(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

**(19) World Intellectual Property Organization**
International Bureau

**(43) International Publication Date**
**20 July 2017 (20.07.2017)**

**(10) International Publication Number**
**WO 2017/123741 A1**

**(51) International Patent Classification:**
*A61K 39/395* (2006.01) *G01N 33/53* (2006.01)
*C07K 16/28* (2006.01) *G01N 33/68* (2006.01)
*C12N 15/12* (2006.01)

**(21) International Application Number:**
PCT/US2017/013169

**(22) International Filing Date:**
12 January 2017 (12.01.2017)

**(25) Filing Language:** English

**(26) Publication Language:** English

**(30) Priority Data:**
62/277,801 12 January 2016 (12.01.2016) US
62/300,708 26 February 2016 (26.02.2016) US

**(71) Applicant: INSTITUTE FOR MYELOMA & BONE CANCER RESEARCH** [US/US]; 9201 Sunset Boulevard, Suite 300, West Hollywood, California 90069 (US).

**(72) Inventor; and**
**(71) Applicant : BERENSON, James Richard** [US/US]; 519 North Elm Drive, Beverly Hills, California 90210 (US).

**(74) Agents: MAJUMDAR, Romit** et al.; Cooley LLP, 1299 Pennsylvania Avenue, NW, Suite 700, Washington, District of Columbia 20004 (US).

**(81) Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

**(84) Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ,

*[Continued on next page]*

**(54) Title**: IMPROVED METHODS FOR MONITORING IMMUNE STATUS OF A SUBJECT

**(57) Abstract**: The invention generally provides improved compositions and methods for monitoring immune status of a subject. In particular, the invention provides methods for detecting BCMA in subjects to reliably monitor immune status of the subject.

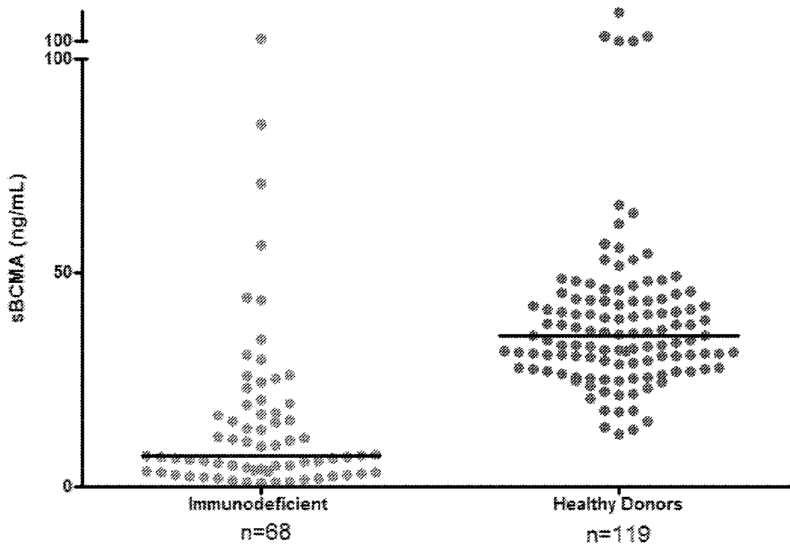



*FIG. 7*

WO 2017/123741 A1

TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

**Published**:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

# IMPROVED METHODS FOR MONITORING IMMUNE STATUS OF A SUBJECT

## CROSS-REFERENCES TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 62/300,708, filed February 26, 2016 and U.S. Provisional Application No. 62/277,801, filed January 12, 2016, both of which are incorporated herein by reference in their entireties.

## STATEMENT REGARDING SEQUENCE LISTING

The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is IMBC_008_01WO_SeqList_ST25.txt. The text file is 6 KB, was created on January 12, 2017, and is being submitted electronically via EFS-Web.

## BACKGROUND

The compositions and methods of the invention relate generally to detection of biomarkers for the monitoring of immune status. In particular, the invention relates to compositions and methods for detection of B-cell maturation antigen for the monitoring of immune status of a subject.

The immune system is a system of many biological structures and processes within a subject that protects against disease. To function properly, an immune system must detect a wide variety of agents, known as pathogens, from viruses to parasitic worms, and distinguish them from a subject's own healthy tissue. Therefore, proper functioning of the immune system requires that all components of the immune system work in a coordinated manner to neutralize pathogens. An impairment of a subject's immune system leads to infections by opportunistic pathogens that may eventually prove to be fatal. Conversely, a hyperactive immune system (*e.g.*, in autoimmune diseases) causes the immune system to attack a subject's normal tissues as

if they were foreign organisms. The overall efficiency (*i.e.*, normal, hyperactive, or impaired) of a subject's immune system is referred to as the subject's immune status.

The immune system can be classified into subsystems – innate immunity and adaptive immunity. Defense against pathogens is mediated by the early reactions of innate immunity and the later responses of adaptive immunity. There are two types of adaptive immune responses, called cell-mediated immunity (mediated by T cells) and humoral immunity (mediated by B cells).

B cells or B lymphocytes are a type of white blood cell of the lymphocyte subtype. They function in the humoral immunity component of the adaptive immune system by secreting antibodies. Additionally, B cells present antigens (they are also classified as professional antigen-presenting cells (APCs)) and secrete cytokines. In mammals, B cells mature in the bone marrow. B cells, unlike the other two classes of lymphocytes, T cells and natural killer cells, express B cell receptors (BCRs) on their cell membrane. BCRs allow the B cell to bind a specific antigen, against which it will initiate an antibody response.

Tumor necrosis factor receptor superfamily, member 17 (TNFRSF17, also designated as B-cell maturation antigen (BCMA) or CD269) is a receptor that was first identified in a T-cell tumor line (Laabi *et al.*, 1992) and subsequently shown to be expressed in B lymphocytes as they mature (Laabi *et al.*, 1994). BCMA ligands include BAFF (B cell-activating factor; TNFSF13B) and APRIL (a proliferation-inducing ligand; TNFSF13) (Rennert *et al.*, 2000; Thompson *et al.*, 2000). In multiple myeloma (MM) cell lines, these ligands activate cell proliferation pathways and upregulate anti-apoptotic proteins (Moreaux *et al.*, 2004). Both ligands also bind the receptor TACI (transmembrane activator and CAML interactor; TNFRSF13B) (Gross *et al.*, 2000; Wu *et al.*, 2000; Yu *et al.*, 2000). Additionally, BAFF binds to a third receptor, called BAFF-receptor (BAFFR; TNFRSF13C), whereas APRIL does not (Thompson *et al.*, 2001; Day *et al.*, 2005). The ligands BAFF and APRIL are members of the tumor necrosis family (TNF) and binding of TNF members to their receptors can lead to apoptosis, differentiation or proliferation (Smith *et al.*, 1994).

BCMA has been shown to be located intracellularly in plasma cell lines (Laabi *et al.*, 1992, 1994). Surface expression of BCMA was found on human tonsilar

B-cells (Thompson *et al.*, 2000), and on human CD138-expressing MM cells (Novak *et al.*, 2004). Malignant cells from Hodgkin lymphoma and Waldenstrom macroglobulinemia (WM) patients also express this protein (Elsawa *et al.*, 2006; Chiu *et al.*, 2007).

5 The present inventors have previously demonstrated that BCMA is present in the serum of patients having various B-cell malignancies, *e.g.,* multiple myeloma (MM), chronic lymphocytic leukemia (CLL), and B-cell non-Hodgkin's lymphomas (NHL) and correlates with the patient's response to therapy and overall survival. In addition, the present inventors have discovered that BCMA levels are 10 increased in the serum of MM, CLL, and NHL patients compared to normal healthy subjects not afflicted with these cancers.

Additional studies indicate that BCMA is expressed as a cell surface receptor protein on activated B lymphocytes and is subsequently cleaved by a γ-secretase enzyme, which results in the release of the extracellular part of BCMA as a 15 soluble BCMA form (Laurent *et al.*, 2015, *Nature Communications*, 6:7333-7344).

Currently, an objective test to determine the immune status of a subject is not available, and the existence of a disease or infection is determined by a physician's observation of the subject's physical symptoms (*e.g.*, body temperature or physical discomfort such as pain). However, such observations are subjective and can 20 vary from one physician to another. Furthermore, a rapid and reliable determination of a subject's response to treatment is also currently not available, and would be greatly facilitated by a test that could reliably monitor a subject's immune status at different time points during the course of a treatment regimen. Therefore, there is a need in the art to design a fast, reproducible, inexpensive, and reliable test that can indicate the 25 immune status of a subject.

The present inventors have now surprisingly found that levels of BCMA polypeptide or a fragment thereof in a biological sample (*e.g.*, serum) of a subject correlate with the subject's overall immune status. The present inventors have found that a decreased amount of BCMA polypeptide or a fragment thereof in a biological 30 sample obtained from a subject compared to BCMA polypeptide or a fragment thereof in a control biological sample obtained from a normal healthy subject is indicative of an

impaired immune system as indicated by a reduction in their immunoglobulin levels, while an increased amount of BCMA polypeptide or a fragment thereof in a biological sample obtained from a subject compared to amounts of BCMA polypeptide or a fragment thereof in a biological sample obtained from a normal healthy subject
5 indicates that the subject is suffering from an infection or a disease.

## BRIEF SUMMARY

In accordance with the purpose of this invention, as embodied and broadly described herein, this invention generally provides compositions and methods for reliably and reproducibly monitoring the immune status of a subject. The levels of
10 BCMA polypeptide or a fragment thereof in a biological sample obtained from a subject can be detected and/or measured and compared against a baseline or control to reliably and reproducibly monitor the immune status of the subject.

In various embodiments, a method of monitoring immune status of a subject is provided. In some embodiments, a method of monitoring immune status of a
15 subject, comprises: (a) detecting an amount of BCMA polypeptide or a fragment thereof in a biological sample obtained from the subject; and (b) comparing the amount of BCMA polypeptide or fragment thereof detected in (a) to a predetermined cut-off value or to an amount detected in a control serum sample, wherein a decreased amount of BCMA polypeptide or fragment in the biological sample of the subject as compared
20 to the predetermined cut-off value or amount in the control serum sample is indicative of an impaired immune system, wherein the biological sample is a serum sample or supernatant obtained from a culture of the subject’s bone marrow mononuclear cells or peripheral blood mononuclear cells.

In other embodiments, a method of monitoring immune status of a
25 subject, comprises: (a) detecting an amount of BCMA polypeptide or a fragment thereof in a biological sample obtained from the subject; and (b) comparing the amount of BCMA polypeptide or fragment thereof detected in (a) to a predetermined cut-off value or to an amount detected in a control serum sample, wherein an increased amount of BCMA polypeptide or fragment in the biological sample of the subject as compared
30 to the predetermined cut-off value or amount in the control serum sample indicates that

the subject is suffering from or at higher risk to develop an infection or an immune deficiency-related disease, wherein the biological sample is a serum or plasma sample or supernatant obtained from a culture of the subject's bone marrow mononuclear cells or peripheral blood mononuclear cells.

5 In additional embodiments, a method of monitoring immune status of a subject, comprises: (a) detecting an amount of BCMA polypeptide or a fragment thereof in a biological sample obtained from the subject; and (b) comparing the amount of BCMA polypeptide or fragment thereof detected in (a) to a predetermined cut-off value or to an amount detected in a control serum or plasma sample, wherein a 10 decreased amount of BCMA polypeptide or fragment in the biological sample of the subject as compared to the predetermined cut-off value or amount in the control serum or plasma sample is indicative of an impaired immune system, and an increased amount of BCMA polypeptide or fragment in the biological sample of the subject as compared to the predetermined cut-off value or amount in the control serum or plasma sample 15 indicates that the subject is suffering from or at higher risk to develop an infection or an immune deficiency-related disorder, wherein the biological sample is a serum or plasma sample or supernatant obtained from a culture of the subject's bone marrow mononuclear cells or peripheral blood mononuclear cells.

In other aspects, a method of monitoring response to a treatment of a 20 subject is provided. In some embodiments, the method of monitoring response to a treatment of a subject comprises: (a) detecting an amount of BCMA or a fragment thereof in a biological sample, obtained from a subject a time point prior to start of the treatment; (b) detecting an amount of BCMA or a fragment thereof in the biological sample, obtained from the subject a time point subsequent to start of the treatment; and 25 (c) comparing the amount of BCMA polypeptide or a fragment thereof detected in (a) to the amount of BCMA polypeptide or a fragment thereof detected in (b), wherein an decreased amount of BCMA polypeptide or a fragment thereof detected in (b) as compared to the amount of BCMA polypeptide or a fragment thereof detected in (a) indicates that the subject is responding to treatment, and wherein an increased or 30 unchanged amount of BCMA polypeptide or a fragment thereof detected in (b) as compared to the amount of BCMA polypeptide or a fragment thereof detected in (a)

indicates that the subject is not responding to treatment, wherein the biological sample is a serum or plasma sample or supernatant obtained from a culture of the subject's bone marrow mononuclear cells or peripheral blood mononuclear cells.

In some embodiments, the biological sample includes, without limitation, cells in culture, cell supernatants, cell lysates, serum, plasma, urine, cerebral spinal fluid, biological fluid, and tissue samples. In certain embodiments, the biological sample is a serum sample. In other embodiments, the biological sample is supernatant obtained from culture of the subject's bone marrow mononuclear cells. In yet other embodiments, the biological sample is supernatant obtained from culture of the subject's peripheral blood mononuclear cells.

In some embodiments, the BCMA fragment is a cleaved BCMA polypeptide. In some embodiments, the cleaved BCMA polypeptide is a soluble form of the BCMA polypeptide. In certain embodiments, the BCMA polypeptide or a fragment thereof comprises the amino acid sequence of SEQ ID NO:1:

MetLeuGlnMetAlaGlyGlnCysSerGlnAsnGluTyrPheAspSerLeu
LeuHisAlaCysIleProCysGlnLeuArgCysSerSerAsnThrProProLeu
ThrCysGlnArgTyrCysAsnAlaSerValThrAsnSerValLysGlyThrAsnAla

In other embodiments, the BCMA polypeptide or a fragment thereof comprises an amino acid sequence having at least about 20% identity, at least about 30% identity, at least about 40% identity, at least about 50% identity, at least about 60% identity, at least about 70% identity, at least about 75% identity, at least about 80% identity, at least about 90% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, or at least about 99% identity with SEQ ID NO:1.

In some embodiments, the BCMA polypeptide or a fragment thereof comprises at least about 50, least about 45, least about 40, least about 30, least about 20, least about 10, or least about 5 amino acids. In specific embodiments, the BCMA polypeptide or a fragment thereof comprises 54 amino acids.

In additional embodiments, the BCMA polypeptide or a fragment thereof is detected using a detection system selected from the group consisting of: an immunohistochemistry, enzyme-linked immunosorbent assay (ELISA),

radioimmunoassay (RIA), enzyme immunoassay (EIA), fluorescence immunoassay (FIA), luminescence immunoassay (LIA), lateral flow assay, or strip assay. In some embodiments, the detection system is an ELISA assay. In other embodiments, the detection system is a lateral flow assay.

5 In some embodiments, the detection is performed using an antibody specific for BCMA polypeptide or a fragment thereof. In certain embodiments, the antibody specific for BCMA polypeptide or a fragment thereof is a monoclonal antibody. In other embodiments, the antibody specific for BCMA polypeptide or a fragment thereof is a polyclonal antibody.

10 In some aspects, the impaired immune system is the result of an immunodeficiency disease. In some embodiments, the immunodeficiency disease includes, but is not limited to, Acquired Immune Deficiency Syndrome (AIDS), Ataxia telangiectasia, Chediak Higashi Syndrome, Common Variable Immune Deficiency (CVID), Combined Immunodeficiency Disease, Complement deficiencies, DiGeorge 15 Syndrome, Hypogammaglobulinemia, Job Syndrome, Leukocyte Adhesion Deficiency, Panhypogammaglobulinemia, X-linked Agammaglobulinemia Disease (Bruton's disease), Congenital Agammaglobulinemia, Selective Deficiency of IgA, Wiskott Aldrich Syndrome, Chronic Granulomatous Disease, Severe Combined Immunodeficiency Disease, Hyper Immunoglobulin E Syndrome (Job's Syndrome), 20 Hyper IgM Syndrome, X-linked agammaglobulinemia (XLA), Crohn's disease, Thymoma, immunodeficiencies associated with mutations in the LRBA gene (encoding the lipopolysaccharide-responsive and beige-like anchor protein), or immunodeficiencies associated with phosphatidylinositol 3-kinase δ (PI3KD).

