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(54) Title: METHODS AND COMPOSITIONS FOR PREDICTING AND TREATING MYELOMA

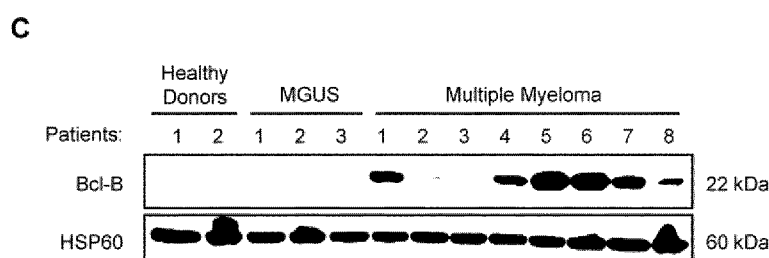


Figure 1C

(57) **Abstract:** The inventors have used the Eμ-bcl-b transgenic mouse model to study the effect of the Bcl-B protein on the evolution of monoclonal gammopathy of undetermined significance (MGUS) to myeloma. They have performed the Bcl-B-positive plasma cell quantification by flow cytometry analysis of bone marrow cells from an MGUS and an multiple myeloma (MM) patient. They have showed that a subject having a high concentration of Bcl-B than a predetermined value is at risk of having myeloma. Accordingly, the invention relates to a method for predicting the risk of having myeloma in a subject suffering from MGUS comprising the steps of: i) measuring the concentration of Bcl-B in a biological sample obtained from said subject, ii) comparing the concentration obtained at step (i) to a predetermined value, and iii) concluding that the subject is at risk of having myeloma when the concentration of Bcl-B is higher than the predetermined value.



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METHODS AND COMPOSITIONS FOR PREDICTING AND TREATING MYELOMA

5 **FIELD OF THE INVENTION:**

The invention is in the field of oncology, particularly the invention relates to use of Bcl-B as a biomarker to predict the evolution of MGUS to myeloma.

BACKGROUND OF THE INVENTION:

10 Myeloma, also known as Multiple myeloma (MM), is a type of bone marrow cancer arising from plasma cells. It corresponds to a malignant condition which evolves from monoclonal gammopathy of undetermined significance (MGUS) to indolent myeloma then to myeloma. Each myeloma evolution step is characterized by the percentage of plasma cells: at step MGUS, the percentage is about to 5 -10%; at stage myeloma indolent, the percentage is about 10% and at step myeloma, the percentage is higher than 10%, the subject presents
15 kidneys and bone damages.

Autologous stem cell transplantation remains a good option for treating MM; however, all patients will unavoidably relapse. Therefore, considerable efforts have been devoted to the development of new therapeutic options for the treatment of patients suffering from MM. Although drugs such as lenalidomide (Revlimid®) and bortezomib (Velcade®) have
20 significantly improved the overall survival rates of patients, MM remains an incurable disease, and there is an urgent need for new therapies (Ludwig et al., 2010; Strobeck, 2007).

Among the 6 human anti-apoptotic Bcl-2 family proteins (Bcl-2 [BCL2], Bcl-XL [BCL2L1], Mcl-1 [MCL1], Bcl-W [BCL2L2], Bfl-1 [BCL2A1], Bcl-B [BCL2L10]) (Adams and Cory, 1998), Bcl-B was the last anti-apoptotic member to be identified, and its
25 physiological function is only partially understood (Ke et al., 2001; Zhai et al., 2003). Bcl-B is expressed predominantly in normal human B lymphocytes and is pathologically overexpressed in malignant plasma cells and many types of solid tumors such as prostate, mammary, colorectal and lung carcinomas (Krajewska et al., 2008; Luciano et al., 2007). Mice lack the Bcl-B gene; however, the closest homolog (Diva/Boo) is predominantly
30 expressed in ovary and testis and exhibits a pro-apoptotic rather than an anti-apoptotic phenotype (Inohara et al., 1998).

MGUS is an asymptomatic plasma cell associated with an overproduction of plasma cell generated monoclonal protein, but is otherwise asymptomatic. While the diagnosis of MGUS requires the absence of anemia, hypercalcemia, renal failure, and lytic bone lesions

related to the plasma cell, MGUS is an asymptomatic myeloproliferative disorder characterized by monoclonal plasma cell proliferation. It is most often found when a routine blood test finds a high level of protein in the blood and further testing shows the protein is a monoclonal antibody. In MGUS, the number of plasma cells may be increased, but they still
5 make up less than 10% of the cells in the bone marrow. As MGUS is asymptomatic, many subjects are not treated to avoid to develop MM. Thus, some people with MGUS will develop multiple myeloma. Each year, about 1% of people with MGUS develops multiple myeloma. There is a need to diagnose the subjects suffering from MGUS and treat them at the early stage to avoid myeloma.

10

SUMMARY OF THE INVENTION:

The present invention relates to a method for predicting the risk of having myeloma in a subject suffering from MGUS comprising the steps of:

- i) measuring the concentration of Bcl-B in a biological sample obtained from said
15 subject,
- ii) comparing the concentration obtained at step (i) to a predetermined value, and
- iii) concluding that the subject is at risk of having myeloma when the concentration of Bcl-B higher than the predetermined value or concluding that the subject is not at risk of having myeloma when the concentration of Bcl-B lower than
20 the predetermined value. In particular, the present invention is defined by the claims.

DETAILED DESCRIPTION OF THE INVENTION:

The inventors have shown that the Bcl-B could be used as a biomarker to predict whether a subject suffering from MGUS will have myeloma. Indeed, they have surprisingly
25 observed that the subject suffering from MGUS has a concentration of Bcl-B higher than healthy subject and develop myeloma.

Method of prediction of the risk to have myeloma

Accordingly, the present invention relates to a method for predicting the risk of having
30 myeloma in a subject suffering from MGUS comprising the steps of:

- i) measuring the concentration of Bcl-B in a biological sample obtained from said subject,
- ii) comparing the concentration obtained at step (i) to a predetermined value, and
- iii) concluding that the subject is at risk of having myeloma when the concentration of Bcl-B

is higher than the predetermined value or concluding that the subject is not at risk of having myeloma when the concentration of Bcl-B is lower than the predetermined value.

As used herein, the term “predicting” means that the subject to be analyzed by the method of the invention is allocated either into the group of subjects who will develop MM, or into a group of subjects who will not develop MM. Develop MM referred to in accordance with the invention, particularly, means that the subject will have higher risk to develop MM. Typically, said risk is elevated as compared to the average risk in a cohort of subjects suffering from MGUS.

In the context of the invention, the risk of having the myeloma in a subject suffering from MGUS shall be predicted. The term “predicting the risk”, as used herein, refers to assessing the probability according to which the patient as referred to herein will develop myeloma.

As will be understood by those skilled in the art, such an assessment is usually not intended to be correct for 100% of the subjects to be investigated. The term, however, requires that prediction can be made for a statistically significant portion of subjects in a proper and correct manner. 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. Preferred confidence intervals are at least 90%, at least 95%, at least 97%, at least 98% or at least 99 %. The p-values are, preferably, 0.1, 0.05, 0.01, 0.005, or 0.0001. Preferably, the probability envisaged by the invention allows that the prediction of an increased risk will be correct for at least 60%, at least 70%, at least 80%), or at least 90% of the subjects of a given cohort or population. The term, preferably, relates to predicting whether or not there is an increased risk of developing myeloma compared to the average risk of myeloma in a population of subjects rather than giving a precise probability for the said risk.

