain and the IgC-like domain.

Figure 3 shows that the anti-B7-H6 clone 1.18 reacts with B7-H6 using an enzyme-linked immunoabsorbant assay (ELISA).

Figure 4a depicts that the anti-B7-H6 clone 1.18 binds to B7-H6 on transfectants (BA/F3-B7-H6) using fluorescence-activated cell sorting (FACS). Figure 4b shows that the anti-B7-H6 clone 1.18 binds to B7-H6 on cell lines (haematopoietic and solid tumor origin), but not to healthy peripheral blood mononuclear cells (PBMCs).

Figure 5 shows that a portion of the IgV domain of B7-H6 is involved in binding of anti-B7-H6 clone 1.18.

Figure 6 depicts that cell surface expression of B7-H6 determined by fluorescence-activated cell sorting (FACS) and mRNA expression in the different cell lines. Figure 6a shows the expression of B7-H6 in tumor cell lines of hematopoietic origin. Figure 6b shows the expression of B7-H6 in tumor cell lines of solid tumor origin.

Figure 7 shows that the anti-B7-H6 mAb 1.18 detects B7-H6 on cytospins (frozen sections) of BA/F-3-B7-H6 transfectants.

Figure 8 shows that primary natural killer (NK) cells degranulate upon co-culture with BA/F3-B7-H6 transfectants.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to an antibody which specifically binds to an epitope formed by a portion of the extracellular domain of the B7-H6 polypeptide, said portion having an

amino acid sequence as shown in SEQ ID NO: 22. Preferably, said sequence represents an IgV-like domain.

The term "antibody" refers to all types of antibodies which specifically bind to an epitope comprised in a portion of the extracellular domain of the B7-H6 polypeptide. Epitopes as referred to herein are, preferably, defined by stretches of 7 to 15, preferably 8 to 11 contiguous amino acids in length. However, an epitope in accordance with the present invention can also be formed by a certain three-dimensional structure and such structural epitopes are also envisaged herein. Specific binding in this context means that the antibody of the invention essentially binds to the epitope without significant cross-reactivity (i.e. binding) to other epitopes either on the B7-H6 polypeptide or other polypeptides. Specific binding can be determined by techniques well known in the art. Preferably, the antibody binds specifically to the said epitope. The aforementioned epitope shall be located in a portion of the extracellular domain of the B7-H6 polypeptide. Preferably, the B7-H6 polypeptide has an amino acid sequence as shown in SEQ ID NO: 2 and the said extracellular domain corresponds to amino acids 58 to 300 of said sequence (see also Figures 1 and 2). It will be understood that the B7-H6 polypeptide may also be represented by a variant sequence of SEQ ID NO: 2 which differs therefrom by substitution, addition and/or deletion of one or more amino acids. Such variant sequences may be orthologous amino acid sequences from other species as well as paralogous or other homologous sequences of the aforementioned specific B7-H6. Preferably, such variant sequences are at least 70%, at least 80%, at least 90%, at least 95% or at least 99% identical over the entire length or at least 50% of SEQ ID NO: 2 with the said sequence. The term "sequence identity" as used herein refers to a relationship between two or more polypeptide sequence, namely a reference sequence and a given sequence to be compared with the reference sequence. Sequence identity can be determined by comparing the given sequence to the reference sequence after the sequences have been aligned optimally to produce the highest degree of sequence similarity which can be determined by the match between strings of such sequences. Said alignment can be performed by a skilled artisan without further ado. Accordingly, sequence identity provides information on the total number of said matches. Sequence identity can be, preferably, calculated using publicly available computer programs which are known by a skilled artisan, e.g., BLAST and FASTA. Other sequence variants envisaged in accordance with the present invention are those which are encoded by nucleic acid molecules capable of hybridizing under stringent hybridization conditions to the nucleic acid sequence encoding B7-H6 shown in SEQ ID NO: 1. Preferably, the B7-H6 polypeptide is encoded by the nucleic acid sequence shown in SEQ ID NO: 1. Stringent hybridization conditions referred to in accordance wit the present invention are equivalent to hybridization in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO4, 1 mM

EDTA at 50°C with washing in 1 X SSC, 0.1 % SDS at 50°C or 65°C, wherein nucleic acid molecule probe comprising at least 100, more preferably at least 150, even more preferably at least 200, most preferably at least 250 consecutive nucleotides of SEQ ID NO:1 or its reverse complement is used. It will be understood that the first and the last amino acid of the extracellular domain in such sequence variants may differ from the positions indicated for SEQ ID NO: 2, above. However, the extracellular domain will start and end at positions corresponding to the said positions. Such corresponding positions can be determined by sequence analysis tools by the skilled artisan without further ado.

Preferably, an antibody as referred to in accordance with the present invention encompasses a monoclonal antibody, a single chain antibody, a chimeric antibody or any fragment or derivative of such antibodies having the above mentioned binding properties. Such fragments and derivatives comprised by the term antibody as used herein encompass a synthetic antibody, an Fab, $F(ab)_2$ Fv or scFv fragment, or a chemically modified derivative of any of these antibodies. Chemical modifications envisaged preferably by the present invention include those which aim to couple the antibody to a detectable marker as specified elsewhere in this specification. Antibodies or fragments thereof, in general, can be obtained by using methods which are described, e.g., in Harlow and Lane "Antibodies, A Laboratory Manual", CSH Press, Cold Spring Harbor, 1988.

Advantageously, the antibody of the present invention specifically binds to B7-H6 with a high affinity. In the studies underlying the present invention it has been found that compared to other anti-B7-H6 antibodies described or suggested in the prior art (Brandt 2009, J. Exp. Med. 206(7): 1495-1503 and US 2011/0081346), the antibody is particularly useful in in vivo applications such as FACS sorting and cell culture as well as in vitro applications including immunohistochemistry on, e.g., frozen tissue sections. Thanks to the present invention, cancer diagnosis based on the determination of B7-H6 will improve. Moreover, therapeutic approaches which aim to target anti-tumor drugs to B7-H6 positive cells are feasible.

In a preferred embodiment of the antibody of the present invention, said antibody comprises complementarity determining regions (CDRs) as shown in SEQ ID NOs: 5, 7, 9, 15, 17, and 19. Nucleic acid sequences of the above mentioned CDRs were annotated according to the IMGT-ONTOLOGY (see Giudicelli and Lefranc 1999, Bioinformatics 15:1047-1054).