In some embodiments, the infection includes, but is not limited to, a viral 25 infection, a bacterial infection, a prion infection, or a fungal infection. In some embodiments, the disease includes, but is not limited to, autoimmune diseases including, without limitations, Systemic Lupus Erythematosus (SLE), Multiple Sclerosis (MS), Hashimoto's thyroiditis, rheumatoid arthritis, or diabetes mellitus type 1. In other embodiments, the disease includes genetic diseases such as cancer 30 including, but not limited to, myeloma, lymphoma, or leukemia. In some aspects, the myeloma is multiple myeloma (MM). In other aspects, the lymphoma is non-Hodgkin

lymphoma (NHL). In other embodiments, the leukemia is chronic lymphocytic leukemia (CLL).

In some embodiments, a kit for monitoring immune status of a subject is provided. In certain aspects, the kit for monitoring immune status of a subject comprises a reagent suitable for determining levels of BCMA polypeptide or a fragment thereof in a biological sample obtained from the subject, wherein the biological sample is a serum or plasma sample or supernatant obtained from culture of the subject's bone marrow mononuclear cells or peripheral blood mononuclear cells.

In some embodiments, the kit comprises an antibody that specifically binds BCMA polypeptide or fragment thereof. In certain embodiments, the antibody that specifically binds BCMA polypeptide or fragment thereof is a monoclonal antibody. In other embodiments, the antibody that specifically binds BCMA polypeptide or fragment thereof is a polyclonal antibody.

In some aspects, the kit comprises a detection system selected from the group consisting of: ELISA assay, RIA assay, EIA assay, FIA assay, LIA assay, lateral flow assay, or strip assay. In some embodiments, the kit comprises an ELISA assay. In other embodiments, the kit comprises a lateral flow assay.

## BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments of the disclosed method and compositions and together with the description, serve to explain the principles of the disclosed methods.

Figure 1 shows that BCMA is found in the serum of control human subjects and a patient with a low IgG level. A subject with low IgG levels had low serum BCMA levels (14.6 ng/mL) compared to serum BCMA levels (median=36.0 ng/mL; range=13.45 ng/ml-958.1 ng/mL) in control subjects (N=104).

Figure 2 shows that IgG levels of patients with IgA multiple myeloma (MM) who have achieved complete remission (CR) with no measurable myeloma correlate with their serum BCMA levels. IgA MM patients (N=23) who are in CR who show low serum BCMA (≤10 ng/mL; $p<0.0001$) have significantly decreased IgG

levels (median=319.0 mg/dL) compared to IgG levels (median=535.0 mg/dL) among IgG MM patients (N=40) who are in CR and show higher serum BCMA (>10 ng/mL; p<0.0001).

Figure 3 shows that IgG levels of patients with IgG MM who have achieved complete remission (CR) with no measurable myeloma correlate with their serum BCMA levels. IgG MM patients (N=47) who are in CR who show low serum BCMA (≤10 ng/mL; p<0.0001) have significantly decreased IgG levels (median=402.0 mg/dL) compared to IgG levels (median=643.5 mg/dL) among IgG MM patients (N=84) who are in CR and show higher serum BCMA (>10 ng/mL; p<0.0001).

Figure 4 shows that uninvolved, normal IgA levels of patients with IgG MM who have achieved CR correlate with their serum BCMA levels. IgG MM patients (N=47) who are in CR and low serum BCMA levels (≤10 ng/mL; p<0.0001) show significantly decreased IgA levels (median=26.0 mg/dL) compared to IgA levels (median=61.0 mg/dL) in IgG MM patients (N=84) who are in CR and show higher serum BCMA (>10 ng/mL; p<0.0001).

Figure 5 shows that uninvolved, normal IgM levels of patients with IgG MM who have achieved CR correlate with their serum BCMA levels. IgG MM patients (N=47) who are in CR and show low serum BCMA (≤10 ng/mL; p<0.0001) have significantly decreased IgM levels (median=11.0 mg/dL) compared to IgM levels (median=32.5 mg/dL) in IgG MM patients (N=84) who are in CR and show higher serum BCMA levels (>10 ng/mL; p<0.0001).

Figure 6 shows serum BCMA levels of patients based on Diagnosis of immunodeficiency. Levels of serum BCMA were substantially lower in patients with immunodeficiency (XLA, CVID, IgG deficiency, IgA deficiency, IgM deficiency, Hyper IgM syndrome, PRH, or Crohn's disease) compared to serum BCMA levels in control subjects.

Figure 7 shows that serum BCMA levels of patients with immunodeficiency (N=68) were significant lower (7.3 ng/mL (range; 0.84 ng/mL – 189.5 ng/mL); p<0.0001) compared to serum BCMA levels (35.2 ng/mL (range; 12.2 ng/mL – 958.1 ng/mL); p<0.0001) in normal healthy donors (N=119).

Figures 8 and 10 shows serum BCMA levels of patients based on Diagnosis of immunodeficiency. Levels of serum BCMA were substantially lower in patients with immunodeficiency (XLA, CVID, CVID + Lymphoma, CVID + Tx Lymphoma, IgG deficiency, IgA deficiency, IgM deficiency, Hyper IgM syndrome,
5 PI3KD, LRBA/LRBA, or Thymoma) compared to serum BCMA levels in control subjects.

Figures 9 and 11 shows serum BCMA levels of patients based on Diagnosis of immunodeficiency. Levels of serum BCMA were substantially lower in patients with immunodeficiency (XLA, CVID, CVID + Lymphoma, CVID + Tx
10 Lymphoma, IgG deficiency, IgA deficiency, IgA, IgA + IgG, IgA + IgG2, IgM deficiency, or Hyper IgM syndrome) compared to serum BCMA levels in control subjects.

## DETAILED DESCRIPTION

The present inventors have found that levels of serum BCMA or a
15 fragment thereof correlate with the overall immune status of a subject. The present inventors have found that a decreased amount of BCMA polypeptide or a fragment thereof in a biological sample obtained from a subject compared to BCMA polypeptide or a fragment thereof in a control biological sample obtained from a normal healthy subject is indicative of an impaired immune system, while an increased amount of
20 BCMA polypeptide or a fragment thereof in a biological sample obtained from a subject compared to amounts of BCMA polypeptide or a fragment thereof in a biological sample obtained from a normal healthy subject indicates that the subject is suffering or is at higher risk to suffer from an infection or an immune deficiency-related disease.

25 In various embodiments, methods for reliably monitoring the immune status of a subject are provided. Concentrations of BCMA or a fragment thereof in a biological sample (*e.g.*, a subject's sera) is detected and/or measured and compared against a baseline or control to reliably monitor the immune status of a subject. Without wishing to be bound to a particular theory, it is believed that because high
30 levels of BCMA or a fragment thereof were detected in biological samples of subjects

having active disease compared to biological samples of subjects having indolent disease, whereas low levels of BCMA or a fragment thereof were detected in biological samples of subjects having impaired immune systems compared to biological samples of normal healthy subjects, levels of BCMA or a fragment thereof can be used to reliably monitor the immune status of a subject.

In various other embodiments, methods for reliably monitoring the response of a subject to treatments targeted to improve the immune status of the subject are provided. Levels of BCMA or a fragment thereof are used to monitor the response of a subject to treatments targeted to improve the immune status of a subject. Without wishing to be bound to a particular theory, it is believed that because levels of BCMA or a fragment thereof in a biological sample obtained from a subject correlated with the immune status of the subject, the levels of BCMA or a fragment thereof in the biological sample can be determined at different times point subsequent to start of the treatment and compared to an initial time point prior to start of the treatment to monitor the response of a subject to treatments targeted to improve the immune status of the subject.

The practice of the invention will employ, unless indicated specifically to the contrary, conventional methods of chemistry, biochemistry, organic chemistry, molecular biology, microbiology, recombinant DNA techniques, genetics, immunology, and cell biology that are within the skill of the art, many of which are described below for the purpose of illustration. Such techniques are explained fully in the literature. See, *e.g.*, Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual* (3rd Edition, 2001); Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); Maniatis *et al.*, *Molecular Cloning: A Laboratory Manual* (1982); Ausubel *et al.*, *Current Protocols in Molecular Biology* (John Wiley and Sons, updated July 2008); *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, Greene Pub. Associates and Wiley-Interscience; Glover, *DNA Cloning: A Practical Approach*, vol. I & II (IRL Press, Oxford, 1985); Anand, T*echniques for the Analysis of Complex Genomes*, (Academic Press, New York, 1992); T*ranscription and Translation* (B. Hames & S. Higgins, Eds., 1984); Perbal, *A*

*Practical Guide to Molecular Cloning* (1984); and Harlow and Lane, *Antibodies*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1998).

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

5 **A. Definitions**

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, 10 preferred embodiments of compositions, methods and materials are described herein. For the purposes of the present invention, the following terms are defined below.

The articles “a,” “an,” and “the” are used herein to refer to one or to more than one (*i.e.,* to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

15 As used herein, the term “about” or “approximately” refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by as much as 30, 25, 20, 25, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 % to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length. In particular embodiments, the terms “about” or “approximately” when 20 preceding a numerical value indicates the value plus or minus a range of 15%, 10%, 5%, or 1%.

Throughout this specification, unless the context requires otherwise, the words “comprise”, “comprises” and “comprising” will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion 25 of any other step or element or group of steps or elements. By “consisting of” is meant including, and limited to, whatever follows the phrase “consisting of.” Thus, the phrase “consisting of” indicates that the listed elements are required or mandatory, and that no other elements may be present. By “consisting essentially of” is meant including any elements listed after the phrase, and limited to other elements that do not interfere with 30 or contribute to the activity or action specified in the disclosure for the listed elements.

Thus, the phrase "consisting essentially of" indicates that the listed elements are required or mandatory, but that no other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

5 Reference throughout this specification to "one embodiment," "an embodiment," "embodiment," "a particular embodiment," "a related embodiment," "a certain embodiment," "an additional embodiment," "some embodiments," "other embodiments," "additional embodiments," "Further embodiments," or "a further embodiment" or combinations thereof means that a particular feature, structure or 10 characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, the appearances of the foregoing phrases in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments.

15 As used herein, the term "BCMA" is intended to generically refer to both the wild-type and variant B-cell maturation antigen polypeptides, unless specifically denoted otherwise. BCMA polypeptides are encoded by the BCMA gene. As it is commonly used in the art, the term "gene" is intended to refer to the genomic region encompassing 5′ untranslated region(s) (UTR), exons, introns, and 3′ UTR. 20 Individual segments may be specifically referred to, *e.g.*, promoter, coding region, etc. Combinations of such segments that provide for a complete BCMA protein may be referred to generically as a protein coding sequence. There are four major haplotypes of the BCMA gene in the human genome, in the present disclosure the term "BCMA" is meant to encompass all four (Kawasaki *et al.*, Genes Immun. 2:276-9, 2001).

25 The terms "BCMA" or "BCMA polypeptide" are used interchangeably and encompass an amino acid sequence encoded by an open reading frame (ORF) of a known BCMA polynucleotide, including the full-length native polypeptide and fragments thereof, particularly biologically active fragments and/or fragments corresponding to functional domains, *e.g.*, a region or domain having biological 30 activity, *etc.*; antigenic fragments thereof, and including fusions of the subject polypeptides to other proteins or parts thereof. The amino acid sequences of BCMA

polypeptides have been disclosed. (*See e.g.*, Laabi *et al.*, *Nucleic Acids Research* 22: 1147-1154, 1994; Laabi *et al.*, *EMBO J.,* 11: 3897-3904 (1992); Gras *et al.*, *Int. Immunology,* 7: 1093-1106 (1995); and Madry *et al.*, *Int. Immunology,* 10: 1693-1702 (1998). The BCMA polypeptides of the invention can be isolated from a variety of sources, such as from human tissue types or biological samples such as serum, plasma, bone, marrow, or tissue.

As used herein, the term "fragment thereof" refers to a portion of the full-length native BCMA polypeptide. In some embodiments, the BCMA fragment is a cleaved BCMA polypeptide. In some embodiments, the cleaved BCMA polypeptide is a soluble form of the BCMA polypeptide.

In certain embodiments, the BCMA polypeptide or a fragment thereof comprises an amino acid sequence having at least about 20% identity, at least about 30% identity, at least about 40% identity, at least about 50% identity, at least about 60% identity, at least about 70% identity, at least about 75% identity, at least about 80% identity, at least about 90% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, or at least about 99% identity with the soluble form of the human BCMA polypeptide (SEQ ID NO:1).

In some embodiments, the BCMA polypeptide or a fragment thereof comprises an amino acid sequence having at least about 20% identity, at least about 30% identity, at least about 40% identity, at least about 50% identity, at least about 60% identity, at least about 70% identity, at least about 75% identity, at least about 80% identity, at least about 90% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, or at least about 99% identity with the full-length native human BCMA polypeptide (SEQ ID NO:2).

In other embodiments, the BCMA polypeptide or a fragment thereof comprises at least about 50, least about 45, least about 40, least about 30, least about 20, least about 10, or least about 5 amino acids. In specific embodiments, the BCMA polypeptide or a fragment thereof comprises 54 amino acids.

As used herein, the term "immune status" of a subject refers to the efficiency of the subject's immune system. As such, the immune status of a subject indicates whether the subject's immune system is normal, impaired (for *e.g.*, if the

subject is afflicted with an immune deficiency disease), or hyperactive (for *e.g.*, if the subject is afflicted with a disease, an autoimmune disease, or an illness) compared to a normal healthy subject.

The term "immune system" refers to a system of many biological structures and processes within an organism that protects against disease.

The following are non-limiting embodiments of polynucleotides: a gene or gene fragment, exons, introns, mRNA, tRNA, rRNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A nucleic acid molecule may also comprise modified nucleic acid molecules, such as methylated nucleic acid molecules and nucleic acid molecule analogs. Analogs of purines and pyrimidines are known in the art. Nucleic acids may be naturally occurring, *e.g.*, DNA or RNA, or may be synthetic analogs, as known in the art. Such analogs may be preferred for use as probes because of superior stability under assay conditions. Modifications in the native structure, including alterations in the backbone, sugars or heterocyclic bases, have been shown to increase intracellular stability and binding affinity. Among useful changes in the backbone chemistry are phosphorothioates; phosphorodithioates, where both of the non-bridging oxygens are substituted with sulfur; phosphoroamidites; alkyl phosphotriesters and boranophosphates. Achiral phosphate derivatives include 3′-O′-5′-S-phosphorothioate, 3′-S-5′-O-phosphorothioate, 3′-CH2-5′-O-phosphonate and 3′-NH-5′-O-phosphoroamidate. Peptide nucleic acids replace the entire ribose phosphodiester backbone with a peptide linkage.

The terms "polypeptide" and "protein", used interchangeably herein, refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones. In various embodiments, BCMA polypeptides are contemplated for use within diagnostic, prognostic, or monitoring compositions and methods disclosed herein. The term includes fusion proteins, including, but not limited to, fusion proteins with a heterologous amino acid sequence, fusions with heterologous and homologous leader sequences, with or without N-terminal methionine residues; immunologically tagged proteins; and the like.

A "substantially isolated" or "isolated" substance is one that is substantially free of its associated surrounding materials in nature. By substantially free is meant at least 50%, preferably at least 70%, more preferably at least 80%, and even more preferably at least 90% free of the materials with which it is associated in nature. As used herein, an "isolated" can refer to polynucleotides, polypeptides, cells, samples, and antibodies.

Hybridization reactions can be performed under conditions of different "stringency". Conditions that increase stringency of a hybridization reaction are widely known and published in the art. *See*, for example, Sambrook *et al.* (1989). Examples of relevant conditions include (in order of increasing stringency): incubation temperatures of 25° C., 37° C., 50° C. and 68° C.; buffer concentrations of 10×SSC, 6×SSC, 1×SSC, 0.1×SSC (where SSC is 0.15 M NaCl and 15 mM citrate buffer) and their equivalents using other buffer systems; formamide concentrations of 0%, 25%, 50%, and 75%; incubation times from 5 minutes to 24 hours; 1, 2, or more washing steps; wash incubation times of 1, 2, or 15 minutes; and wash solutions of 6×SSC, 1×SSC, 0.1×SSC, or deionized water. Examples of stringent conditions are hybridization and washing at 50° C. or higher and in 0.1×SSC (9 mM NaCl/0.9 mM sodium citrate).

The term "target cell" includes an individual cell, cell from a biological sample, or cell culture. Target cells include progeny of a single target cell, and the progeny may not necessarily be completely identical (in morphology or in total DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation and/or change. In particular embodiments, target cells include multiple myeloma, chronic lymphocytic leukemia, lymphoma, or Waldenstrom's macroglobulinemia tumor cells, bone marrow or peripheral blood mononuclear cells, B-cells, or plasma cells.