As used herein, the term “Bcl-B” also called as BCL2L10 refers to a protein that in humans is encoded by the BCL2L10 gene. The naturally occurring human Bcl-B gene has a nucleotide sequence as shown in Genbank Accession numbers NM_001306168.1 (variant 1) and NM_020396.3 (variant 2). The naturally occurring human Bcl-B protein has an amino

acid sequence as shown in Genbank Accession numbers NP_001293097.1 (variant1) and NP_065129.1 (variant2).

As used herein, the term “Myeloma”, also known as multiple myeloma (MM), refers to a cancer arising from plasma cells, a type of white blood cell which is made in the bone marrow.

As used herein, the term “MGUS” refers to Monoclonal Gammopathy of Undetermined Significance (MGUS) is a common asymptomatic plasma cell disorder with a variable stable period. It may eventually progress to severe symptomatic multiple myeloma and, therefore, needs periodic monitoring. The incidence of MGUS increases with age, affecting approximately 3% of population more than 50 years of age and up to 10% in those more than 70 years of age. Diagnosis of MGUS is characterized by the presence of a monoclonal immunoglobulin in serum < 30 g/l and $< 10\%$ of plasma cells in bone marrow, in the absence of end organ damage related to the proliferation of monoclonal plasma cell, see Perez-Persona et al. 2007, Blood 110, 2586-2592; Kyle and Rajkumar 2007, British J Haem 139, 730-743.

The “predetermined value” according to the invention can be a single value such as a reference value derived from the concentration of Bcl-B in biological samples obtained from subjects who are at MGUS stages, or a control value derived from the concentration of Bcl-B in blood samples from healthy subjects.

For example the concentration of Bcl-B has been assessed for 100 blood samples of 100 patients. The 100 samples are ranked according to the concentration of Bcl-B. Sample 1 has the highest level and sample 100 has the lowest level. A first grouping provides two subsets: on one side sample Nr 1 and on the other side the 99 other samples. The next grouping provides on one side samples 1 and 2 and on the other side the 98 remaining samples etc., until the last grouping: on one side samples 1 to 99 and on the other side sample Nr 100. According to the information relating to the actual clinical outcome for the corresponding subject suffering from MGUS, for each of the 99 groups of two subsets, estimates of survival were calculated using the Kaplan Meier method (or any other survival analysis method) and tested with the log-rank test (or any other suitable test). The predetermined reference value is then selected such as the discrimination based on the criterion of the minimum p value is the strongest. In other terms, the concentration of Bcl-B corresponding to the boundary between both subsets for which the p value is minimum is considered as the predetermined reference value. It should be noted that the predetermined reference value is not necessarily the median value of concentration of Bcl-B.

The setting of a single "cut-off" value thus allows discrimination between a poor and a good prognosis with respect to the overall survival (OS) for a patient. Practically, high statistical significance values (e.g. low P values) are generally obtained for a range of successive arbitrary quantification values, and not only for a single arbitrary quantification value. Thus, in one alternative embodiment of the invention, instead of using a definite predetermined reference value, a range of values is provided. Therefore, a minimal statistical significance value (minimal threshold of significance, e.g. maximal threshold P value) is arbitrarily set and a range of a plurality of arbitrary quantification values for which the statistical significance value calculated at step g) is higher (more significant, e.g. lower P value) are retained, so that a range of quantification values is provided. This range of quantification values includes a "cut-off" (for which the p value is the lowest) value as described above. For example, on a hypothetical scale of 1 to 10, if the ideal cut-off value (the value with the highest statistical significance) is 5, a suitable (exemplary) range may be from 4-6. Therefore, a subject may be assessed by comparing values obtained by measuring the concentration of Bcl-B, where values greater than 5 reveal a poor prognosis and values less than 5 reveal a good prognosis. In a another embodiment, a subject may be assessed by comparing values obtained by measuring the concentration of Bcl-B and comparing the values on a scale, where values above the range of 4-6 indicate a poor prognosis and values below the range of 4-6 indicate a good prognosis, with values falling within the range of 4-6 indicating an intermediate prognosis.

Accordingly, in a particular embodiment, a concentration of Bcl-B higher than the predetermined value is indicative of an increased risk of having myeloma.

As used herein, the term "concentration of "Bcl-B" refers to an amount or a concentration of a transcription product, for instance mRNA coding for Bcl-B, or of a translation product, for instance the protein Bcl-B.

As used herein, the term "biological sample" refers to a sample obtained from a subject, for example blood, saliva, breast milk, urine, semen, blood plasma, synovial fluid, serum or a tumor tissue.

In a particular embodiment, the biological sample is a tumor tissue sample. As used herein, the term "tumor tissue sample" has its general meaning in the art and encompasses pieces or slices of tissue that have been removed including following a surgical tumor resection. The tumor tissue sample can be subjected to a variety of well-known post-collection preparative and storage techniques (e.g., fixation, storage, freezing, etc.) prior to

determining the cell densities. Typically the tumor tissue sample is fixed in formalin and embedded in a rigid fixative, such as paraffin (wax) or epoxy, which is placed in a mould and later hardened to produce a block which is readily cut. Thin slices of material can be then prepared using a microtome, placed on a glass slide and submitted e.g. to
5 immunohistochemistry (IHC) (using an IHC automate such as BenchMark® XT or Autostainer Dako, for obtaining stained slides). The tumour tissue sample can be used in microarrays, called as tissue microarrays (TMAs). TMA consists of paraffin blocks in which up to 1000 separate tissue cores are assembled in array fashion to allow multiplex histological analysis. This technology allows rapid visualization of molecular targets in tissue specimens
10 at a time, either at the DNA, RNA or protein level. TMA technology is described in WO2004000992, US8068988, Olli et al 2001 Human Molecular Genetics, Tzankov et al 2005, Elsevier; Kononen et al 1198; Nature Medicine.

In a particular embodiment, the tumor tissue sample, in the context of the invention, is bone marrow. Typically, the bone marrow cells are isolated from the subjects suffering from
15 MGUS. A skilled man knows different methods to isolate the bone marrow cells. For example, CD138+ cells from whole bone marrow samples are magnetically labeled with Whole Blood CD138 MicroBeads. Once the pre-set separation program is selected, the sample is automatically loaded onto the autoMACS Column of the autoMACS Pro Separator. CD138+ cells are magnetically retained within the column, while unlabeled cells are collected
20 in the flow-through as the negative fraction. Hence, this cell fraction is now depleted of CD138+ cells. After retraction of the magnet from the column, CD138+ cells are automatically eluted as the positive fraction and can be immediately subjected to downstream applications (Miltenyibiotec).

Once the biological sample from the subject is prepared, the concentration of Bcl-B
25 may be measured by any known method in the art.

Typically, the concentration of Bcl-B is measured at Bcl-B gene level.

In a particular embodiment, the expression of Bcl-B is quantified by determining the expression level of gene Bcl-B at step i). Typically, the expression level of gene Bcl-B may be quantified by determining the quantity of mRNA. Methods for determining the quantity of
30 mRNA are well known in the art. For example the nucleic acid contained in the samples (e.g., cell or tissue prepared from the subject) is first extracted according to standard methods, for example using lytic enzymes or chemical solutions or extracted by nucleic-acid-binding resins following the manufacturer's instructions. The extracted mRNA is then detected by hybridization (e. g., Northern blot analysis) and/or amplification (e.g., RT-PCR). Preferably

quantitative or semi-quantitative RT-PCR is preferred. Real-time quantitative or semi-quantitative RT-PCR is particularly advantageous. Other methods of amplification include ligase chain reaction (LCR), transcription-mediated amplification (TMA), strand displacement amplification (SDA) and nucleic acid sequence based amplification (NASBA).