The term "complementarity determining region" or "CDR" as used herein refers to variable domains of an antibody which are responsible for the specificity in antigen binding. An antigen, usually, comprises three CDRs (CDR1, CDR2 and CDR3). These CDRs are arranged in a non-consecutive manner. Since the antigen recognizing portions of the antibody are typically composed of two variable domains on a heavy and a light chain, six CDRs come into contact with the antigen upon binding. The CDRs can be transferred from one antibody species to another by conventional molecular biology techniques such as CDR grafting (see Ewert 2004, Methods 34(2): 184-199; Benny K.C. Lo in Antibody Engineering – Methods in Molecular Biology 2004, Volume 248, II, 135-159, DOI 10.1385/1-59259-666-5:135).

It will be understood from the above that in another preferred embodiment, the antibody of the present invention is a monoclonal antibody.

Preferably, such a monoclonal antibody can be prepared by applying an immunogenic polypeptide having the portion of the extracellular domain as characterized above to a mammal, preferably a mouse. More preferably, the immunogenic polypeptide is conjugated to a carrier protein, such as bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). Depending on the host species, various adjuvants can be used to increase the immunological response. Such adjuvants encompass, preferably, Freund's adjuvant, mineral gels, e.g., aluminum hydroxide, and surface active substances, e.g., lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. Monoclonal antibodies according to the invention can be subsequently prepared using the well known hybridoma technique, the human B cell hybridoma technique, and the EBV hybridoma technique. Further details on the preparation of an antibody of the invention are described in the accompanying Examples below.

In a more preferred embodiment of the antibody of the present invention, the antibody is the antibody or the antibody produced by the corresponding hybridoma cell clone as deposited under accession number DSM ACC 3117 at the "DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH", 38124 Braunschweig, GERMANY on February 2, 2011 according to the Budapest Treaty by "Deutsches Krebsforschungszentrum", Heidelberg, GERMANY.

The aforementioned anti-B7-H6 mAb shall comprise at least one heavy chain and at least one light chain. Preferably, anti-B7-H6 mAb has an amino acid sequence of the heavy chain (IGHV/IGHD/IGHJ) as shown in SEQ ID NO: 3, whereby the secreted form

(IGHV/IGHD/IGHJ/IGHG1) is shown in SEQ ID NO: 11 and the membrane bound form (IGHV/IGHD/IGHJ/IGHG1) is shown in SEQ ID NO: 12. The nucleic acid sequences of fragments 1-4 of the heavy chain are shown in SEQ ID NO: 4, 6, 8, and 10 and the nucleic acid sequences of CDRs 1-3 of the heavy chain are shown in SEQ ID NO: 5, 7, and 9. Further, said antibody has an amino acid sequence of the light chain (IGLV/IGLJ) as shown in SEQ ID NO: 13, whereby the sequence of IGLV/IGLJ/IGLC is shown in SEQ ID NO: 21. The nucleic acid sequences of fragments 1-4 of the light chain are shown in SEQ ID NO: 14, 16, 18, and 20 and the nucleic acid sequences of CDRs 1-3 of the light chain are shown in SEQ ID NO: 15, 17, and 19. It will be understood that the anti-B7-H6 mAb may also be represented by variant sequences of the aforementioned SEQ ID NOs: 3-21 which differ therefrom by substitution, addition and/or deletion of one or more amino acids. Such variant sequences may be orthologous amino acid sequences from other species as well as paralogous or other homologous sequences of the aforementioned specific anti-B7-H6 mAb. Preferably, such variant sequences are at least 70%, at least 80%, at least 90%, at least 95% or at least 99% identical over the entire length or at least 50% of SEQ ID NOs: 3-21 with said sequences. The term sequence identity has been defined elsewhere in this description and applies mutatis mutandis.

The present invention further relates to an antibody of the invention for use in the treatment or diagnosis of cancer.

The term "treatment" as used herein encompasses the amelioration of a disease referred to herein or its symptoms as well as curing the disease, i.e. the reestablishment of the healthy condition in a subject with respect to the disease or its symptoms. Amelioration as referred to herein refers to a significant improvement of the health condition with respect to the disease or a symptom of the disease. Such a significant improvement is, preferably, clinically apparent in, e.g., staging or grading systems applied in order to investigate a subject. As will be understood by those skilled in the art, treatment as used herein is usually not intended to be correct for all (i.e. 100%) of the subjects under a given treatment. The term, however, requires that a statistically significant portion of subjects can be treated (e.g. a cohort in a cohort study). Whether a portion is statistically significant can be determined without further ado by the person skilled in the art using various well known statistic evaluation tools, e.g., determination of confidence intervals, p-value determination, Student's t-test, Mann- Whitney test etc.. Details are found in Dowdy and Wearden, Statistics for Research, John Wiley & Sons, New York 1983.

Preferably, the antibody of the invention for use in treating cancer is either coupled to a cytotoxic agent or an anti-tumor agent or is capable of recruiting such agents suitable for treating cancer. The term "agent" as used herein refers to an element, compound, or other molecular entity (e.g., a pharmaceutical compound, a therapeutic compound, or a pharmacologic compound). Such an agent can be natural, synthetic or a combination thereof. The term "therapeutic agent" as used herein refers to an agent that either alone or in combination with another agent exhibits a therapeutic or beneficial effect on a cell or a tissue. Preferably, a therapeutic agent in accordance with the present invention shall comprise drugs, toxins, immunomodulators, chelators, boron compounds, photoactive agents or dyes, and radioisotopes. Techniques for coupling therapeutic agents to polypeptides such as to antibodies are well-known by the skilled artisan (e.g., Amon et al. 1985 , "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy," in Monoclonal Antibodies And Cancer Therapy (Reisfeld et al. eds., Alan R. Liss, Inc., 1985)). The term "cytotoxic agent" as used herein refers to an agent that has a cytotoxic or cytostatic effect on a cell, thereby depleting or inhibiting the growth of, respectively, cells within a cell population. Preferably, cytotoxic agents in accordance with the present invention shall comprise anti-tubulin agents (e.g., dolastatins, vinca alkaloids, podophyllatoxins, taxanes, baccatin derivatives, cryptophysins, maytansinoids, and combretastatins), DNA minor groove binding agents, DNA replication inhibitors, alkylating agents (e.g., platinum complexes), anthracyclines, antibiotics, antifolates, antimetabolites, chemotherapy sensitizers, duocarmycins, etoposides, fluorinated pyrimidines, ionophores, lexitropsins, nitro soureas, platinols, pre-forming compounds, purine antimetabolites, puromycins, radiation sensitizers, steroids, taxanes, topoisomerase inhibitors, vinca alkaloids, or the like. The term "anti-tumor agent" as used herein refers to an agent that has a cytotoxic or malign effect on cancer cells, thereby arresting the growth of, respectively, cancer cells within a tumor resulting, preferably, in cell death. Preferably, the antibody of the invention binds to a target cell (e.g., a cancer cell) and specific effector cells expressing receptors for said antibody (e.g., natural killer cells, monocytes, granulocytes) which results in target cell death. In another preferred embodiment of the invention the antibody of the invention is coupled to a cytotoxic agent or an anti-tumor agent via a linker. Preferably, a linker in accordance with the present invention shall comprise linker that are cleavable under intracellular conditions (e.g., a peptide linker cleavable by an intracellular protease, dipeptide linker, disulfide linker, and hydrolysable linker which are e.g., hydrolysable at a pH of less than 5.5). However, the antibody of the invention may also be used for treating cancer due to its blocking and binding properties on B7-H6 as a modulator of signaling cascades involved in cancer.