The detection systems of the invention are based, in part, on the ability of a binding agent to bind BCMA or a fragment thereof. Generally, the invention contemplates the use of a binding agent that specifically binds BCMA or a fragment thereof, resulting in the formation of a detectable complex of BCMA or a fragment thereof and binding agent. In some embodiments, the invention utilizes two binding agents, a capture binding agent and a detection binding agent, both of which bind to

BCMA or a fragment thereof, resulting in the formation of a ternary complex comprising capture binding agent, BCMA, and detection binding agent.

Any of a variety of binding agents may be used, including, for example, polypeptides, sugars, and nucleic acids. In yet other embodiments, the invention further includes the use of an additional binding agent that binds to the detection binding agent. Such an additional binding agent may be useful, *e.g.*, in detecting bound detection binding agent. Accordingly, one example of such an additional binding agent is antibodies specific for a fragment of an antibody, *e.g.*, an $F_c$ fragment, which may be detectably labeled and, therefore used to detect bound detection binding agent, and are particularly useful when the detection binding agent is not itself easily amenable to labeling. In certain embodiments, the binding agent is an antibody specific for bacteria.

The term "binds specifically," in the context of antibody binding, refers to high avidity and/or high affinity binding of an antibody to a specific polypeptide *i.e.*, epitope of a BCMA or a fragment thereof. Antibody binding to an epitope on a specific polypeptide (also referred to herein as "an epitope") is preferably stronger than binding of the same antibody to any other epitope, particularly those which may be present in molecules in association with, or in the same sample, as the specific polypeptide of interest, *e.g.*, binds more strongly to a specific BCMA epitope than to a different BCMA epitope or non-BCMA epitope. Antibodies which bind specifically to a polypeptide of interest may be capable of binding other polypeptides at a weak, yet detectable, level (*e.g.*, 10% or less, 5% or less, 1% or less of the binding shown to the polypeptide of interest). Such weak binding, or background binding, is readily discernible from the specific antibody binding to the compound or polypeptide of interest, *e.g.* by use of appropriate controls. In general, antibodies used in compositions and methods of the invention which bind to a specific BCMA polypeptide or a fragment thereof with a binding affinity of $10^7$ moles/L or more, preferably $10^8$ moles/L or more are said to bind specifically to the specific BCMA polypeptide. In general, an antibody with a binding affinity of $10^6$ moles/L or less is not useful in that it will not bind an antigen at a detectable level using conventional methodology currently used.

In some embodiments, the affinity of specific binding of a BCMA binding agent to BCMA or a fragment thereof is about 2 times greater than background

binding, about 5 times greater than background binding, about 10 times greater than background binding, about 20 times greater than background binding, about 50 times greater than background binding, about 100 times greater than background binding, or about 1000 times greater than background binding or more.

5 In other embodiments, the affinity of specific binding is between about 2 to about 1,000 times greater than background binding, between about 2 to 500 times greater than background binding, between about 2 to about 100 times greater than background binding, between about 2 to about 50 times greater than background binding, between about 2 to about 20 times greater than background binding, between 10 about 2 to about 10 times greater than background binding, between about 5 to about 100 times greater than background binding, between about 5 to about 50 times greater than background binding, between about 5 to about 20 times greater than background binding, between about 10 to about 100 times greater than background binding, between about 10 to about 50 times greater than background binding, between about 50 15 to about 500 times greater than background binding, or any intervening range of affinity.

Accordingly, specific binding occurs between a binding agent and BCMA or a fragment thereof where there is an interaction between the two which produces a bound complex having the characteristics of an antibody/antigen or 20 enzyme/substrate interaction. In some embodiments, specific binding is characterized when one member of a pair substantially binds to a particular species and to no other species within the family of compounds to which the corresponding member of the binding member belongs. In other embodiments, specific binding is characterized when one member of a pair substantially binds to one or more particular species and to no 25 other species within the family of compounds to which the corresponding member of the binding member belongs. In yet other embodiments, specific binding is characterized when one member of a pair substantially binds to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more particular species and to no other species within the family of compounds to which the corresponding member of the binding member belongs.

30 Generally speaking, the binding affinity of a binding agent of the invention (A) to BCMA or a fragment thereof (B) can be generally expressed by the

chemical equilibrium constant $K_d$ resulting from the following reaction: [A] +[B]-[AB]. The chemical equilibrium constant $K_d$ is then given by: $K_d$=[A]x[B]/[AB]. Whether the binding of a binding agent is specific or not can be judged from the difference between the binding affinity ($K_d$ value) of the binding agent to BCMA or a fragment thereof, versus the binding to another polypeptide.

$K_d$ values and differences in $K_d$ values can be measured using, for example, *in vitro* or *in vivo* binding assays and/or assays on other materials such as a polystyrene microtitre plate or a specialized surface in an analytical biosensor. In some embodiments, the difference between the $K_d$ value of a binding agent to BCMA or a fragment thereof, versus the binding to an undesired polypeptide is about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about-9 fold, about 10-fold, about 20-fold, about 50-fold, about 100-fold, about 1000-fold, or more.

In other embodiments, the $K_d$ value is less than $10^{4}$ M, less than $10^{5}$ M, less than $10^{6}$ M, less than $10^{7}$ M, less than $10^{8}$ M, less than $10^{9}$ M, less than $10^{10}$ M and could be $10^{11}$ M, less than $10^{12}$ M, less than $10^{13}$ M, less than $10^{14}$ M, less than $10^{15}$ M or less.

In other aspects, the $K_d$ value is between about $10^{4}$ M and about $10^{15}$ M, between about $10^{4}$ M and about $10^{12}$ M, between about $10^{4}$ M and about $10^{10}$ M, between about $10^{6}$ M and about $10^{15}$ M, between about $10^{6}$ M and about $10^{12}$ M, between about $10^{6}$ M and about $10^{10}$ M, between about $10^{8}$ M and about $10^{15}$ M, between about $10^{8}$ M and about $10^{12}$ M, between about $10^{8}$ M and about $10^{10}$ M, between about $10^{7}$ M and about $10^{10}$ M, or any intervening range of affinity.

The term "antibody" herein is used in the broadest sense and specifically 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.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that can be present in minor amounts.

In some embodiments, the monoclonal antibody is an anti-BCMA monoclonal antibody. In other embodiments, the monoclonal antibody specifically recognizes an epitope present in a fragment of the BCMA polypeptide.

Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al.*, Nature, 256: 495 (1975), or may be made by recombinant DNA methods (see, *e.g.*, U.S. Pat. No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.*, Nature, 352: 624-628 (1991) and Marks *et al.*, J. Mol. Biol., 222: 581-597 (1991), for example.

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

"Humanized" forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab′,

F (ab′) 2 or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin.

For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementarity-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and maximize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence although the FR regions may include one or more amino acid substitutions that improve binding affinity. The number of these amino acid substitutions in the FR is typically no more than 6 in the H chain, and no more than 3 in the L chain. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.*, Nature, 321: 522-525 (1986); Reichmann *et al.*, Nature, 332: 323-329 (1988); and Presta, *Curr. Op. Struct. Biol.*, 2: 593-596 (1992). The humanized antibody includes a PRIMATIZED antibody wherein the antigen-binding region of the antibody is derived from an antibody produced by, *e.g.*, immunizing macaque monkeys with the antigen of interest. Methods of making humanized antibodies are known in the art.

Human antibodies can also be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222: 581 (1991). The techniques of Cole *et al.* and Boerner *et al.* are also available for the preparation of human monoclonal antibodies. Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner *et al.*, *J. Immunol.*, 147(1): 86-95 (1991).

"Functional fragments" of the binding antibodies of the invention are those fragments that retain binding to antigen with substantially the same affinity as the intact full chain molecule from which they are derived.

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than about 95% by weight of antibody as determined by the Lowry method, and most preferably more than about 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

The terms "detectably labeled antibody" refers to an antibody (or antibody fragment which retains binding specificity for a BCMA or a fragment thereof), having an attached detectable label. The detectable label is normally attached by-chemical conjugation, but where the label is a polypeptide, it could alternatively be attached by genetic engineering techniques. Methods for production of detectably labeled proteins are well known in the art. Detectable labels may be selected from a variety of such labels known in the art, including, but not limited to, haptens, radioisotopes, fluorophores, paramagnetic labels, enzymes (*e.g.*, horseradish peroxidase), or other moieties or compounds which either emit a detectable signal (*e.g.*, radioactivity, fluorescence, color) or emit a detectable signal after exposure of the label to its substrate. Various detectable label/substrate pairs (*e.g.*, horseradish peroxidase/diaminobenzidine, avidin/streptavidin, luciferase/luciferin)), methods for labeling antibodies, and methods for using labeled antibodies are well known in the art (*see*, for example, Harlow and Lane, eds. (*Antibodies: A Laboratory Manual* (1988) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.)).

In one technique, an immunogen comprising the polypeptide is initially injected into any of a wide variety of mammals (*e.g.*, mice, rats, rabbits, sheep or goats). Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a
5 suitable solid support. In some embodiments, the antibody is an anti-BCMA polyclonal antibody. In other embodiments, the antibody is polyclonal antibody that recognizes a fragment of the BCMA polypeptide.

A "biological sample" encompasses a variety of sample types obtained from an individual and can be used in a diagnostic or monitoring assay. The definition
10 encompasses blood and other liquid samples of biological origin, solid tissue samples such as a biopsy specimen or tissue cultures or cells derived there from and the progeny thereof. The definition also includes samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components, such as polynucleotides. The term "biological
15 sample" encompasses a clinical sample, and also includes, without limitation, cells in culture, cell supernatants, cell lysates, serum, plasma, urine, cerebral spinal fluid, biological fluid, and tissue samples. The sample may be pretreated as necessary by dilution in an appropriate buffer solution or concentrated, if desired. Any of a number of standard aqueous buffer solutions, employing one of a variety of buffers, such as
20 phosphate, Tris, or the like, preferably at physiological pH can be used. Biological samples can be derived from patients using well known techniques such as venipuncture, lumbar puncture, fluid sample such as saliva or urine, or tissue biopsy and the like.

As used herein, the terms "correlated with" or "associated with" refer to
25 the levels of BCMA or a fragment thereof in a biological sample of a subject that has a statistically significant correlation with a physiologic state, *e.g.,* disease status or extent of the disease, response to treatment, and survival. The strength of the correlation between levels of BCMA or a fragment thereof and the presence or absence of a particular physiologic state may be determined by a statistical test of significance.
30 Methods for determining the strength of a correlation between the expression level of a differentially-expressed gene and a particular physiologic state by assigning a statistical

score to the correlation are reviewed in Holloway *et al.* (2002) *Nature Genetics* Suppl. 32:481-89, Churchill (2002) *Nature Genetics* Suppl. 32:490-95, Quackenbush (2002) *Nature Genetics* Suppl. 32: 496-501; Slonim (2002) *Nature Genetics* Suppl. 32:502-08; and Chuaqui *et al.* (2002) *Nature Genetics* Suppl. 32:509-514; each of which is herein
5 incorporated by reference in its entirety.

A "conjugate" refers to any molecule, *e.g.*, antibody bound or joined covalently or non-covalently to another molecule, *e.g.*, a hapten, small molecule, or label, including fusion proteins and as well as molecules that contain both amino acid or protein portions and non-protein portions. Conjugates may be synthesized by a variety
10 of techniques known in the art including, for example, solid phase synthesis, solution phase synthesis, organic chemical synthetic techniques or a combination of these techniques. The choice of synthesis will depend upon the particular molecule to be generated.

The terms "individual," "subject," and "patient," used interchangeably
15 herein, refer to a mammal, including, but not limited to, murines, simians, humans, mammalian farm animals, mammalian sport animals, and mammalian pets. In some embodiments, the subject is a human subject.

The term "mammal" refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as
20 dogs, horses, cats, cows, etc. In some embodiments, the mammal herein is human.

## B. Methods of Monitoring of Immune Status of a Subject

The present inventors have discovered that BCMA polypeptide levels or levels of a fragment thereof correlate with the immune status of a subject. As such, BCMA polypeptide levels or levels of a fragment thereof are decreased in biological
25 samples obtained from subjects with impaired immune systems, and increased in biological samples obtained from subjects suffering from an infection or disease. Accordingly, particular embodiments of the invention provide methods for the monitoring the immune status of a subject as well as monitoring the response of the subject to treatment, based upon the level of BCMA polypeptide or a fragment thereof
30 in a biological sample obtained from a patient, including, *e.g.*, a patient's bloodstream,

serum, bone marrow, or tissue at different time points. A variety of methods of determining BCMA levels are known and available in the art. In certain embodiments, these involve the use of a BCMA binding agent, such as a BCMA specific antibody. As discussed elsewhere herein, there are a variety of assay formats known to those of ordinary skill in the art and suitable for using a binding agent to detect polypeptide markers in a sample. *E.g.*, ELISA assays, lateral flow assays, etc.; *see also*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988.

In general, a condition leading to impairment of the immune system is monitored by the presence of at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 20-fold, at least about 50-fold, at least about 100-fold, at least about 1000-fold, or lower levels of BCMA as compared to those in a normal control subject. In general, a infective or disease state is monitored by the presence of at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 20-fold, at least about 50-fold, at least about 100-fold, at least about 1000-fold, or higher levels of BCMA as compared to those in a normal control subject.

In some embodiments, methods of monitoring immune status of a subject comprise: (a) detecting an amount of BCMA or a fragment thereof in a biological sample, *e.g.,* serum, obtained from a subject; and (b) comparing the amount of BCMA polypeptide or fragment thereof detected in (a) to a predetermined cut-off value or to an amount detected in a control serum sample, wherein a decreased amount of BCMA polypeptide or a fragment thereof in the biological sample of the subject as compared to the predetermined cut-off value or amount in the control serum sample is indicative of an impaired immune system, and an increased amount of BCMA polypeptide or a fragment thereof in the biological sample of the subject as compared to the predetermined cut-off value or amount in the control serum sample indicates that the subject is at higher risk of or is suffering from an infection or a disease, wherein the biological sample is a serum or plasma sample or supernatant obtained from a culture of the subject's bone marrow mononuclear cells or peripheral blood mononuclear cells.

In other embodiments, methods of monitoring immune status of a subject comprise: (a) detecting an amount of BCMA or a fragment thereof in a biological sample, *e.g.,* serum, obtained from a subject; and (b) comparing the amount

of BCMA polypeptide or a fragment thereof detected in (a) to a predetermined cut-off value or to an amount detected in a control serum sample, wherein a decreased amount of BCMA polypeptide or a fragment thereof in the biological sample of the subject as compared to the predetermined cut-off value or amount in the control serum or plasma sample is indicative of an impaired immune system, wherein the biological sample is a serum or plasma sample or supernatant obtained from a culture of the subject's bone marrow mononuclear cells or peripheral blood mononuclear cells.

In yet other embodiments, methods of monitoring immune status of a subject comprise: (a) detecting an amount of BCMA or a fragment thereof in a biological sample obtained from a subject at an initial time point; (b) detecting an amount of BCMA or a fragment thereof in the biological sample obtained from the subject at a later time point; and (c) comparing the amount of BCMA polypeptide or a fragment thereof detected in (a) to the amount of BCMA polypeptide or a fragment thereof detected in (b), wherein an decreased amount of BCMA polypeptide or a fragment thereof detected in (b) as compared to the amount of BCMA polypeptide or a fragment thereof detected in (a) indicates that the subject is responding to treatment, and wherein an increased or unchanged amount of BCMA polypeptide or a fragment thereof detected in (b) as compared to the amount of BCMA polypeptide or a fragment thereof detected in (a) indicates that the subject is not responding to treatment, wherein the biological sample is a serum sample or supernatant obtained from a culture of the subject's bone marrow mononuclear cells or peripheral blood mononuclear cells.

In some embodiments, the immune status of a subject may be determined by (a) contacting a biological sample obtained from a subject with a BCMA binding agent; (b) detecting in the sample a level of BCMA polypeptide that binds to the binding agent; and (c) comparing the level of BCMA polypeptide with a predetermined cut-off value or with the value obtained from a normal control subject. In certain embodiments, the cut-off value for the detection of impairment of the immune system and/or an infection or disease state is the average mean signal obtained when the immobilized antibody is incubated with samples from patients not suffering from an immune system impairment and not suffering from an infection or disease state.

In various embodiments, the biological sample includes, without limitation, cells in culture, cell supernatants, cell lysates, serum, plasma, urine, cerebral spinal fluid, biological fluid, and tissue samples. In certain embodiments, the biological sample is supernatant obtained from a culture of the subject's cells. In some aspects, 5 the cells are the subject's bone marrow mononuclear cells. In other aspects, the cells are the subject's peripheral blood mononuclear cells.

In particular embodiments, detection involves measuring BCMA mRNA levels present in the biological sample. In other embodiments, detection involves determining BCMA polypeptide levels present in the biological sample. In some 10 embodiments, detection is performed using one or more primers specific for BCMA. In other embodiments, detection is performed using an antibody specific for BCMA or a fragment thereof.