5 Nucleic acids having at least 10 nucleotides and exhibiting sequence complementarity or homology to the mRNA of Bcl-B find utility as hybridization probes or amplification primers. It is understood that such nucleic acids need not be identical, but are typically at least about 80% identical to the homologous region of comparable size, more preferably 85% identical and even more preferably 90-95% identical. In certain embodiments, it will be
10 advantageous to use nucleic acids in combination with appropriate means, such as a detectable label, for detecting hybridization. A wide variety of appropriate indicators are known in the art including, fluorescent, radioactive, enzymatic or other ligands (e. g. avidin/biotin).

Probes typically comprise single-stranded nucleic acids of between 10 to 1000
15 nucleotides in length, for instance of between 10 and 800, more preferably of between 15 and 700, typically of between 20 and 500. Primers typically are shorter single-stranded nucleic acids, of between 10 to 25 nucleotides in length, designed to perfectly or almost perfectly match a nucleic acid of interest, to be amplified. The probes and primers are "specific" to the nucleic acids they hybridize to, i.e. they preferably hybridize under high stringency
20 hybridization conditions (corresponding to the highest melting temperature T_m , e.g., 50 % formamide, 5x or 6x SCC. SCC is a 0.15 M NaCl, 0.015 M Na-citrate). The nucleic acid primers or probes used in the above amplification and detection method may be assembled as a kit. Such a kit includes consensus primers and molecular probes. A preferred kit also includes the components necessary to determine if amplification has occurred. The kit may
25 also include, for example, PCR buffers and enzymes; positive control sequences, reaction control primers; and instructions for amplifying and detecting the specific sequences.

In a particular embodiment, the methods of the invention comprise the steps of providing total RNAs extracted from cumulus cells and subjecting the RNAs to amplification and hybridization to specific probes, more particularly by means of a quantitative or semi-
30 quantitative RT-PCR.

In another preferred embodiment, the expression level of gene Bcl-B is determined by DNA chip analysis. Such DNA chip or nucleic acid microarray consists of different nucleic acid probes that are chemically attached to a substrate, which can be a microchip, a glass slide or a microsphere-sized bead. A microchip may be constituted of polymers, plastics, resins,

polysaccharides, silica or silica-based materials, carbon, metals, inorganic glasses, or nitrocellulose. Probes comprise nucleic acids such as cDNAs or oligonucleotides that may be about 10 to about 60 base pairs. To determine the expression level, a sample from a test subject, optionally first subjected to a reverse transcription, is labelled and contacted with the microarray in hybridization conditions, leading to the formation of complexes between target nucleic acids that are complementary to probe sequences attached to the microarray surface. The labelled hybridized complexes are then detected and can be quantified or semi-quantified. Labelling may be achieved by various methods, e.g. by using radioactive or fluorescent labelling. Many variants of the microarray hybridization technology are available to the man skilled in the art (see e.g. the review by Hoheisel, Nature Reviews, Genetics, 2006, 7:200-210).

In a particular embodiment, the concentration of Bcl-B is measured by determining the expression level of protein Bcl-B.

Methods for determining protein Bcl-B level in a sample are well-known in the art. Examples of such methods include, but are not limited to, Multiplex methods (Luminex), western blot, enzyme-linked immunosorbent assay (ELISA), sandwich ELISA, fluorescent-linked immunosorbent assay (FLISA), enzyme immunoassay (EIA), radioimmunoassay (RIA) and the like. A reference value can be relative to a number or value derived from population studies, including without limitation, such subjects having similar age range, subjects in the same or similar ethnic group, and the like. In one embodiment, the reference value is constructed using algorithms and other methods of statistical and structural classification. In one embodiment of the invention, the reference value is derived from the measurement of the protein level of Bcl-B in a control sample derived from one or more substantially healthy subjects. As used herein, a "substantially healthy subject" has not been previously diagnosed or identified as having or suffering from myeloma.

In a particular embodiment, the measurement of the concentration of Bcl-B protein is performed by flow cytometry (immunophenotyping) on sorted CD138 positive plasmocytes. This technique allows the analysis and sorting according to one or more parameters of the cells. Usually one or multiple secretion parameters can be analyzed simultaneously in combination with other measurable parameters of the cell, including, but not limited to, cell type, cell surface antigens, DNA content, etc. The data can be analyzed and cells can be sorted using any formula or combination of the measured parameters. Cell sorting and cell analysis methods are known in the art and are described in, for example, (The handbook of experimental immunology, Volumes 1 to 4, (D.N. Weir, editor) and flow

cytometry and cell sorting (A. Radbruch, editor, Springer Verlag, 1992). Cells can also be analyzed using microscopy techniques including, for example, laser scanning microscopy, fluorescence microscopy; techniques such as these may also be used in combination with image analysis systems. Other methods for cell sorting include, for example, panning and
5 separation using affinity techniques, including those techniques using solid supports such as plates, beads and columns.

Some methods for cell sorting utilize magnetic separations, and some of these methods utilize magnetic beads. Different magnetic beads are available from a number of sources, including for example, Miltenyi Biotec GmbH (Germany).

Method of preventing the risk of having myeloma

In another aspect, the invention relates to a method of preventing the risk of having (the prophylactic treatment) myeloma in a subject suffering from MGUS comprising a step of administering to said subject a therapeutically amount of Bcl-B antagonist.

As used herein, the terms "treating" or "treatment" refer to both prophylactic or preventive treatment as well as curative or disease modifying treatment, including treatment of subject at risk of contracting the disease or suspected to have contracted the disease as well as subject who are ill or have been diagnosed as suffering from a disease or medical
20 condition, and includes suppression of clinical relapse. The treatment may be administered to a subject having a medical disorder or who ultimately may acquire the disorder, in order to prevent, cure, delay the onset of, reduce the severity of, or ameliorate one or more symptoms of a disorder or recurring disorder, or in order to prolong the survival of a subject beyond that expected in the absence of such treatment. By "therapeutic regimen" is meant the pattern of
25 treatment of an illness, e.g., the pattern of dosing used during therapy. A therapeutic regimen may include an induction regimen and a maintenance regimen. The phrase "induction regimen" or "induction period" refers to a therapeutic regimen (or the portion of a therapeutic regimen) that is used for the initial treatment of a disease. The general goal of an induction regimen is to provide a high level of drug to a subject during the initial period of a treatment
30 regimen. An induction regimen may employ (in part or in whole) a "loading regimen", which may include administering a greater dose of the drug than a physician would employ during a maintenance regimen, administering a drug more frequently than a physician would administer the drug during a maintenance regimen, or both. The phrase "maintenance regimen" or "maintenance period" refers to a therapeutic regimen (or the portion of a

therapeutic regimen) that is used for the maintenance of a subject during treatment of an illness, e.g., to keep the subject in remission for long periods of time (months or years). A maintenance regimen may employ continuous therapy (e.g., administering a drug at a regular intervals, e.g., weekly, monthly, yearly, etc.) or intermittent therapy (e.g., interrupted
5 treatment, intermittent treatment, treatment at relapse, or treatment upon achievement of a particular predetermined criteria [e.g., pain, disease manifestation, etc.]).

The term "Bcl-B antagonist" refers to any Bcl-B antagonist that is currently known in the art or that will be identified in the future, and includes any chemical entity that, upon
10 administration to a patient, results in inhibition of a biological activity associated with activation of the Bcl-B in the subject. Typically, such antagonist inhibits the biological activity of Bcl-B, which is anti-apoptotic. Thus, the antagonist of Bcl-B induces apoptosis of cells overexpressing Bcl-B.