The term "diagnosis" as used herein means the assessment whether a subject suffers from a disease referred to herein, or not. As will be understood by those skilled in the art, such an assessment is usually not intended to be correct for all (i.e. 100%) of the subjects to be identified. The term, however, requires that a statistically significant portion of subjects can be identified (e.g., a cohort in a cohort study). Whether a portion is statistically significant can be determined without further ado by the person skilled in the art using various well known statistic evaluation tools referred to elsewhere herein. Diagnosis according to the present invention includes applications of the method in monitoring, confirmation, and sub-classification of the relevant disease. Moreover, the establishment of a diagnosis as used herein also includes establishing a prognosis for a subject. Such a prognosis is a predictive indicator for the further development of the disease in a future time window, i.e. the predictive window. Thus, a diagnosis as used herein, preferably, encompasses a prediction of whether a subject will improve with respect to the disease or diseases symptoms in the future or whether the disease or symptoms will become worse. Accordingly, the antibody of the invention can be also applied for risk stratification approaches and, thus, for determining the amount of intensive care and hospitalization which will be required for an individual subject suffering from a disease referred to herein.

Preferably, the antibody of the invention for use in diagnosis is either coupled to a detection agent or is capable of recruiting such an agent. A detection agent as used herein encompasses a radioactive isotope (e.g., radioactive isotopes of Iodide Technetium), fluorescent or chemoluminescent agents (e.g., FITC, rhodamin), an enzyme which is capable of generating a detectable signal by converting a substrate (e.g., horseradish peroxidase, firefly luciferase, or beta galactosidase), a fluorescent protein (e.g., green-, blue- or red- fluorescent protein). Suitable detection agents are well known in the art. Also preferably, the antibody to be applied in the method of the present invention can be coupled to an agent that is capable of attracting a detection agent. Such an agent may be biotin. In such a case an avidin- or streptavidin coupled detection agent can be used which upon binding of the biotin of the bound antibody will serve as a detectable marker. Suitable detectable markers in such a case are those referred to above, more preferably, an enzyme shall be used as a detectable marker in such a case. Furthermore, a secondary antibody may be used for detection of the first antibody, i.e. the antibody to be applied in the method of the present invention which is bound to the B7-H6 polypeptide of the sample. Such a secondary antibody shall be coupled to a detectable marker as describe above. Thus, in the latter case, the secondary antibody will upon binding to the first antibody generate a detectable signal and thereby enables the detection of the bound first antibody. The principle of detection of bound antibodies with a secondary antibody is well known in the art and routinely applied, e.g., for determining antibody binding on tissue

sections. Dependent on the type of detectable marker, different detection methods can be applied using a reader system for the signal generated by the detectable marker. Such systems include automatic signal reader device, such as an ELISA or RIA reader, but also microscopic device for manual or automatic detection of the detectable signal. Moreover, the reader system may determine additional information of the sample, e.g., a microscopic system may display the cells of a tissue section optically or an automated signal reader may determine further biomarkers comprised by the sample in addition.

The term "cancer" as used herein refers to any malignant neoplasm. The malignant neoplasm refers to diseases resulting from the undesired growth, the invasion, and under certain conditions metastasis of impaired cells in an organism. The cells giving rise to cancer are genetically impaired and have usually lost their ability to control cell division, cell migration behavior, differentiation status and/or cell death machinery. Most cancers form a tumor but some hematopoietic cancers, such as leukemia, do not. The cancer in accordance with the present invention shall comprise cancer cells expressing a B7-H6 polypeptide as specified elsewhere herein. Preferred types of cancer are selected from the group consisting of: T cell lymphoma, myeloid leukemia, colon carcinoma, B cell lymphoma, melanoma, or cervical carcinoma. Symptoms and staging systems for the different cancers are well known in the art and described in standard text books of pathology. Cancer as used herein encompasses any stage, grade, morphological feature, invasiveness, aggressiveness or malignancy of the cancer or the tissue or organ affected thereby.

The present invention further relates to an antibody of the invention for use in the treatment or diagnosis of an inflammatory disease.

Preferably, the antibody of the invention for use in treating an inflammatory disease is either coupled to an anti-inflammation agent or is capable of recruiting such an agent as specified elsewhere herein. However, the antibody of the invention may also be used for an inflammatory disease due to its blocking and binding properties on B7-H6 as a modulator of signaling cascades involved in inflammation.

Preferably, the antibody of the invention for use in diagnosis is either coupled to a detection agent or is capable of recruiting such an agent as specified elsewhere herein.

The term "inflammatory disease" as used herein refers to a tissue response involving inflammatory cytokines and inflammatory cell infiltrates in response to an injury or

destruction of tissue. The inflammatory disease in accordance with the present invention shall comprise a viral infection, and bacterial infection. In addition, autoimmune diseases such as diabetes, multiple sclerosis and inflammatory bowl disease are included.