In certain embodiments, a sample generating a signal that is statistically stronger than the predetermined cut-off value is considered positive for an infection or 15 disease condition, whereas a sample generating a signal that is statistically weaker than the predetermined cut-off value is considered positive for an impaired immune system. In certain embodiments, the sample generates a signal that is up to about two standard deviations, up to about three standard deviations, up to about five standard deviations, up to about ten standard deviations, up to about twenty standard deviations, up to about 20 thirty standard deviations, up to about forty standard deviations, up to about fifty standard deviations, up to about sixty standard deviations, up to about seventy standard deviations, up to about eighty standard deviations, up to about ninety standard deviations, or up to about hundred standard deviations above the predetermined cut-off. In other embodiments, the sample generates a signal that is up to about two standard 25 deviations, up to about three standard deviations, up to about five standard deviations, up to about ten standard deviations, up to about twenty standard deviations, up to about thirty standard deviations, up to about forty standard deviations, up to about fifty standard deviations, up to about sixty standard deviations, up to about seventy standard deviations, up to about eighty standard deviations, up to about ninety standard 30 deviations, or up to about hundred standard deviations below the predetermined cut-off.

In other embodiments, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett *et al.*, *Clinical Epidemiology: A Basic Science for Clinical Medicine*, Little Brown and Co., 1985, p. 106-7. Briefly, in these embodiments, the cut-off value may be determined from a plot of pairs of true positive rates (*i.e.*, sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut--off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (*i.e.*, the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for an infection or disease condition, whereas a sample generating a signal that is three standard deviations below the predetermined cut-off value is considered positive for an impaired immune system.

In some embodiments, the assay involves the use of a BCMA binding agent immobilized on a solid support to bind to and remove the BCMA polypeptide from the remainder of the sample. The bound BCMA polypeptide may then be detected using a detection reagent that contains a reporter group and specifically binds to the binding agent/polypeptide complex. Such detection reagents may comprise, for example, a binding agent that specifically binds to the BCMA polypeptide or an antibody or other agent that specifically binds to the binding agent, such as an anti-immunoglobulin, protein G5 protein A or a lectin. In some embodiments, the BCMA detection reagent, *e.g.*, antibody, is bound to biotin which recognizes and specifically binds a streptavidin or avidin binding agent.

In certain embodiments, the assay is performed in a lateral flow or strip test format, as discussed elsewhere herein, wherein the BCMA binding agent, *e.g.*, antibody, is immobilized on a membrane, such as nitrocellulose. In the lateral flow test, BCMA polypeptides within the sample bind to the immobilized binding agent as the sample passes through the membrane. A second, labeled binding agent then binds to the BCMA binding agent-polypeptide complex as a solution containing the second

binding agent flows through the membrane. The detection of bound second binding agent may then be performed as described above. In the strip test format, one end of the membrane to which BCMA binding agent is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a region containing second binding agent and to the area of immobilized binding agent. Concentration of second binding agent at the area of immobilized antibody indicates the immune status of a subject.

In several embodiments, the invention provides similar methods for determining response of the immune status of a subject to treatment. Since serum BCMA levels correlate with the immune status, response to treatment or therapy is monitored by comparing BCMA levels in a subject's serum (or other biological sample) at different time points during the course of a treatment regimen. Thus, the present invention provides a rapid and reliable method of monitoring immune status of a subject and response to treatment of immune status, using, for *e.g.*, a serum or plasma sample obtained from the subject's bloodstream. In particular embodiments, the method is practiced by ELISA assay, lateral flow assay, or strip test assay using an antibody specific for BCMA.

The invention further provides systems and kits for monitoring immune status of a subject, comprising a reagent suitable for determining levels of BCMA polypeptide or a fragment thereof in a biological sample obtained from the subject, wherein the biological sample is a serum or plasma sample or supernatant obtained from culture of the subject's bone marrow mononuclear cells or peripheral blood mononuclear cells. In some embodiments, the kit includes reagents for performing ELISA, lateral flow, or strip test assays such as an antibody specific for BCMA. Detection systems and kits of the invention are described in further detail below.

## C. Detection Systems and Kits

In various embodiments, the present invention provides detection systems and kits for monitoring immune status of a subject. A detection system or kit of the present invention may be used for monitoring immune status of a subject using a biological sample, *e.g.*, serum, of a subject. The diagnostic kit could include the

method for the detection of antigen-antibody reaction in addition to the material. The detection method is preferably selected from the group consisting of flow cytometry, immunohistochemistry, and enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), enzyme immunoassay (EIA), fluorescence immunoassay (FIA), luminescence immunoassay (LIA), lateral flow assay, and strip assay. The reactivity of the antigen recognition material could be confirmed using device detecting an enzyme reaction, fluorescence, luminescence, or radiation. In some embodiments, monitoring the immune status of a subject can be performed with a flow cytometry kit, immunohistochemistry kit, ELISA kit or lateral flow or strip kit including the anti-BCMA antibody or an antigen binding fragment thereof.

In some embodiment, monitoring of the immune status of a subject can be performed with a flow cytometry kit, immunohistochemistry kit, ELISA kit or lateral flow or strip kit including an antibody that is specific for BCMA or a fragment thereof.

In some embodiments, a kit or system may comprise one or more or all of the following components: 1) one or more standards comprised of one or more of the biomarker(s) of the invention, such as BCMA or a fragment thereof; 2) a binding agent, such as an antibody or a plurality of antibodies, that are specific for the biomarker(s) that are to be assayed for using the kit; 3) written instructions; 4) diluents for samples and the standards; 5) a wash buffer; 6) color reagents; 7) stop solution; and 8) a carrier, such as an antibody carrier, for example, a lateral flow device, or a microplate with bound antibody, or polystyrene beads.

In some embodiments, the detection system or kit used to monitor immune status of a subject is a quantitative ELISA (enzyme-linked immunosorbent assay) that determines the concentration or concentrations of the biomarker or biomarker(s) in accordance with methods embodied by the invention. The principle of the assay is to use the quantitative sandwich enzyme immunoassay technique wherein a monoclonal or polyclonal antibody selective for a biomarker is pre-coated onto a carrier such as a microplate into its wells. The standards and sample are then pipetted into the wells and any of the biomarker that is present is bound to this immobilized antibody. Next, the wells are washed with washing buffer, and an enzyme-linked monoclonal or polyclonal antibody that is specific for the biomarker is added to the wells. Washing is

again performed, and then a substrate solution is added to the wells. Color subsequently develops in proportion to the amount of polypeptide of the invention that is bound in the first step. The color development is stopped using a stop solution, and the intensity of the color is measured by a microplate reader.

5 In other embodiments, the monitoring of the immune status of a subject may be carried out using, for example, a lateral flow assay. Such lateral flow assays have the potential to be a cost-effective, fast, simple, and sensitive method, for instance for on-site screening assays. The lateral flow assay comprises a carrier that allows a lateral flow to occur wherein either the sample or the detection reagent is displaced
10 form one location on the carrier to another. There are many formats of lateral flow assays suitable for use in the methods embodied by the invention, and the skilled person will readily know how to select and optimize a particular format. An example of a lateral flow test strip of the invention comprises, for example, the following components: sample pad; an absorbent pad onto which the test sample is applied; a
15 conjugate or reagent pad that contains antibodies specific to the target analyte and conjugated to colored particles (usually colloidal gold particles, or latex microspheres); a reaction membrane, typically a hydrophobic nitrocellulose or cellulose acetate membrane onto which anti-target analyte antibodies are immobilized in a line across the membrane as a capture zone or test line (a control zone may also be present, containing
20 antibodies specific for the conjugate antibodies); and a wick or waste reservoir, a further absorbent pad designed to draw the sample across the reaction membrane by capillary action and collect it.

There are a number of variations on lateral flow technology. The capture zone on the membrane may contain immobilized antigens or enzymes
25 depending on the target analyte rather than antibodies. It is also possible to apply multiple capture zones to create a multiplex test. For example, in particular embodiments, test strips able to detect BCMA or a fragment thereof and separately in the same sample additional biomarkers of a specific disease, *e.g.*, multiple myeloma, *e.g.*, β2M, IL-6, C-reactive protein, and serum monoclonal protein are contemplated.
30 Lateral flow immunoassays are simple to use by untrained operators and generally produce a result within 15 minutes. They are very stable and robust, have a long shelf

life and do not usually require refrigeration. They are also relatively inexpensive to produce. These features make them ideal for use at the point-of-care and for testing samples in the field, as well as in the laboratory.

While most lateral flow immunoassays are only capable of providing a qualitative result, it is possible to obtain some degree of quantification by measuring the amount of conjugate bound to the capture zone. This can be done using a dedicated reader to measure the intensity of the colored test line. For example, the Neogen Corporation has developed the Accuscan™ lateral flow reader for use with its range of Reveal® assay kits and Charm Sciences also supplies a reader for its Rosa® range of test strips. More sophisticated techniques, such as fluorescent dye labeled conjugates, have also been developed to improve the quantitative potential of lateral flow assays.

A detection system in kit form can include, for example, in an amount sufficient for at least one assay a polyclonal antibody composition or a monoclonal antibody composition that binds BCMA or a fragment thereof, as a packaged reagent. Instructions for use of the packaged reagent are also typically included.

A detection system in kit form can also include, for example, a means for combining the test sample with a buffering system (Reagent 1) containing viscosity controllers and stabilizers into a reaction vessel and mixing the solution. A detection system in kit form can also include a means for reading the a parameter of the reaction vessel with sample and buffer, and further means for combining the test sample and buffer mixture with a fluorescence-labeled ligand (Reagent 2) to said biological substance in the reaction vessel, mixing the solution to produce an assay solution. Furthermore, Reagent 2 may be delivered to the reaction vessel without further dilution volume of the assay solution.

As used herein, the term “package” refers to a solid matrix or material such as glass, plastic, paper, foil and the like capable of holding within fixed limits an antibody composition or monoclonal antibody composition. Thus, for example, a package can be a glass vial used to contain milligram quantities of a contemplated polypeptide or it can be a microtiter plate well to which microgram quantities of a contemplated polypeptide or antibody have been operatively affixed.

"Instructions for use" typically include a tangible expression describing the reagent concentration or at least one assay method parameter such as the relative amounts of reagent and sample to be admixed, maintenance time periods for reagent/sample admixtures, temperature, buffer conditions and the like.

5 In particular embodiments, a detection system of the present invention further includes a label or indicating means capable of signaling the formation of a complex containing a polypeptide or antibody molecule of the present invention.

"Complex" as used herein refers to the product of a specific binding reaction such as an antibody-antigen or receptor-ligand reaction. Exemplary complexes 10 are immunoreaction products.

As used herein, the terms "label" and "indicating means" in their various grammatical forms refer to single atoms and molecules that are either directly or indirectly involved in the production of a detectable signal to indicate the presence of a complex. Any label or indicating means can be linked to or incorporated in an 15 expressed protein, polypeptide, or antibody molecule that is part of an antibody or monoclonal antibody composition of the present invention, or used separately, and those atoms or molecules can be used alone or in conjunction with additional reagents such labels are themselves well-known in clinical diagnostic chemistry and constitute a part of this invention only insofar as they are utilized with otherwise novel proteins 20 methods and/or systems.

The labeling means can be a fluorescent labeling agent that chemically binds to antibodies or antigens without denaturing them to form a fluorochrome (dye) that is a useful immunofluorescent tracer. Suitable fluorescent labeling agents are fluorochromes such as fluorescein isocyanate (FIC), fluorescein isothiocyante (FITC), 25 5-dimethylamine-1-naphthalenesulfonyl chloride (DANSC), tetramethylrhodamine isothiocyanate (TRITC), lissamine, rhodamine 8200 sulphonyl chloride (RB 200 SC) and the like. A description of immunofluorescence analysis techniques is found in DeLuca, "Immunofluorescence Analysis", in *Antibody As a Tool*, Marchalonis, *et al.*, eds., John Wiley & Sons, Ltd., pp. 189-231 (1982), which is incorporated herein by 30 reference.

In certain embodiments, the indicating group is an enzyme, such as horseradish peroxidase (HRP), glucose oxidase, or the like. In such cases where the principal indicating group is an enzyme such as HRP or glucose oxidase, additional reagents are required to visualize the fact that a receptor-ligand complex
5 (immunoreactant) has formed. Such additional reagents for HRP include hydrogen peroxide and an oxidation dye precursor such as diaminobenzidine. An additional reagent useful with glucose oxidase is 2,2′-azino-di-(3-ethyl-benzthiazoline-G-sulfonic acid) (ABTS).

In other embodiments, the indicating group is a green fluorescent protein
10 (GFP).

Radioactive elements are also useful labeling agents and are used illustratively herein. An exemplary radiolabeling agent is a radioactive element that produces gamma ray emissions. Elements which themselves emit gamma rays, such as $^{124}I$, $^{125}I$, $^{128}I$, $^{132}I$ and $^{51}Cr$ represent one class of gamma ray emission-producing
15 radioactive element indicating groups. Particularly preferred is $^{125}I$. Another group of useful labeling means are those elements such as $^{11}C$, $^{18}F$, $^{15}O$ and $^{13}N$ which themselves emit positrons. The positrons so emitted produce gamma rays upon encounters with electrons present in the animal's body. Also useful is a beta emitter, such $^{111}$indium or $^{3}H$.

20 The linking of labels, *i.e.*, labeling of, polypeptides and proteins is well known in the art. For instance, antibody molecules produced by a hybridoma can be labeled by metabolic incorporation of radioisotope-containing amino acids provided as a component in the culture medium. *See*, for example, Galfre *et al.*, *Meth. Enzymol.*, 73:3-46 (1981). The techniques of protein conjugation or coupling through activated
25 functional groups are particularly applicable. *See*, for example, Aurameas, *et al.*, *Scand. J. Immunol.*, Vol. 8 Suppl. 7:7-23 (1978), Rodwell *et al.*, *Biotech.*, 3:889-894 (1984), and U.S. Pat. No. 4,493,795, which are all incorporated herein by reference.

The detection systems or kits of the present invention can be used in an "ELISA" format to detect, for example, the presence or quantity of BCMA or a
30 fragment thereof in a body fluid sample such as the bloodstream, plasma, serum, bone marrow, or tissue, etc. "ELISA" refers to an enzyme-linked immunosorbent assay that

employs an antibody or antigen bound to a solid phase and an enzyme-antigen or enzyme-antibody conjugate to detect and quantify the amount of an antigen or antibody present in a sample. Thus, for example, a polypeptide, antibody molecule composition or monoclonal antibody molecule composition of the present invention can be affixed to a solid matrix to form a solid support that comprises a package in the subject diagnostic systems. The reagent is typically affixed to the solid matrix by adsorption from an aqueous medium although other modes of affixation, well known to those skilled in the art, can be used.

Useful solid matrices are also well known in the art. Such materials are water insoluble and include cross-linked dextran; agarose; beads of polystyrene beads about 1 micron to about 5 millimeters in diameter; polyvinyl chloride, polystyrene, cross-linked polyacrylamide, nitrocellulose- or nylon-based webs such as sheets, strips or paddles; or tubes, plates or the wells of a microtiter plate such as those made from polystyrene or polyvinylchloride.

The reagent species, labeled specific binding agent or amplifying reagent of any detection system described herein can be provided in solution, as a liquid dispersion or as a substantially dry power, *e.g.*, in lyophilized form. Where the indicating means is an enzyme, the enzyme's substrate can also be provided in a separate package of a system. A solid support such as the before-described microtiter plate and one or more buffers can also be included as separately packaged elements in this detection assay system.

The packaging materials discussed herein in relation to detection systems are those customarily utilized in diagnostic systems. Such materials include glass and plastic (*e.g.*, polyethylene, polypropylene and polycarbonate) bottles, vials, plastic and plastic-foil laminated envelopes and the like. In some embodiments, a detection system of the present invention is useful for assaying for the presence of BCMA or a fragment thereof. In certain embodiments, such a system comprises, in kit form, a package containing an antibody to BCMA or a fragment thereof.

All publications, patent applications, and issued patents cited in this specification are herein incorporated by reference as if each individual publication,

patent application, or issued patent were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims. The following examples are provided by way of illustration only and not by way of limitation. Those of skill in the art will readily recognize a variety of noncritical parameters that could be changed or modified to yield essentially similar results.