In a particular embodiment, the Bcl-B antagonist is an antibody.

As used herein, the term "antibody" is used in the broadest sense and specifically
15 covers monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity. In natural antibodies, two heavy chains are linked to each other by disulfide bonds and each heavy chain is linked to a light chain by a disulfide
20 bond. There are two types of light chain, lambda (l) and kappa (k). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE. Each chain contains distinct sequence domains. The light chain includes two domains, a variable domain (VL) and a constant domain (CL). The heavy chain includes four domains, a variable domain (VH) and three constant domains (CH1, CH2 and
25 CH3, collectively referred to as CH). The variable regions of both light (VL) and heavy (VH) chains determine binding recognition and specificity to the antigen. The constant region domains of the light (CL) and heavy (CH) chains confer important biological properties such as antibody chain association, secretion, trans-placental mobility, complement binding, and binding to Fc receptors (FcR). The term includes antibody fragments that comprise an antigen
30 binding domain such as Fab', Fab, F(ab')₂, single domain antibodies (DABs), TandAbs dimer, Fv, scFv (single chain Fv), dsFv, ds-scFv, Fd, linear antibodies, minibodies, diabodies, bispecific antibody fragments, bibody, tribody (scFv-Fab fusions, bispecific or trispecific, respectively); sc-diabody; kappa(lamda) bodies (scFv-CL fusions); BiTE (Bispecific T-cell Engager, scFv-scFv tandems to attract T cells); DVD-Ig (dual variable domain antibody,

bispecific format); SIP (small immunoprotein, a kind of minibody); SMIP ("small modular immunopharmaceutical" scFv-Fc dimer; DART (ds-stabilized diabody "Dual Affinity ReTargeting"); small antibody mimetics comprising one or more CDRs and the like. The techniques for preparing and using various antibody-based constructs and fragments are well known in the art (see Kabat et al., 1991, specifically incorporated herein by reference). Diabodies, in particular, are further described in EP 404, 097 and WO 93/1 1 161; whereas linear antibodies are further described in Zapata et al. (1995). Antibodies can be fragmented using conventional techniques. For example, F(ab')₂ fragments can be generated by treating the antibody with pepsin. The resulting F(ab')₂ fragment can be treated to reduce disulfide bridges to produce Fab' fragments. Papain digestion can lead to the formation of Fab fragments. Fab, Fab' and F(ab')₂, scFv, Fv, dsFv, Fd, dAbs, TandAbs, ds-scFv, dimers, minibodies, diabodies, bispecific antibody fragments and other fragments can also be synthesized by recombinant techniques or can be chemically synthesized. Techniques for producing antibody fragments are well known and described in the art. For example, each of Beckman et al., 2006; Holliger & Hudson, 2005; Le Gall et al., 2004; Reff & Heard, 2001 ; Reiter et al., 1996; and Young et al., 1995 further describe and enable the production of effective antibody fragments. In some embodiments, the antibody is a "chimeric" antibody as described in U.S. Pat. No. 4,816,567. In some embodiments, the antibody is a humanized antibody, such as described U.S. Pat. Nos. 6,982,321 and 7,087,409. In some embodiments, the antibody is a human antibody. A "human antibody" such as described in US 6,075,181 and 6,150,584. In some embodiments, the antibody is a single domain antibody such as described in EP 0 368 684, WO 06/030220 and WO 06/003388.

In a particular embodiment, the Bcl-B antibody is a monoclonal antibody.

Monoclonal antibodies can be prepared and isolated using any technique that provides for the production of antibody molecules by continuous cell lines in culture. Techniques for production and isolation include but are not limited to the hybridoma technique, the human B-cell hybridoma technique and the EBV-hybridoma technique.

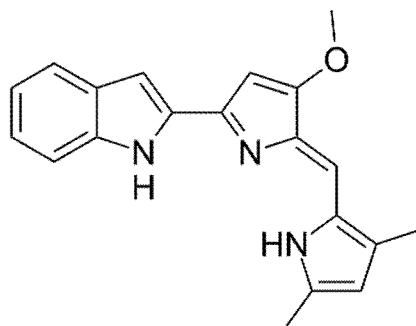
In some embodiments, the Bcl-B antagonist is a small molecule.

The term "small organic molecule" as used herein, refers to a molecule of a size comparable to those organic molecules generally used in pharmaceuticals. The term excludes biological macro molecules (e. g. proteins, nucleic acids, etc.). Typically, small organic molecules range in size up to about 5000 Da, more preferably up to 2000 Da, and most

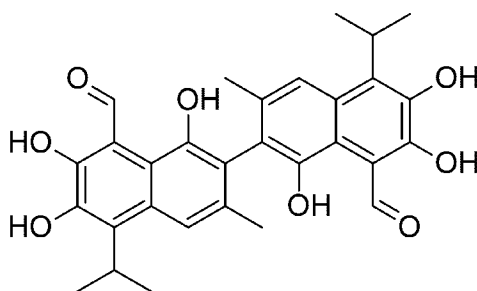
preferably up to about 1000 Da. The small organic molecules interfere with transduction pathway which induces apoptosis of malignant cells overexpressing Bcl-B.

In a particular embodiment, the small molecule is GX15-070 also called as Obatoclax (Parikh et al 2010; Goard et al 2013; Peter et al 2014) having the following chemical structure

5 2-(2-((3,5-Dimethyl-1H-pyrrol-2-yl)methylene)-3-methoxy-2H-pyrrol-5-yl)-1H-indole:



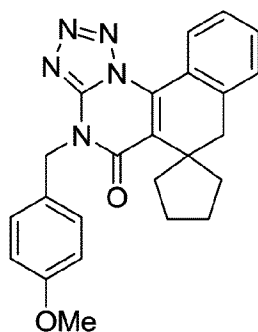
In a particular embodiment, the small molecule is Gossypol having the following chemical structure in the art (RM et al 2005; GAO et al 2010) 2,2'-Bis(formyl-1,6,7-trihydroxy-5-isopropyl-3-methylnaphthalene), C₃₀H₃₀O₈:



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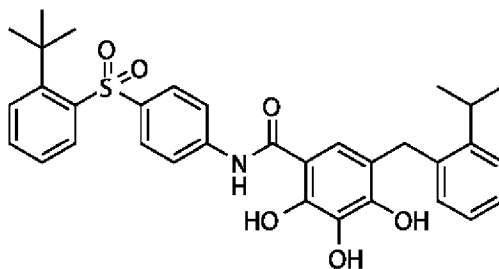
In a particular embodiment, the small molecule is ML-258 having the following chemical structure in the art (Zou et al 2013) 4-(4-methoxybenzyl)-4H-spiro[benzo[h]tetrazolo[1,5-a]quinazoline- 6,1'-cyclopentan]-5(7H)-one:

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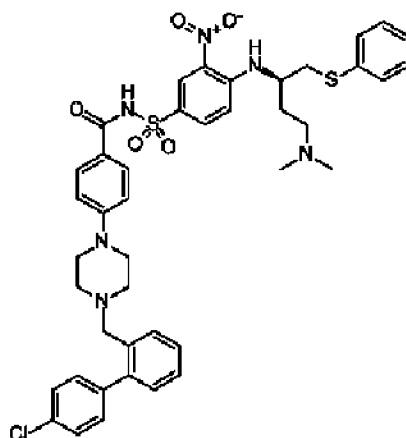