If follows from the above that the present invention also relates to a method for diagnosing cancer in a sample of a subject suspected to suffer from cancer comprising:

a) contacting the sample with the antibody of the invention under conditions which allow for binding of said antibody to its epitope on the B7-H6 polypeptide; and

b) determining binding of the antibody to the said epitope, whereby cancer is diagnosed.

The term "diagnosing" as used herein means the assessment whether a subject suffers from a disease referred to herein, or not. As will be understood by those skilled in the art, such an assessment is usually not intended to be correct for all (i.e. 100%) of the subjects to be identified. The term, however, requires that a statistically significant portion of subjects can be identified (e.g., a cohort in a cohort study). Whether a portion is statistically significant can be determined without further ado by the person skilled in the art using various well known statistic evaluation tools referred to elsewhere herein. Diagnosis according to the present invention includes applications of the method in monitoring, confirmation, and sub-classification of the relevant disease. Moreover, the establishment of a diagnosis as used herein also includes establishing a prognosis for a subject. Such a prognosis is a predictive indicator for the further development of the disease in a future time window, i.e. the predictive window. Thus, a diagnosis as used herein, preferably, encompasses a prediction of whether a subject will improve with respect to the disease or diseases symptoms in the future or whether the disease or symptoms will become worse. Accordingly, the antibody of the invention can be also applied for risk stratification approaches and, thus, for determining the amount of intensive care and hospitalization which will be required for an individual subject suffering from a disease referred to herein.

The aforementioned method for diagnosing cancer in a sample of the subject also, preferably, encompasses the step of recommending an anti-cancer therapy for a subject based on the diagnostic result obtained by the method. The term "recommending" as used herein refers to making a recommendation for an anti-cancer therapy or excluding (i.e. not recommending) a certain anti cancer therapy for a subject. Such a recommendation shall serve optionally together with other information, e.g., information from histopathological investigations, as a basis for a clinician to apply a certain anti-cancer therapy for an individual subject, or not. Based on the diagnosis of the present invention, i.e. the

diagnosis of cancer or no cancer, a recommendation for an anti-cancer therapy will be made. It will be understood that only in cases where the diagnosis of cancer has been established by the method of the present invention, the recommendation for the anti-cancer therapy shall be made. In cases where no cancer is established as diagnosis based on the method of the present invention, the recommendation would be to refrain from an anti-cancer therapy. As set forth above, further information from the subject from which the sample originates can be used as well for improving the recommendation. In an aspect, a combined anti-cancer therapy, e.g., with different anti tumor drugs, can be recommended if the method of the present invention identifies cancer cells but if further cancer cells which are not identified by the method of the present invention are detected in the investigated cancer, e.g., by histopathological analyses.

The term "sample" refers to a sample of separated cells or to a sample from a tissue or an organ. Tissue or organ samples may be obtained from any tissue or organ by, e.g., biopsy. Separated cells may be obtained from the body fluids, such as lymph, blood, plasma, serum, liquor and other, or from the tissues or organs by separating techniques such as centrifugation or cell sorting. Preferably, the sample is a tissue or body fluid sample which expresses or produces the polypeptides referred to herein. The sample can be obtained from the subject by routine techniques which are well known to the person skilled in the art, e.g., open biopsy including aspiration of tissue or cellular material from a subject. For those areas which cannot be easily reached via an open biopsy, a surgery and, preferably, minimal invasive surgery can be performed.

The term "subject" as used herein relates to animals, preferably mammals, and, more preferably, humans. The method of the present invention shall be applied for subjects suspected to suffer from cancer. A subject suspect to suffer from cancer is either a subject exhibiting clinically apparent symptoms of the cancer or is a subject having an increased predisposition for cancer. In the context of large scale diagnostic screening trials, a subject suspected to suffer from cancer can be even a healthy subject, i.e., a subject who does not show symptoms of the disease nor a subject having a predisposition therefor.
The terms "contacting" and "contacting the sample" as used herein refer to bringing the antibody and the sample into physical contact thereby allowing specific binding of the antibody to the epitope on the B7-H6 polypeptide if comprised by the sample. It will be understood that contacting as meant herein is carried out for a time and under conditions sufficient for allowing the antibody to bind specifically to the B7-H6 polypeptide. Depending on the nature of the sample, pre-treatment steps might be necessary in order to release the B7-H6 polypeptide or to de-mask the epitope in the B7-H6 polypeptide so that the antibody has access and can specifically bind thereto. Moreover, dependent on the kind

of sample, the handling might be different. For example, a tissue sample which shall be analyzed for the presence or absence of a B7-H6 polypeptide is, preferably, homogenized and the proteins comprised by the tissue are isolated and separated, e.g., by SDS PAGE or other protein separation methods known by a skilled artisan. The separated proteins are analyzed for the presence or absence of the B7-H6 polypeptide by immunological methods such as Western Blot using the antibody defined herein above. These methods also include incubation steps which allow specific binding of the antibody to the B7-H6 polypeptide. In order to increase the specificity washing steps are to be carried out. How to carry out such measures is well known to the person skilled in the art. If a tissue section is used as a sample (i.e. a tissue section sample), it will be understood that it is envisaged to analyze not only the presence or absence of the B7-H6 polypeptide but also the cellular or sub cellular localization thereof. Accordingly, the tissue shall be kept intact and may be also stained by histochemical staining techniques prior or after antibody binding. Suitable techniques which allow for immunostaining of tissue sections are well known to the person skilled in the art. Dependent on whether the tissue section sample has been embedded in an embedding medium, such as paraffin, removal of said embedding medium might be necessary. The relevant techniques are also well known in the art.