## EXAMPLES

### Example 1: Enzyme-linked immunosorbent assay for determination of BCMA concentrations in serum and supernatant fluid from BMMC cultures

Serum and supernatant samples were analyzed by BCMA enzyme-linked immunosorbent assay (ELISA) obtained from R&D Systems, Minneapolis, MN, USA (catalogue #DY193E). Serum samples were diluted 1:50 or 1:500 and the BCMA ELISA assay carried out according to the manufacturer's protocol. The ELISA plates were analysed using a μQuant (Biotek Industries, Winooski, VT, USA) plate reader set to 450 nm with KC Junior software. Values represent the mean of triplicate samples on each specimen. This BCMA ELISA kit does not cross react with recombinant human APRIL or BAFF, recombinant human TACI/Fc or recombinant mouse BCMA/Fc or mouse BCMA.

*Polyclonal anti-BCMA antibody (Ab) blocking experiment*

B-cell maturation antigen standards were incubated with another polyclonal goat anti-human BCMA Ab (catalogue #AF193; R&D Systems) or control Ab at a high (400 ng/ml) or low (40 ng/ml) concentration overnight at 4°C. Polyclonal goat IgG Ab was used as an isotype control (catalogue # AB-108-C; R&D Systems). We also tested the ability of this polyclonal anti-BCMA Ab to block detection of BCMA from the serum of MM Patient 1056 following an overnight incubation and BCMA levels were assessed using the BCMA ELISA protocol described above.

*Detection of BCMA with a monoclonal anti-BCMA Ab*

B-cell maturation antigen standards or serum (diluted 1:50 OR 1:500) from MM patients were incubated using a murine monoclonal anti-human BCMA Ab (catalogue # WH0000608M1; Sigma-Aldrich), instead of the polyclonal "capture Ab"
5 used in the BCMA ELISA. The samples were then assayed according to the BCMA ELISA protocol.

*MM xenograft studies*

Six-week old CB17 SCID mice were obtained from Charles River Laboratories (Wilmington, MA, USA). Animal studies were conducted according to
10 protocols approved by the Institutional Animal Care and Use Committee. To establish the CD38 and CD138–expressing LAGκ–2 tumor, a BM biopsy from a MM patient showing IgGκ paraprotein was implanted into the hind limb of a SCID mouse (Campbell & Berenson, 2008). Sera from mice containing the xenograft did not show human IgG or free κ light chains; and, thus, this xenograft was characterized as non-
15 secretory. However, κ chains were observed in the cytosol of tumor cells using immunhistochemical (IHC) staining. The LAGκ-1A tumor was developed from a patient with an IgGκ-producing MM resistant to lenalidomide (Campbell & Berenson, 2008). The LAGλ-1 tumor was developed from a MM patient who showed IgGλ paraprotein (Campbell & Berenson, 2008). The xenografts were excised, sectioned into
20 20–40 $mm^3$ pieces, and implanted into the muscle. Seven days post-tumor implantation, mice were randomized into treatment groups. Animals were euthanized when the tumors reached 2.5 cm in diameter.

The proteasome inhibitor (PI) bortezomib (Millennium Pharmaceuticals, Cambridge, MA, USA) was used as a 1 mg/ml stock solution and diluted using 0.9%
25 sodium chloride (NaCl). Bortezomib was administered i.v. at 0.75 mg/kg twice weekly. Cyclophosphamide (Florida Infusion, Palm Harbor, FL, USA) was dissolved from a stock solution of 20 mg/mL with NaCl and administered at 10 mg/kg via oral gavage once weekly. Melphalan (Sigma-Aldrich) at 3 mg was dissolved in 100 μL Acid-EtOH (47 μl concentrated HCl and 1 ml 100% EtOH) and diluted to 1 mL with phosphate-
30 buffered saline (PBS) to generate a 3 mg/mL stock solution. The drug was administered via intraperitoneal (i.p.) injection twice weekly at a dose of 3 mg/kg.

Tumors were measured using standard calipers and the formula for an ellipsoid volume was applied ($4/3\pi$ x [width/2]$^2$ x [length/2]). Tumor growth and IgG curves were analyzed in terms of treatment group means and standard error.

Mice were bled weekly via retro-orbital sinus to determine human IgG and BCMA levels. Samples were spun at 10,000 rpm for 5 min and serum was collected. The human IgG ELISA kit (Bethyl Laboratories, Montgomery, TX, USA) was used according to the manufacturer's specifications. Absorbance at 450 nm with a reference wavelength of 550 nm was determined on a μQuant microplate spectrophotometer with KC Junior software (Bio-Tek Instruments, Winooski, VT, USA). The human BCMA ELISA kit (R&D Systems) was used to determine serum protein levels.

*Immunohistochemical analysis*

BCMA protein expression was determined in MM and normal BMMCs and in our human MM xenografts. For the xenografts, 5 μm sections were cut after fixation in 4% paraformaldehyde. For the BMMCs, the cells were fixed with 1% paraformaldehyde and 1 x $10^5$ cells/slide were cytopsun. The slides were blocked with 0.05% Tween-20 PBS (PBST) and 3% bovine serum albumin (BSA) for 1 h at room temperature (RT). The samples were exposed to the anti-human BCMA Ab (5 μg/mL) at 4°C overnight. The slides were washed three times with TBST and treated with horseradish peroxidase conjugated with either anti-mouse, anti-rabbit or anti-goat antibodies (KPL, Gaithersburg, MD, USA) diluted 1:500 in TBST at RT for 2 h. The slides were washed three times in TBST and placed in 3-amino-9-ethylcarbazole (AEC) buffer for 5 min, and color was detected using an AEC kit (Vector Laboratories, Burlingame, CA, USA). For light chain staining, BMMCs were resuspended in 100 μL PBS and cytospun on slides. The samples were blocked with 3% BSA before the Ab was added to prevent non-specific binding. Goat anti-human λ light chain Ab (Sigma-Aldrich), anti-human κ light chain Ab (Sigma-Aldrich) or isotype control Ab (R&D System) was added to the corresponding samples. These antibodies were incubated overnight at 4°C. On the following day, the antibodies were washed with 0.05 mol/L TBST buffer. The samples were then treated with 10% $H_2O_2$ methanol before the secondary Ab. The samples were then incubated with peroxidase-labeled rabbit anti-

goat Ab (KPL) for 2 h at RT and then washed. Peroxidase substrate (Vector Laboratories) was added to the samples for 30 min. The cells were stained with haematoxylin for 1 min, and the samples were mounted. BCMA and λ and κ light chain expression was determined using a light microscope (Olympus BX51; Olympus, San Diego, CA, USA). Haematoxylin and eosin (H&E) staining was performed on BMMCs using standard staining procedures.

*Statistical analyses*

Statistical significance of differences observed in supernatant, serum and xenograft studies was determined using a Student's t-test. The minimal level of significance was $P < 0.05$. Statistical analysis was determined using GRAPHPAD PRISM version 4.03 for Windows (GraphPad Software, San Diego, CA, USA).

In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

**Example 2: BCMA is found in the serum of control human subjects and a patient with a low IgG**

Serum from patients with low IgG and control healthy human subjects was obtained and analyzed for the presence of BCMA. A subject with low IgG levels had low serum BCMA levels (14.6 ng/mL) compared to serum BCMA levels (median=36.0 ng/mL; range=13.45 ng/ml-958.1 ng/mL) in control subjects (N=104).

**Example 3: Serum IgG Levels of Patients With IgA MM Who Have Achieved Complete Remission Based on Their Serum BCMA Levels**

Serum from patients with IgA multiple myeloma (MM) who have achieved complete remission (CR) was obtained and the serum IgG and BCMA levels were analyzed. IgG levels of patients with IgA MM who have achieved CR with no measurable myeloma correlate with their serum BCMA levels. IgA MM patients (N=23) who are in CR who show low serum BCMA (≤10 ng/mL; $p<0.0001$) have

significantly decreased IgG levels (median=319.0 mg/dL) compared to IgG levels (median=535.0 mg/dL) among IgG MM patients (N=40) who are in CR and show higher serum BCMA (>10 ng/mL; p<0.0001).

5 **Example 4: Serum IgG Levels of Patients With IgG MM Who Have Achieved Complete Remission Based on Their Serum BCMA Levels**

Serum from patients with IgG MM who have achieved CR was obtained and the serum IgG and BCMA levels were analyzed. IgG levels of patients with IgG MM who have achieved CR with no measurable myeloma correlate with their serum
10 BCMA levels. IgG MM patients (N=47) who are in CR who show low serum BCMA (≤10 ng/mL; p<0.0001) have significantly decreased IgG levels (median=402.0 mg/dL) compared to IgG levels (median=643.5 mg/dL) among IgG MM patients (N=84) who are in CR and show higher serum BCMA (>10 ng/mL; p<0.0001).

15 **Example 5: Serum IgA Levels of Patients With IgG MM Who Have Achieved Complete Remission Based on Their Serum BCMA Levels**

Serum from patients with IgG MM who have achieved CR was obtained and the serum IgA and BCMA levels were analyzed. Uninvolved, normal IgA levels of patients with IgG MM who have achieved CR correlate with their serum BCMA levels.
20 IgG MM patients (N=47) who are in CR and low serum BCMA levels (≤10 ng/mL; p<0.0001) show significantly decreased IgA levels (median=26.0 mg/dL) compared to IgA levels (median=61.0 mg/dL) in IgG MM patients (N=84) who are in CR and show higher serum BCMA (>10 ng/mL; p<0.0001).

25 **Example 6: Serum IgM Levels of Patients With IgG MM Who Have Achieved Complete Remission Based on Their Serum BCMA Levels**

Serum from patients with IgG MM who have achieved CR was obtained and the serum IgM and BCMA levels were analyzed. Uninvolved, normal IgM levels of patients with IgG MM who have achieved CR correlate with their serum BCMA
30 levels. IgG MM patients (N=47) who are in CR and show low serum BCMA (≤10 ng/mL; p<0.0001) have significantly decreased IgM levels (median=11.0 mg/dL)

compared to IgM levels (median=32.5 mg/dL) in IgG MM patients (N=84) who are in CR and show higher serum BCMA levels (>10 ng/mL; p<0.0001).

**Example 7: Serum BCMA Levels of Patients Based on Diagnosis of**
5 **Immunodeficiency**

Serum from patients with immunodeficiency diseases and control healthy human subjects was obtained, and serum BCMA levels were analyzed and compared. Levels of serum BCMA were substantially lower in patients with immunodeficiency (XLA, CVID, IgG deficiency, IgA deficiency, IgM deficiency,
10 Hyper IgM syndrome, PRH, or Crohn's disease) compared to serum BCMA levels in control subjects.

| *Immunodeficiency Disease* | *Median Serum BCMA Levels (ng/mL)* |
|---|---|
| XLA (N=8) | 2.3 |
| CVID (n=48) | 7.16 |
| IgG Deficiency (N=3) | 19.1 |
| IgA Deficiency (N=4) | 37.58 |
| IgM Deficiency (N=1) | 25.89 |
| Hyper IgM Syndrome (N=2) | 13.2 |
| Crohn's Disease (N=1) | 70.83 |
| Control (N=119) | 35.20 |

15 **Example 8: Serum BCMA Levels of Patients With Immunodeficiency vs. Normal Healthy Donors**

Serum from patients with immunodeficiency diseases and normal healthy donors was obtained, and serum BCMA levels were analyzed and compared. Serum BCMA levels of patients with immunodeficiency (N=68) were significant lower
20 (7.3 ng/mL (range; 0.84 ng/mL – 189.5 ng/mL); p<0.0001) compared to serum BCMA levels (35.2 ng/mL (range; 12.2 ng/mL – 958.1 ng/mL); p<0.0001) in normal healthy donors (N=119).

**Example 9: Serum BCMA Levels of Patients Based on Diagnosis of Immunodeficiency**

Serum from patients with immunodeficiency diseases and control healthy human subjects was obtained, and serum BCMA levels were analyzed and
5 compared. Levels of serum BCMA were substantially lower in patients with immunodeficiency (XLA, CVID, CVID + Lymphoma, CVID + Tx Lymphoma, IgG deficiency, IgA deficiency, IgM deficiency, Hyper IgM syndrome, PI3KD, LRBA/LRBA, or Thymoma) compared to serum BCMA levels in control subjects.

10

| *Immunodeficiency Disease* | *Median Serum BCMA Levels (ng/mL)* |
|---|---|
| XLA (N=1) | 2.21 |
| CVID (N=27) | 6.33 |
| CVID + Lymphoma (N=1) | 40.12 |
| CVID + Tx Lymphoma (N=1) | 8.25 |
| IgG Deficiency (N=3) | 5.57 |
| IgA Deficiency (N=1) | 56.64 |
| IgM Deficiency (N=1) | 9.35 |
| Hyper IgM Syndrome (N=1) | 5.54 |
| PI3KD (N=1) | 1.38 |
| LRBA/LRBA (N=1) | 17.39 |
| Thymoma (N=1) | 15.25 |
| Control (N=4) | 20.72 |

| *Immunodeficiency Disease* | *Median Serum BCMA Levels (ng/mL)* |
|---|---|
| XLA (N=9) | 2.21 |
| CVID (N=75) | 7.11 |
| CVID + Lymphoma (N=1) | 40.12 |
| CVID + Tx Lymphoma (N=1) | 8.25 |
| IgG Deficiency (N=6) | 16.28 |

| | |
|---|---|
| IgA Deficiency (N=5) | 44.24 |
| IgM Deficiency (N=2) | 17.62 |
| Hyper IgM Syndrome (N=3) | 10.94 |
| PI3KD (N=1) | 1.38 |
| LRBA/LRBA (N=1) | 17.39 |
| Thymoma (N=1) | 15.25 |
| Control (N=123) | 34.11 |

**Example 10: Serum BCMA Levels of Patients Based on Diagnosis of Immunodeficiency**

Serum from patients with immunodeficiency diseases and control healthy human subjects was obtained, and serum BCMA levels were analyzed and
5 compared. Levels of serum BCMA were substantially lower in patients with immunodeficiency (XLA, CVID, CVID + Lymphoma, CVID + Tx Lymphoma, IgG deficiency, IgA deficiency, IgA, IgA + IgG, IgA + IgG2, IgM deficiency, or Hyper IgM syndrome) compared to serum BCMA levels in control subjects.

| *Immunodeficiency Disease* | *Median Serum BCMA Levels (ng/mL)* |
|---|---|
| XLA (N=1) | 2.21 |
| CVID (N=27) | 6.33 |
| CVID + Lymphoma (N=1) | 40.12 |
| CVID + Tx Lymphoma (N=1) | 8.25 |
| IgG Deficiency (N=3) | 5.57 |
| IgA Deficiency (N=1) | 56.64 |
| IgM Deficiency (N=1) | 9.35 |
| Hyper IgM Syndrome (N=1) | 5.54 |
| IgA (N=5) | 33.09 |
| IgA + IgG (N=1) | 7.85 |
| IgA + IgG2 (N=3) | 14.03 |
| Control (N=4) | 20.72 |

10

| *Immunodeficiency Disease* | *Median Serum BCMA Levels (ng/mL)* |
|---|---|
| XLA (N=9) | 2.21 |
| CVID (N=75) | 7.11 |
| CVID + Lymphoma (N=1) | 40.12 |
| CVID + Tx Lymphoma (N=1) | 8.25 |
| IgG Deficiency (N=6) | 16.28 |
| IgA Deficiency (N=5) | 44.24 |
| IgA (N=1) | 33.09 |
| IgA + IgG (N=1) | 7.85 |
| IgA + IgG2 (N=1) | 14.03 |
| IgM Deficiency (N=2) | 17.62 |
| Hyper IgM Syndrome (N=3) | 10.94 |
| Control (N=123) | 34.11 |

## CLAIMS

1. A method of monitoring immune status of a subject, comprising:

(a) detecting an amount of BCMA polypeptide or a fragment thereof in a biological sample obtained from the subject; and

(b) comparing the amount of BCMA polypeptide or a fragment thereof detected in (a) to a predetermined cut-off value or to an amount detected in a control serum sample, wherein a decreased amount of BCMA polypeptide or a fragment thereof in the biological sample of the subject as compared to the predetermined cut-off value or amount in the control serum or plasma sample is indicative of an impaired immune system,

wherein the biological sample is a serum or plasma sample or supernatant obtained from a culture of the subject's bone marrow mononuclear cells or peripheral blood mononuclear cells.

2. The method of claim 1, wherein the BCMA fragment is a cleaved BCMA polypeptide.

3. The method of claim 1, wherein the BCMA polypeptide or a fragment thereof comprises the amino acid sequence of SEQ ID NO:1.

4. The method of claim 1, wherein the BCMA polypeptide or a fragment thereof comprises an amino acid sequence having at least about 90% identity with SEQ ID NO:1.

5. The method of claim 1, wherein the BCMA polypeptide or a fragment thereof comprises an amino acid sequence having at least about 80% identity with SEQ ID NO:1.

6. The method of claim 1, wherein the BCMA polypeptide or a fragment thereof comprises an amino acid sequence having at least about 75% identity with SEQ ID NO:1.