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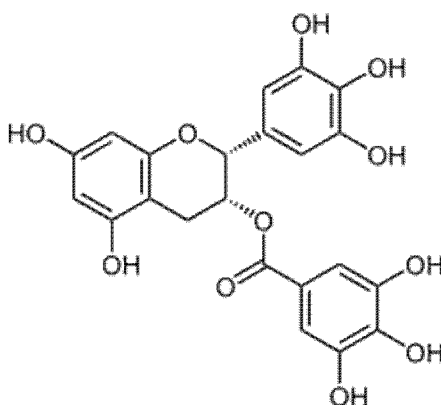
In a particular embodiment, the small molecule is TW-37 (Wang et al 2006; Al-Katib et al 2009) having the following chemical structure in the art N-[(2-tert-butylbenzenesulfonyl)-phenyl]-2,3,4-trihydroxy-5-(2-isopropyl-benzyl)-benzamide:



- 5 In a particular embodiment, the small molecule is ABT-737 having the following chemical structure in the art (Van Delft et al 2006; Rosswinkel et al 2012) 4-[4-[[2-(4-chlorophenyl)phenyl]methyl]piperazin-1-yl]-N-[4-[[[(2R)-4-(dimethylamino)-1-phenylsulfanylbutan-2-yl]amino]-3-nitrophenyl] sulfonyl]benzamide:



- 10 In a particular embodiment, the small molecule is EGCG (Green tea catechin, epigallocatechin-3-gallate) having the following formula in the art (Singh et al 2011): 3,4,5-trihydroxybenzoate de (2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphényl)-3,4-dihydro-2H-chromén-3-yle:

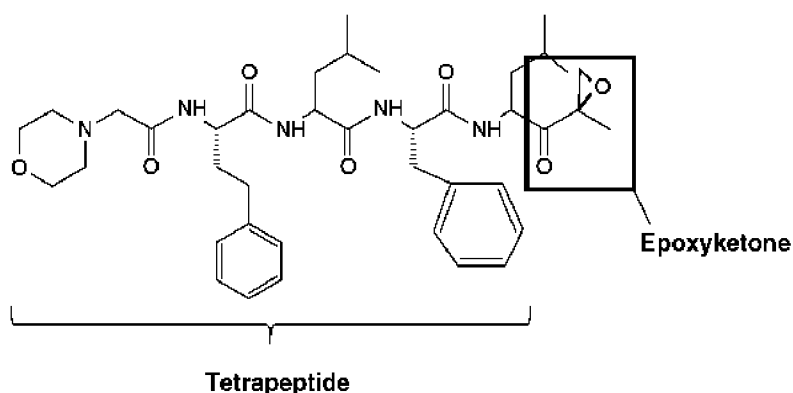


In a particular embodiment, the method of the invention comprising i) a first step consisting in determining whether a subject is at risk of having myeloma by the method as described above and ii) administering to said subject a therapeutically amount of Bcl-B antagonist, conventional treatment or a combination of thereof when the concentration of Bcl-B is higher than the predetermined value.

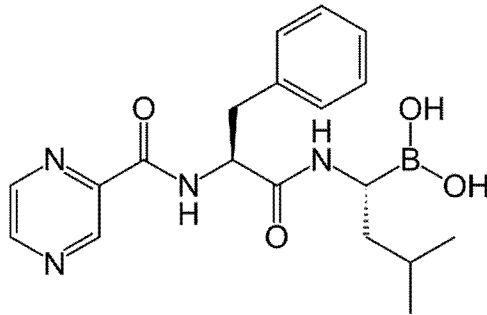
As used herein, the term "conventional treatment" refers to the treatments already existing to treat myeloma. For example, conventional treatment refers to chemotherapy, steroids and biological therapies.

In a particular embodiment, proteasome inhibitors could be used as conventional treatment. The term "proteasome inhibitor" refers to any substance which directly or indirectly inhibits the 20S and/or 26S proteasome or an activity thereof. Such proteasome inhibitors could be bortezomib, carfilzomib, oprozomib, ixazomib citrate, marizomib, delanzomib, and syringolin A (Chauhan et al 2005; Crawford et al 2011; Zang et al 2012, WO2015191668).

In a particular embodiment, the proteasome inhibitor is carfilzomib, having the following structure in the art (Vieyra et al 2014; Khan et al 2014):



In a particular embodiment, the proteasome inhibitor is Velcade[®], also called bortezomib, refers to a type of chemotherapy that is approved by the FDA for the treatment of multiple myeloma. This molecule is an inhibitor of proteasome and has the following structure in the art, (1R)-3-methyl-1-((2S)-3-phenyl-2-[(pyrazin-2-ylcarbonyl)-amino]propanoyl)amino)butyl]boronic acid:



In the context of the invention, the prophylactic treatment of myeloma depends of the subject age.

5

In a particular embodiment, the method of the invention comprising i) a first step consisting in determining whether the subject is at risk of having myeloma by the method of as described above, ii) identifying the age of said subject and ii) administering to said subject a therapeutically amount of Bcl-B antagonist when the concentration of Bcl-B is higher than the predetermined value.

10

In a particular embodiment, the method according to the invention wherein the method comprising a step administering to said young subject a therapeutically amount of Velcade® when the concentration of Bcl-B is higher than the predetermined value.

15

As used herein, the term “young subject” refers to a subject having less than 70 years old.

In a particular embodiment, the method according to the invention, wherein the method comprising a step of administering to said subject a therapeutically amount of a combination of a Bcl-B antagonist and Velcade®.

20

In a particular embodiment, the method according to the invention, wherein the method comprising a step of administering to said subject a therapeutically amount of a combination of a Velcade® with thalidomide and dexamethasone (VTD). Such treatment is called tri-therapy.

25

In a particular embodiment, the said young subject could be transplanted with his own stem cells (allogenic transplantation also called autogenous, autogeneic, or autogenic stem-cell transplantation). Typically, an autologous stem cell transplantation consists, following the destruction of all patient's tumoral plasmocytes, in the administration of patient's own stem cells by perfusion with a central venous catheter. Typically, hematopoietic stem cells (HSC)

are harvested from the patient prior to intensive chemotherapy, and such HSC are subsequently reinfused to rescue HSC function in the patient. The aim of autologous transplantation is to bypass the toxicity of chemotherapy to HSC, enabling higher dosis of chemotherapy to be administered, and higher efficiency of treatment towards tumoral cell eradication to be achieved.

Alternatively to Velcade[®], Carfilzomib could be used in the context of the invention.

In a further embodiment, the method according to the invention wherein the subject is elderly comprising a step of administering to said subject a therapeutically amount of a combination of a Bcl-B antagonist and Velcade[®].

As used herein, the term "elderly" refers to a subject having 70 years old and more.

In a particular embodiment, the method according to the invention, wherein the method comprising a step of administering to said subject a therapeutically amount of a combination of a Velcade[®] with thalidomide or dexamethasone (VTD).

In a further aspect, the invention relates to a method of preventing of myeloma comprising i) a first step consisting in determining whether the subject is at risk of having myeloma by the method of as described above, and ii) administering to said subject a therapeutically amount of DARZALEX[®] when the concentration of Bcl-B is higher than the predetermined value.

As used, herein, the term "DARZALEX[®]" is a CD38 Monoclonal Antibody which is the first human monoclonal antibody available for the treatment of Multiple Myeloma. It has obtained FDA to treat myeloma on 2015.