The term "determining" as used herein refers to the detection of the antibody which is specifically bound to the B7-H6 polypeptide comprised by the sample, if any. Detection methods for antibodies which are specifically bound to an antigen are also well known in the art. Preferably, the antibody to be applied in the method of the present invention itself can be coupled to a detectable marker such as a radioactive isotope (e.g., radioactive isotopes of Iodide Technetium), fluorescent or chemoluminescent agents (e.g., FITC, rhodamin), an enzyme which is capable of generating a detectable signal by converting a substrate (e.g., horseradish peroxidase, firefly luciferase, or beta galactosidase), a fluorescent protein (e.g., green-, blue- or red- fluorescent protein). Suitable detectable markers are well known in the art. Also preferably, the antibody to be applied in the method of the present invention can be coupled to an agent that is capable of attracting a detection agent. Such an agent may be biotin. In such a case an avidin- or streptavidin coupled detection agent can be used which upon binding of the biotin of the bound antibody will serve as a detectable marker. Suitable detectable markers in such a case are those referred to above, more preferably, an enzyme shall be used as a detectable marker in such a case. Furthermore, a secondary antibody may be used for detection of the first antibody, i.e. the antibody to be applied in the method of the present invention which is bound to the B7-H6 polypeptide of the sample. Such a secondary antibody shall be coupled to a detectable marker as describe above. Thus, in the latter case, the secondary antibody will upon binding to the first antibody generate a detectable signal and thereby

enables the detection of the bound first antibody. The principle of detection of bound antibodies with a secondary antibody is well known in the art and routinely applied, e.g., for determining antibody binding on tissue sections. Dependent on the type of detectable marker, different detection methods can be applied using a reader system for the signal generated by the detectable marker. Such systems include automatic signal reader device, such as an ELISA or RIA reader, but also microscopic device for manual or automatic detection of the detectable signal. Moreover, the reader system may determine additional information of the sample, e.g., a microscopic system may display the cells of a tissue section optically or an automated signal reader may determine further biomarkers comprised by the sample in addition.

In a preferred embodiment of the method of the present invention, the cancer is T cell lymphoma, myeloid leukemia, colon carcinoma, B cell lymphoma, melanoma, or cervical carcinoma.

The present invention also provides a method for diagnosing an inflammatory disease in a sample of a subject suspected to suffer from an inflammatory disease comprising:

a) contacting the sample with the antibody of invention under conditions which allow for binding of said antibody to its epitope on the B7-H6 polypeptide; and
b) determining binding of the antibody to the said epitope, whereby the inflammatory disease is diagnosed.

Explanations of the terms made in connection with the method for diagnosing cancer or other embodiments elsewhere herein apply mutatis mutandis for the terms in connection with the aforementioned method except as specified otherwise herein below.

The term "subject" as used herein relates to animals, preferably mammals, and, more preferably, humans. The method of the present invention shall be applied for subjects suspected to suffer from an inflammatory disease. A subject suspect to suffer from an inflammatory disease is either a subject exhibiting clinically apparent symptoms of the an inflammatory disease or is a subject having an increased predisposition for an inflammatory disease. In the context of large scale diagnostic screening trials, a subject suspected to suffer from an inflammatory disease can be even a healthy subject, i.e. a subject who does not show symptoms of the disease nor a subject having a predisposition therefor.

As discussed elsewhere herein, the inflammatory disease referred to above is, preferably, a viral infection.

The invention also relates to a device for diagnosing cancer or an inflammatory disease in a sample comprising:

a) an analyzing unit comprising the antibody of the invention; and

b) a detector which detects binding of the antibody in the analyzing unit to its epitope on the B7-H6 polypeptide.

The term "device" as used herein relates to a system comprising at least the aforementioned analyzing unit and the evaluation unit operatively linked to each other. How to link the units of the device in an operating manner will depend on the type of units included into the device. For example, where units for automatic analysis of a sample are applied, the data obtained by said automatically operating analyzing unit can be processed by, e.g., a computer program in order to obtain the desired results by the evaluation unit. Preferably, the units are comprised by a single device in such a case. The analyzing unit may comprise the antibody in immobilized form on a solid support. Such an analyzing unit is particular useful for liquid samples. The sample to be investigated with the device of the present invention is preferably a tissue sample and, more preferably, a tissue section sample. Thus, in another aspect, the antibody may be comprised in a detection solution which will be applied to tissue samples such as tissue section by the analyzing unit. The detection solution can be stored in the analyzing unit or a separate vial, even outside the device. The evaluation unit, preferably a computer or data processing device, comprises implemented rules, i.e. an algorithm, for evaluating the binding determined by the analyzing unit whereby the binding is evaluated into significant or non-significant binding based on the signal type, strength and, in the case of tissue samples, position of the signal with respect to the tissue. For samples which are evaluated to show non-significant binding the diagnosis "no cancer" will be established. If significant binding is obtained as result of the evaluation, the diagnosis cancer shall be established.

Preferably, the device in its evaluation unit also comprises an implemented expert system with an algorithm that is adapted for making recommendations based on the established diagnosis for a suitable therapy or treatment as set forth elsewhere herein in more detail.

In a preferred embodiment of the device of the present invention, said sample is a tissue or body fluid sample.

Finally, the present invention relates to a kit for diagnosing cancer or an inflammatory disease comprising the antibody of the invention and, preferably, an agent for detection of binding of said antibody to its epitope on the B7-H6 polypeptide.

The term "kit" as used herein refers to a collection of the aforementioned antibody and instructions provided in a ready-to-use manner for diagnosing cancer in a sample. The antibody and the instructions are, preferably, provided in a single container. Preferably, the kit also comprises further components which are necessary for carrying out the diagnosis. Such components may be auxiliary agents which are required for the detection of the antibody binding, agents for pre-treating the sample to be analyzed or calibration standards.

All references cited in this specification are herewith incorporated by reference with respect to their entire disclosure content and the disclosure content specifically mentioned in this specification.

EXAMPLES

The following Examples shall merely illustrate the invention. They shall not be construed, whatsoever, to limit the scope of the invention.

Example 1: Method for Immunization to obtain anti-B7-H6 monoclonal Antibody (mAb) 1.18: Six weeks old BALB/c mice were immunized with 100 µg of a B7-H6-Ig-fusionprotein consisting of the extracellular domain of B7-H6 fused to an IgG1-Fc domain (B7-H6-Ig-FP) shown in Fig. 1 in complete Freud's Adjuvant injected s.c. at four different sites. Three weeks later, 100 µg B7-H6-Ig-FP was injected i.p. in PBS. After three weeks, BA/F3 (pro-B cells)-B7-H6 transfectants (2 x 10^7 cells) in PBS were injected i.p.. Two months later, 100 µg B7-H6-Ig-FP in PBS was applied i.p.. After three weeks, injection with BA/F3-B7-H6 transfectants (2 x 10^7 cells) in PBS i.p. was performed and five days later spleen cells were fused with Ag8 mouse myeloma cells. 910 hybridoma were screened by flow cytometry for binding of produced immunoglobulins to BA/F3-B7-H6 cells. Additionally, 480 clones were screened by ELISA for binding to the B7-H6-Ig-FP. Anti-B7-H6 clone 1.18 was selected for further studies, because it stained BA/F3-B7-H6 transfectants and not control vector transduced BA/F3 cells at high levels and it bound to cell lines expressing B7-H6 endogenously at high levels.