7. The method of claim 1, wherein the BCMA polypeptide or a fragment thereof is detected using a detection system selected from the group consisting of: an immunohistochemistry, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), enzyme immunoassay (EIA), fluorescence immunoassay (FIA), luminescence immunoassay (LIA), lateral flow assay, or strip assay.

8. The method of claim 7, wherein the detection system is a lateral flow assay.

9. The method of claim 1, wherein the detection is performed using an antibody specific for BCMA polypeptide or a fragment thereof.

10. The method of claim 9, wherein the antibody specific for BCMA polypeptide or a fragment thereof is a monoclonal antibody.

11. The method of claim 9, wherein the antibody specific for BCMA polypeptide or a fragment thereof is a polyclonal antibody.

12. The method of claim 1, wherein the impaired immune system is the result of an immunodeficiency disease.

13. The method of claim 12, wherein the immunodeficiency disease includes, but is not limited to, Acquired Immune Deficiency Syndrome (AIDS), Ataxia telangiectasia, Chediak Higashi Syndrome, Common Variable Immune Deficiency (CVID), Combined Immunodeficiency Disease, Complement deficiencies, DiGeorge Syndrome, Hypogammaglobulinemia, Job Syndrome, Leukocyte Adhesion Deficiency, Panhypogammaglobulinemia, X-linked Agammaglobulinemia Disease (Bruton's disease), Congenital Agammaglobulinemia, Selective Deficiency of IgA, Wiskott Aldrich Syndrome, Chronic Granulomatous Disease, Severe Combined Immunodeficiency Disease, Hyper Immunoglobulin E Syndrome (Job's Syndrome), Hyper IgM Syndrome, X-linked

agammaglobulinemia (XLA), Crohn's disease, Thymoma, immunodeficiencies associated with mutations in the LRBA gene, or immunodeficiencies associated with PI3KD.

14. A method of monitoring immune status of a subject, comprising:

(a) detecting an amount of BCMA polypeptide or a fragment thereof in a biological sample obtained from the subject; and

(b) comparing the amount of BCMA polypeptide or a fragment thereof detected in (a) to a predetermined cut-off value or to an amount detected in a control serum sample, wherein an increased amount of BCMA polypeptide or a fragment thereof in the biological sample of the subject as compared to the predetermined cut-off value or amount in the control serum sample indicates that the subject is at higher risk of or suffering from an infection or an immune deficiency-related disease,

wherein the biological sample is a serum or plasma sample or supernatant obtained from a culture of the subject's bone marrow mononuclear cells or peripheral blood mononuclear cells.

15. A method of monitoring immune status of a subject, comprising:

(a) detecting an amount of BCMA polypeptide or a fragment thereof in a biological sample obtained from the subject; and

(b) comparing the amount of BCMA polypeptide or a fragment thereof detected in (a) to a predetermined cut-off value or to an amount detected in a control serum or plasma sample, wherein a decreased amount of BCMA polypeptide or a fragment thereof in the biological sample of the subject as compared to the predetermined cut-off value or amount in the control serum or plasma sample is indicative of an impaired immune system, and an increased amount of BCMA polypeptide or fragment in the biological sample of the subject as compared to the predetermined cut-off value or amount in the control serum sample indicates that the subject is at higher risk of or suffering from an infection or a disease,

wherein the biological sample is a serum or plasma sample or supernatant obtained from a culture of the subject's bone marrow mononuclear cells or peripheral blood mononuclear cells.

16. A kit for monitoring immune status of a subject, comprising a reagent suitable for determining levels of BCMA polypeptide or a fragment thereof in a biological sample obtained from the subject, wherein the biological sample is a serum sample or supernatant obtained from culture of the subject's bone marrow mononuclear cells or peripheral blood mononuclear cells.

17. The kit of claim 16, comprising an antibody specific for BCMA polypeptide or fragment thereof.

18. The kit of claim 17, wherein the antibody specific for BCMA polypeptide or fragment thereof is a monoclonal antibody.

19. The kit of claim 17, wherein the antibody specific for BCMA polypeptide or fragment thereof is a polyclonal antibody.

20. The kit of claim 16, wherein the kit comprises a detection system selected from the group consisting of: ELISA assay, RIA assay, EIA assay, FIA assay, LIA assay, lateral flow assay, or strip assay.

21. A method of monitoring response to a treatment of a subject, comprising:

(a) detecting an amount of BCMA or a fragment thereof in a biological sample obtained from a subject at a time point prior to start of the treatment;

(b) detecting an amount of BCMA or a fragment thereof in the biological sample obtained from the at a time point subsequent to start of the treatment; and

(c) comparing the amount of BCMA polypeptide or a fragment thereof detected in (a) to the amount of BCMA polypeptide or a fragment thereof detected in (b), wherein an decreased amount of BCMA polypeptide or a fragment thereof detected in (b) as compared to the amount of BCMA polypeptide or a fragment thereof detected in (a) indicates that the subject is responding to treatment, and wherein an increased or unchanged amount of BCMA polypeptide or a fragment thereof detected in (b) as compared to the amount of

BCMA polypeptide or a fragment thereof detected in (a) indicates that the subject is not responding to treatment,

wherein the biological sample is a serum sample or supernatant obtained from a culture of the subject's bone marrow mononuclear cells or peripheral blood mononuclear cells.

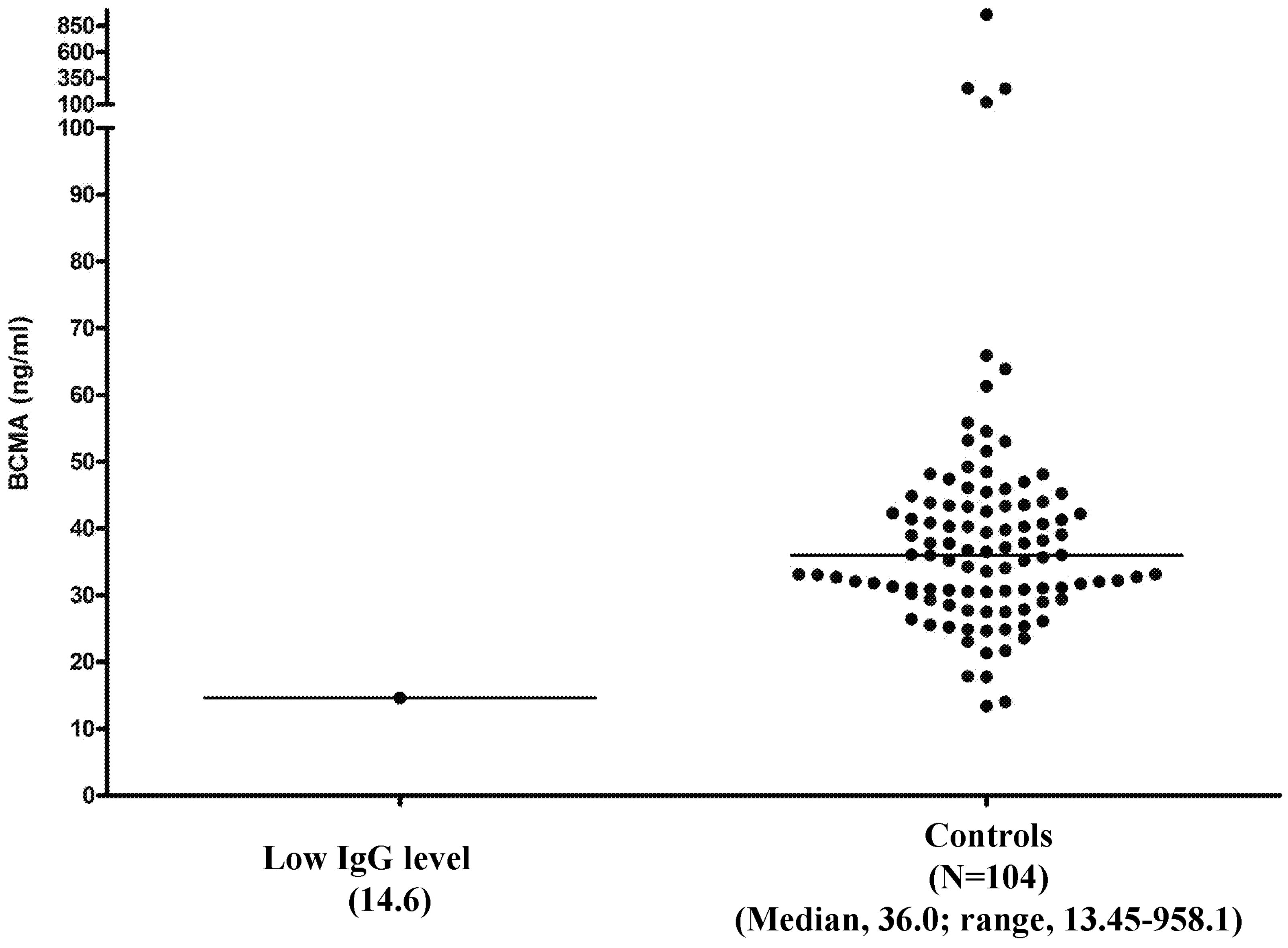
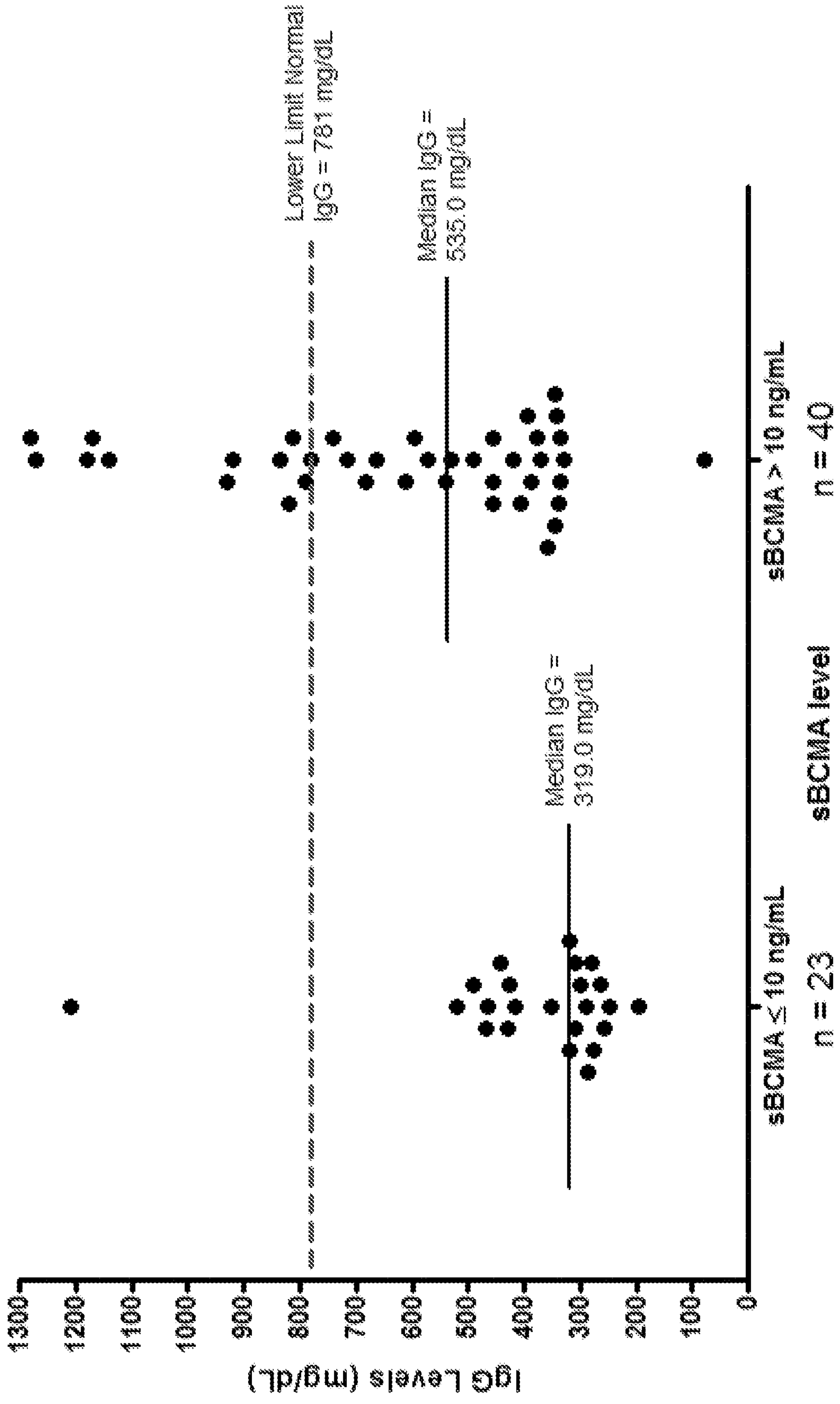
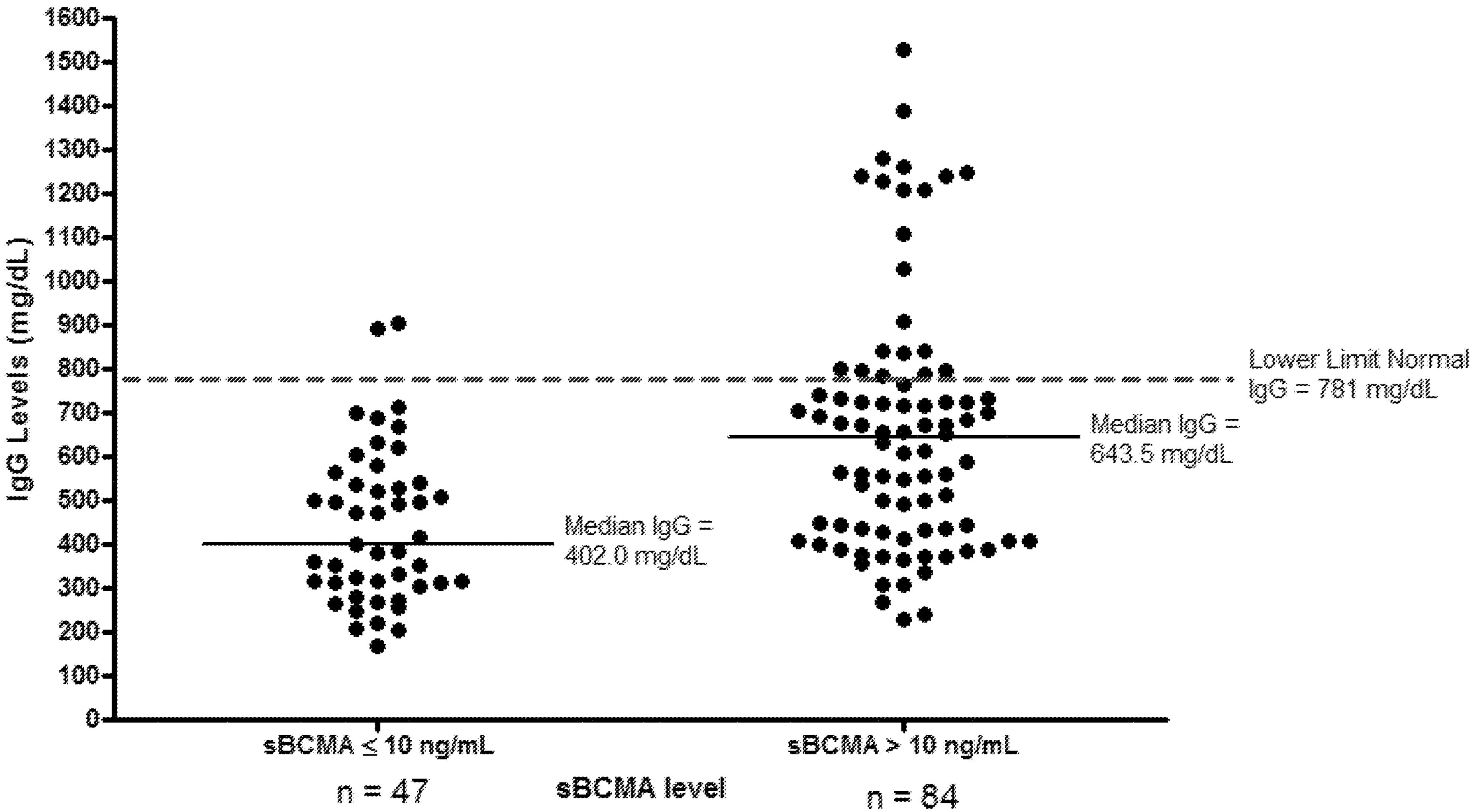