As used herein the terms "administering" or "administration" refer to the act of injecting or otherwise physically delivering a substance as it exists outside the body (e.g., an antagonist of Bcl-B) into the subject, such as by mucosal, intradermal, intravenous, subcutaneous, intramuscular delivery and/or any other method of physical delivery described herein or known in the art. When a disease, or a symptom thereof, is being treated, administration of the substance typically occurs after the onset of the disease or symptoms thereof. When a disease or symptoms thereof, are being prevented, administration of the substance typically occurs before the onset of the disease or symptoms thereof.

A "therapeutically effective amount" is intended for a minimal amount of active agent which is necessary to impart therapeutic benefit to a subject. For example, a "therapeutically effective amount" to a subject is such an amount which induces, ameliorates or otherwise causes an improvement in the pathological symptoms, disease progression or physiological conditions associated with or resistance to succumbing to a disorder. It will be understood that the total daily usage of the compounds of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed, the age, body weight, general health, sex and diet of the subject; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. However, the daily dosage of the products may be varied over a wide range from 0.01 to 1,000 mg per adult per day. Typically, the compositions contain 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100, 250 and 500 mg of the active ingredient for the symptomatic adjustment of the dosage to the subject to be treated. A medicament typically contains from about 0.01 mg to about 500 mg of the active ingredient, preferably from 1 mg to about 100 mg of the active ingredient. An effective amount of the drug is ordinarily supplied at a dosage level from 0.0002 mg/kg to about 20 mg/kg of body weight per day, especially from about 0.001 mg/kg to 7 mg/kg of body weight per day.

Pharmaceutical composition

The antagonists of Bcl-B, conventional treatment or combination of thereof as described above may be combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form pharmaceutical compositions. "Pharmaceutically" or "pharmaceutically acceptable" refer to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

The pharmaceutical compositions of the present invention for oral, sublingual, subcutaneous, intramuscular, intravenous, transdermal, local or rectal administration, the active principle, alone or in combination with another active principle, can be administered in a unit administration form, as a mixture with conventional pharmaceutical supports, to animals and human beings. Suitable unit administration forms comprise oral-route forms such as tablets, gel capsules, powders, granules and oral suspensions or solutions, sublingual and buccal administration forms, aerosols, implants, subcutaneous, transdermal, topical, intraperitoneal, intramuscular, intravenous, subdermal, transdermal, intrathecal and intranasal administration forms and rectal administration forms. Typically, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. Solutions comprising compounds of the invention as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. The polypeptide (or nucleic acid encoding thereof) can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol,

propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetables oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought
5 about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin. Sterile injectable
10 solutions are prepared by incorporating the active polypeptides in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile
15 powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The
20 formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed. For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for
25 intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion. Some variation in dosage will necessarily occur depending on
30 the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

Kit

In some embodiments, the invention relates to a kit for performing the method of the invention, said kit comprising means for measuring the level of Bcl-B in a biological sample obtained from a subject.

5 The kit may also contain other suitably packaged reagents and materials needed for the particular detection protocol, including solid-phase matrices, if applicable, and standards.

In a particular embodiment, the invention relates to a kit comprising means for measuring the level of Bcl-B in a biological sample obtained from a subject affected with MGUS for performing a method for predicting the risk of developing myeloma.

10

Method of screening

A further object of the invention relates to a method of screening a candidate compound for use in the prevention of myeloma in a subject in need thereof, wherein the method comprises the steps of: i) providing a plurality of candidate compounds, ii) bringing
15 the candidate compounds into contact with Bcl-B cell lines expressing Bcl-B, iii) determining the level of the Bcl-B expressed by the cancer cells, iv) comparing the level determined at step iii) with the level determined in the absence of the candidate compounds and v) positively selecting the candidate compounds when the level determined at step iii) is lower than the level determined in the absence of the candidate compounds.

20 In a further embodiment, the method of screening may be performed by a multiplexed bead-based flow cytometry high-throughput assay as described in Zou et al 2013.

For example, the method may involve contacting cells expressing Bcl-B with the candidate compound, and measuring the Bcl-B mediated transcription (e.g., inactivation of promoters containing Bcl-B binding sites), and comparing the cellular response to a standard
25 cellular response. Typically, the standard cellular response is measured in absence of the candidate compound. An increased cellular response over the standard indicates that the candidate compound is able to inactivate Bcl-B gene expression. Determination of the expression level of a gene can be performed by a variety of techniques. Typically, the determination comprises contacting the cells with selective reagents such as probes, primers
30 or ligands, and thereby detecting the presence, or measuring the amount, of polypeptide or nucleic acids of interest originally in the sample. Contacting may be performed in any suitable device, such as a plate, microtiter dish, test tube, well, glass, column, and so forth. In specific embodiments, the contacting is performed on a substrate coated with the reagent, such as a nucleic acid array or a specific ligand array. The substrate may be a solid or semi-solid

substrate such as any suitable support comprising glass, plastic, nylon, paper, metal, polymers and the like. The substrate may be of various forms and sizes, such as a slide, a membrane, a bead, a column, a gel, etc. The contacting may be made under any condition suitable for a detectable complex, such as a nucleic acid hybrid or an antibody-antigen complex, to be formed between the reagent and the nucleic acids or polypeptides of the sample. In a preferred embodiment, the expression level may be determined by determining the quantity of mRNA. Methods for determining the quantity of mRNA are well known in the art. For example the nucleic acid contained in the samples (e.g., cell or tissue prepared from the subject) is first extracted according to standard methods, for example using lytic enzymes or chemical solutions or extracted by nucleic-acid-binding resins following the manufacturer's instructions. The extracted mRNA is then detected by hybridization (e. g., Northern blot analysis) and/or amplification (e.g., RT-PCR). Preferably quantitative or semi-quantitative RT-PCR is preferred. Real-time quantitative or semi-quantitative RT-PCR is particularly advantageous. Other methods of Amplification include ligase chain reaction (LCR), transcription- mediated amplification (TMA), strand displacement amplification (SDA) and nucleic acid sequence based amplification (NASBA).

In a particular embodiment, the candidate compounds may be selected from a library of compounds previously synthesised, or a library of compounds for which the structure is determined in a database, or from a library of compounds that have been synthesised de novo or natural compounds. The candidate compound may be selected from the group of (a) proteins or peptides, (b) nucleic acids and (c) organic or chemical compounds (natural or not).

Bcl-B inactivation with the candidate compound can be tested by various known methods of the man skilled in the art.

The method may be performed in vitro or ex vivo. When performed ex vivo, it can be performed for example on a sample from a subject who has been administered with a test compound. The method as described herein comprising determining the ability of a test compound to modify the expression of Bcl-B, or compensate an abnormal expression thereof.

In a particular embodiment, the candidate compounds may be selected from small organic molecules.

In some embodiments, the candidate compounds may be selected from aptamers.

In still another particular embodiment, the candidate compound may be selected from molecules that block expression of a gene of Bcl-B. Also within the scope of the invention is the use of oligoribonucleotide sequences that include anti-sense RNA and DNA molecules and ribozymes that function to inhibit the translation of mRNA of a nuclear protein required

for Notch 1 transcriptional activity. Anti- sense RNA and DNA molecules act to directly block the translation of mRNA by binding to targeted mRNA and preventing protein translation. In regard to antisense DNA, oligodeoxyribonucleotides derived from the translation initiation site. Ribozymes are enzymatic RNA molecules capable of catalysing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridisation of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage. To inhibit the activity of the gene of interest or the gene product of the gene of interest, custom-made techniques are available directed at three distinct types of targets: DNA, RNA and protein. For example, the gene or gene product of a nuclear protein required for Notch 1 transcriptional activity of the invention can be altered by homologous recombination, the expression of the genetic code can be inhibited at the RNA levels by antisense oligonucleotides, interfering RNA (RNAi) or ribozymes, and the protein function can be altered by antibodies or drugs.