Example 2: Binding of anti-B7-H6 mAb 1.18 to B7-H6-Ig-FP by ELISA and to BA/F3-B7-H6 transfected cells by flow cytometry:

For ELISA: B7-H6-Ig-FP (3 µg/ml) was immobilized on ELISA plates and incubated with the indicated concentrations of anti-B7-H6 mAb 1.18 and developed with HRP-conjugated mAbs.

For flow cytometry: BA/F3 or BA/F3-B7-H6 transfectants were stained with anti-B7-H6 mAb 1.18 (2 µg/ml), the isotype controls, NKp30-FP and a control FP and PE-conjugated secondary mAbs.

The data depict binding of anti-B7-H6 mAb 1.18 to B7-H6-Ig-FP by ELISA and to BA/F3-B7-H6 transfected cells by flow cytometry.

Example 3: Binding of anti-B7-H6 1.18 mAb involves the IgV domain of B7-H6: The following constructs based on the pcDNA3.1 with CD8-leader peptide and a C-terminal HA-tag encoding for the following portions of B7-H6 were prepared:

B7-H6_1 (amino acids 24-454)
B7-H6_2 (amino acids 83-454)
B7-H6_3 (amino acids 141-454)
B7-H6_4 (amino acids 190-454)
B7-H6_5 (amino acids 239-454)

Resulting plasmids were transiently transfected in HEK cells and subsequently stained with the anti-B7-H6 1.18 mAb as described in Example 2. As can be seen in Figure 5, the anti-B7-H6 1.18 mAb bound to B7-H6_1 (amino acids 24-454) and B7-H6_2 (amino acids 83-454), but not to B7-H6_3 (amino acids 141-454) indicating that amino acids 83-141 of B7-H6 **(GDHQEAFRPGAIVSPWRLKSGDASLRLPGIQLEEAGEYRCEVVVTPLKAQGT VQLEVV**, as shown in SEQ ID NO: 22 and Figures 1 and 2) are involved in the binding of anti-B7-H6 mAb 1.18. All proteins of truncated B7-H6 were expressed and were detectable by western blotting using the anti-HA-tag mAb.

Example 4: Binding of anti-B7-H6 mAb 1.18 to cell lines of different origin: Cell lines of different origin were stained with anti-B7-H6 mAb 1.18 and analyzed by flow cytometry as described in Example 2. The data reveal binding of anti-B7-H6 mAb 1.18 to cell lines of different origin.

Example 5: Quantitative real-time PCR to determine B7-H6 mRNA expression: RNA was isolated from tumor cell lines using the RNeasy Mini kit (Qiagen), contaminating

DNA was removed using TURBO DNase (Ambion) and the RNA was reverse transcribed using the ProtoScript M-MuLV First Strand cDNA synthesis kit (NEB). Quantitative real-time PCR was performed using the SYBR Green I Master and LightCycler480 (Roche). Specific primers for B7-H6 (GACCTGGAGCCATTGTGTCT as shown in SEQ ID NO: 23 and AAGCTGGACTGTTCCCTGTG as shown in SEQ ID NO: 24) and the housekeeping gene GAPDH (GCAAATTCCATGGCACCGT as shown in SEQ ID NO: 25 and TCGCCCCACTTGATTTTGG as shown in SEQ ID NO: 26) were used in order to calculate the B7-H6 mRNA expression level relative to GAPDH. The data depict that cell lines of different origin that are stained with anti-B7-H6 mAb 1.18 express mRNA of B7-H6 in different amounts.

Example 6: Immunohistochemical staining of B7-H6 on cytospins of Ba/F3-B7-H6 transfectants. Acetone-fixed cytospins of a 1:1 mixture of Ba/F3 and Ba/F3-B7-H6 cells were stained using the Dual Envision+ System-HRP (Dako). After blocking endogenous peroxidase activity, cytospins were blocked with 10% goat serum and 0.1 mg/ml human IgG. The cytospins were incubated with 5 µg/ml anti-B7-H6 mAb 1.18 or a mouse IgG1 isotype control (clone 11711, R&D) in Dako antibody diluent, washed and incubated with Dako Peroxidase labeled polymer conjugated to goat anti-mouse and goat anti-rabbit immunoglobulins. After incubation with 3,3'-diaminobenzidine (DAB) substrate solution, cell nuclei were counterstained with Hematoxylin and mounted cyotospins were analyzed by light microscopy. The data reveal that anti-B7-H6 mAb 1.18 stains B7-H6 Ba/F3-B7-H6 transfectants on cytospins.

Example 7: Degranulation of primary NK cells after co-culture with BA/F3 cells transduced with B7-H6. Primary NK cells expanded with IL-2 for 14 days were cultured in media, with BA/F3, BA/F3-B7-H6 (ligand for NKp30) or BA/F3-MICA (ligand for the activating receptor NKG2D) cells in the presence of a PE-conjugated anti-CD107 mAb for 5h. Degranulation of NK cells was determined as percentages of CD107-positive NK cells after co-culture by flow cytometry. Error bars depict mean+/- SD of triplicate cultures. The data reveal that BA/F3-B7-H6 cells induce degranulation of primary NK cells.

Claims

1. An antibody which specifically binds to an epitope formed by a portion of the extracellular domain of the B7-H6 polypeptide, said portion having an amino acid sequence as shown in SEQ ID NO: 22.

2. The antibody of claim 1, wherein said antibody comprises complementarity determining regions (CDRs) as shown in SEQ ID NOs: 5, 7, 9, 15, 17, and 19.

3. The antibody of claims 1 or 2, wherein said antibody is a monoclonal antibody.

4. The antibody of any one of claims 1 to 3, wherein said antibody is the antibody deposited under accession number DSM ACC 3117 at the DSMZ, Braunschweig, Germany under the Budapest treaty on February 02, 2011.

5. An antibody of any one of claims 1 to 4 for use in the treatment or diagnosis of cancer.

6. The antibody of claim 4, wherein the cancer is T cell lymphoma, myeloid leukemia, colon carcinoma, B cell lymphoma, melanoma, or cervical carcinoma.