*FIG. 1*




*sBCMA ≤ 10 ng/mL vs sBCMA > 10 ng/mL; p<0.0001

*FIG. 2*




*sBCMA ≤ 10 ng/mL vs. sBCMA > 10 ng/mL: p<0.0001

*FIG. 3*

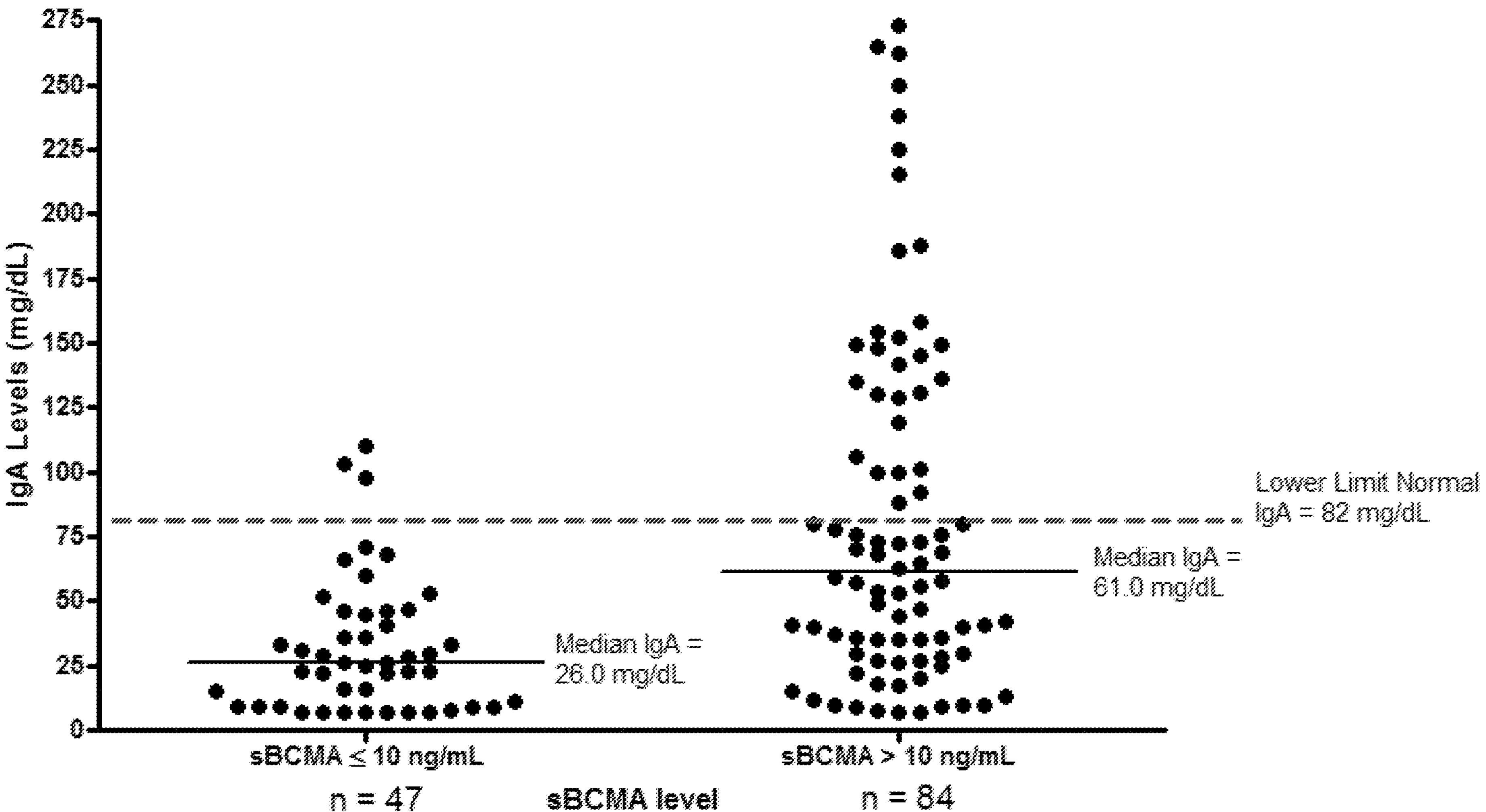



*FIG. 4*

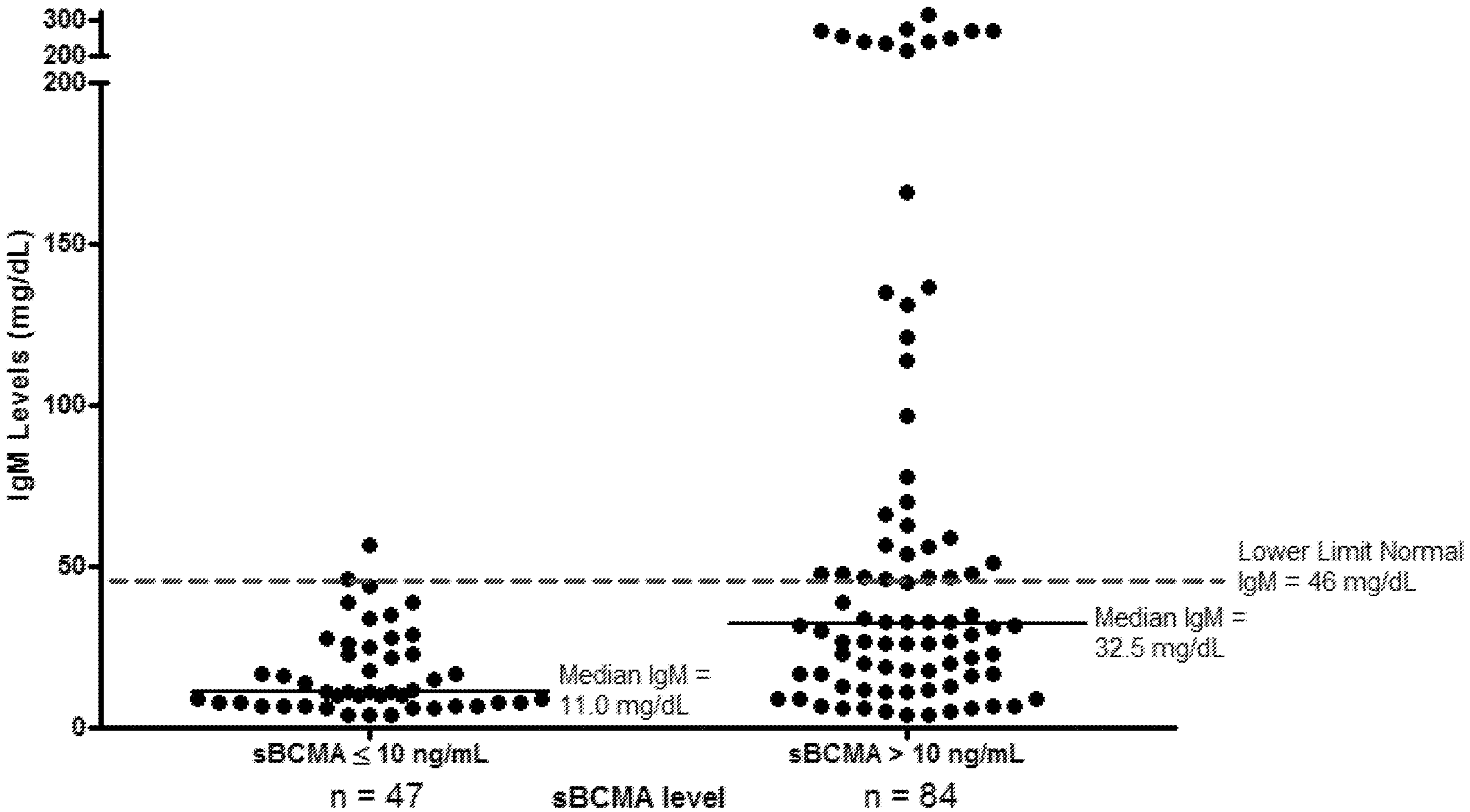



*FIG. 5*

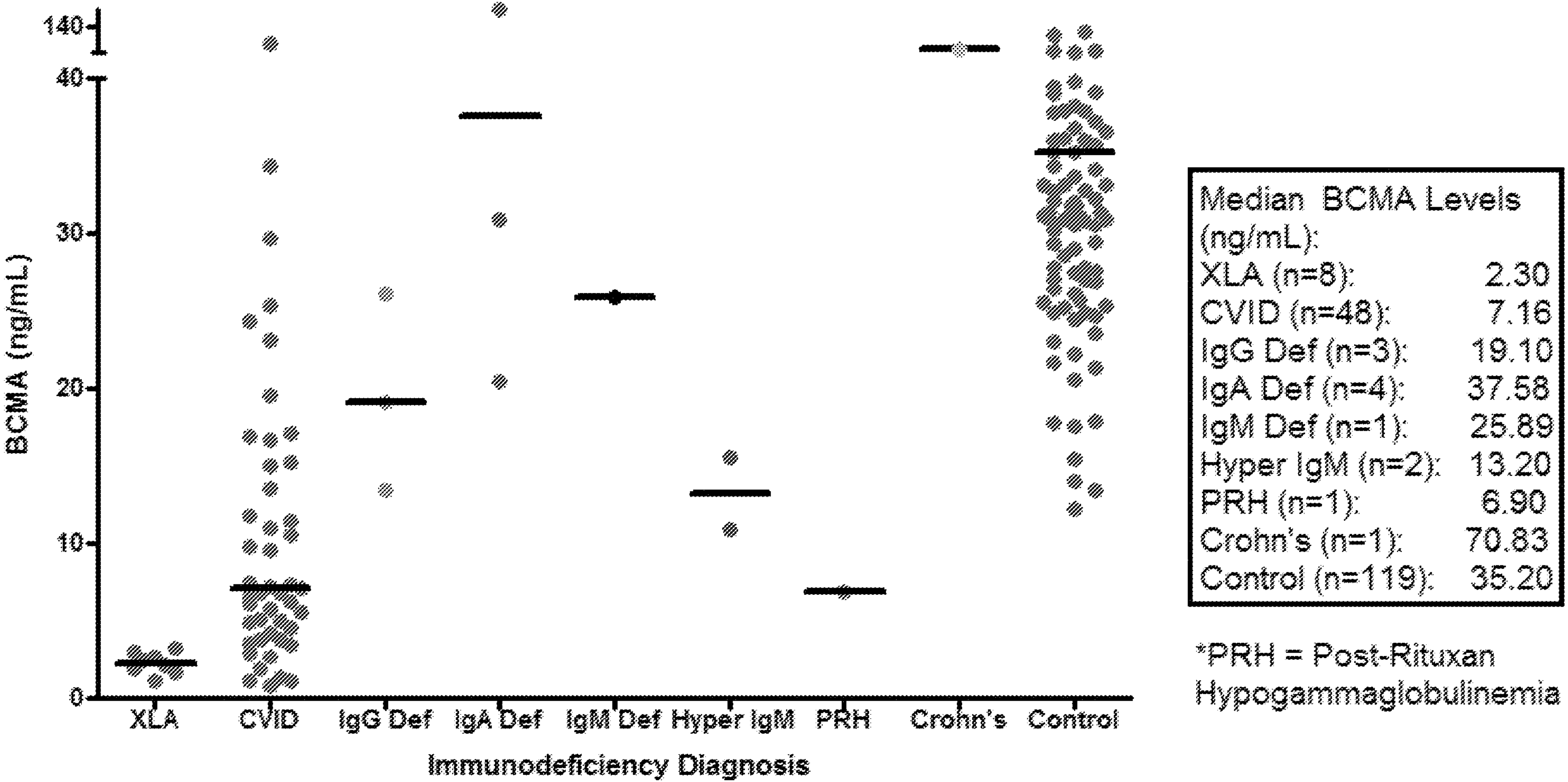



*FIG. 6*

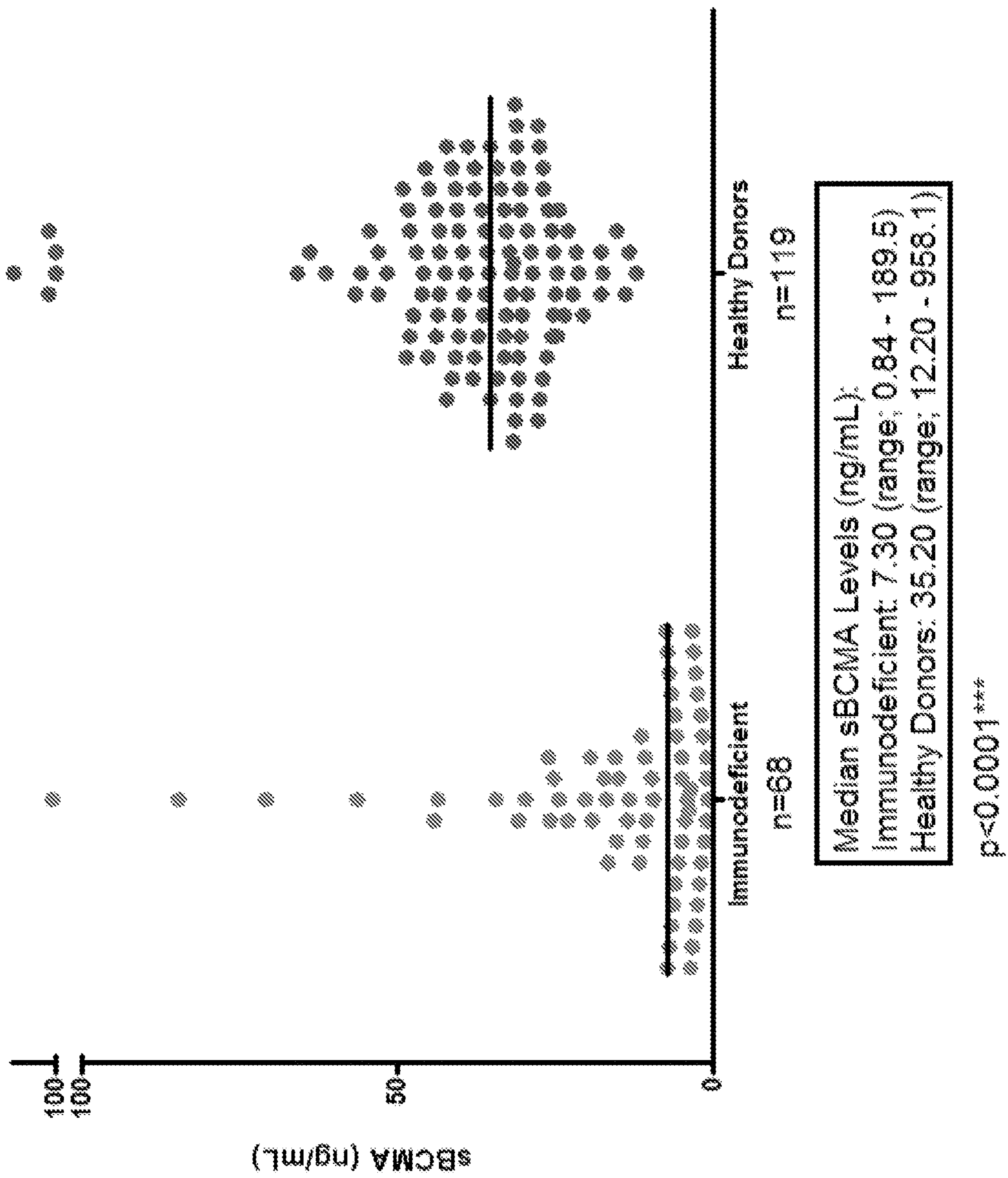



*FIG. 7*

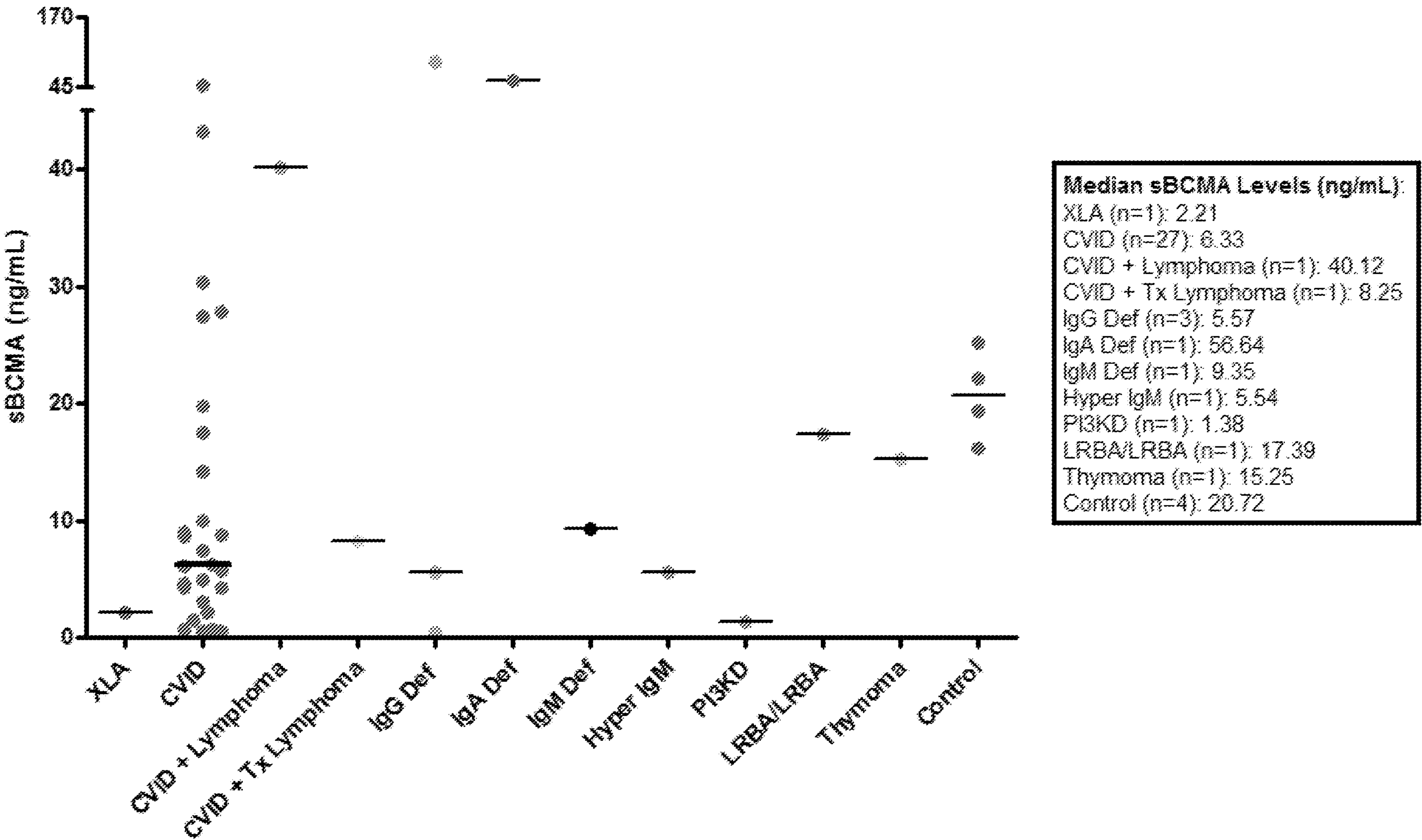



*FIG. 8*

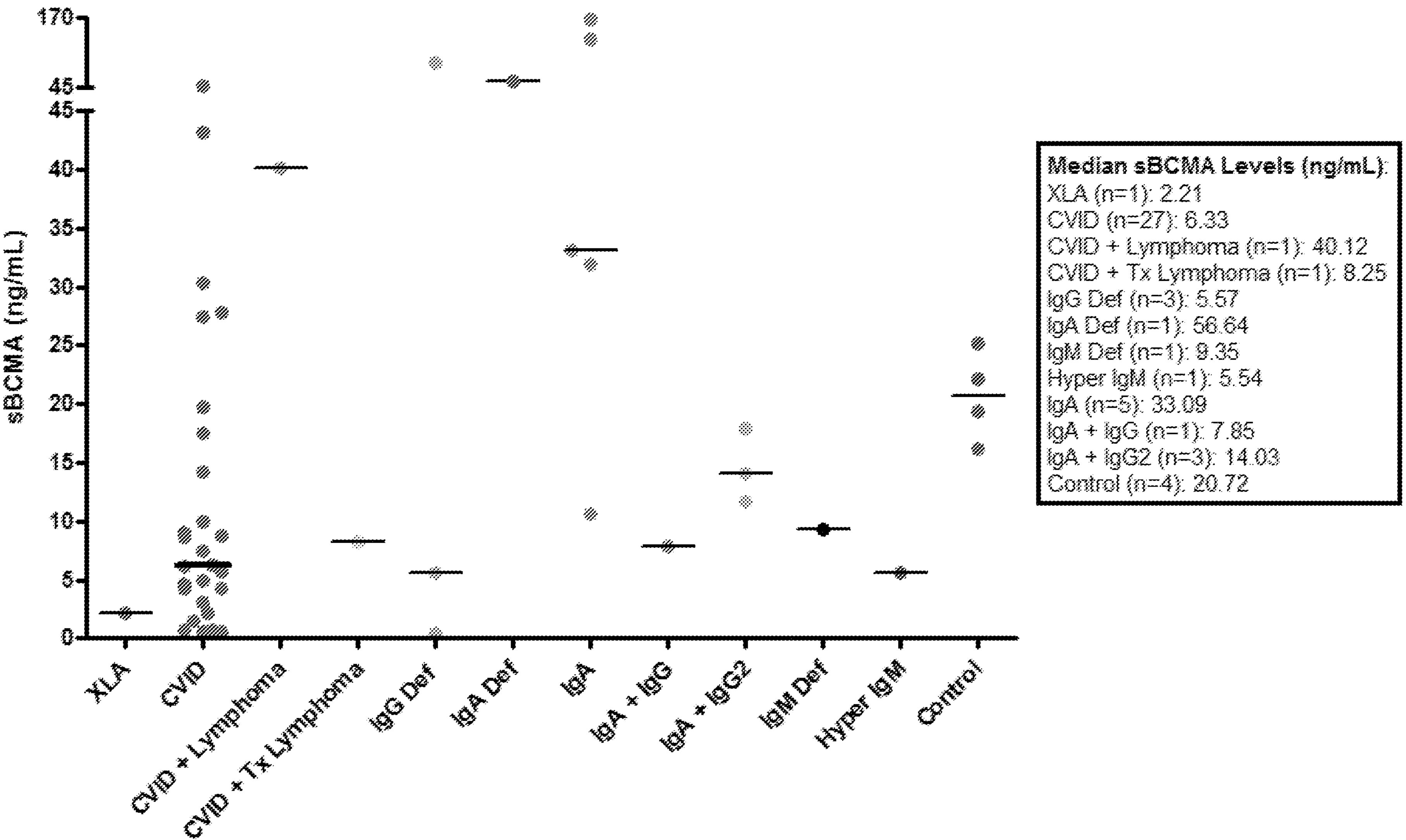



*FIG. 9*

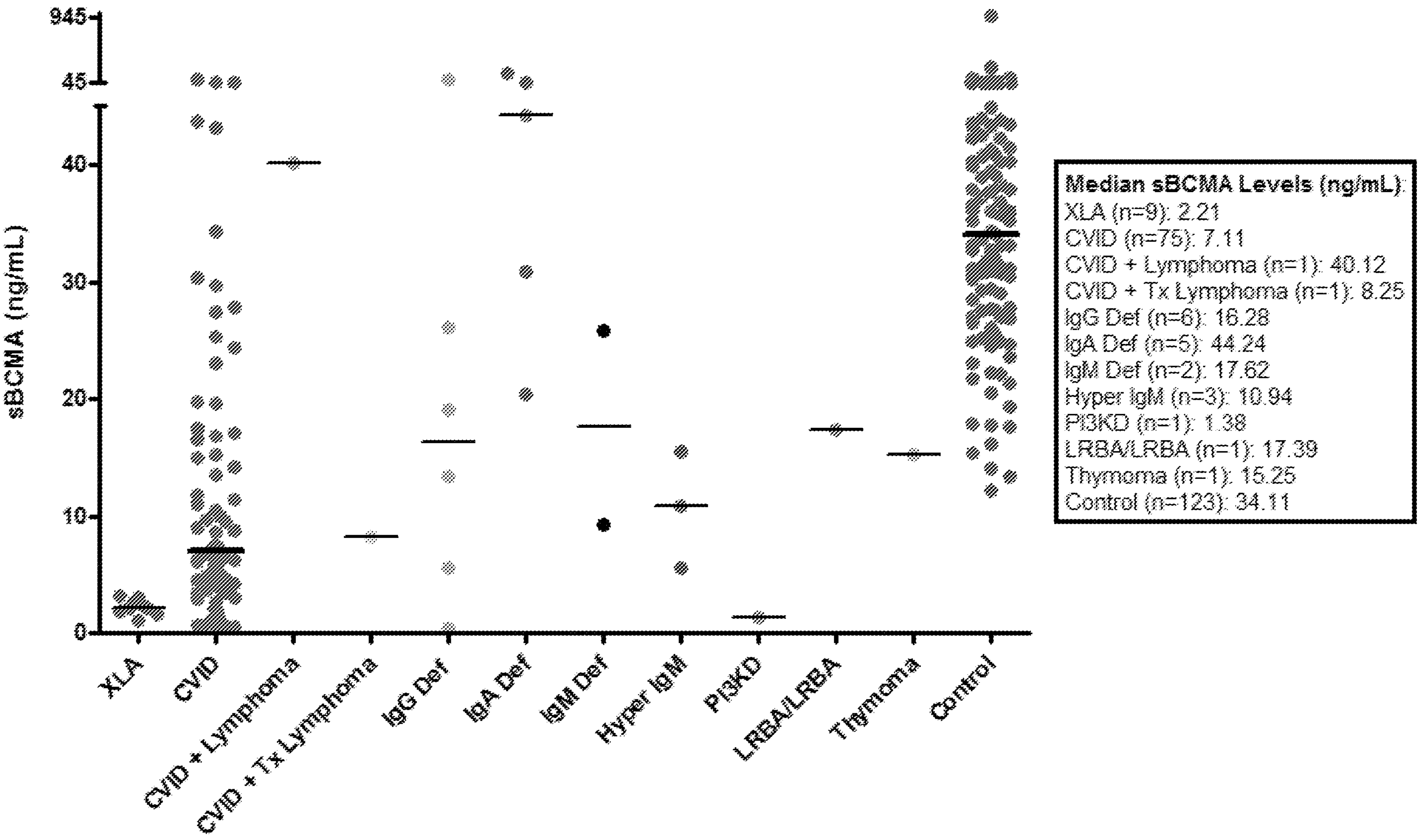



*FIG. 10*

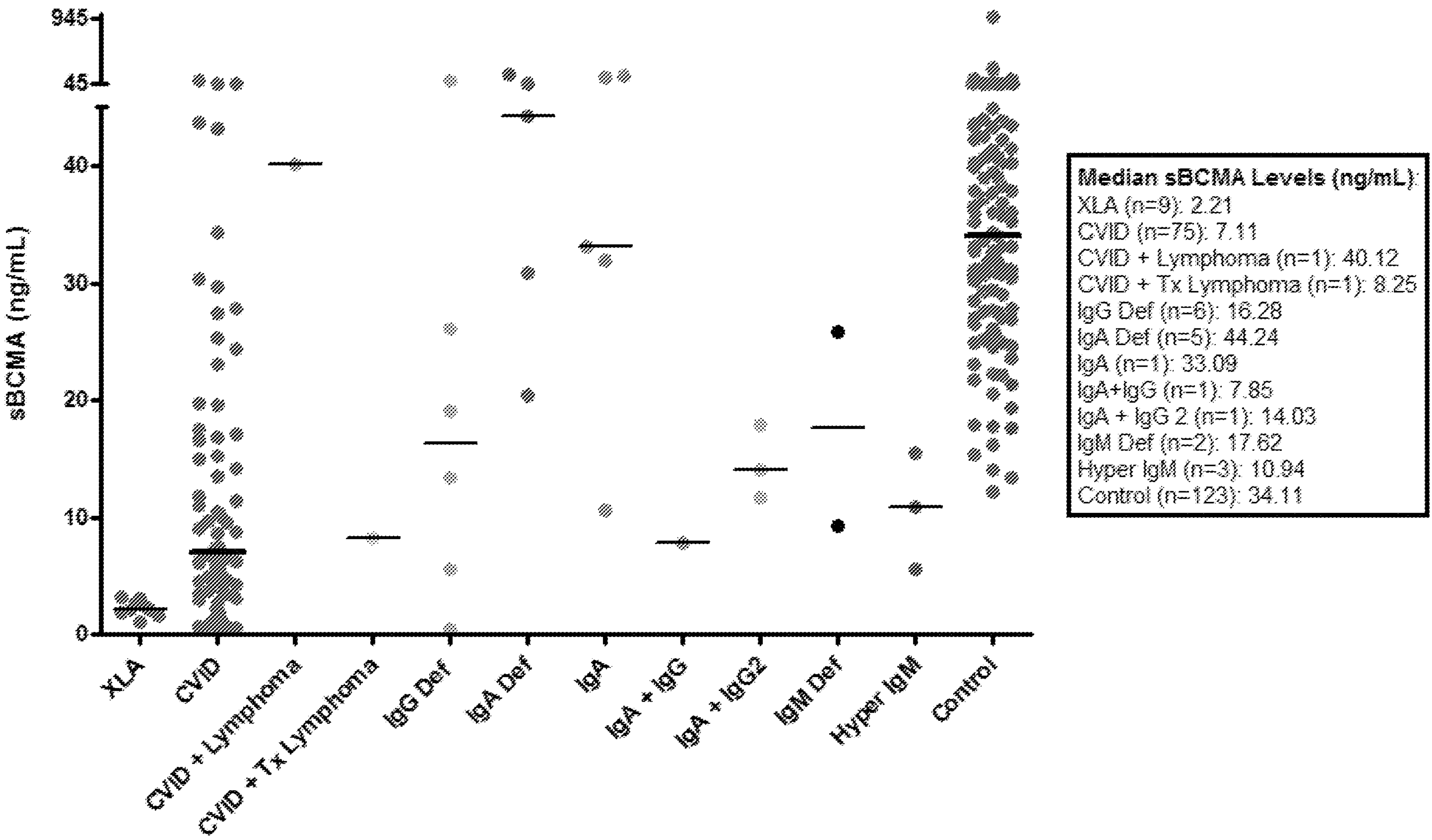



*FIG. 11*

## INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/013169

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/395; C07K 16/28; C12N 15/12; G01N 33/53; G01N 33/68 (2017.01)
CPC - A61K 2039/505; C07K 14/70578; C07K 16/2878; G01N 33/53; G01N 2800/52 (2017.02)

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)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/130.1; 435/7.1; 530/387.1 (keyword delimited)

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>--<br>Y | WO 2014/124280 A1 (INSTITUTE FOR MYELOMA & BONE CANCER RESEARCH) 14 August 2014 (14.08.2014) entire document | 16-21<br>------<br>14 |
| Y | HAMBLIN et al. "The Immunodeficiency of Chronic Lymphocytic Leukaemia," British Medical Bulletin, 27 August 2008 (27.08.2007), Vol. 87, Pgs. 49-62. entire document | 14 |