The screening method of the invention is particularly suitable for identifying a compound that is an inhibitor of Bcl-B activity or expression. In some cases, a candidate therapeutic agent has been identified and further testing may be required. In some embodiments the further testing is to evaluate a candidate therapeutic agent (or an agent that has been confirmed to be therapeutic) for quality control and/or safety concerns. In some embodiments, methods of the invention include a method of assaying a therapeutic agent (or candidate therapeutic agent) for efficacy against myeloma in a relevant animal model suffering from MGUS.

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

FIGURES:

Figure 1: Bcl-B protein is predominantly expressed in the bone marrow of patients suffering from MM and is widely expressed in human MM cell lines.

(A) Representative cytometric profiles as examples of Bcl-B-positive plasma cell quantification by flow cytometry analysis of bone marrow cells from an MGUS and an MM patient. Total bone marrow cells were quantified and Bcl-B protein expression was measured by gating the CD138-positive fraction corresponding to medullar plasmocytes. NR Ig: non relevant rabbit Immunoglobulin. (B) The percentage of Bcl-B-positive plasma cells was

quantified by flow cytometry analysis of bone marrow cells from healthy donors (n=3), MGUS patients (n=20) and patients with newly diagnosed MM (n=18). (C) Plasma cells (PCs) were isolated from the bone marrow of healthy donors (lanes 1 and 2), MGUS patients (lanes 3 to 5) and MM patients (lanes 6 to 13) using CD138 magnetic beads. Total protein lysates (35 µg per lane) were subjected to western blot analysis using anti-Bcl-B or Hsp60 antibodies. p values were determined by one-way ANOVA. NS, no significant difference was observed. **p<0.01.

EXAMPLE:

Material & Methods

Isolation of plasma cells from patients

CD138+ bone marrow cells were isolated from patients using magnetic CD138 microbeads (Miltenyi Biotec) according to the manufacturer's instructions. Briefly, patient samples were collected in tubes containing EDTA. Then, the cells were passed through a filter (100-µm pore size) to remove bone fragments or cell clumps. The cells were then centrifuged at 445 x g for 10 min at room temperature in a swinging bucket rotor with the brake off. The supernatant was aspirated, and the cells were incubated with 50 µl of whole blood CD138 microbeads per 1 ml of anticoagulated bone marrow for 15 min at 4°C. The cells were then washed and resuspended in autoMACS running buffer. The magnetic separation was performed using the Possel WB program on the AutoMACS Pro Separator. The purity of the isolated plasma cells was assessed by flow cytometry using an anti-human CD138 antibody.

Results

Bcl-B is predominantly expressed in the bone marrow of MM patients

We previously reported Bcl-B overexpression in histological bone marrow (BM) sections from MM patients (Krajewska et al., 2008; Luciano et al., 2007). To confirm Bcl-B protein expression we used a dedicated flow cytometry assay. Figure 1A shows an example of Bcl-B protein expression measurement by flow cytometry in an MGUS patient and an MM patient with 3.6% and 28.2% of medullar plasmocytes, respectively. We found that most of the plasmocytes from the MM patient highly expressed the Bcl-B protein, whereas a negligible proportion of the plasmocytes from the MGUS patient weakly expressed the Bcl-B protein. In patients with newly diagnosed MM (n=18), 20-100% of sorted CD138+ BM cells were positive for Bcl-B whereas only a very small percentage (0-10%) of CD138+ BM cells from healthy donors (n=3) and MGUS patients (n=20) were positive for this protein (Figure

1B). The level of Bcl-B expression was next analyzed by western blot using plasmocytes from two healthy donors, three MGUS patients and eight patients with newly diagnosed MM. All MM patients expressed Bcl-B, whereas the protein was almost undetectable in healthy donors and MGUS patients (Figure 1C). These data suggest that Bcl-B protein could be a biomarker to predict the evolution of MGUS to myeloma.

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Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

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CLAIMS:

1. A method for predicting the risk of having myeloma in a subject suffering from MGUS comprising the steps of:
 - 5 i) measuring the concentration of Bcl-B in a biological sample obtained from said subject,
 - ii) comparing the concentration obtained at step (i) to a predetermined value, and
 - iii) concluding that the subject is at risk of having myeloma when the concentration of Bcl-B is higher than the predetermined value or concluding
10 that the subject is not at risk of having myeloma when the concentration of Bcl-B is lower than the predetermined value.
2. A method of preventing the risk of having myeloma in a subject suffering from MGUS comprising a step of administering to said subject a therapeutically amount of Bcl-B antagonist.
- 15 3. The method according to claim 2, wherein the method comprising i) a first step consisting in determining whether the subject is at risk of having myeloma by the method of claim 1 and ii) administering to said subject a therapeutically amount of Bcl-B antagonist, conventional treatment or a combination of thereof when the concentration of Bcl-B is higher than the predetermined value.