7. An antibody of any one of claims 1 to 4, for use in the treatment or diagnosis of inflammatory disease.

8. The antibody of claim 7, wherein the inflammatory disease is a viral infection.

9. A method for diagnosing cancer in a sample of a subject suspected to suffer from cancer comprising:
   a) contacting the sample with the antibody of any one of claims 1 to 4 under conditions which allow for binding of said antibody to its epitope on the B7-H6 polypeptide; and
   b) determining binding of the antibody to the said epitope, whereby cancer is diagnosed.

10. The method of claim 9, wherein the cancer is T cell lymphoma, myeloid leukemia, colon carcinoma, B cell lymphoma, melanoma, or cervical carcinoma.

11. A method for diagnosing an inflammatory disease in a sample of a subject suspected to suffer from an inflammatory disease comprising:
    a) contacting the sample with the antibody of any one of claims 1 to 4 under conditions which allow for binding of said antibody to its epitope on the B7-H6 polypeptide; and
    b) determining binding of the antibody to the said epitope, whereby the inflammatory disease is diagnosed.

12. The method of any one of claims 9 to 11, wherein said sample is a tissue or body fluid sample.

13. A device for diagnosing cancer or an inflammatory disease in a sample comprising:
    a) an analyzing unit comprising the antibody of any one of claims 1 to 4; and
    b) a detector which detects binding of the antibody in the analyzing unit to its epitope on the B7-H6 polypeptide.

14. The device of claim 13, wherein said sample is a tissue or body fluid sample.

15. A kit for diagnosing cancer or an inflammatory disease comprising the antibody of any one of claims 1 to 4 and, preferably, an agent for detection of binding of said antibody to its epitope on the B7-H6 polypeptide.

SEQUENCE LISTING

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      285


Ile Met His Gln Asp Trp Leu Asn Gly Lys
Glu Phe Lys Cys Arg Val
    290                 295
  300


Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu
Lys Thr Ile Ser Lys Thr
305                 310
315                 320


Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr
Thr Ile Pro Pro Pro Lys
                325                 330
              335
```

```
Glu Gln Met Ala Lys Asp Lys Val Ser Leu
Thr Cys Met Ile Thr Asp
            340                 345
          350


Phe Phe Pro Glu Asp Ile Thr Val Glu Trp
Gln Trp Asn Gly Gln Pro
        355                 360
      365


Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile
Met Asn Thr Asn Gly Ser
    370                 375
  380


Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln
Lys Ser Asn Trp Glu Ala
385                 390
395                 400


Gly Asn Thr Phe Thr Cys Ser Val Leu His
Glu Gly Leu His Asn His
                405                 410
              415


His Thr Glu Lys Ser Leu Ser His Ser Pro
Gly Lys
            420                 425
```

<210> 12
<211> 498
<212> PRT
<213> Artificial Sequence

<220>
<223> Amino acid sequence of the heavy chain of anti-B7-H6 clone 1.18 (IGHV/IGHD/IGHJ/IGHG1), membrane bound form

<400> 12

Asp Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Thr Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
35 40 45

```
Ala Thr Ile Asn Asn Gly Gly Ser Tyr Thr
Tyr Tyr Pro Asp Ser Val
    50                  55
   60


Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn
Ala Lys Asn Thr Leu Tyr
65                  70
75                  80


Leu Gln Met Ser Ser Leu Lys Ser Glu Asp
Thr Ala Ile Tyr Tyr Cys
                 85                  90
                95


Tyr Gly Tyr Asp Pro Ala Trp Phe Ala Tyr
Trp Gly Gln Gly Thr Leu
             100                 105
           110


Val Thr Val Ser Ala Ala Lys Thr Thr Pro
Pro Ser Val Tyr Pro Leu
         115                 120
       125


Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser
Met Val Thr Leu Gly Cys
```

130 135
140

Leu Val Lys Gly Tyr Phe Pro Glu Pro Val
Thr Val Thr Trp Asn Ser
145 150
155 160

Gly Ser Leu Ser Ser Gly Val His Thr Phe
Pro Ala Val Leu Glu Ser
165 170
175

Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr
Val Pro Ser Ser Pro Arg
180 185
190

Pro Ser Glu Thr Val Thr Cys Asn Val Ala
His Pro Ala Ser Ser Thr
195 200
205

Lys Val Asp Lys Lys Ile Val Pro Glu Val
Ser Ser Val Phe Ile Phe
210 215
220

```
Pro Pro Lys Pro Lys Asp Val Leu Thr Ile
Thr Leu Thr Pro Lys Val
225                 230
235                 240


Thr Cys Val Val Val Asp Ile Ser Lys Asp
Asp Pro Glu Val Gln Phe
                245                 250
               255


Ser Trp Phe Val Asp Asp Val Glu Val His
Thr Ala Gln Thr Gln Pro
            260                 265
           270


Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg
Ser Val Ser Glu Leu Pro
        275                 280
       285


Ile Met His Gln Asp Trp Leu Asn Gly Lys
Glu Phe Lys Cys Arg Val
    290                 295
   300


Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu
Lys Thr Ile Ser Lys Thr
```

305 310
315 320

Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr
Thr Ile Pro Pro Pro Lys
325 330
335

Glu Gln Met Ala Lys Asp Lys Val Ser Leu
Thr Cys Met Ile Thr Asp
340 345
350

Phe Phe Pro Glu Asp Ile Thr Val Glu Trp
Gln Trp Asn Gly Gln Pro
355 360
365

Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile
Met Asn Thr Asn Gly Ser
370 375
380

Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln
Lys Ser Asn Trp Glu Ala
385 390
395 400

```
Gly Asn Thr Phe Thr Cys Ser Val Leu His
Glu Gly Leu His Asn His
                405                 410
            415


His Thr Glu Lys Ser Leu Ser His Ser Pro
Gly Lys Leu Gln Leu Asp
            420                 425
        430


Glu Thr Cys Ala Glu Ala Gln Asp Gly Glu
Leu Asp Gly Leu Trp Thr
        435                 440
    445


Thr Ile Thr Ile Phe Ile Ser Leu Phe Leu
Leu Ser Val Cys Tyr Ser
    450                 455
460


Ala Ala Val Thr Leu Phe Lys Val Lys Trp
Ile Phe Ser Ser Val Val
465                 470
475                 480