| A | US 2009/0191203 A1 (BELLOIR et al) 30 July 2009 (30.07.2009) entire document | 1-21 |
| A | US 2014/0193433 A1 (BORGES et al) 10 July 2014 (10.07.2014) entire document | 1-21 |
| A | WO 2015/166073 A1 (MAX-DELBRÜCK-CENTRUM FÜR MOLEKULARE MEDIZIN ) 05 November 2015 (05.11.2015) entire document | 1-21 |
| A | US 2014/0220014 A1 (UNIVERSITY OF WASHINGTON et al) 07 August 2014 (07.08.2014) entire document | 1-21 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 12 March 2017 | 10 APR 2017 |

| Name and mailing address of the ISA/US | Authorized officer |
|---|---|
| Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, VA 22313-1450<br>Facsimile No. 571-273-8300 | Blaine R. Copenheaver<br>PCT Helpdesk: 571 272-4300<br>PCT OSP: 571-272-7774 |

patsnap

| 专利名称(译) | 用于监测受试者免疫状态的改进方法 | | |
| --- | --- | --- | --- |
| 公开(公告)号 | EP3402515A1 | 公开(公告)日 | 2018-11-21 |
| 申请号 | EP2017738918 | 申请日 | 2017-01-12 |
| [标]申请(专利权)人(译) | 贝伦森JAMES - [R | | |
| 申请(专利权)人(译) | 贝伦森，詹姆斯R. | | |
| 当前申请(专利权)人(译) | 贝伦森，詹姆斯R. | | |
| [标]发明人 | BERENSON JAMES R | | |
| 发明人 | BERENSON, JAMES, R. | | |
| IPC分类号 | A61K39/395 C07K16/28 C12N15/12 G01N33/53 G01N33/68 | | |
| CPC分类号 | G01N33/5091 C07K16/2878 G01N33/6893 G01N2800/24 | | |
| 优先权 | 62/300708 2016-02-26 US<br>62/277801 2016-01-12 US | | |
| 其他公开文献 | EP3402515A4 | | |
| 外部链接 | Espacenet | | |

**摘要(译)**

本发明一般提供用于监测受试者免疫状态的改进的组合物和方法。特别地，本发明提供了用于检测受试者中的BCMA以可靠地监测受试者的免疫状态的方法。