1/3

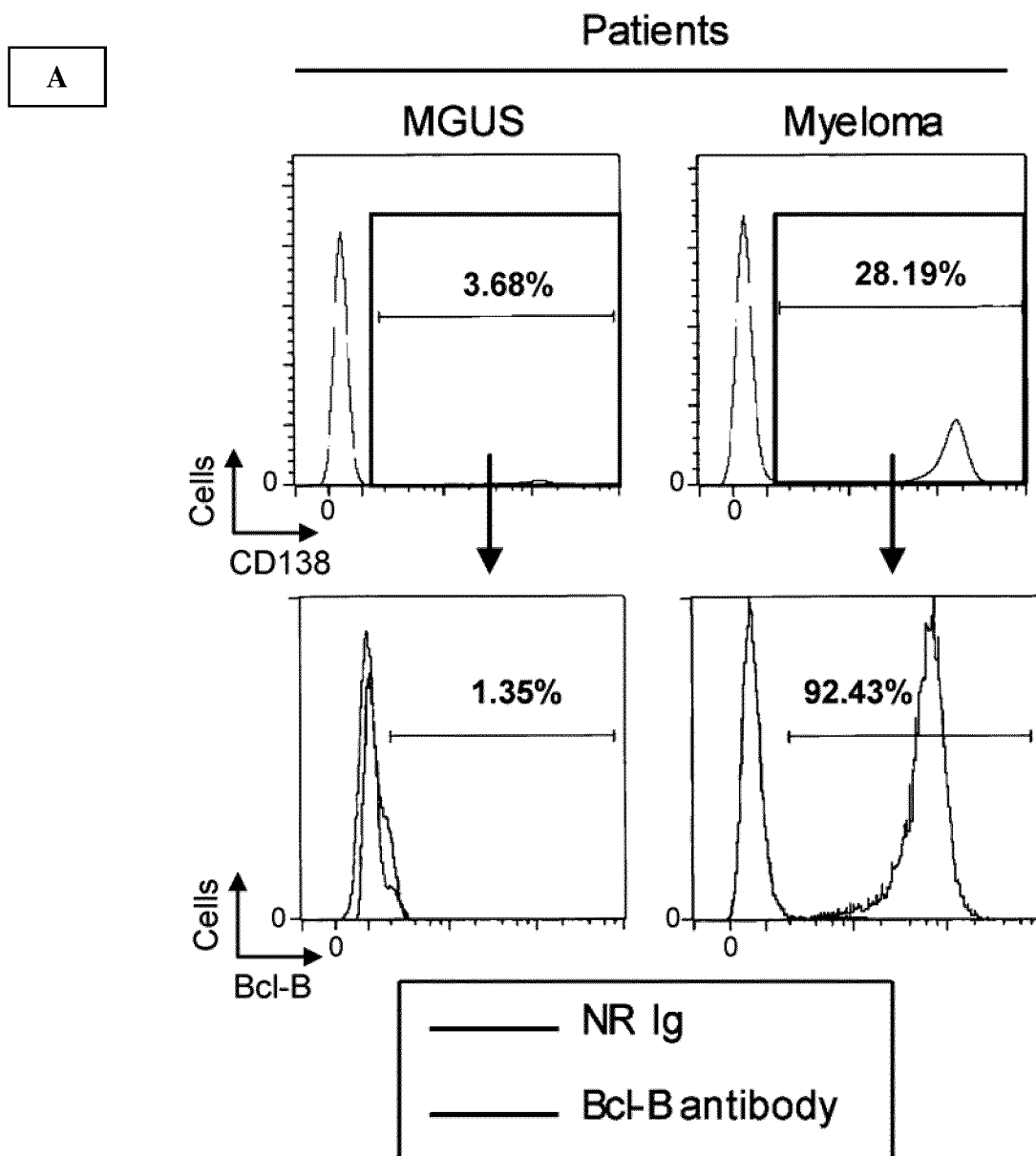


Figure 1A

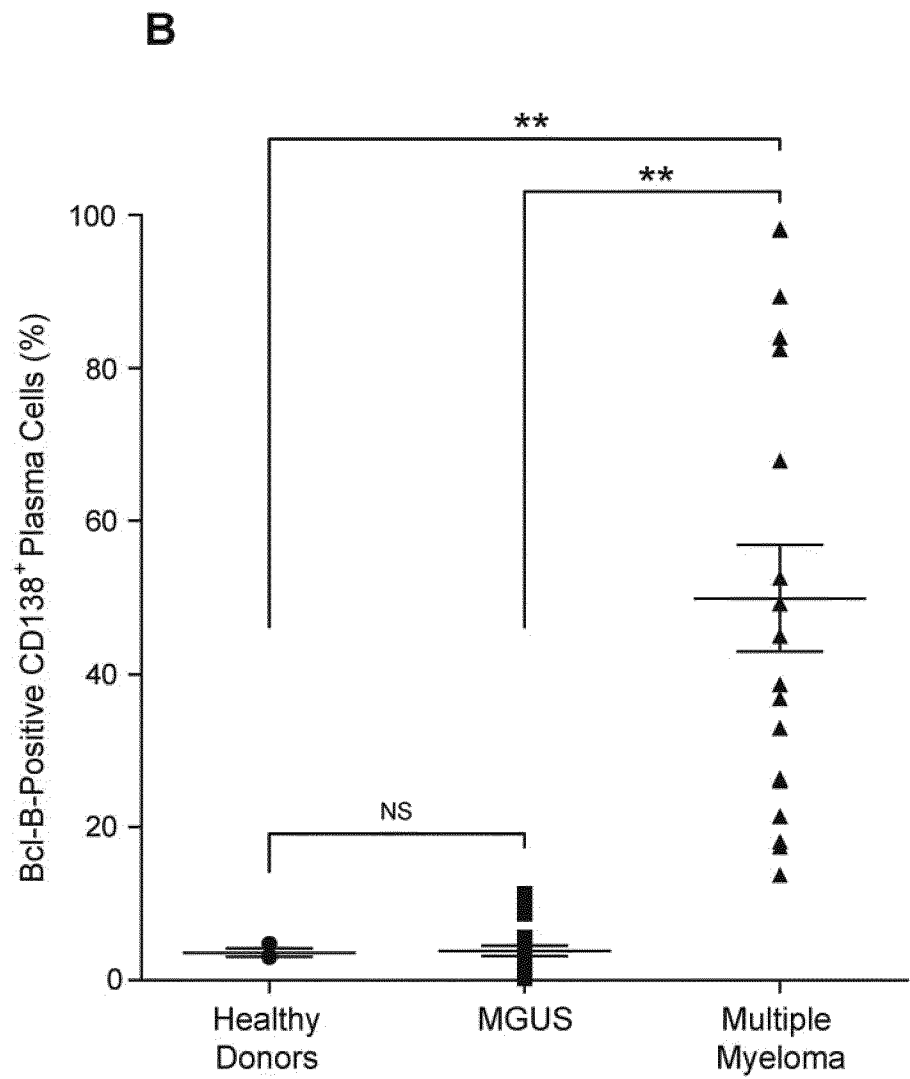


Figure 1B

C

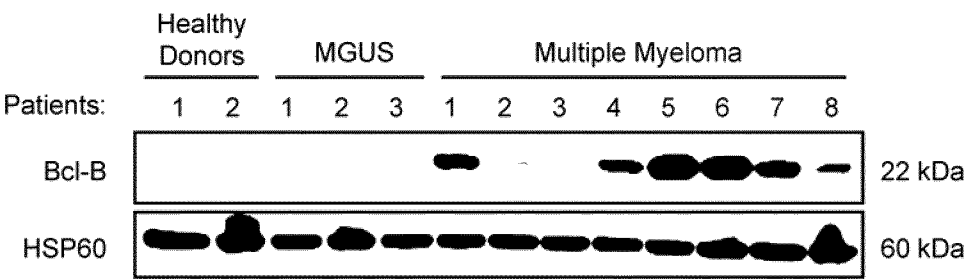


Figure 1C

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/062504

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/574
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

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

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

EPO-Internal, BIOSIS, COMPENDEX, EMBASE, FSTA, 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	VALENTINA JR RICHEL ET AL: "Implication of the Anti-Apoptotic Protein Bcl-B (BCL2L10) in the Pathogenesis of Multiple Myeloma", 57TH ANNUAL MEETING OF THE AMERICAN-SOCIETY-OF-HEMATOLOGY, vol. 126(23), 30 October 2015 (2015-10-30), XP055319297, the whole document	1,3
X	US 2004/214902 A1 (WANG SHAOMENG [US] ET AL) 28 October 2004 (2004-10-28) the whole document abstract paragraph [0418] - paragraph [0419] claims 1, 27-28 ----- -/--	2



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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"O" document referring to an oral disclosure, use, exhibition or other means

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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

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Date of the actual completion of the international search

15 June 2017

Date of mailing of the international search report

27/06/2017

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/062504

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	HAMOUDA MOHAMED-AMINE ET AL: "BCL-B (BCL2L10) is overexpressed in patients suffering from multiple myeloma (MM) and drives an MM-like disease in transgenic mice", JOURNAL OF EXPERIMENTAL MED, ROCKEFELLER UNIV. PRESS, NEW YORK, NY, USA, vol. 213, no. 9, 22 August 2016 (2016-08-22), pages 1705-1722, XP008182280, ISSN: 1540-9538, DOI: 10.1084/JEM.20150983 [retrieved on 2016-07-25] the whole document abstract	
T	----- Patrick Auberger: "Cancer de la moelle osseuse : un nouveau marqueur pour diagnostiquer et prédire l'évolution de la maladie - Université Nice Sophia Antipolis", 29 September 2016 (2016-09-29), XP055322541, Retrieved from the Internet: URL:http://filuns.unice.fr/fil/service-communication/actualites/cancer-de-la-moelle-osseuse-un-nouveau-marqueur-pour-predire-l-evolution-de-la-maladie/@@article_view#.WDaqCHrdfsJ [retrieved on 2016-11-24] the whole document -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2017/062504

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		US 2009082424 A1	26-03-2009
		US 2009092684 A1	09-04-2009
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