Glu Leu Lys Gln Thr Leu Val Pro Glu Tyr
Lys Asn Met Ile Gly Gln
```

485 490
495

Ala Pro

<210> 13
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> Amino acid sequence of the light chain of anti-B7-H6 clone 1.18 (IGLV/IGLJ)

<400> 13

Gln Ala Val Val Thr Gln Glu Ser Ala Leu Thr Thr Ser Pro Gly Glu
1 5 10 15

Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30

Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro

Asp His Leu Phe Thr Gly
35 40
45

Leu Ile Gly Gly Thr Asn Asn Arg Ala Pro
Gly Val Pro Ala Arg Phe
50 55
60

Ser Gly Ser Leu Ile Gly Asp Lys Ala Ala
Leu Thr Ile Thr Gly Ala
65 70
75 80

Gln Thr Glu Asp Glu Ala Ile Tyr Phe Cys
Ala Leu Trp Tyr Ser Asn
85 90
95

His Trp Val Phe Gly Gly Gly Thr Lys Leu
Thr Val Leu
100 105

<210> 14
<211> 75
<212> PRT
<213> Artificial Sequence

<220>
<223> Nucleic acid sequence of the light chain of anti-B7-H6 clone 1.18 (IGLV/IGLJ), FR1

<400> 14

Cys Ala Gly Gly Cys Thr Gly Thr Thr Gly Thr Gly Ala Cys Thr Cys
1   5   10   15

Ala Gly Gly Ala Ala Thr Cys Thr Gly Cys Ala Cys Thr Cys Ala Cys
            20   25   30

Cys Ala Cys Ala Thr Cys Ala Cys Cys Thr Gly Gly Thr Gly Ala Ala
        35   40   45

Ala Cys Ala Gly Thr Cys Ala Cys Ala Cys Thr Cys Ala Cys Thr Thr
    50   55   60

Gly Thr Cys Gly Cys Thr Cys Ala Ala Gly

Thr
65 70
75

<210> 15
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleic acid sequence of the light chain of anti-B7-H6 clone 1.18 (IGLV/IGLJ), CDR1

<400> 15
actggggctg ttacaactag taactat
27

<210> 16
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleic acid sequence of the light chain of anti-B7-H6 clone 1.18 (IGLV/IGLJ), FR2

<400> 16
gccaactggg tccaagaaaa accagatcat
ttattcactg gtctaatagg t

51

<210> 17
<211> 9
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleic acid sequence of the light chain of anti-B7-H6 clone 1.18 (IGLV/IGLJ), CDR2

<400> 17
ggtaccaac 9

<210> 18
<211> 108
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleic acid sequence of the light chain of anti-B7-H6 clone 1.18 (IGLV/IGLJ), FR3

<400> 18
aaccgagctc caggtgttcc tgccagattc tcaggctccc tgattggaga caaggctgcc 60

ctcaccatca caggggcaca gactgaggat gaggcaatat atttctgt
108

<210> 19
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleic acid sequence of the light chain of anti-B7-H6 clone 1.18 (IGLV/IGLJ), CDR3

<400> 19
gctctatggt acagcaacca ctgggtg
27

<210> 20
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleic acid sequence of the light chain of anti-B7-H6 clone 1.18 (IGLV/IGLJ), FR4

<400> 20
ttcggtggag gaaccaaact gactgtcctg
30

<210> 21
<211> 215
<212> PRT
<213> Artificial Sequence

<220>
<223> Amino acid sequence of the light chain of anti-B7-H6 clone 1.18 (IGLV/IGLJ/IGLC)

<400> 21

Gln Ala Val Val Thr Gln Glu Ser Ala Leu Thr Thr Ser Pro Gly Glu
1 5 10 15

Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30

Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Asp His Leu Phe Thr Gly
35 40 45

Leu Ile Gly Gly Thr Asn Asn Arg Ala Pro

```
Gly Val Pro Ala Arg Phe
     50                  55
    60


Ser Gly Ser Leu Ile Gly Asp Lys Ala Ala
Leu Thr Ile Thr Gly Ala
65                  70
75                  80


Gln Thr Glu Asp Glu Ala Ile Tyr Phe Cys
Ala Leu Trp Tyr Ser Asn
                  85                  90
                95


His Trp Val Phe Gly Gly Gly Thr Lys Leu
Thr Val Leu Gly Gln Pro
             100                 105
           110


Lys Ser Ser Pro Ser Val Thr Leu Phe Pro
Pro Ser Ser Glu Glu Leu
         115                 120
       125


Glu Thr Asn Lys Ala Thr Leu Val Cys Thr
Ile Thr Asp Phe Tyr Pro
     130                 135
    140
```

```
Gly Val Val Thr Val Asp Trp Lys Val Asp
Gly Thr Pro Val Thr Gln
145                 150
155                 160


Gly Met Glu Thr Thr Gln Pro Ser Lys Gln
Ser Asn Asn Lys Tyr Met
                165                 170
               175


Ala Ser Ser Tyr Leu Thr Leu Thr Ala Arg
Ala Trp Glu Arg His Ser
            180                 185
           190


Ser Tyr Ser Cys Gln Val Thr His Glu Gly
His Thr Val Glu Lys Ser
        195                 200
       205


Leu Ser Arg Ala Asp Cys Ser
    210                 215


<210>  22
<211>  58
<212>  PRT
```

<213> Homo sapiens

<400> 22

Gly Asp His Gln Glu Ala Phe Arg Pro Gly
Ala Ile Val Ser Pro Trp
1 5 10
15

Arg Leu Lys Ser Gly Asp Ala Ser Leu Arg
Leu Pro Gly Ile Gln Leu
20 25
30

Glu Glu Ala Gly Glu Tyr Arg Cys Glu Val
Val Val Thr Pro Leu Lys
35 40
45

Ala Gln Gly Thr Val Gln Leu Glu Val Val
50 55

<210> 23
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Forward primer for B7-H6

<400> 23
gacctggagc cattgtgtct
20

<210> 24
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Reverse primer for B7-H6

<400> 24
aagctggact gttccctgtg
20

<210> 25
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Forward primer for GADPH

<400> 25
gcaaattcca tggcaccgt
19

<210> 26

<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Reverse primer for GADPH

<400> 26
tcgccccact tgattttgg
19